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Techniques to increase lumbar puncture success in newborn babies: the NeoCLEAR RCT

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Abstract

Techniques to increase lumbar puncture success in newborn babies: the NeoCLEAR RCT

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Background: Lumbar puncture is an essential tool for diagnosing meningitis. Neonatal lumbar puncture, although frequently performed, has low success rates (50–60%). Standard technique includes lying infants on their side and removing the stylet 'late', that is, after the needle is thought to have entered the cerebrospinal fluid. Modifications to this technique include holding infants in the sitting position and removing the stylet 'early', that is, following transection of the skin. To the best of our knowledge, modified techniques have not previously been tested in adequately powered trials.

Objectives: The aim of the Neonatal Champagne Lumbar punctures Every time – An RCT (NeoCLEAR) trial was to compare two modifications to standard lumbar puncture technique, that is, use of the lying position rather than the sitting position and of 'early' rather than 'late' stylet removal, in terms of success rates and short-term clinical, resource and safety outcomes.

Methods: This was a multicentre 2 × 2 factorial pragmatic non-blinded randomised controlled trial. Infants requiring lumbar puncture (with a working weight ≥ 1000 g and corrected gestational age from 27^{+0} to 44^{+0} weeks), and whose parents provided written consent, were randomised by web-based allocation to lumbar puncture (1) in the sitting or lying position and (2) with early or late stylet removal. The trial was powered to detect a 10% absolute risk difference in the primary outcome, that is, the percentage of infants with a successful lumbar puncture (cerebrospinal fluid containing < 10,000 red cells/mm^3). The primary outcome was analysed by modified intention to treat.

Results: Of 1082 infants randomised (sitting with early stylet removal, *n* = 275; sitting with late stylet removal, *n* = 271; lying with early stylet removal, *n* = 274; lying with late stylet removal, *n* = 262), 1076 were followed up until discharge. Most infants were term born (950/1076, 88.3%) and were aged < 3 days (936/1076, 87.0%) with a working weight > 2.5 kg (971/1076, 90.2%). Baseline characteristics

were balanced across groups. In terms of the primary outcome, the sitting position was significantly more successful than lying [346/543 (63.7%) vs. 307/533 (57.6%), adjusted risk ratio 1.10 (95% confidence interval 1.01 to 1.21); p = 0.029; number needed to treat = 16 (95% confidence interval 9 to 134)]. There was no significant difference in the primary outcome between early stylet removal and late stylet removal [338/545 (62.0%) vs. 315/531 (59.3%), adjusted risk ratio 1.04 (95% confidence interval 0.94 to 1.15); p = 0.447]. Resource consumption was similar in all groups, and all techniques were well tolerated and safe.

Limitations: This trial predominantly recruited term-born infants who were < 3 days old, with working weights > 2.5 kg. The impact of practitioners' seniority and previous experience of different lumbar puncture techniques was not investigated. Limited data on resource use were captured, and parent/practitioner preferences were not assessed.

Conclusion: Lumbar puncture success rate was higher with infants in the sitting position but was not affected by timing of stylet removal. Lumbar puncture is a safe, well-tolerated and simple technique without additional cost, and is easily learned and applied. The results support a paradigm shift towards sitting technique as the standard position for neonatal lumbar puncture, especially for term-born infants during the first 3 days of life.

Future work: The superiority of the sitting lumbar puncture technique should be tested in larger populations of premature infants, in those aged > 3 days and outside neonatal care settings. The effect of operators' previous practice and the impact on family experience also require further investigation, alongside in-depth analyses of healthcare resource utilisation. Future studies should also investigate other factors affecting lumbar puncture success, including further modifications to standard technique.

Trial registration: This trial is registered as ISRCTN14040914 and as Integrated Research Application System registration 223737.

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List of abbreviations

aRR	adjusted risk ratio
b.p.m.	beats per minute
CGA	corrected gestational age
CI	confidence interval
CONSORT	Consolidated Standards of Reporting Trials
CRF	clinical research fellow
CRP	C-reactive protein
CSF	cerebrospinal fluid
CTU	Clinical Trials Unit
DMC	Data Monitoring Committee
eCRF	electronic case report form
ESR	early stylet removal
HR	heart rate
IQR	interquartile range
LP	lumbar puncture
LSR	late stylet removal
MD	mean difference
Med D	median difference
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health and Care Research
NNT	number needed to treat
NPEU	National Perinatal Epidemiology Unit
PCR	polymerase chain reaction
PI	principal investigator
PMG	Project Management Group
PMN	polymorphonuclear leucocyte
PPI	patient and public involvement
RBC	red blood cell
RCT	randomised controlled trial
RR	risk ratio
SAE	serious adverse event
SD	standard deviation
SOP	standard operating procedure
SpO_2	oxygen saturation
SSNAP	Support for the Sick Newborn And their Parents
STAI-S	State–Trait Anxiety Inventory Subscale
TSC	Trial Steering Committee
WBC	white blood cell

Plain language summary

Newborn babies are more susceptible to getting meningitis, and this can be fatal or have lifelong complications. A lumbar puncture is an essential test for diagnosing meningitis. Lumbar puncture involves taking a small amount of spinal fluid from the lower back using a needle. Analysing the fluid confirms or excludes meningitis, allowing the right treatment to be given. Lumbar punctures are commonly performed in newborns, but are technically difficult. In 50–60% of lumbar punctures in newborns, either no fluid is obtained or the sample is mixed with blood, making analysis less reliable. No-one knows which is the best technique, and so practice varies. The baby can be held lying on their side or sat up, and the 'stylet', which is a thin piece of metal that sits inside (and aids insertion of) the needle, can be removed either soon after passing through the skin (i.e. 'early stylet removal') or once the tip is thought to have reached the spinal fluid (i.e. 'late stylet removal').

We wanted to find the best technique for lumbar puncture in newborns. Therefore, we compared sitting with lying position, and 'early' with 'late' stylet removal.

We carried out a large trial in newborn care and maternity wards in 21 UK hospitals. With parental consent, we recruited 1082 full-term and premature babies who needed a lumbar puncture. Our results demonstrated that the sitting position was more successful than lying position, but the timing of stylet removal did not affect success.

In summary, the sitting position is an inexpensive, safe, well-tolerated and easily learned way to improve lumbar puncture success rates in newborns. Our results strongly support using this technique in newborn babies worldwide.

Scientific summary

Background

The neonatal period carries the highest risk of bacterial meningitis (≈ 1 per 4000–5000 births), which is associated with significant mortality (≈ 10%) and morbidity (20–50%). Meningitis is diagnosed by analysis of cerebrospinal fluid (CSF), obtained via lumbar puncture (LP). LPs are frequently performed in newborns because of the non-specific clinical features of neonatal meningitis. However, LP success rates in newborns are much lower (50–60%) than in older children (78–87%). Unsuccessful LPs include those with heavily blood-stained CSF or LPs that fail to obtain CSF at all. Treatment for suspected or confirmed neonatal meningitis involves intravenous antibiotics, typically for 14–21 days. Unsuccessful LPs lead to repeated attempts, whereas LPs with equivocal or uninterpretable CSF results often prompt cautious treatment with extended courses of antibiotics. Prolonged antibiotic use is associated with a range of complications, including induced antimicrobial resistance. Consequently, interventions to improve neonatal LP success rates should allow more accurate diagnosis of meningitis, which will help reduce unnecessary courses of antibiotic therapy and extended hospitalisation, and will save healthcare resources.

There have been few modifications to the original LP technique. Thus far, the sitting position, as employed for older patients, and 'early stylet removal' (ESR) have been suggested. ESR promises advantages because, in neonates, a 'loss of resistance' on entering the CSF is often indistinguishable and a needle advanced too far can cause venous puncture and a blood-stained tap, impairing CSF interpretation.

We conducted the Neonatal Champagne Lumbar punctures Every time – An RCT (NeoCLEAR) trial to determine the optimal LP technique in neonates in terms of the effect of infant position (sitting vs. lying) and timing of stylet removal [ESR vs. late stylet removal (LSR)] on success [i.e. a CSF red blood cell (RBC) count of < 10,000/mm^3] of first LP.

