BMJ Open

Clonidine as analgesia during retinopathy of prematurity screening in preterm infants (cloROP), a protocol for a randomized controlled trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-064251
Article Type:	Protocol
Date Submitted by the Author:	26-Apr-2022
Complete List of Authors:	Carlsen Misic, Martina; Örebro University, Faculty of Medicine and Health; Örebro Universitet, Department of Pediatrics Eriksson, Mats; Örebro University, Sweden, Faculty of Medicine and Health, School of Health Sciences Normann, Erik; Uppsala Universitet Medicinska fakulteten, Department of Womens and Childrens health Pettersson, Miriam; Örebro University, Blomqvist, Y; Uppsala Universitet Medicinska fakulteten, Department of Womens and Childrens health Olsson, Emma ; Örebro University, Faculty of Medicine and Health; Örebro Universitet, Department of Pediatrics
Keywords:	Neonatal intensive & critical care < INTENSIVE & CRITICAL CARE, PAIN MANAGEMENT, Paediatric ophthalmology < OPHTHALMOLOGY

Clonidine as analgesia during retinopathy of prematurity screening in preterm infants (cloROP), a protocol for a randomized controlled trial

Martina Carlsen Misic[1,2], Mats Eriksson[2], Erik Normann[3,4], Miriam Pettersson[1,5], Ylva Thernström Blomqvist[3,4], Emma Olsson[1,2]

1. Department of Pediatrics, Faculty of Medicine and Health, Örebro University, Örebro, Sweden
2. Faculty of Medicine and Health, School of Health Sciences, Örebro University, Örebro, Sweden
3. University Hospital, Neonatal Intensive Care Unit, Uppsala, Sweden
4. Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden
5. Faculty of Medicine and Health, School of Medical Sciences, Örebro University, Örebro, Sweden

Corresponding author:

Emma Olsson

Adress: Faculty of Medicine and Health, School of Health Sciences, Örebro University, S-701 82, Örebro, Sweden

Email: emma.olsson@oru.se

Abstract

Introduction

Preterm infants are at risk of negative consequences from stress and pain at the same time as they often are in need of intensive care that includes painful interventions. One of the frequent painful procedures preterm infants undergo is eye examination screening to detect early signs of ROP (retinopathy of prematurity). These examinations are both stressful and painful, and despite a multitude of research studies no conclusive pain-relieving treatment has been demonstrated. The main aim of this trial is to investigate the analgesic effect of clonidine during ROP eye examinations.

Methods and analysis

The planned study is a multi-center randomized controlled trial with a crossover design. Infants will be recruited from two different neonatal intensive care units in Sweden. Infants born before gestation week 30 (and therefor eligible for ROP screening) and cared for in either of the NICUs will be eligible for inclusion in the study. The primary outcome will be PIPP-R (Premature Infant Pain Profile – revised) score within 30 seconds after starting the examination. Secondary outcomes will be changes in the GSR (Galvanic Skin Response)-parameters (area small peaks, area huge peaks, peaks per second and average rise time) within 30 seconds after starting the eye examination together with the number and evaluation of adverse events reported within 72 hours after the examination and the examining physician´s assessment of how easy the infant was to examine.

Ethics and dissemination

Approval from the Swedish Ethical Review Authority has been obtained for the study. Parents of eligible infants will be getting both verbal and written information about the study including that participation is voluntary. Data will be collected and treated in accordance with the European General Data Protection regulation. The results will be reported on group level and published in a scientific journal.

Strengths and limitation of this study

- To date there are no practical and effective pain relieving methods for ROP screening described.
- The study aims att evaluating a promising method to reduce pain in premature infants.
- The randomized cross-over design is the most effective and scientific sound method to investigate pain relief for repetitive painful procedures.

Key-words:

Trial registration

ClinicalTrials NCT04902859

EudraCT 2021-003005-21

Introduction

Preterm infants are sensitive to stimuli and are at risk for short- and long-term negative consequences from stress and pain. They are often in need of intensive care that includes painful interventions. Repeated painful experiences during the neonatal period can result in future negative effects in pain response [1], brain development [2, 3], cognitive functioning [4] and cortisol levels [5]. These infants are also at risk of secondary complications from their prematurity, one being retinopathy of prematurity (ROP). This condition is caused by abnormal blood vessel growth that can progress to retinal detachment and blindness. A low gestational age and low birthweight are risk factors for ROP [6] and all infants born before gestation week 30 are therefore screened with regular eye examinations until the retina is fully developed [7].

The eye examinations are painful and stressful for the preterm infant. The infant need to be restrained during the examination and bright light as well as physical manipulation of the eye with eye speculum are some of the aspects that can be painful and stressful [8]. The mydriatic eyedrops used before the examination also causes pain [9].

Considerable amount of research has examined a variety of potentially pain-relieving interventions during ROP eye examinations without finding a effective treatment. Studies on oral sweet solutions that are effective as pain relief during other painful procedures have shown inconclusive results [10, 11, 12, 13] and a few that have shown significantly lower pain scores with sweet solutions are still showing pain scores within the limits for severe pain in the experiment group [14, 15]. Nonpharmacological interventions such as skin-to-skin contact [16] and facilitated tucking [17] have also shown an inadequate pain relief as well as inhaled nitrous oxide [18]. A randomized controlled trial investigating morphine as pain relief during ROP screening had to be terminated due to adverse effects [19]. Despite multimodal techniques the pain relief during ROP eye examinations seem to be insufficient [20, 21, 22, 23], leaving preterm infants exposed to repeated untreated pain.

Clonidine is an alpha2-agonist that is used increasingly in neonatal care due to its safe sedating and pain-relieving properties [24], without the risk of respiratory depression [25, 24]. The use of clonidine

can also reduce the need for other sedation and pain-relieving medication [26] and some data also indicates neuroprotective capacities [27].

Considering the pain and stress during ROP eye examinations and as of today the lack of effective pain relief methods the aim of this study will be to investigate the pain-relieving effect of clonidine during ROP eye examinations.

Methods and analysis

Study design

This study is a multi-center randomized controlled trial with a crossover design. Infants will be recruited from two different neonatal intensive care units (NICUs) in university hospitals the central part of Sweden (NICU A and NICU B). Infants born before gestation week 30 (and therefore undergoing ROP screening) and cared for in either of the NICUs will be eligible for inclusion in the study. With a power of 0.8 and a significance level of 0.05 eighteen infants are needed to detect a difference of 2 steps or more in the primary outcome variable PIPP-R (Premature Infant Pain Profile – Revised), and with consideration of potential dropouts the aim is to include 25 infants. Because of different examination techniques at the respective units, we plan to include 25 infants at each unit. In unit A RetCam (Clarity Medical Systems, Pleasanton, CA, USA) is used for ROP screening while direct ophthalmoscopy is used almost exclusively at unit B.

Exclusion criteria

- Infants that have received pain relieving analgetics, sedatives or β-blockers within 24 hours before the eye examination
- Previous documented renal failure
- Infants without gastric tube
- Infants with known heart arrythmias or neurologic deficit

- Infants with circulatory instability (assessed as a mean arterial blood pressure below infants' gestation age in weeks)

The study will be monitored by an independent monitor appointed by the sponsor. Monitoring will be performed according to risk- based monitoring and a monitoring plan.

Procedure

Parents of eligible infants will receive written and verbal information about the study before the first scheduled eye examination. The principal investigator on each site will obtain informed consent from the parents. The infant will serve as his or her own control and will be examined according to the units' policy. After parental consent the infant´s first two eye examinations will be included in the study. To minimize potential effects from previous experience the order of the treatment (clonidine or placebo) will be randomized for each infant using an online random sequence generator that gives the order. The allocation for the treatments will be kept in opaque envelopes and an unblinded nurse or pharmacist, not involved in the study, will prepare the study solution (clonidine or sterile water) in a syringe marked with the patient's study ID. A document with the included patients´ allocations will be kept in a closed envelope inside a locked room in the respective NICUs in order to make unblinding possible if needed.