Methods

Trial design and oversight

The NeoCLEAR trial was a 2 × 2 factorial open-label multicentre randomised controlled trial (RCT), with an internal pilot. The study protocol was published previously. The NeoCLEAR trial was co-ordinated by the National Perinatal Epidemiology Unit – Clinical Trials Unit. The University of Oxford (Oxford, UK) sponsored the trial. Trial oversight was conducted by the Trial Steering Committee and an independent Data Monitoring Committee. The funder [i.e. the National Institute for Health and Care Research (NIHR)] did not have a role in study design, conduct, data collection, analysis or interpretation. Ethics approval was obtained.

Trial population

Eligible infants were inpatients in UK neonatal units and maternity wards who required a LP at a corrected gestational age (CGA) of 27^{+0} to 44^{+0} weeks and with a working weight of ≥ 1000 g. Infants were excluded if they had already had a LP for the same indication, if they were unable to be held in sitting position or if sitting was deemed unsafe.

Trial procedures

Infants whose parents had provided consent were randomised 1 : 1 : 1 : 1 by a one-based system to the following four groups: (1) lying position and LSR, (2) lying position and ESR, (3) sitting position and LSR or (4) sitting position and ESR. Block randomisation was stratified according to site and CGA (four categories: 27^{+0} to 31^{+6} weeks, 32^{+0} to 36^{+6} weeks, 37^{+0} to 40^{+6} weeks and ≥ 41 weeks).

Staff were trained in all four techniques. Any second LP required followed the same allocated technique. The need for any further LPs and the techniques used were determined by the clinical team. Blinding of practitioners was impossible, but the primary outcome was based on laboratory tests performed blinded to allocation.

Outcomes

Participants were followed up until discharge. The primary outcome was the proportion of infants with a successful first LP, defined as a CSF RBC count of < 10,000/mm^3. Secondary outcomes included short-term clinical measures (e.g. number of procedures/attempts per infant, proportions with different CSF-based diagnoses, time taken per procedure, infant movement), resources (e.g. duration of antibiotics, length of stay) and safety (e.g. complications, adverse event reporting).

Statistics and analysis

The NeoCLEAR trial was designed with 90% power to detect a 10% absolute difference in the primary outcome (estimated comparator group event rate 59%), with a 5% two-sided significance level. Four hundred and eighty-three infants were required for each arm of each comparison (i.e. sitting vs. lying position and ESR vs. LSR). Allowing for 5% attrition, the recruitment target was 1020 infants.

Outcomes were analysed by modified intention to treat (excluding participants who were withdrawn before collection of trial data or who did not undergo LP). For infant positioning, we compared groups (1) lying/LSR plus (2) lying/ESR with groups (3) sitting/LSR plus, (4) sitting/ESR. To assess the timing of stylet removal, we compared groups (1) plus (3) with groups (2) plus (4). We estimated risk ratios (RRs) for the primary outcome and all other dichotomous outcomes, the mean difference for normally distributed continuous outcomes and the median difference (Med D) for skewed continuous variables. The 95% confidence intervals (CIs) were calculated for all effect estimates. Groups were compared using regression analysis, adjusting for the stratification factors used at randomisation (i.e. centre and CGA) and allocation to the other intervention. The latter adjustment was advised after the final statistical analysis plan was signed off and is a noted deviation. Adjusted risk ratios (aRRs) were estimated using log-binomial regression, or using a Poisson regression model with a robust variance estimator in the event of non-convergence. Linear regression was used for normally distributed outcomes and quantile regression for skewed continuous outcomes.

To mitigate multiple testing, inference was restricted to prespecified tested outcomes. A descriptive multiarm analysis was also performed for the primary outcome, other tested outcomes and baseline characteristics (i.e. for each of the four randomised groups). Effect modification between position (i.e. sitting/lying) and the timing of stylet removal (i.e. ESR/LSR) was investigated for the primary outcome using the statistical test for interaction. Prespecified subgroup analyses were conducted for working weight, day of life and CGA at trial entry. Two-sided p-values of ≤ 0.05 were considered to indicate statistical significance.

Results

From August 2018 to August 2020, 1082 participants from 21 centres in the UK were randomised in a 2 × 2 factorial design, resulting in two principal comparisons: (1) sitting position (n = 546) compared with lying position (n = 536) and (2) ESR (n = 549) compared with LSR (n = 533).

A total of 1079 infants had a ‘first’ LP, and 166 (15.4%) infants had a second LP (each of these LP ‘procedures’ involved one or more ‘attempts’). Nine infants were withdrawn during the trial, but in the case of only one of these participants was consent withdrawn before data collection for the primary outcome. Three infants did not receive a LP, and in the case of a further two infants the consent form was missing. Overall, six infants were excluded, leaving 1076 infants for the final (modified intention-to-treat) analysis: (1) sitting position (n = 543) compared with lying position (n = 533) and (2) ESR (n = 545) compared with LSR (n = 531). All infants were followed up until discharge.

Baseline characteristics were similar for the two groups in both comparisons, as recorded at trial entry and at time of first LP. The majority of infants were born at term, were < 3 days old and were not receiving respiratory support. Raised C-reactive protein was the most common indication for LP.

First comparison: sitting position compared with lying position

The primary outcome – a successful first LP – was achieved in 346 of 543 (63.7%) infants in the sitting arm and 307 of 533 (57.6%) infants in the lying arm [aRR 1.10 (95% CI 1.01 to 1.21); $p = 0.03$; adjusted absolute risk difference 6.1% (95% CI 0.7% to 11.4%), adjusted number needed to treat (NNT) 16 (95% CI 9 to 134)].

Infants allocated to the sitting position were less likely than infants allocated to the lying position to exhibit moderate or severe struggling at the time of needle insertion [169/541 (31.2%) vs. 202/527 (38.4%), aRR 0.82 (95% CI 0.71 to 0.94); p = 0.006]. Other secondary outcomes did not reach statistical significance, but predominantly favoured the sitting position.

Based on microscopy of CSF extracted from the first and second LPs (and any culture/polymerase chain reaction results), infants who were sitting were more likely to be 'negative' for meningitis than infants who were lying [396/537 (73.7%) vs. 359/521 (68.9%)], and a result of 'uninterpretable CSF' (i.e. no sample obtained or CSF not possible to analyse, usually due to a **heavily blood contaminated or clotted sample**) was more likely to be recorded for infants who were lying than for infants who were sitting [139/521 (26.7%) vs. 114/537 (21.2%)].

Median duration of antibiotic treatment and length of stay were not significantly different in the sitting and lying arms {median 5 [interquartile range (IQR) 4–6] days in each arm, for both duration of antibiotic treatment and length of stay}.

Four (0.3%) of 1241 first or second LPs, 1 in ESR and 1 in LSR arms, respectively, were abandoned because of cardiovascular deterioration. Lowest oxygen saturation (SpO_2) during the first LP averaged 93% (IQR 89–96%) in the sitting arm and 90% (IQR 85–94%) in the lying arm (adjusted Med D 3.0%, 95% CI 2.1% to 3.9%; $p < 0.001$). Three of 1075 (0.3%) infants required increased respiratory support within 1 hour of their first LP (sitting arm, $n = 1$; lying arm, $n = 2$; not significantly different). The proportion of infants whose lowest SpO_2 fell below 80% during the first LP (analysed post hoc) was 6.6% (35 of 532) in the sitting arm and 14.2% (72 of 508) in the lying LP arm, and this pattern was consistent in preterm and term-born babies.

In 47 of 543 (8.7%) first LPs in infants allocated to the sitting position, at least one attempt involved switching to the lying position [compared with 4/533 (0.8%) infants allocated to the lying allocation, who were switched to sitting]. Of the 47 LPs where there was at least one attempt in which the allocated technique was not adhered to, the decision to change position was mostly made on the second (22/247) or third (24/257) attempt. The decision to change position was usually made by a clinical (45/47). Similarly, for the second LP, the sitting allocation was less often followed [for at least one attempt in 16/76 (22.5%) of infants allocated to sitting vs. 6/90 (7.0%) of infants allocated to lying]. There were no obvious differences in baseline infant characteristics between LPs that were carried out in the allocated position and those that were not.

In prespecified subgroup analyses, the effect of position on the proportion of infants with a successful first LP was consistent across working weight and CGA at trial entry, but a difference in effect was observed between infants enrolled within 3 days of life ($n = 836$, RR 1.14, 95% CI 1.04 to 1.25) and those enrolled after 3 days ($n = 140$, RR 0.9, 95% CI 0.78 to 1.05; $p = 0.001$).

Second comparison: early stylet removal compared with late stylet removal

The primary outcome was achieved in 338 of 545 (62.0%) infants following ESR and in 315 of 531 (59.3%) infants following LSR. There was no significant difference between the groups (aRR 1.04, 95% CI 0.94 to 1.15; $p = 0.45$). There were also no obvious differences between the groups in any of the secondary outcomes.

Either the first or the second LP (4/1242, 0.3%) was abandoned in four infants receiving LP (two in each arm) because of cardiovascular deterioration. Three of 1076 (0.3%) infants required increased respiratory support within 1 hour of their first LP (ESR, *n* =1; LSR, *n* =2; not significantly different). There were no differences in lowest SpO_2, lowest heart rate (HR) or highest HR between the two arms.

The allocated technique was not adhered to in 35 of 1076 (3.3%) first LPs, with similar numbers in each arm (untested outcome). In 13 of 78 infants allocated to ESR, the second LP involved at least one attempt without ESR, compared with 6 of 79 allocated to LSR; however, denominators were small and this was an untested outcome.

The effect of timing of stylet removal on the proportion of infants with successful first LP was consistent across working weight at trial entry, CGA at randomisation and day of life at trial entry.

Multiarm analysis: comparing four randomised groups (sitting plus early stylet removal, sitting plus late stylet removal, lying plus early stylet removal and lying plus late stylet removal)

No significant interaction between infant position and timing of stylet removal was detected ($p = 0.14$). Multiarm baseline characteristics and analyses did not reveal any new obvious differences (only those previously described for the sitting position compared with the lying position; see *First comparison: sitting position compared with lying position*).

Serious adverse events

Four serious adverse events (SAEs) were reported during the trial. Three SAEs were deemed unrelated to LP. One infant, from the sitting/LSR group, developed a scrotal haematoma 2 days after LP. The infant did not undergo further investigations to identify a cause for this, and so a relationship with the LP could not be ruled out. Therefore, this event was deemed 'possibly related'.

Discussion

The NeoCLEAR trial is, to the best of our knowledge, the first adequately powered RCT examining different LP techniques in newborns. The sitting position was superior to the lying position for achieving a successful first LP, with a NNT of 16. Sitting LP was also better tolerated in terms of infant struggling, SpO_2 and HR. Timing of stylet removal did not influence LP success.

Our results might be explained by the anatomical advantages of sitting position described in neonates: (1) the intervertebral spaces widen when the infants lean forward to adopt a (natural) kyphotic position; (2) the CSF passively sinks to the lowest point of the spinal canal and close to the entry site of the needle; and (3) this position is more comfortable for the baby, as evidenced by the reduced struggling we observed. At the time of the study, the only other paediatric RCT of sitting compared with lying position, by Hanson *et al*., involved 168 infants who were < 90 days of age in a paediatric emergency room setting.[32] In that trial, the success rate did not differ significantly between groups (lateral group 63/82, 77%; sitting group, 61/85, 72%; difference 5.1%, 95% CI −8.2% to 18.3%).

The suggestion that higher LP success rates could be achieved with ESR was based on reports of increased success rates with non-styletted needles. However, non-styletted needles are associated with iatrogenic intraspinal epidermoid tumour formation and, therefore, the technique of ESR was introduced. Subsequent observational studies suggested that ESR was associated with increased success rates in infants. At the time of the study, to the best of our knowledge, the NeoCLEAR trial is the first RCT to have investigated ESR and has demonstrated no significant benefit in it for neonatal LP. Therefore, we cannot advise for or against early or late stylet removal in neonates.

Our safety analysis showed greater physiological stability for sitting LP, in keeping with previous observations. Other secondary outcomes lacked statistical significance, including resource outcomes.

The NeoCLEAR trial has several strengths. To the best of our knowledge, it is the largest RCT, to date, investigating modifications to traditional LP technique, and the only such RCT in newborns. We chose to investigate modifications that came at no additional cost and were easily learned. The results are clear-cut, showing a significantly higher success rate for LPs with more interpretable CSF results when infants were held in a sitting position.

Limitations include the fact that many practitioners were unfamiliar with sitting LP before the NeoCLEAR training sessions, and this may have led to more practitioners switching from the allocated sitting position to the lying position following an initially unsuccessful attempt. It could be speculated that success rates would have been even higher if there had been more experience of sitting position LP among practitioners, and if fewer practitioners had switched position. We did not investigate LPs carried out in infants with a CGA of under 27 weeks or over 44 weeks. Furthermore, most infants were born at or near term and had working weights above 2.5 kg and, therefore, we cannot extrapolate our results to infants born extremely pre or post term or to those of significantly different working weights. In addition, in the subgroup analyses of gestational ages, although the inconsistent effect of sitting position may well be a chance finding and/or due to confounders, the NeoCLEAR trial was underpowered to detect a significant difference for those beyond day 3 of life.

In conclusion, the NeoCLEAR trial demonstrates that sitting position is superior to lying position for neonatal LP success rates (NNT 16), with no significant benefit for ESR. Adopting the sitting position is cost neutral, safe, well tolerated, and easy to learn. The results would be applicable in similar settings worldwide and should promote the sitting technique becoming the standard for neonatal lumbar puncture.

Trial registration

This trial is registered as ISRCTN14040914 and as Integrated Research Application System registration 223737.

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Chapter 1 Introduction

Background neonatal lumbar punctures

In the UK, between 15,000 and 30,000 newborns undergo a lumbar puncture (LP) each year to rule out meningitis.[1] Neonatal meningitis is associated with high mortality and morbidity.[1] Symptoms and signs are subtle, and the diagnosis can be confirmed only by analysis of the cerebrospinal fluid (CSF), obtained by performing a LP. Thus, LPs are an essential part of the diagnostic work-up for meningitis.[1,2] LP techniques vary in current neonatal practice, with no level 1 evidence to determine the best approach. Owing to the technical challenges of neonatal LP, thousands of infants each year undergo unsuccessful LPs, resulting in repeat procedures, causing distress to infants and parents and often necessitating prolonged courses of antibiotics and hospitalisation for the infant, and, oftentimes, rooming-in for the accompanying mother (*Box 1*).

The LP procedure aims to obtain CSF from the lower spine for laboratory analysis to confirm the presence/absence of meningitis and to identify the causative organism. Infants with meningitis typically require 14–21 days of inpatient intravenous antibiotics, incurring significant financial costs, and often receive hospital follow-up because of the risk of long-term neurological sequelae.[3] Prolonged antibiotic use is associated with significant complications, such as necrotising enterocolitis,[4] and a potential for the development of antibiotic resistance.[5] If meningitis can be excluded, then antibiotics are usually stopped after 5 days, allowing discharge with no further follow-up.

The definition of successful LP varies, but usually encompasses the acquisition of 'clear' CSF (colloquial medical term: a 'champagne clear tap'). However, in neonates, CSF samples are often pink/red due to red blood cells (RBCs) sampled unintentionally from nearby blood vessels. Significant numbers of RBCs hinder CSF interpretation, and the presence/absence of meningitis cannot be confirmed. Therefore, LP often needs to be repeated, and many infants are treated with extended courses of antibiotics because meningitis cannot be excluded. Repeated procedures and concern about meningitis understandably lead to heightened parental anxiety.[6]

The success rates of LP are much lower in neonates (50–60%)[7,8] than in older children (78–87%).[9,10] Modifications to 'traditional' LP technique have been studied, but most data thus far are observational and have a high risk of bias,[11] and so no improvements have been incorporated into widespread routine practice.

In 2017, it was believed that a trial aiming at establishing the most successful LP technique would be particularly timely. The, then recent, 2016 National Institute for Health and Care Excellence (NICE) guidance,[12] although aiming to avoid delays in diagnosing meningitis, had resulted in many more neonatal LPs being performed,[12] and this led to an increase in antibiotic use, which came at a time of growing concerns about antimicrobial resistance caused by unnecessary use of antibiotics.