Sixty minutes before the first eye examination the infant will receive clonidine 4 mcg/kg (intervention) or sterile water (placebo) in equivalent volumes in the nasogastric tube by a nurse blinded for the content. Everyone else in the room will be blinded for the content of the syringe as well as the researcher performing the subsequent analyses. Mydriatic eye drops (cyclopentolate 0.5% and phenylephrine 0.5%) will be administered according to clinical routine at 45 and 30 min before the procedure. The infant will be connected to a probe measuring oxygen saturation and heart rate on one foot and three electrodes measuring GSR (galvanic skin response) on the other foot. The ophthalmologist will then examine the eyes with either RetCam or direct ophthalmoscopy according to the respective units' guidelines. In addition to the study solution the infant will receive standard care with facilitated tucking and a pacifier. The infants' face, expressions, and heart rate and oxygen

saturation values from the monitor display, will be videorecorded by two cameras before, during and after the examination for subsequent PIPP-R assessments. Afterwards the examining ophthalmologist will estimate how easy the infant was to examine by marking an "X" on a 10 cm long horizontal line where one end indicates "very easy to examine" and the other end "very difficult to examine". The ophthalmologist can also write any other comments in free text in the same document. The same procedure will then be executed during the infants' second eye examination with the study solution (intervention or placebo) he or she did not receive the first time.

Primary outcome

The primary outcome is the PIPP-R (Premature Infant Pain Profile – Revised) [28] score within 30 seconds after starting the eye examination. PIPP-R is a pain assessment scale for procedural pain consisting of three behavioral parameters (brow bulge, eye squeeze and nasolabial furrow), two physiological parameters (oxygen saturation and heart rate) and two contextual parameters (gestational age and behavioral state). An assessment will result in a score between 0 and 21 where a higher score indicats a higher level of pain. A Swedish version of the scale will be used in the study [29]. The PIPP-R assessments will be performed by one of the researchers (MCM) and 20% of them will also be assessed by another researcher with extensive experience in pain assessment (EO) in order to assess interrater reliability.

Secondary outcomes

The secondary outcomes are changes in the GSR (Galvanic Skin Response) parameters (area small peaks, area huge peaks, peaks per second and average rise time) within 30 seconds after starting the eye examination. GSR is recorded with the Pain Monitor (MedStorm AS, Oslo, Norway) and reflects changes in activity in the sweat glands in response to sensory stimuli such as pain [30].

The number and evaluation of AEs (adverse events), SAEs (serious adverse events) and SUSARs (sudden unexpected serious adverse reactions) reported within 72 hours after the examination will also be measured as secondary outcomes as well as the examining physician´s assessment of how easy the infant was to examine.

Demographic and medical data about the infant such as gestational age, birth weight, apgar, current weight and time since last feeding will also be recorded.

Data analysis

The primary outcome (PIPP-R) will be analyzed and reported with the Mann-Whitney U-test. The secondary outcomes (VAS, GSR) will be analyzed and reported with parametric statistics (mean, standard deciation, pairied t-test) when the data is normally distributed and non-parametric statistics (median, interquartile range and Mann-whitney U-test) when not normally distributed. Level of significance is set to 0.05.

The population will be all randomized patients according to intention to treat (ITT). Drop-outs and missing data will be reported in the final report.

Patient and public involvement

There will be no patient or public involvement in this study.

Ethics and dissemination

The study is registered in ClinicalTrials database (NCT04902859) and European Union Drug Regulating Authorities Clinical Trials Database (EudraCT 2021-003005-21).

The study protocol adheres to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) guidelines and checklist [31]. Approval from the Swedish Ethical Review Authority (2021-03610) has been obtained for the study. Parents of eligible infants will receive both verbal and written information about the study including that participation is voluntary. Data will be collected and treated in accordance with the European data protection regulations. To protect the identity of the individual patients all data will be registered with a uniqe code. The key for the code will be stored in a safe place where no unauthorized persons will have access.

Individual, coded data will be collected and registered in a case report form (CRF) that will only be accessible for the researchers involved in the project. The recorded videos on the infant´s faces and

saturation/heart rate values as well as GSR values will be collected, registered with the same code and stored on a safe database for the study. Data will be stored for a minimum of 10 years after the completion of the study. Results from the study will be published in a scientific journal and reported on group level.

Discussion

The purpose of this multi-center randomized controlled trial is to investigate the pain-relieving effect of clonidine during ROP eye examinations. Numerous non-pharmacological as well as pharmacological methods of pain management during ROP eye examinations have been tested in previous studies without finding an adequate pain relief. To the best of our knowledge, this will be the first study investigating the pain-relieving properties of clonidine during these examinations. Clonidine is used clinically during other painful events in neonatal care and has both pain-relieving and sedative components. By including infants from two NICUs with different screening routines the results from this study will provide insights of the pain relief of clonidine both during RetCam and indirect ophthalmoscopy.

Authors contributions

All authors has been involved in planning the study and writing the protocol.

Funding statement

This work was supported by the Research committee at Region Örebro county, grant number OLL-934822 and Lilla Barnets fond grant number YE1353.

Competing interests statement

None of the authors have any competing interests.

References

1. Valeri B, Ranger M, Chau C, et al. Neonatal Invasive Procedures Predict Pain Intensity at School Age in Children Born Very Preterm. *Clin J Pain* 2016; 32:1086-93.
2. Brummelte S, Grunau R, Chau V, et al. Procedural pain and brain development in premature newborns. *Ann Neurol* 2012;71:385-9.
3. Ranger M, Chau C, Garg A, et al. Neonatal pain-related stress predicts cortical thickness at age 7 years in children born very preterm. *PLoS One* 2013; 18:e76702.
4. Vinall J, Grunau R. Impact of repeated procedural pain-related stress in infants born very preterm. *Pediatr Res* 2014; 75:584-587.
5. Brummelte S, Chau C, Cepeda I, et al. Cortisol levels in former preterm children at school age are predicted by neonatal procedural pain-related stress. *Psychoneuroendocrinology* 2015;51:151-63.
6. Bashinsky A. Retinopathy of prematurity. *NCMJ* 2017;78:124-128.
7. Holmström G, Hellström A, Gränse L, et al. New modifications of Swedish ROP guidelines based on 10-year data from the SWERDROP register. *Br J Ophtalmol* 2019; 0:1-7. doi:10.1136/bjophthalmol-2019-314874.
8. Rush R, Rush S, Ighani F, et al. The pain response in preterm infants undergoing screening for retinopathy of prematurity. *Retina* 2005; 25:59-62.
9. Cohen AM, Cook N, Harris MC, et al. The pain response to mydriatic eyedrops in preterm infants. *J Perinatol* 2013;33:462-5, doi: 10.1038/jp.2012.149.
10. Olsson E, Eriksson M. Oral glucose for pain relief during eye examinations for retinopathy of prematurity. *Journal of clinical nursing* 2011;20:1054-1059.
11. Grabska J, Walden P, Lerer T, et al. Can oral sucrose reduce the pain and distress associated with screening for retinopathy of prematurity? *J Perinatol* 2005; 25:33-5.

12. Nesargi S, Nithyanandam S, Rao S, et al. Topical anesthesia or oral dextrose for the relief of pain in screening for retinopathy of prematurity: a randomized controlled double-blinded trial. *J Trop Pediatr* 2014; 61:20-4.

13. Gal P, Kissling G, Young W, et al. Efficacy of sucrose to reduce pain in premature infants during eye examinations for retinopathy of prematurity. *Ann Pharmacother* 2005; 39:1029-33.

14. Dilli D, Ilarslan N, Kabatas E, et al. Oral sucrose and non-nutritive sucking goes some way to reducing pain during retinopathy of prematurity eye examinations. *Acta Paediatrica* 2014;103:e76-9.

15. Taplak S, Erdem E. A comparison of breast milk and sucrose in reducing neonatal pain during eye exam for retinopathy of prematurity. *Breastfeed Med* 2017;12:305-310.

16. Kristoffersen L, Stoen R, Bergsend H, et al. Skin-to-skin contact during eye examination did not reduce pain compared to standard care with parental support in preterm infants. *Acta Paediatrica* 2019;108:1434-40.

17. Rush R, Rush S, Nicolau J, et al. Systemic manifestations in response to mydriasis and physical examination during screening for retinopathy of prematurity. *Retina* 2004; 24: 242-5.

18. Mandel R, Ali N, Chen J, et al. Nitrous oxide analgesia during retinopathy screening: a randomized controlled trial. *Arch Dis Child Fetal Neonatal Ed* (2012;97:F83-7.

19. Hartley C, Moultrie F, Hoskin A, et al. Analgesic efficacy and safety of morphine in the Procedural Pain in Premature Infants (Poppi) study: randomized placebo-controlled trial. *Lancet* 2018; 392:2595-605.

20. Sun X, Lemyre B, Barrowman N, et al. Pain management during eye examinations for retinopathy of prematurity in preterm infants: a systematic review. *Acta Paediatrica* 2010; 99:329-34.

21. Francis K. What is best practice for providing pain relief during retinopathy of prematurity eye examinations. *Adv Neonatal Care* 2016;16: 220-8.

22. Disher T, Cameron C, Mitra S, et al. Pain-Relieving Interventions for Retinopathy of Prematurity: A Meta-analysis. *Pediatrics* 2018; 142:1.

23. Pirelli A, Savant Levet P, Garetti E, et al. Literature review informs clinical guidelines for pain management during screening and laser photocoagulation for retinopathy of prematurity. *Acta Paediatrica* 2019; 188:593-99.

24. Läkemedelsverket (2014) Behandling av barn i samband med smärtsamma procedurer i hälso- och sjukvård – kunskapsdokument. Collected 220424 from: https://www.lakemedelsverket.se/sv/behandling-och-forskrivning/behandlingsrekommendationer/sok-behandlingsrekommendationer/behandling-av-barn-och-ungdomar-i-samband-med-smartsamma-procedurer--kunskapsdokument

25. Pichot C, Ghignone M, Quintin L. Dexmedetomidine and clonidine: from second- to first-line sedative agents in the critical care setting? *J Intensive Care Med* 2012; 27:219-37.

26. Hunseler C, Trieschmann U, Möller O, et al. Continuous Infusion of Clonidine in Ventilated Newborns and Infants: A Randomized Controlled Trial. *Pediatric Critical Care Medicine* 2014;15:512-522.

27. Zhang Y. Clonidine preconditioning decreases infarct size and improves neurological outcome from transient forebrain ischemia in the rat. *Neuroscience*. 2004;125:625-31.

28. Stevens B, Gibbins S, Yamada J, et al.The premature infant pain profile-revised (PIPP-R): initial validation and feasibility. *Clinical journal of pain* 2014; 30:238-43.

29. Olsson E, Anderzén-Carlsson A, Atladottir S, et al. Cultural adaptation and harmonization of four Nordic translations of the revised premature infant pain profile (PIPP-R). *BMC Pediatrics* 2018; 8:349.

30. Storm H. Changes in skin conductance as a tool to monitor nociceptive stimulation and pain. *Curr Opin Anaesthesiol* 2008; 21: 96-804.

31. Chan AW, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200-7.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	
Funding	4	Sources and types of financial, material, and other support	13
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	2, 5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5-6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7-8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	5
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	8-9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	8
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	7
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	8
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8-9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	9
	31b	Authorship eligibility guidelines and any intended use of professional writers	
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Clonidine as analgesia during retinopathy of prematurity screening in preterm infants (cloROP): Protocol for a randomized controlled trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-064251.R1
Article Type:	Protocol
Date Submitted by the Author:	27-Jul-2022
Complete List of Authors:	Carlsen Misic, Martina; Örebro University, Faculty of Medicine and Health; Örebro Universitet, Department of Pediatrics Eriksson, Mats; Örebro University, Sweden, Faculty of Medicine and Health, School of Health Sciences Normann, Erik; Uppsala Universitet Medicinska fakulteten, Department of Womens and Childrens health Pettersson, Miriam; Örebro University, Blomqvist, Y; Uppsala Universitet Medicinska fakulteten, Department of Womens and Childrens health Olsson, Emma ; Örebro University, Faculty of Medicine and Health; Örebro Universitet, Department of Pediatrics
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Ophthalmology
Keywords:	Neonatal intensive & critical care < INTENSIVE & CRITICAL CARE, PAIN MANAGEMENT, Paediatric ophthalmology < OPHTHALMOLOGY

Clonidine as analgesia during retinopathy of prematurity screening in preterm infants (cloROP): Protocol for a randomized controlled trial

Martina Carlsen Misic[1,2], Mats Eriksson[2], Erik Normann[3,4], Miriam Pettersson[1,5], Ylva Thernström Blomqvist[3,4], Emma Olsson[1,2]

1. Department of Pediatrics, Faculty of Medicine and Health, Örebro University, Örebro, Sweden
2. Faculty of Medicine and Health, School of Health Sciences, Örebro University, Örebro, Sweden
3. University Hospital, Neonatal Intensive Care Unit, Uppsala, Sweden
4. Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden
5. Faculty of Medicine and Health, School of Medical Sciences, Örebro University, Örebro, Sweden

Corresponding author:

Emma Olsson

Adress: Faculty of Medicine and Health, School of Health Sciences, Örebro University, S-701 82, Örebro, Sweden

Email: emma.olsson@oru.se

Abstract

Introduction

Preterm infants are at risk of negative consequences from stress and pain at the same time as they often are in need of intensive care that includes painful interventions. One of the frequent painful procedures preterm infants undergo is eye examination screening to detect early signs of ROP (retinopathy of prematurity). These examinations are both stressful and painful, and despite a multitude of research studies no conclusive pain-relieving treatment has been demonstrated. The main aim of this trial is to investigate the analgesic effect of clonidine during ROP eye examinations.

Methods and analysis

The planned study is a multi-center randomized controlled trial with a crossover design. Infants will be recruited from two different neonatal intensive care units in Sweden. Infants born before gestation week 30 (and therefor eligible for ROP screening) and cared for in either of the NICUs will be eligible for inclusion in the study. The primary outcome will be PIPP-R (Premature Infant Pain Profile – revised) score within 30 seconds after starting the examination. Secondary outcomes will be changes in the GSR (Galvanic Skin Response)-parameters (area small peaks, area huge peaks, peaks per second and average rise time) within 30 seconds after starting the eye examination together with the number and evaluation of adverse events reported within 72 hours after the examination and the examining physician´s assessment of how easy the infant was to examine.

Ethics and dissemination

Approval from the Swedish Ethical Review Authority and the Swedish Medical Products Agency has been obtained for the study. Parents of eligible infants will be getting both verbal and written information about the study including that participation is voluntary. Data will be collected and treated in accordance with the European General Data Protection regulation. The results will be reported on group level and published in a scientific journal.