BOX 1 Advantages of improved LP technique

- Fewer uninterpretable CSF samples.
- Fewer repeated LP procedures.
- Reduced distress and anxiety for infants and their families.
- Decreased antibiotic use and risk of antibiotic resistance.
- Reduced NHS costs due to fewer procedures, reduced length of stay, shorter antibiotic courses and minimised antibiotic-associated complications.

It was thought that a large-scale randomised controlled trial (RCT) of LP technique would serve the neonatal knowledge base in several ways. First, optimising LP technique will mitigate the impact of the above-mentioned change in practice on NHS resources. Second, as the dangers of antibiotic resistance become ever more pressing, technologies that have the capacity to reduce hospital antibiotic use are invaluable in preventing antibiotic-resistance problems in the future. Third, it addresses the need, identified in a recent systematic review,[11] for an investigation into alternative LP techniques.

Outcomes affected by lumbar puncture technique

If a large-scale RCT could demonstrate an improved LP technique, then its incorporation into clinical practice across the UK should follow, hopefully improving neonatal LP success over the current expected event rate of 59%, which is based on local cohort data.[13] During trial planning, a 10% improvement in LP success was deemed clinically significant. Such an increase in LP success rate can be expected to translate, each year, into 1600 fewer infants having repeat procedures, 14,400 fewer doses of intravenous antibiotics (with fewer complications) and 2680 fewer bed-days for mothers and infants. Parental anxiety would be reduced, as would healthcare costs through reduced hospitalisation and reduced antibiotic use (limiting the ongoing rise of antibiotic-resistant pathogens[13]), and efficiency of neonatal services would be improved.

Existing evidence

Summary of literature review at trial inception

After performing a structured systematic review of the literature, we found no formal systematic reviews or meta-analyses on LP technique in children or neonates. A limited structured review by Hart *et al*.,[11] published in August 2016, investigated various positions of LP. This review, published in the Archimedes section (i.e. a summary of brief structured reviews that is led by a clinical question) of *Archives of Disease in Childhood*, examined the sitting position in both children and neonates and concluded that current evidence suggests that 'Positions other than the lateral decubitus may be equal or superior in terms of lumbar puncture success' and 'Positions other than the lateral decubitus appear as safe'.[11] Hart *et al*.[11] further concluded that 'A large-scale prospective clinical trial directly addressing LP success and safety in different positions would clarify the need to change current practice'.[11]

Following the 2016 update of the NICE guidance, an increase in the frequency of neonatal LP was reported nationally.[2] The imperative to optimise this technique was, therefore, stronger than ever. To expand on the question raised by Hart *et al*.,[11] we conducted our own systematic review in neonates and children (summarised below), investigating any method for improving LP success rate.

Methods

The following electronic databases were searched on 1 February 2016 via Ovid® (Wolters Kluwer, Alphen aan den Rijn, the Netherlands): MEDLINE (1946–present), EMBASE™ (Elsevier, Amsterdam, the Netherlands) (1974–present) and Global Health (1973–present). The search strategy included the keywords {[neonat* OR newborn OR pediatri* OR paediatr* OR infan*] AND 'lumbar puncture'}. The search generated 56 records. Abstracts were screened for any studies comparing factors relating to LP technique, and four studies of relevance were found. Outcomes included success rates or those predicting success, for example number of attempts, anatomical benefits and safety outcomes. Searching the bibliographies of the studies identified by the electronic search strategy identified 21 further studies.

Results

We found eight studies that were RCTs and 17 that were observational studies. Interventions/factors with no consistent evidence of significant benefit were training in LP,[14,15] seniority of practitioner,[9,10,16–19]

sedation,[18,20] use of local anaesthetic,[9,10,15,18,21,22] formulae for needle insertion depth (except certain subgroup analyses)[23] and ultrasound assistance.[24–26] Trials investigating different body position during neonatal LP found that sitting was as safe as lying,[27–29] with increased space for LP needles[27,29,30] and equally effective in obtaining CSF availability.[14] In one study,[31] sitting was associated with a 25% higher chance of a successful first LP attempt in infants aged < 90 days (p = 0.03). The hollow LP needle shaft contains a 'stylet'. Most practitioners aim to insert the needle into the CSF space and then remove the stylet [i.e. late stylet removal (LSR)]. If the needle has advanced too far, then unintentional blood vessel puncture can cause RBC contamination. With early stylet removal (ESR), the stylet is removed after passing through the subcutaneous tissues, and the needle slowly advanced until CSF flows. Two studies[9,10] found that ESR was associated with increased LP success compared with LSR {odds ratio 2.4 [95% confidence interval (CI) 1.1 to 5.2] and odds ratio 1.3 (95% CI 1.04 to 1.7), respectively}.

Updated literature search

Updated search

The above search was repeated most recently on 7 May 2017. One RCT[32] was found, comparing the sitting and lying LP position in a paediatric accident and emergency setting. The trial, reporting only 167 infants, detected no statistically significant difference between groups. The authors concluded that 'further studies are needed to establish stronger statistical power'.[32] Our trial met that recommendation, being appropriately powered, and complements this study[32] by investigating a similar population (i.e. neonates) in whom the need for high-quality evidence is greater because of lower baseline success rates.[7–10] Three studies[33–35] with small sample sizes and/or wide CIs found that ultrasound assistance was associated with increased LP success.

Other trials

The International Clinical Trials Registry Platform was searched (last updated on 25 July 2017) with the key words 'lumbar puncture', and screened as above. The trials listed in *Box 2* have investigated local anaesthetic (n = 7), ultrasound assistance (n = 5), pressure transduction (n = 2), restraint (n = 1) and sedation (n = 1). None overlap with our proposal (see *Box 2*).

Brief summary of the evidence review at trial inception

No LP technique was backed by high-quality evidence from studies conducted in the neonatal period. LP techniques warranting further investigation, which can be studied most efficiently and reliably with a RCT, were (1) sitting position and (2) ESR. Both techniques are free 'existing technologies' that are already used by some practitioners. Observational evidence for these techniques has not led to a change of routine clinical practice. Randomised evidence was felt to be important and highly necessary to provide robust and convincing data. If either technique was shown to be beneficial, it would be free and easy to introduce nationwide.

BOX 2 Summary findings of limited literature review

- Four observational case–control studies[14,27,30,36] (total n = 155 infants and children).
- Two retrospective cohort studies[16,31] (total n = 259 infants).
- Two prospective cohort studies[10,20] (total n = 1639 infants and children).
- One RCT[32] (total n = 168 infants).

Objective

We aimed to determine the optimal technique for LP in newborn infants by evaluating the success rate and short-term clinical, resource and safety outcomes of two modifications to traditional LP technique: a change of infant position (i.e. from lying to sitting) and a change in the timing of stylet removal (i.e. from LSR to ESR). This would be, to the best of our knowledge, the first appropriately powered RCT investigating different neonatal LP techniques, one with the potential to make a significant contribution to current knowledge and even change practice.

Chapter 2 Methods

Design

The trial rationale and design were closely discussed with, and received input from, parent representatives from the Oxford-based charity Support for the Sick Newborn And their Parents (SSNAP) [Oxford, UK; URL: www.ssnap.org.uk (accessed 14 June 2022)]. The final trial design and its protocol are published elsewhere.[37] Briefly, the Neonatal Champagne Lumbar punctures Every time – An RCT (NeoCLEAR) trial was planned as a pragmatic non-blinded multicentre 2 × 2 factorial RCT to compare the proportion of infants in whom CSF with a RBC count < 10,000/mm^3 was successfully obtained on the first LP procedure. Investigated techniques included a combination of (1) the infant's position (i.e. sitting vs. lying) and (2) the timing of stylet removal (ESR vs. LSR) [URL: www.npeu.ox.ac.uk/neoclear (accessed 14 June 2022)]. Following written parental consent, infants requiring LP [with a working weight of > 1000 g and corrected gestational age (CGA) of between 27^{+0} and 44^{+0} weeks] were randomised by web-based allocation to LP (1) in the sitting or lying position and (2) with ESR or LSR. The trial was powered to detect a 10% absolute risk difference in the primary outcome of an interpretable CSF sample, defined as containing a RBC count < 10,000/mm^3. Clinicians undertaking LPs received practical training in the different trialled techniques. The application of topical anaesthetic cream prior to LP was encouraged. A minimum set of vital signs were monitored throughout the procedure [i.e. oxygen saturation (SpO_2) and heart rate (HR)] by pulse oximetry. In addition, the duration of the procedure was timed and the clinical monitoring data were documented for the purpose of the trial. All other technical and, especially, patient management decisions were in accordance with local protocols.