Strengths and limitations of this study

- To our knowledge, this will be the first study investigating the pain-relieving effect of Clonidine during ROP eye examinations of preterm infants.
- The study will investigate the pain relieving effect from both indirect ophthalmoscopy and RetCam.
- The cross-over design with every infant as its own control will reduce many confounding factors.
- Since there are no previous successful studies decreasing the pain from ROP eye examinations, the sample size calculations is built on finding from studies on other procedural pain

Trial registration

ClinicalTrials NCT04902859

EudraCT 2021-003005-21

Introduction

Preterm infants are sensitive to stimuli and are at risk for short- and long-term negative consequences from stress and pain. They are often in need of intensive care that includes painful interventions. Repeated painful experiences during the neonatal period can result in future negative effects in pain response [1], brain development [2, 3], cognitive functioning [4] and cortisol levels [5]. These infants are also at risk of secondary complications from their prematurity, one being retinopathy of prematurity (ROP). This condition is caused by abnormal blood vessel growth that can progress to retinal detachment and blindness. A low gestational age and low birthweight are risk factors for ROP [6] and all infants born before gestation week 30 are therefore screened with regular eye examinations until the retina is fully developed [7].

The eye examinations are painful and stressful for the preterm infant. The infant need to be restrained during the examination and bright light as well as physical manipulation of the eye with eye speculum are some of the aspects that can be painful and stressful [8]. The mydriatic eyedrops used before the examination also causes pain [9].

Considerable amount of research has examined a variety of potentially pain-relieving interventions during ROP eye examinations without finding an effective treatment. Studies on oral sweet solutions that are effective as pain relief during other painful procedures have shown inconclusive results [10, 11, 12, 13] and a few that have shown significantly lower pain scores with sweet solutions are still showing pain scores within the limits for severe pain in the experiment group [14, 15]. Nonpharmacological interventions such as skin-to-skin contact [16] and facilitated tucking [17] have also shown an inadequate pain relief as well as inhaled nitrous oxide [18]. A randomized controlled trial investigating morphine as pain relief during ROP screening had to be terminated due to adverse effects [19]. Despite multimodal techniques the pain relief during ROP eye examinations seem to be insufficient [20, 21, 22, 23], leaving preterm infants exposed to repeated untreated pain.

Clonidine is an alpha2-agonist that is used increasingly in neonatal care due to its safe sedating and pain-relieving properties [24], without the risk of respiratory depression [25, 24]. The use of clonidine

can also reduce the need for other sedation and pain-relieving medication [26] and some data also indicates neuroprotective capacities [27].

Considering the pain and stress during ROP eye examinations and as of today the lack of effective pain relief methods the aim of this study will be to investigate the pain-relieving effect of clonidine during ROP eye examinations.

Methods and analysis

Study design

This study is a multi-center randomized controlled trial with a crossover design. Infants will be recruited from two different neonatal intensive care units (NICUs) in university hospitals the central part of Sweden (NICU A and NICU B). Infants born before gestation week 30 (and therefore undergoing ROP screening) and cared for in either of the NICUs will be eligible for inclusion in the study. Previous studies on ROP-screening induced pain revealed a mean PIPP-score of 10.3 (SD 4.2) (16) and 16.4 (1.8) (14) in the control groups. Based on the literature (28) a difference of 2 steps in the primary outcome variable PIPP-R (Premature Infant Pain Profile – Revised) could discriminate between painful and non painful situation, and thus is considered clinically significant in this study. With a power of 0.8 and a significance level of 0.05 eighteen infants are needed to detect this difference and with consideration of potential dropouts the aim is to include 25 infants. Because of different examination techniques at the respective units, we plan to include 25 infants at each unit. In unit A RetCam (Clarity Medical Systems, Pleasanton, CA, USA) is used for ROP screening while direct ophthalmoscopy is used almost exclusively at unit B.

Exclusion criteria

- Infants that have received pain relieving analgetics, sedatives or β-blockers within 24 hours before the eye examination
- Previous documented renal failure

- Infants without gastric tube
- Infants with known heart arrythmias or neurologic deficit
- Infants with circulatory instability (assessed as a mean arterial blood pressure below infants' gestation age in weeks)

The study will be monitored by an independent monitor appointed by the physician responsible for the study (MP). Monitoring will be performed according to risk- based monitoring and a monitoring plan.

Procedure

Parents of eligible infants will receive written and verbal information about the study before the first scheduled eye examination. The principal investigator on each site will obtain informed consent from the parents. The infant will serve as his or her own control and will be examined according to the units' policy. After parental consent the infant´s first two eye examinations will be included in the study. To minimize potential effects from previous experience the order of the treatment (clonidine or placebo) will be randomized for each infant using an online random sequence generator that gives the order. The allocation for the treatments will be kept in opaque envelopes and an unblinded trained nurse or pharmacist, not involved in the study, will prepare the study solution (clonidine or sterile water) in a syringe marked with the patient's study ID. Both clonidine and sterile water will be retrieved from the units ordinary medical supply. A document with the included patients´ allocations will be kept in a closed envelope inside a locked room in the respective NICUs in order to make unblinding possible if needed.

Sixty minutes before the first eye examination the infant will receive clonidine 4 mcg/kg (intervention) or sterile water (placebo) in equivalent volumes in the nasogastric tube by a nurse blinded for the content. Everyone else in the room will be blinded for the content of the syringe as well as the researcher performing the subsequent analyses. Mydriatic eye drops (cyclopentolate 0.5% and phenylephrine 0.5%) will be administered according to clinical routine at 45 and 30 min before the procedure. The infant will be connected to a probe measuring oxygen saturation and heart rate on one foot and three electrodes measuring GSR (galvanic skin response) on the other foot before the

procedure. The ophthalmologist will then examine the eyes with either RetCam or direct ophthalmoscopy according to the respective units' guidelines. In addition to the study solution the infant will receive standard care with facilitated tucking and a pacifier. The infants' face, expressions, and heart rate and oxygen saturation values from the monitor display, will be videorecorded by two cameras before, during and after the examination for subsequent PIPP-R assessments. From these videorecordings one researcher will perform the PIPP-R pain assessments including noting the baseline values as well as the values within 30 seconds after the beginning of the eye examination according to the instructions of the PIPP-R scale (28). Afterwards the examining ophthalmologist will estimate how easy the infant was to examine by marking an "X" on a 10 cm long horizontal line where one end indicates "very easy to examine" and the other end "very difficult to examine". The ophthalmologist can also write any other comments in free text in the same document. The same procedure will then be executed during the infants' second eye examination with the study solution (intervention or placebo) he or she did not receive the first time.

Primary outcome

The primary outcome is the PIPP-R (Premature Infant Pain Profile – Revised) [28] score within 30 seconds after starting the eye examination see table 1.

Primary outcome	**Secondary outcomes**
PIPP-R (Premature Infant Pain Profile – revised) score within 30 seconds after starting the eye examination.	Changes in GSR (Galvanic Skin Response)-parameters within 30 seconds after starting the eye examination.
	The number, and evaluation, of: - Adverse events - Serious adverse events - Sudden unexpected serious adverse reactions reported within 72 hours after the examination will be measured.
	The examining physician's assessment of how "easy" the infant was to examine.

Table 1 Overview of outcomes

PIPP-R is a pain assessment scale for procedural pain consisting of three behavioral parameters (brow bulge, eye squeeze and nasolabial furrow), two physiological parameters (oxygen saturation and heart rate) and two contextual parameters (gestational age and behavioral state). An assessment will result in

a score between 0 and 21 where a higher score indicats a higher level of pain. A Swedish version of the scale will be used in the study [29]. The PIPP-R assessments will be performed by one of the researchers (MCM) and 20% of them will also be assessed by another researcher with extensive experience in pain assessment (EO) in order to assess interrater reliability.

Secondary outcomes

The secondary outcomes are changes in the GSR (Galvanic Skin Response) parameters (area small peaks, area huge peaks, peaks per second and average rise time) within 30 seconds after starting the eye examination. GSR is recorded with the Pain Monitor (MedStorm AS, Oslo, Norway) and reflects changes in activity in the sweat glands in response to sensory stimuli such as pain [30].

The number and evaluation of AEs (adverse events), SAEs (serious adverse events) and SUSARs (sudden unexpected serious adverse reactions) reported within 72 hours after the examination will also be measured as secondary outcomes as well as the examining physician´s assessment of how easy the infant was to examine. Any AEs, SAEs and SUSARs will be recorded in the patients´ case report form (CRF) in accordance with regulations from the Swedish Medical Products Agency.

Demographic and medical data about the infant such as gestational age, birth weight, apgar, current weight and time since last feeding will also be recorded.

Data analysis

The data from the respective units will be analyzed independently for each unit. The primary outcome (PIPP-R) will be analyzed and reported with the Mann-Whitney U-test. The secondary outcomes (VAS, GSR) will be analyzed and reported with parametric statistics (mean, standard deciation, pairied t-test) when the data is normally distributed and non-parametric statistics (median, interquartile range and Mann-whitney U-test) when not normally distributed. Level of significance is set to 0.05.

The population will be all randomized patients according to intention to treat (ITT). Drop-outs and missing data will be reported in the final report.

Patient and public involvement

There will be no patient or public involvement in this study.

Ethics and dissemination

The study is registered in ClinicalTrials database (NCT04902859) and European Union Drug Regulating Authorities Clinical Trials Database (EudraCT 2021-003005-21).

The study protocol adheres to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) guidelines and checklist [31]. Approval from the Swedish Ethical Review Authority (2021-03610) and the Swedish Medical Products Agency (2021-91712) has been obtained for the study. Parents of eligible infants will receive both verbal and written information about the study including that participation is voluntary. Data will be collected and treated in accordance with the European data protection regulations. To protect the identity of the individual patients all data will be registered with a uniqe code. The key for the code will be stored in a safe place where no unauthorized persons will have access.

Individual, coded data will be collected and registered in a case report form (CRF) that will only be accessible for the researchers involved in the project. The recorded videos on the infant´s faces and saturation/heart rate values as well as GSR values will be collected, registered with the same code and stored on a safe database for the study. Data will be stored for a minimum of 10 years after the completion of the study. Results from the study will be published in a scientific journal and reported on group level.

Authors contributions

All authors (MCM, ME, EN, MP, YTB and EO) have planned and designed the study. EO wrote the first draft of the manuscript and MCM, ME, EN, MP and YTB critically appraised and revised it.

Funding statement

This work was supported by the Research committee at Region Örebro county, grant number OLL-934822 and Lilla Barnets fond grant number YE1353.

Competing interests statement

None of the authors have any competing interests.

References

1. Valeri B, Ranger M, Chau C, et al. Neonatal Invasive Procedures Predict Pain Intensity at School Age in Children Born Very Preterm. *Clin J Pain* 2016; 32:1086-93.
2. Brummelte S, Grunau R, Chau V, et al. Procedural pain and brain development in premature newborns. *Ann Neurol* 2012;71:385-9.
3. Ranger M, Chau C, Garg A, et al. Neonatal pain-related stress predicts cortical thickness at age 7 years in children born very preterm. *PLoS One* 2013; 18:e76702.
4. Vinall J, Grunau R. Impact of repeated procedural pain-related stress in infants born very preterm. *Pediatr Res* 2014; 75:584-587.
5. Brummelte S, Chau C, Cepeda I, et al. Cortisol levels in former preterm children at school age are predicted by neonatal procedural pain-related stress. *Psychoneuroendocrinology* 2015;51:151-63.
6. Bashinsky A. Retinopathy of prematurity. *NCMJ* 2017;78:124-128.
7. Holmström G, Hellström A, Gränse L, et al. New modifications of Swedish ROP guidelines based on 10-year data from the SWERDROP register. *Br J Ophtalmol* 2019; 0:1-7. doi:10.1136/bjophthalmol-2019-314874.
8. Rush R, Rush S, Ighani F, et al. The pain response in preterm infants undergoing screening for retinopathy of prematurity. *Retina* 2005; 25:59-62.
9. Cohen AM, Cook N, Harris MC, et al. The pain response to mydriatic eyedrops in preterm infants. *J Perinatol* 2013;33:462-5, doi: 10.1038/jp.2012.149.
10. Olsson E, Eriksson M. Oral glucose for pain relief during eye examinations for retinopathy of prematurity. *Journal of clinical nursing* 2011;20:1054-1059.
11. Grabska J, Walden P, Lerer T, et al. Can oral sucrose reduce the pain and distress associated with screening for retinopathy of prematurity? *J Perinatol* 2005; 25:33-5.

12. Nesargi S, Nithyanandam S, Rao S, et al. Topical anesthesia or oral dextrose for the relief of pain in screening for retinopathy of prematurity: a randomized controlled double-blinded trial. *J Trop Pediatr* 2014; 61:20-4.

13. Gal P, Kissling G, Young W, et al. Efficacy of sucrose to reduce pain in premature infants during eye examinations for retinopathy of prematurity. *Ann Pharmacother* 2005; 39:1029-33.

14. Dilli D, Ilarslan N, Kabatas E, et al. Oral sucrose and non-nutritive sucking goes some way to reducing pain during retinopathy of prematurity eye examinations. *Acta Paediatrica* 2014;103:e76-9.

15. Taplak S, Erdem E. A comparison of breast milk and sucrose in reducing neonatal pain during eye exam for retinopathy of prematurity. *Breastfeed Med* 2017;12:305-310.

16. Kristoffersen L, Stoen R, Bergsend H, et al. Skin-to-skin contact during eye examination did not reduce pain compared to standard care with parental support in preterm infants. *Acta Paediatrica* 2019;108:1434-40.

17. Rush R, Rush S, Nicolau J, et al. Systemic manifestations in response to mydriasis and physical examination during screening for retinopathy of prematurity. *Retina* 2004; 24: 242-5.

18. Mandel R, Ali N, Chen J, et al. Nitrous oxide analgesia during retinopathy screening: a randomized controlled trial. *Arch Dis Child Fetal Neonatal Ed* (2012;97:F83-7.

19. Hartley C, Moultrie F, Hoskin A, et al. Analgesic efficacy and safety of morphine in the Procedural Pain in Premature Infants (Poppi) study: randomized placebo-controlled trial. *Lancet* 2018; 392:2595-605.

20. Sun X, Lemyre B, Barrowman N, et al. Pain management during eye examinations for retinopathy of prematurity in preterm infants: a systematic review. *Acta Paediatrica* 2010; 99:329-34.

21. Francis K. What is best practice for providing pain relief during retinopathy of prematurity eye examinations. *Adv Neonatal Care* 2016;16: 220-8.

22. Disher T, Cameron C, Mitra S, et al. Pain-Relieving Interventions for Retinopathy of Prematurity: A Meta-analysis. *Pediatrics* 2018; 142:1.

23. Pirelli A, Savant Levet P, Garetti E, et al. Literature review informs clinical guidelines for pain management during screening and laser photocoagulation for retinopathy of prematurity. *Acta Paediatrica* 2019; 188:593-99.