In practice, parents were approached for written consent when an eligible infant needed to undergo LP. Once consent was given, computerised randomisation proceeded. Staff were advised to make one or two attempts, defined as the needle passing through the skin once, per 'procedure'. The randomised technique was to be used for up to two procedures. The requirement for and timing of a second procedure or any further procedures were determined by the clinical team. Patient characteristics and demographic data, as well as trial-relevant clinical data, were sourced by the local study teams from hospital records and recorded on trial-specific electronic case report forms (eCRFs). All CSF samples were sent to the local laboratories, as per recruiting site procedures, and only data from laboratory reports that were immediately relevant to the trial were entered into eCRFs.

To optimise study processes around recruitment, intervention delivery, training and outcome assessments, the trial included an internal pilot, which was carried out over 8 months at centres recruiting the first 250 randomised infants. The Trial Steering Committee (TSC) reviewed the pilot data, made recommendations and approved continuation. Details of TSC members are provided in *Appendix 1*.

Ethics approval and research governance

The NeoCLEAR RCT received approval from the NHS Health Research Authority, South Central Hampshire B Research Ethics Committee on 12 June 2018 (reference 18/SC/0222, nrescommittee.southcentral-hampshireb@nhs.net).

Trust confirmation of capacity and capability was obtained at each site. The chief investigator or delegate submitted an annual progress report, an end of study notification and the final report to the Research Ethics Committee, Health Research Authority, host organisation and sponsor, as required by the respective organisations.

The study was sponsored by the University of Oxford (Oxford, UK). The trial was run by a designated Project Management Group (PMG) at the National Perinatal Epidemiology Unit (NPEU) Clinical Trials Unit (CTU), Nuffield Department of Population Health, University of Oxford. The core PMG met once a month, either remotely or face to face. An extended PMG (i.e. the Co-Investigator Group) met every 3 months initially and then every 4 months subsequently to troubleshoot, review progress and forward plan. The PMG reported to the TSC. Meetings were held either remotely or face to face, as permitted by COVID-19 restrictions.

The trial was overseen by the TSC, which had the ultimate responsibility for considering and, as appropriate, acting on the recommendations of the Data Monitoring Committee (DMC). The TSC included an independent chairperson, at least one clinician, statistician and patient and public involvement (PPI) representative, the chief investigator and the senior co-investigator. The TSC met annually and reviewed the progress of the trial. Contributions on documentation were also received from the baby charity Bliss (London, UK).

A DMC reviewed the study data and outcomes. The DMC ensured the safety and well-being of the trial participants and, if appropriate, made recommendations regarding continuance of the study or modification of the protocol. The DMC was, therefore, also responsible for reviewing the safety reports of serious adverse events (SAEs), but, ultimately, the TSC would have the responsibility for stopping the trial on safety grounds. Lists of the members of the DMC and the TSC are in *Appendices 1* and *2*, respectively.

Patient and public involvement

Patient and public involvement was integral in the trial design, with feedback and input coming from several channels, including (1) early involvement of a PPI representative and co-applicant, (2) advice from the National Institute for Health and Care Research (NIHR) PPI division and information/details for a focus group sent to the NIHR PPI online mailing list, (3) feedback from a PPI representative for the NIHR Neonatal Clinical Study Group, (4) information/details for a focus group via SSNAP and (5) parental written feedback from a survey of parents of babies who previously received care on a neonatal unit.

As a direct result, we (1) confirmed the importance of this trial in improving the clinical care provided to newborns and their families, (2) prioritised our clinical outcomes relating to LP success, number of procedures and length of stay (as those were reported to be the most important outcomes for parents), (3) developed a procedural pain relief protocol aiming to provide better analgesia than current standard practice, (4) had assurance that the timing of our consenting process and randomisation was appropriate and acceptable to parents surveyed and (5) enhanced our plans for the LP training provided to each unit.

Participants

Participants of the trial were infants who were having a LP in UK neonatal units and their associated maternity wards.

Inclusion and exclusion criteria are shown in *Box 3*.

BOX 3 Inclusion and exclusion criteria

Inclusion criteria

- Parent(s) willing and able to give informed consent.
- Infants of CGA from 27^{+0} weeks to 44^{+0} weeks and with a working weight of ≥ 1000 g.
- First LP for current indication.

Exclusion criteria

- Unable to be held in sitting position (including infants intubated and mechanically ventilated) or other clinical conditions that are likely, in the opinion of the treating clinician, to make sitting difficult or that are likely to be compromised by sitting (open gastroschisis, etc.).
- Previously randomised to the trial schedule of study procedures.

Setting

The NeoCLEAR trial was set in UK neonatal units and their associated maternity wards. The following centres were included:

- recruiting sites, where parental consent was obtained, infants were enrolled by randomisation and participation in the trial was commenced (n = 21) (see *Appendix 3*)
- 'continuing care sites', that is other units to which babies were discharged from the recruiting unit and where data collection continued until discharge (see *Appendix 4*).

Infants were eligible to participate in other clinical trials at the same time as taking part in the NeoCLEAR trial, depending on the nature of the interventions in the other trial. Other trials running concurrently were discussed by the chief investigators or their delegated representative, who then agreed if joint recruitment was appropriate.

Screening and eligibility assessment

Infants whom the clinical teams deemed necessary to undergo LP were screened for eligibility. Anonymised screening data were recorded via the randomisation website for the co-ordinating centre to review rates of ineligibility and participant uptake rates.

Informed consent and recruitment

The clinical teams provided the parents of eligible infants with both verbal information and written information, in the form of a parent information leaflet. The teams approached parents with legal parental responsibility to discuss the trial, to answer any questions the parents may have and to request consent. Parents had as much time as they needed to consider the information provided, to discuss it with the research team or other independent parties, and to decide whether or not to participate in the NeoCLEAR trial. Written informed consent for the study was then obtained by a suitably qualified member of the study team. During the study pilot parents were given a copy of a parent anxiety score [using the State–Trait Anxiety Inventory Subscale (STAI-S)] (see *Parent questionnaire*).[38] Parents completing the STAI-S were also asked to provide written consent for their participation (use of the STAI-S was stopped following the review of the internal pilot because completion rates were

low). Consent was given with enough time to allow randomisation and to enable the clinical team to prepare for the procedure. Where this was not possible, the LP was not delayed if the infant's clinician deemed any delay to be clinically unsound, and, in such cases, the infant was not recruited to the NeoCLEAR trial.

Site and staff training

Before undertaking procedures as part of the trial, all 'operators' (i.e. practitioners inserting the needle for the LP) received face-to-face training in trial background and overview and how to perform all four LP techniques to be investigated in the trial. Training included safety monitoring and recommendations for analgesia. Training included demonstration and practice on a neonatal LP manikin (LumbarPunctureBaby, EMD Services, Uttoxeter, UK). Operators were asked to sign a training log to confirm that they were confident in performing any of the four trial techniques on completion of training. Only after this were operators entered onto the delegation log. Assistants (i.e. practitioners holding the baby for LP and/or collecting CSF) were offered the same training or a shortened positioning-focused training session. Uptake of training was variable between sites. Narrated videos of each trial technique were available on the trial website throughout the recruitment period and were signposted during all training sessions. The regular trainee rotation across sites made regular training sessions necessary, and this was facilitated by the clinical research fellow (CRF) and local principal investigators (PIs).

Training the trainers

Owing to junior doctor rotation, the number of participating centres and the resulting distances between the primary study sites, the number of training sessions required to ensure ongoing recruitment became large. Additional manikins were purchased and a 'train the trainer' competency document was constructed to allow PIs and sub-PIs to become trainers. Sign-off of this competency document was carried out by the CRF and local PI. LP manikins were couriered to the sites participating in 'train the trainer' to ensure that all operators had experience in practising on the manikins. Uptake of 'train the trainer' made restarting recruitment after the COVID-19 hiatus possible when face-to-face site visits were not permitted. Mechanical upkeep of the training manikin was ensured by the CRF together with the manufacturer.

Interventions

Infants requiring LP were randomly allocated to one of four combinations of interventions: (1) LP in the lying (lateral decubitus) position and LSR, (2) LP in the lying position and ESR, (3) LP in the sitting position and LSR or (4) LP in the sitting position and ESR. The trial protocol suggested that term-born infants were treated with local anaesthetic cream prior to LP. Infants were clinically monitored throughout the procedures through detailed clinical observation, aided by pulse oximetry (i.e. continuous measurement of peripheral SpO_2 and HR). The duration of procedures was timed and recorded as part of the trial documentation.

Randomisation and blinding

The NeoCLEAR trial used 1 : 1 : 1 : 1 randomisation to one of the four arms [i.e. (1) lying (lateral decubitus) position and LSR, (2) lying position and ESR, (3) sitting position and LSR or (4) sitting position and ESR] using a 24 hours a day, 7 days a week, secure web-based randomisation facility, which ensured balance between the groups. A telephone back-up system was available 24 hours a day.

Stratified block randomisation was used to ensure balance between the groups with respect to the collaborating hospital and CGA at trial entry (four groups: 27^{+0} to 31^{+6} weeks, 32^{+0} to 36^{+6} weeks, 37^{+0} to 40^{+6} weeks and ≥ 41 weeks). If repeat LPs were warranted for the same infant for the same indication after an initial unsuccessful attempt or procedure, then the infant received the same allocated technique. Infants who had more than one indication for LP during the trial recruitment period were not re-randomised. Multiple births were randomised separately, with their study identification numbers linked on the database prior to analysis.

A statistician who was independent of the trial generated the randomisation schedule, and the senior trials programmer wrote the web-based randomisation program; both were independently validated. The implementation of the randomisation procedure was monitored by the senior trials programmer throughout the trial, and reports were provided to the DMC.

The NeoCLEAR trial was an open-label trial, as blinding of the practitioner and nursing staff to the allocated technique was not possible. The assessment of the primary outcome and major secondary outcomes was based on laboratory tests (effectively blinded). Parents were not usually told which technique their infant had been allocated to and were not routinely present for the procedure; however, if they requested this information, it was shared with them, and they were able to observe the LP, at the discretion of the practitioner.

Primary and secondary outcomes

Primary outcome

The primary outcome was proportion of infants in whom the first LP procedure was successful (i.e. a RBC count in CSF of < 10,000/mm^3). We chose the cut-off point as 10,000 RBC count in accordance with previous studies[7,19,23] investigating LP success, as identified in our literature research (*Boxes 4* and *5*).

BOX 4 Definition interpretable LP (laboratory)

- CSF obtained and RBC count < 10,000/mm^3.
- A CSF WBC count not requiring a correction (regardless of the RBC count).
- If the RBC count is ≥ 500, then the WBC count would be reduced by 1 for every 500 RBCs to give a 'corrected' WBC count.

WBC, white blood cell.

BOX 5 Definition of sample, indicating meningitis/confirmative diagnosis of meningitis

- A CSF WBC count of ≥ 20.
- True-positive bacterial CSF culture.
- Positive PCR.

PCR, polymerase chain reaction; WBC, white blood cell.

Secondary outcomes

The following short-term clinical, resource and safety outcomes were defined.

- The proportion of infants with:
  - no CSF obtained, or pure-blood/clotted blood, blood-stained or clear CSF
  - CSF obtained and a RBC count of < 500/mm^3, < 5000/mm^3, < 10,000/mm^3 or < 25,000/mm^3, or any RBC count
  - a CSF white blood cell (WBC) count not requiring a correction (regardless of the RBC count).
- The total number of procedures and attempts performed per infant.
- The proportion of infants diagnosed (by WBC count criteria, culture, Gram stain and/or clinically) via CSF with the following:
  - Meningitis, with a WBC count of ≥ 20 in CSF or a true-positive culture/polymerase chain reaction (PCR). If the RBC count is ≥ 500, then the WBC count will be reduced by 1 for every 500 RBCs to give a 'corrected' WBC count.
  - Equivocal, with a WBC count (or corrected WBC) of < 20 and negative (or contaminated/incidental) culture and PCR with either:
    - polymorphonuclear leucocytes (PMNs) > 2 (and a RBC count < 500), or
    - organism found on Gram stain.
  - Negative, with a WBC (or corrected WBC) count of < 20, PMN ≤ 2 (if the RBC count is < 500) and negative (or contaminated/incidental) cultures, PCR and Gram stain.
  - Uninterpretable, with no CSF obtained, CSF too bloody, blood clotted, or CSF insufficient to perform a cell count.
- CSF WBC, RBC, corrected WBC counts, PMNs and lymphocytes from the clearest sample.
- Time taken on first procedure from start of cleaning skin to removing needle at end of all attempts.
- Infant movement on first procedure using a basic 4-point scale.
- Outcomes relating to cost, resource consumption and safety:
  - In all infants, according to CSF-defined and clinically defined diagnostic criteria:
    - duration of the antibiotic course
    - length of stay in surviving infants.
  - Immediate complications related to LP:
    - cardiovascular instability, including SpO_2 and HR
    - respiratory deterioration (escalating respiratory support) post LP.
- For the pilot phase, parental anxiety (assessed using the STAI-S).

The decision on treatment for any study participant was in accordance with the individual centre's guidance. Choice of antibiotic, route of administration and length of treatment were as per the local protocols, many of which were based on those issued by NICE[12] (*Box 6*).

BOX 6 National Institute for Health and Care Excellence treatment guidance for neonatal meningitis

Early- and late-onset meningitis (babies in neonatal units)

1.14.1 If a baby is in a neonatal unit and meningitis is suspected but the causative pathogen is unknown (e.g. because the CSF Gram stain is uninformative), treat with intravenous amoxicillin and cefotaxime. [2012, amended 2021.]

1.14.2 If a baby is in a neonatal unit and meningitis is shown (by either CSF Gram stain or culture) to be caused by Gram-negative infection, stop amoxicillin and treat with cefotaxime alone. [2012, amended 2021.]

1.14.3 If a baby is in a neonatal unit and meningitis is shown (by CSF Gram stain) to be caused by a Gram-positive bacterium then continue treatment with intravenous amoxicillin and cefotaxime while waiting for the CSF culture result and seek expert microbiological advice. [2012, amended 2021.]

1.14.4 If the CSF culture is positive for group B streptococcus, then consider changing the antibiotic treatment to benzylpenicillin 50 mg/kg every 12 hours, normally for at least 14 days and gentamicin, with:

- a starting dosage of 5 mg/kg every 36 hours
- subsequent doses and intervals adjusted, if necessary, based on clinical judgement and blood gentamicin concentrations
- treatment lasting for 5 days.

Parent questionnaire

During the pilot phase (i.e. after consent but before the first LP), parents were asked 'How have you felt physically during the last couple of days?'. Parents used a five-point Likert scale to answer the question. In addition, parents were asked to complete the STAI-S questionnaire.[38,39] The STAI-S is a well-validated measure that consists of 20 questions that identify how stressed/anxious someone is feeling at the time of assessment. All items on the STAI-S are rated on a four-point scale, and the mean score can be used in analyses. Discontinuation of the STAI-S was recommended by the TSC based on a review of the pilot.

Sample size

The NeoCLEAR trial was designed with 90% power to detect a 10% absolute difference in the primary outcome (with an estimated comparator group event rate of 59%), with a 5% two-sided significance level. A total of 483 infants were required for each arm of each comparison (i.e. sitting position vs. lying position and ESR vs. LSR). Allowing for 5% attrition, the recruitment target was 1020 infants. Initial recruitment was planned at 10 hospitals and this was later extended to 21 centres across England (see *Appendix 3*).

Statistical analyses

A statistical analysis plan was agreed prior to data lock. The analysis and presentation of results followed recommendations of the CONSORT (Consolidated Standards of Reporting Trials) group.