24. Läkemedelsverket (2014) Behandling av barn i samband med smärtsamma procedurer i hälso- och sjukvård – kunskapsdokument. Collected 220424 from: https://www.lakemedelsverket.se/sv/behandling-och-forskrivning/behandlingsrekommendationer/sok-behandlingsrekommendationer/behandling-av-barn-och-ungdomar-i-samband-med-smartsamma-procedurer--kunskapsdokument

25. Pichot C, Ghignone M, Quintin L. Dexmedetomidine and clonidine: from second- to first-line sedative agents in the critical care setting? *J Intensive Care Med* 2012; 27:219-37.

26. Hunseler C, Trieschmann U, Möller O, et al. Continuous Infusion of Clonidine in Ventilated Newborns and Infants: A Randomized Controlled Trial. *Pediatric Critical Care Medicine* 2014;15:512-522.

27. Zhang Y. Clonidine preconditioning decreases infarct size and improves neurological outcome from transient forebrain ischemia in the rat. *Neuroscience*. 2004;125:625-31.

28. Stevens B, Gibbins S, Yamada J, et al.The premature infant pain profile-revised (PIPP-R): initial validation and feasibility. *Clinical journal of pain* 2014; 30:238-43.

29. Olsson E, Anderzén-Carlsson A, Atladottir S, et al. Cultural adaptation and harmonization of four Nordic translations of the revised premature infant pain profile (PIPP-R). *BMC Pediatrics* 2018; 8:349.

30. Storm H. Changes in skin conductance as a tool to monitor nociceptive stimulation and pain. *Curr Opin Anaesthesiol* 2008; 21: 96-804.

31. Chan AW, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200-7.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	3	Date and version identifier	n/a
Funding	4	Sources and types of financial, material, and other support	13
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	6
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	n/a
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	6

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	2, 5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5-6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	n/a
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	n/a
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7-8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	5
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	8-9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n/a
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	8
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	7
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	6
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	8
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	n/a

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8-9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	9
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Suppl consent form
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	9
	31b	Authorship eligibility guidelines and any intended use of professional writers	9
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Suppl consent form
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Clonidine as analgesia during retinopathy of prematurity screening in preterm infants (cloROP): Protocol for a randomized controlled trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-064251.R2
Article Type:	Protocol
Date Submitted by the Author:	14-Sep-2022
Complete List of Authors:	Carlsen Misic, Martina; Örebro University, Faculty of Medicine and Health; Örebro Universitet, Department of Pediatrics Eriksson, Mats; Örebro University, Sweden, Faculty of Medicine and Health, School of Health Sciences Normann, Erik; Uppsala Universitet Medicinska fakulteten, Department of Womens and Childrens health Pettersson, Miriam; Örebro University, Blomqvist, Y; Uppsala Universitet Medicinska fakulteten, Department of Womens and Childrens health Olsson, Emma ; Örebro University, Faculty of Medicine and Health; Örebro Universitet, Department of Pediatrics
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Ophthalmology
Keywords:	Neonatal intensive & critical care < INTENSIVE & CRITICAL CARE, PAIN MANAGEMENT, Paediatric ophthalmology < OPHTHALMOLOGY

Clonidine as analgesia during retinopathy of prematurity screening in preterm infants (cloROP): Protocol for a randomized controlled trial

Martina Carlsen Misic[1,2], Mats Eriksson[2], Erik Normann[3,4], Miriam Pettersson[1,5], Ylva Thernström Blomqvist[3,4], Emma Olsson[1,2]

1. Department of Pediatrics, Faculty of Medicine and Health, Örebro University, Örebro, Sweden
2. Faculty of Medicine and Health, School of Health Sciences, Örebro University, Örebro, Sweden
3. University Hospital, Neonatal Intensive Care Unit, Uppsala, Sweden
4. Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden
5. Faculty of Medicine and Health, School of Medical Sciences, Örebro University, Örebro, Sweden

Corresponding author:

Emma Olsson

Adress: Faculty of Medicine and Health, School of Health Sciences, Örebro University, S-701 82, Örebro, Sweden

Email: emma.olsson@oru.se

Abstract

Introduction

Preterm infants are at risk of negative consequences from stress and pain at the same time as they often are in need of intensive care that includes painful interventions. One of the frequent painful procedures preterm infants undergo is eye examination screening to detect early signs of ROP (retinopathy of prematurity). These examinations are both stressful and painful, and despite a multitude of research studies no conclusive pain-relieving treatment has been demonstrated. The main aim of this trial is to investigate the analgesic effect of clonidine during ROP eye examinations.

Methods and analysis

The planned study is a multi-center randomized controlled trial with a crossover design. Infants will be recruited from two different neonatal intensive care units in Sweden. Infants born before gestation week 30 (and therefor eligible for ROP screening) and cared for in either of the NICUs will be eligible for inclusion in the study. The primary outcome will be PIPP-R (Premature Infant Pain Profile – revised) score within 30 seconds after starting the examination. Secondary outcomes will be changes in the GSR (Galvanic Skin Response)-parameters (area small peaks, area huge peaks, peaks per second and average rise time) within 30 seconds after starting the eye examination together with the number and evaluation of adverse events reported within 72 hours after the examination and the examining physician´s assessment of how easy the infant was to examine.

Ethics and dissemination

Approval from the Swedish Ethical Review Authority and the Swedish Medical Products Agency has been obtained for the study. Parents of eligible infants will be getting both verbal and written information about the study including that participation is voluntary. Data will be collected and treated in accordance with the European General Data Protection regulation. The results will be reported on group level and published in a scientific journal.

Strengths and limitations of this study

- To our knowledge, this will be the first study investigating the pain-relieving effect of Clonidine during ROP eye examinations of preterm infants.
- The study will investigate the pain relieving effect from both indirect ophthalmoscopy and RetCam, whereas the results from the two examination techniques/sites cannot be compared.
- Recording facial expressions during eye examinations can be difficult but still gives us the opportunity to go back and review over again.
- The cross-over design with every infant as its own control will reduce many confounding factors.
- Since there are no previous successful studies decreasing the pain from ROP eye examinations, the sample size calculations is built on findings from studies on other procedural pain

Trial registration

ClinicalTrials.gov NCT04902859

EudraCT 2021-003005-21

Introduction

Preterm infants are sensitive to stimuli and are at risk for short- and long-term negative consequences from stress and pain. They are often in need of intensive care that includes painful interventions. Repeated painful experiences during the neonatal period can result in future negative effects in pain response [1], brain development [2, 3], cognitive functioning [4] and cortisol levels [5]. These infants are also at risk of secondary complications from their prematurity, one being retinopathy of prematurity (ROP). This condition is caused by abnormal blood vessel growth that can progress to retinal detachment and blindness. A low gestational age and low birthweight are risk factors for ROP [6] and all infants born before gestation week 30 are therefore screened with regular eye examinations until the retina is fully developed [7].

The eye examinations are painful and stressful for the preterm infant. The infant need to be restrained during the examination and bright light as well as physical manipulation of the eye with eye speculum are some of the aspects that can be painful and stressful [8]. The mydriatic eyedrops used before the examination also causes pain [9].

Considerable amount of research has examined a variety of potentially pain-relieving interventions during ROP eye examinations without finding an effective treatment. Studies on oral sweet solutions that are effective as pain relief during other painful procedures have shown inconclusive results [10, 11, 12, 13] and a few that have shown significantly lower pain scores with sweet solutions are still showing pain scores within the limits for severe pain in the experiment group [14, 15]. Nonpharmacological interventions such as skin-to-skin contact [16] and facilitated tucking [17] have also shown an inadequate pain relief as well as inhaled nitrous oxide [18]. A randomized controlled trial investigating morphine as pain relief during ROP screening had to be terminated due to adverse effects [19]. Despite multimodal techniques the pain relief during ROP eye examinations seem to be insufficient [20, 21, 22, 23], leaving preterm infants exposed to repeated untreated pain.