Descriptive analysis

The flow of participants through each stage of the trial is summarised by principal comparison and by randomised group using a CONSORT diagram (see *Figure 1*).

Baseline characteristics of the infants at trial entry and characteristics of the infants at first LP are summarised by principal comparison group. Counts and percentages are presented for binary and categorical variables. The mean and standard deviation (SD) or median and interquartile range (IQR) are presented for continuous variables, as well as range, if appropriate. There were no tests of statistical significance of differences between groups on any baseline variable.

The numbers of losses to follow-up are reported for each group, with reasons, and deaths are reported separately. Missing data are described by presenting the number of individuals with missing data for each outcome.

Primary analysis

Outcomes are analysed by modified intention to treat (i.e. excluding participants who were withdrawn before collection of trial data or did not undergo LP). For infant positioning, we compared the lying/ LSR group (i.e. group 1) plus the lying/ESR group (i.e. group 2) with the sitting/LSR group (i.e. group 3) plus the sitting/ESR group (i.e. group 4). To assess the timing of stylet removal, we compared groups 1 and 3 with groups 2 and 4. Lying position and LSR were considered the reference groups.

We estimated risk ratios (RRs) for the primary outcome and all other dichotomous outcomes, the mean difference (MD) for normally distributed continuous outcomes and the median difference (Med D) for skewed continuous variables. The absolute risk difference was calculated for tested dichotomous clinical outcomes. Groups were compared using regression analysis, adjusting for the stratification variables used at randomisation (i.e. centre and CGA), with CGA as a fixed effect and centre as a random effect, where possible. Comparative analyses were also adjusted for the allocation to the other intervention (i.e. the sitting and lying comparison was adjusted for allocation to ESR/LSR, and vice versa). This adjustment was advised because of potential correlation between comparison arms after the final statistical analysis plan was signed off, and is a noted deviation. Adjusted risk ratios (aRRs) were estimated using log-binomial regression or using a Poisson regression model with a robust variance estimator in the event of non-convergence. Linear regression was used for normally distributed outcomes, and quantile regression was used for skewed continuous outcomes. Both crude and adjusted estimates are presented, with the primary inference based on the adjusted estimate. Ninety-five per cent CIs were calculated for all effect estimates, and two-sided *p*-values of 0.05 or less were considered to indicate statistical significance.

To mitigate multiple testing, inference was restricted to prespecified tested outcomes.

Secondary clinical outcomes tested

- The proportion of infants with:
    - no CSF obtained, or pure-blood/clotted, blood-stained or clear CSF from clearest sample of the first procedure (any attempt)
    - CSF obtained with any RBC count on first procedure (any attempt)
    - CSF obtained with a WBC count not requiring correction on first procedure, that is, a WBC count < 20 regardless of the RBC count, or a RBC count < 500 (any attempt).
- The proportion of infants diagnosed by the clinical team at discharge – in relation to their LP(s) – with:
    - definite/probable meningitis

- possible meningitis or equivocal CSF result
- negative CSF result
- uninterpretable CSF result (e.g. very high RBC or clotted CSF)
- no CSF obtained.

- WBC count, RBC count, corrected WBC count, PMN and lymphocytes from clearest CSF sample.
- Total number of procedures performed per infant.
- Total number of attempts performed per infant.
- Time taken to complete the first procedure, from start of cleaning skin to removing needle at end of all attempts.
- Level of infant struggling movement on first attempt of first procedure.

Secondary clinical outcomes not tested

- For the first attempt of the first procedure, for any attempt of first procedure if not in Secondary clinical outcomes tested and for any attempt of second procedure:
  - CSF appearance (clear CSF/blood-stained CSF/pure-blood or clotted CSF/no CSF sample obtained)
  - CSF obtained and any RBC count
  - CSF obtained and RBC count < 500/mm^3
  - CSF obtained and RBC count < 5000/mm^3
  - CSF obtained and RBC count < 10,000/mm^3
  - CSF obtained and RBC count < 25,000/mm^3
  - CSF obtained with WBC count not requiring correction (i.e. a WBC count < 20 regardless of the RBC count, or a RBC count < 500).
- Number of attempts for first and second procedure per infant.
- From first two procedures, the proportion of infants diagnosed by CSF with following:
  - Meningitis, with a WBC count of ≥ 20 in CSF or a true-positive culture/PCR. If the RBC count is ≥ 500, then theWBC count will be reduced by 1 for every 500 RBCs, to give a 'corrected' WBC count.
  - Equivocal, with a WBC count (or corrected WBC) of < 20 and negative (or contaminated/incidental) culture and PCR with either:
    - PMNs > 2 (and a RBC count < 500), or
    - organism found on Gram stain.
  - Negative, with a WBC (or corrected WBC) count of < 20, PMN ≤ 2 (if the RBC count is < 500) and negative (or contaminated/incidental) cultures, PCR and Gram stain.
- Uninterpretable, with no CSF obtained, clotted CSF or CSF too bloody or insufficient to enable a cell count.

Cost/resource consumption tested

- Duration of the antibiotic course from trial entry to discharge home.
- Length of stay in hospital in surviving infants from trial entry until discharge home.

Safety outcomes tested

- Immediate complications related to first procedure:
  - procedure abandoned because of cardiovascular deterioration
  - infant's lowest SpO_2 (%)

- infant's lowest HR [beats per minute (b.p.m.)]
- infant's highest HR (b.p.m.)
- respiratory deterioration post LP (a requirement for escalating respiratory support within 1 hour of the LP).

Safety outcomes not tested

- Immediate complications related to second procedure:
  - procedure abandoned because of cardiovascular deterioration
  - respiratory deterioration post LP (a requirement for escalating respiratory support within 1 hour of the LP).

Secondary analysis

A descriptive multiarm analysis was performed for the primary outcome, other tested outcomes and baseline characteristics (i.e. for each of the four randomised groups). Effect modification between positions (i.e. sitting/lying) and the timing of stylet removal (i.e. ESR/LSR) was investigated for the primary outcome using the statistical test for interaction.

Subgroup analysis

Prespecified subgroup analyses were conducted on the primary outcome for working weight at trial entry (i.e. < 2500 g, 2500–3500 g and > 3500 g), day of life (i.e. < 3 days and ≥ 3 days) and CGA at trial entry (27^{+0} to 36^{+6} weeks, 37^{+0} to 40^{+6} weeks and ≥ 41 weeks). It was planned to use four subgroups of CGA, but 27^{+0} to 31^{+6} weeks and 32^{+0} to 36^{+6} weeks were collapsed into a single group because of low numbers in each, which was a deviation from the statistical analysis plan. RRs and 95% CIs are presented for each subgroup, along with the interaction *p*-value.

Data collection

All trial data were collected from hospital records and recorded on trial-specific eCRFs (see *Appendix 6*). Trial entry data included details to confirm eligibility and confirmation of parental written consent. The completion of data entry was monitored through the CTU. Most outcome data consisted of routinely recorded clinical items obtained from the clinical notes. Non-routinely collected data included procedural details, such as time taken for LP, infant movement, SpO_2 and HR. No additional blood or tissue samples were required. Outcome data were collected until discharge home. Parents completing the STAI-S were asked to complete a second questionnaire within 48 hours of the first LP (pilot phase only).

Adverse event reporting

Box 7 lists known, but rare, complications of LP. Any occurrence of a complication following the LP was reported as a SAE.

BOX 7 Reportable SAEs

- Iatrogenic meningitis.
- Iatrogenic haemorrhage: spinal haematoma (symptomatic), intraventricular, intracerebral and subarachnoid haemorrhage.
- Cerebral herniation.

The full protocol and guidance sheets provided to sites also prespecified expected SAEs that were foreseeable and should not have been reported unless thought to be causally related to trial procedures. All unforeseeable SAEs occurring after consent until discharge home had to be reported.

Parents had the right to withdraw consent for any aspect of the study, including their infant's future procedures and data collection, as well as their own questionnaire completion. The treating clinician was permitted to discontinue a participant if they considered it to be in the best interests of the infant's health and well-being.