Clonidine is an alpha2-agonist that is used increasingly in neonatal care due to its safe sedating and pain-relieving properties [24], without the risk of respiratory depression [25, 24]. The use of clonidine can also reduce the need for other sedation and pain-relieving medication [26] and some data also indicates neuroprotective capacities [27].

Considering the pain and stress during ROP eye examinations and as of today the lack of effective pain relief methods the aim of this study will be to investigate the pain-relieving effect of clonidine during ROP eye examinations.

Methods and analysis

Study design

This study is a multi-center randomized controlled trial with a crossover design. Infants will be recruited from two different neonatal intensive care units (NICUs) in university hospitals the central part of Sweden (NICU A and NICU B). Infants born before gestation week 30 (and therefore undergoing ROP screening) and cared for in either of the NICUs will be eligible for inclusion in the study. Previous studies on ROP-screening induced pain revealed a mean PIPP-score of 10.3 (SD 4.2) (16) and 16.4 (1.8) (14) in the control groups. Based on the literature (28) a difference of 2 steps in the primary outcome variable PIPP-R (Premature Infant Pain Profile – Revised) could discriminate between painful and non painful situation, and thus is considered clinically significant in this study. With a power of 0.8 and a significance level of 0.05 eighteen infants are needed to detect this difference and with consideration of potential dropouts the aim is to include 25 infants. Because of different examination techniques at the respective units, we plan to include 25 infants at each unit. In unit A RetCam (Clarity Medical Systems, Pleasanton, CA, USA) is used for ROP screening while direct ophthalmoscopy is used almost exclusively at unit B.

Exclusion criteria

- Infants that have received pain relieving analgetics, sedatives or β-blockers within 24 hours before the eye examination
- Previous documented renal failure
- Infants without gastric tube
- Infants with known heart arrythmias or neurologic deficit
- Infants with circulatory instability (assessed as a mean arterial blood pressure below infants' gestation age in weeks)

The study will be monitored by an independent monitor appointed by the physician responsible for the study (MP). Monitoring will be performed according to risk- based monitoring and a monitoring plan.

Procedure

Parents of eligible infants will receive written and verbal information about the study before the first scheduled eye examination. The principal investigator on each site will obtain informed consent from the parents. The infant will serve as his or her own control and will be examined according to the units' policy. After parental consent the infant´s first two eye examinations will be included in the study. To minimize potential effects from previous experience the order of the treatment (clonidine or placebo) will be randomized for each infant using an online random sequence generator that gives the order. The allocation for the treatments will be kept in opaque envelopes and an unblinded trained nurse or pharmacist, not involved in the study, will prepare the study solution (clonidine or sterile water) in a syringe marked with the patient's study ID. Both clonidine and sterile water will be retrieved from the units ordinary medical supply. A document with the included patients´ allocations will be kept in a closed envelope inside a locked room in the respective NICUs in order to make unblinding possible if needed.

Sixty minutes before the first eye examination the infant will receive clonidine 4 mcg/kg (intervention) or sterile water (placebo) in equivalent volumes in the nasogastric tube by a nurse blinded for the content. Everyone else in the room will be blinded for the content of the syringe as well as the researcher performing the subsequent analyses. Mydriatic eye drops (cyclopentolate 0.5%

and phenylephrine 0.5%) will be administered according to clinical routine at 45 and 30 min before the procedure. The infant will be connected to a probe measuring oxygen saturation and heart rate on one foot and three electrodes measuring GSR (galvanic skin response) on the other foot before the procedure. The ophthalmologist will then examine the eyes with either RetCam or direct ophthalmoscopy according to the respective units' guidelines. In addition to the study solution the infant will receive standard care with facilitated tucking and a pacifier. The infants' face, expressions, and heart rate and oxygen saturation values from the monitor display, will be videorecorded by two cameras before, during and after the examination for subsequent PIPP-R assessments. From these videorecordings one researcher will perform the PIPP-R pain assessments including noting the baseline values as well as the values within 30 seconds after the beginning of the eye examination according to the instructions of the PIPP-R scale (28). The research group has previous experience of video recording and assessing pain with PIPP-R during eye-examinations (10). Afterwards the examining ophthalmologist will estimate how easy the infant was to examine by marking an "X" on a 10 cm long horizontal line where one end indicates "very easy to examine" and the other end "very difficult to examine". Though this is not a validated measure for examining difficulties, it will give an indication if clonidine or placebo affects the examining procedure. The ophthalmologist can also write any other comments in free text in the same document. The same procedure will then be executed during the infants' second eye examination with the study solution (intervention or placebo) he or she did not receive the first time.

Primary outcome

The primary outcome is the PIPP-R (Premature Infant Pain Profile – Revised) [28] score within 30 seconds after starting the eye examination see table 1.

Primary outcome	**Secondary outcomes**
PIPP-R (Premature Infant Pain Profile – revised) score within 30 seconds after starting the eye examination.	Changes in GSR (Galvanic Skin Response)-parameters within 30 seconds after starting the eye examination.
	The number, and evaluation, of: - Adverse events - Serious adverse events - Sudden unexpected serious adverse reactions

	reported within 72 hours after the examination will be measured.
	The examining physician's assessment of how "easy" the infant was to examine.

Table 1 Overview of outcomes

PIPP-R is a pain assessment scale for procedural pain consisting of three behavioral parameters (brow bulge, eye squeeze and nasolabial furrow), two physiological parameters (oxygen saturation and heart rate) and two contextual parameters (gestational age and behavioral state). An assessment will result in a score between 0 and 21 where a higher score indicats a higher level of pain. A Swedish version of the scale will be used in the study [29]. The PIPP-R assessments will be performed by one of the researchers (MCM) and 20% of them will also be assessed by another researcher with extensive experience in pain assessment (EO) in order to assess interrater reliability.

Secondary outcomes

The secondary outcomes are changes in the GSR (Galvanic Skin Response) parameters (area small peaks, area huge peaks, peaks per second and average rise time) within 30 seconds after starting the eye examination. GSR is recorded with the Pain Monitor (MedStorm AS, Oslo, Norway) and reflects changes in activity in the sweat glands in response to sensory stimuli such as pain [30].

The number and evaluation of AEs (adverse events), SAEs (serious adverse events) and SUSARs (sudden unexpected serious adverse reactions) reported within 72 hours after the examination will also be measured as secondary outcomes as well as the examining physician´s assessment of how easy the infant was to examine. Any AEs, SAEs and SUSARs will be recorded in the patients´ case report form (CRF) in accordance with regulations from the Swedish Medical Products Agency.

Demographic and medical data about the infant such as gestational age, birth weight, apgar, current weight and time since last feeding will also be recorded.

Data analysis

The data from the respective units will be analyzed independently for each unit. The primary outcome (PIPP-R) will be analyzed and reported with the Mann-Whitney U-test. The secondary outcomes (VAS, GSR) will be analyzed and reported with parametric statistics (mean, standard deciation,

pairied t-test) when the data is normally distributed and non-parametric statistics (median, interquartile range and Mann-whitney U-test) when not normally distributed. Level of significance is set to 0.05.

The population will be all randomized patients according to intention to treat (ITT). Drop-outs and missing data will be reported in the final report.

Patient and public involvement

There will be no patient or public involvement in this study.

Ethics and dissemination

The study is registered in the ClinicalTrials.gov database (NCT04902859) and European Union Drug Regulating Authorities Clinical Trials Database (EudraCT 2021-003005-21).

The study protocol adheres to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) guidelines and checklist [31]. Approval from the Swedish Ethical Review Authority (2021-03610) and the Swedish Medical Products Agency (2021-91712) has been obtained for the study. Parents of eligible infants will receive both verbal and written information about the study including that participation is voluntary. Data will be collected and treated in accordance with the European data protection regulations. To protect the identity of the individual patients all data will be registered with a uniqe code. The key for the code will be stored in a safe place where no unauthorized persons will have access.

Individual, coded data will be collected and registered in a case report form (CRF) that will only be accessible for the researchers involved in the project. The recorded videos on the infant´s faces and saturation/heart rate values as well as GSR values will be collected, registered with the same code and stored on a safe database for the study. Data will be stored for a minimum of 10 years after the completion of the study. Results from the study will be published in a scientific journal and reported on group level.

Authors contributions

All authors (MCM, ME, EN, MP, YTB and EO) have planned and designed the study. EO wrote the first draft of the manuscript and MCM, ME, EN, MP and YTB critically appraised and revised it.

Funding statement

This work was supported by the Research committee at Region Örebro county, grant number OLL-934822 and Lilla Barnets fond grant number YE1353.

Competing interests statement

None of the authors have any competing interests.

References

1. Valeri B, Ranger M, Chau C, et al. Neonatal Invasive Procedures Predict Pain Intensity at School Age in Children Born Very Preterm. *Clin J Pain* 2016; 32:1086-93.
2. Brummelte S, Grunau R, Chau V, et al. Procedural pain and brain development in premature newborns. *Ann Neurol* 2012;71:385-9.
3. Ranger M, Chau C, Garg A, et al. Neonatal pain-related stress predicts cortical thickness at age 7 years in children born very preterm. *PLoS One* 2013; 18:e76702.
4. Vinall J, Grunau R. Impact of repeated procedural pain-related stress in infants born very preterm. *Pediatr Res* 2014; 75:584-587.
5. Brummelte S, Chau C, Cepeda I, et al. Cortisol levels in former preterm children at school age are predicted by neonatal procedural pain-related stress. *Psychoneuroendocrinology* 2015;51:151-63.
6. Bashinsky A. Retinopathy of prematurity. *NCMJ* 2017;78:124-128.
7. Holmström G, Hellström A, Gränse L, et al. New modifications of Swedish ROP guidelines based on 10-year data from the SWERDROP register. *Br J Ophtalmol* 2019; 0:1-7. doi:10.1136/bjophthalmol-2019-314874.
8. Rush R, Rush S, Ighani F, et al. The pain response in preterm infants undergoing screening for retinopathy of prematurity. *Retina* 2005; 25:59-62.
9. Cohen AM, Cook N, Harris MC, et al. The pain response to mydriatic eyedrops in preterm infants. *J Perinatol* 2013;33:462-5, doi: 10.1038/jp.2012.149.
10. Olsson E, Eriksson M. Oral glucose for pain relief during eye examinations for retinopathy of prematurity. *Journal of clinical nursing* 2011;20:1054-1059.
11. Grabska J, Walden P, Lerer T, et al. Can oral sucrose reduce the pain and distress associated with screening for retinopathy of prematurity? *J Perinatol* 2005; 25:33-5.

12. Nesargi S, Nithyanandam S, Rao S, et al. Topical anesthesia or oral dextrose for the relief of pain in screening for retinopathy of prematurity: a randomized controlled double-blinded trial. *J Trop Pediatr* 2014; 61:20-4.

13. Gal P, Kissling G, Young W, et al. Efficacy of sucrose to reduce pain in premature infants during eye examinations for retinopathy of prematurity. *Ann Pharmacother* 2005; 39:1029-33.

14. Dilli D, Ilarslan N, Kabatas E, et al. Oral sucrose and non-nutritive sucking goes some way to reducing pain during retinopathy of prematurity eye examinations. *Acta Paediatrica* 2014;103:e76-9.

15. Taplak S, Erdem E. A comparison of breast milk and sucrose in reducing neonatal pain during eye exam for retinopathy of prematurity. *Breastfeed Med* 2017;12:305-310.

16. Kristoffersen L, Stoen R, Bergsend H, et al. Skin-to-skin contact during eye examination did not reduce pain compared to standard care with parental support in preterm infants. *Acta Paediatrica* 2019;108:1434-40.

17. Rush R, Rush S, Nicolau J, et al. Systemic manifestations in response to mydriasis and physical examination during screening for retinopathy of prematurity. *Retina* 2004; 24: 242-5.

18. Mandel R, Ali N, Chen J, et al. Nitrous oxide analgesia during retinopathy screening: a randomized controlled trial. *Arch Dis Child Fetal Neonatal Ed* (2012;97:F83-7.

19. Hartley C, Moultrie F, Hoskin A, et al. Analgesic efficacy and safety of morphine in the Procedural Pain in Premature Infants (Poppi) study: randomized placebo-controlled trial. *Lancet* 2018; 392:2595-605.

20. Sun X, Lemyre B, Barrowman N, et al. Pain management during eye examinations for retinopathy of prematurity in preterm infants: a systematic review. *Acta Paediatrica* 2010; 99:329-34.

21. Francis K. What is best practice for providing pain relief during retinopathy of prematurity eye examinations. *Adv Neonatal Care* 2016;16: 220-8.

22. Disher T, Cameron C, Mitra S, et al. Pain-Relieving Interventions for Retinopathy of Prematurity: A Meta-analysis. *Pediatrics* 2018; 142:1.

23. Pirelli A, Savant Levet P, Garetti E, et al. Literature review informs clinical guidelines for pain management during screening and laser photocoagulation for retinopathy of prematurity. *Acta Paediatrica* 2019; 188:593-99.

24. Läkemedelsverket (2014) Behandling av barn i samband med smärtsamma procedurer i hälso- och sjukvård – kunskapsdokument. Collected 220424 from: https://www.lakemedelsverket.se/sv/behandling-och-forskrivning/behandlingsrekommendationer/sok-behandlingsrekommendationer/behandling-av-barn-och-ungdomar-i-samband-med-smartsamma-procedurer--kunskapsdokument

25. Pichot C, Ghignone M, Quintin L. Dexmedetomidine and clonidine: from second- to first-line sedative agents in the critical care setting? *J Intensive Care Med* 2012; 27:219-37.

26. Hunseler C, Trieschmann U, Möller O, et al. Continuous Infusion of Clonidine in Ventilated Newborns and Infants: A Randomized Controlled Trial. *Pediatric Critical Care Medicine* 2014;15:512-522.

27. Zhang Y. Clonidine preconditioning decreases infarct size and improves neurological outcome from transient forebrain ischemia in the rat. *Neuroscience*. 2004;125:625-31.

28. Stevens B, Gibbins S, Yamada J, et al.The premature infant pain profile-revised (PIPP-R): initial validation and feasibility. *Clinical journal of pain* 2014; 30:238-43.

29. Olsson E, Anderzén-Carlsson A, Atladottir S, et al. Cultural adaptation and harmonization of four Nordic translations of the revised premature infant pain profile (PIPP-R). *BMC Pediatrics* 2018; 8:349.

30. Storm H. Changes in skin conductance as a tool to monitor nociceptive stimulation and pain. *Curr Opin Anaesthesiol* 2008; 21: 96-804.

31. Chan AW, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200-7.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	3	Date and version identifier	n/a
Funding	4	Sources and types of financial, material, and other support	13
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	6
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	n/a
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	6

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	2, 5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5-6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	n/a
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	n/a
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7-8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	5
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	8-9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n/a
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	8
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	7
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	6
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	8
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	n/a

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8-9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	9
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Suppl consent form
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	9
	31b	Authorship eligibility guidelines and any intended use of professional writers	9
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Suppl consent form
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.