Adverse events were recorded locally and in the eCRF, and were followed up by the trials team. The DMC reviewed the study data and outcomes, including safety reports of SAEs. SAEs were collected until the infant was discharged home, as SAEs occurring after this time point were unlikely to be related to the trial intervention. As parental participation was limited to the STAI-S questionnaire, no SAE recording was conducted for this group. For the duration of the trial, the DMC ensured the safety and well-being of the trial participants and would have made recommendations to the TSC regarding discontinuance of the study or modification of the protocol, if required; however, this was not necessary throughout the NeoCLEAR trial.

Reporting procedures

All expected SAEs (see *Box 7*) were recorded on the eCRF and reviewed by the DMC at regular intervals throughout the trial. Any unexpected SAEs were reported by trial sites to the NeoCLEAR Coordinating Centre as soon as possible after the event had been recognised as a SAE that was not included in the list of expected SAEs. Information on each SAE was recorded on a SAE reporting form, which was electronically transferred to the NeoCLEAR Coordinating Centre. A standard operating procedure (SOP) outlining the reporting procedure for clinicians was provided with the SAE form and in the trial handbook. The NeoCLEAR Coordinating Centre processed and reported the events as specified in the CTU's SOPs. The chief investigator informed all investigators concerned of relevant information about unexpected SAEs that could adversely affect the safety of participants. Once a year, during the recruiting period of the trial, a safety report was submitted to the sponsor and Research Ethics Committee.

Governance and monitoring

The NPEU CTU operates on a stringent set of accredited SOPs. Within this framework, the CTU oversaw the trial governance and conduct, as well as the monitoring of the trial centres. The CTU guidance comprised structured face-to-face induction visits with trial-specific training for investigators, their support team and research staff at site initiation. Written trial-specific materials were shared in printed and electronic forms. Local study centre staff were given access to multiple online training resources, which were also made available to staff at continuing care sites, together with other supporting material [URL: www.npeu.ox.ac.uk/neoclear (accessed 14 June 2022)]. A 24-hour telephone line was available to help with randomisation issues, including the option of telephone randomisation in the case of unresolvable information technology issues at the recruiting site.

Trial monitoring continued throughout the recruitment phase with review of investigator site files, the delegation logs, certificates of good clinical practice and the research curricula vitae of the local site staff. Trial data management was performed to the standards of the NPEU CTU's SOPs, following prespecified schedules. Data from the recruiting centres were closely monitored for quality assurance. The monitoring included regular review of consent forms and reassurance of correctly assessed participant eligibility. Additional validation checks of data were carried out in intervals. Whenever needed, queries were issued to study centres for resolution. In accordance with best data management

practice, final data validation checks were carried out before the database lock. Open questions were sought to be resolved through discussion with the site PI and their local teams.

The independent DMC was continuously informed of study progress in the form of written reports and at regularly scheduled physical DMC meetings. Incidents where the study statistician might raise concerns regarding the data quality were reported to the study data management staff, who would query these incidents if deemed appropriate, or followed up by further routine data validation checks, or both. DMC meetings were also used to provide external independent review of summary data, where necessary.

Summary of changes to the study protocol

A summary of the changes made to the original protocol is presented in *Appendix 7*.

Chapter 3 Results

The NeoCLEAR trial investigators sought to optimise LP technique in newborns by evaluating three modifications to traditional LP technique in an adequately powered randomised controlled clinical trial. An overview of the trial is given in *Figure 1*.

Results are presented in three parts (i.e. parts A–C). In part A, we compare the impact of infant position on LP success. In part B, we report on the LP outcomes, focusing on differences between ESR and LSR. In part C, we report on the multiarm analysis. The study flow of participants per principal comparison is outlined in *Figures 2–5*. Post hoc exploratory analyses are reported in *Appendix 9*. Further information on data collection forms (*Table 29*), details of changes to the study protocol (*Table 30*), group allocation per recruiting site (*Table 31*), baseline characteristics by non-adherence to position allocation (any attempt in first LP) (*Table 32*), baseline characteristics by non-adherence to Stylet Removal allocation (any attempt in first LP) (*Table 33*), characteristics at baseline/ first LP by age at randomisation (*Table 34*), and infant's lowest oxygen saturation <80% at first LP by position allocation (*Table 35*), will also be found in the appendix.

Recruitment and retention

The NeoCLEAR trial recruited infants from August 2018 to August 2020. COVID-19 regulations forced a pause of recruitment between March and July 2020. Trial activity restarted without difficulty thereafter

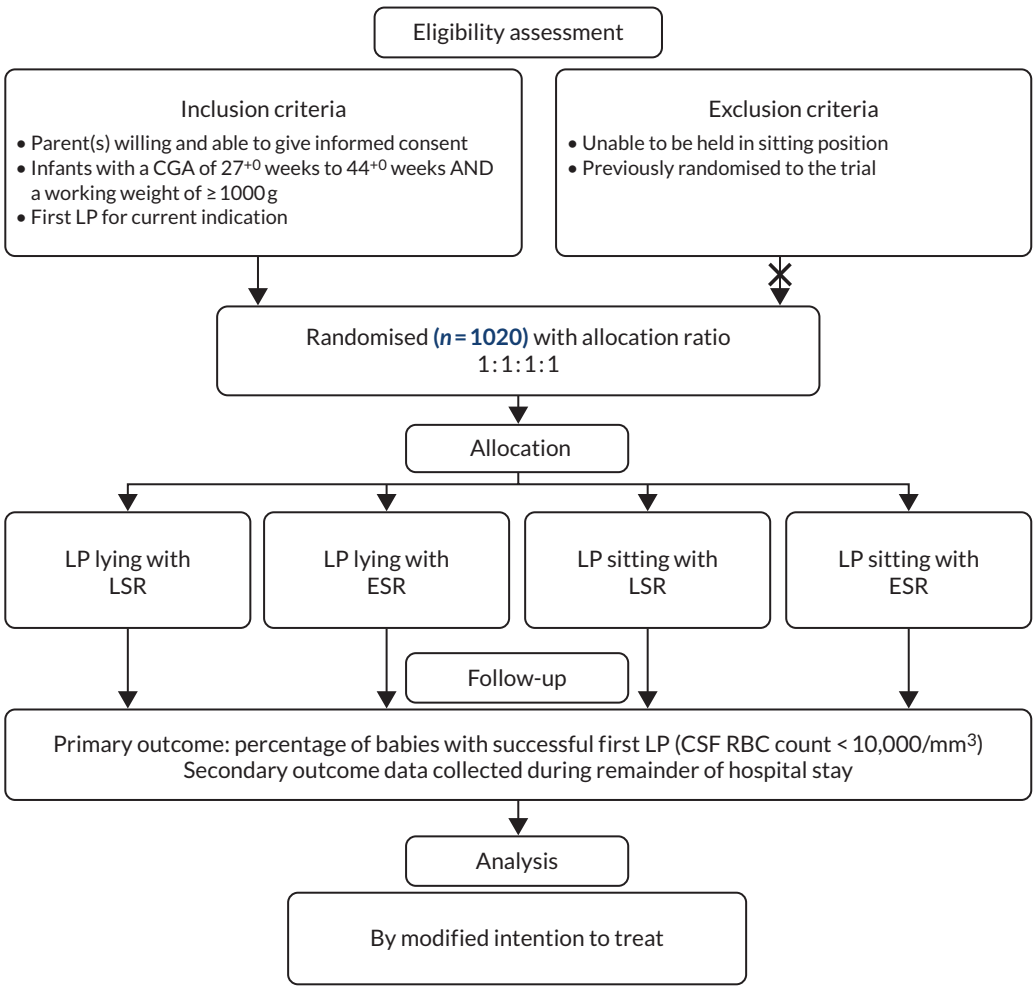

FIGURE 1 Study participant flow diagram.

until completion. The trial recruited in 21 hospitals. Infant and parent characteristics at trial entry and at first LP are detailed in *Tables 1* and *2*, respectively. See *Table 27* for the list of participating centres.

Participants

From August 2018 to August 2020, 1082 participants from 21 centres were randomised to the principal comparisons: (1) sitting position (*n* = 546) compared with lying position (*n* = 536) and (2) ESR (*n* = 549) compared with LSR (*n* = 533).

Demographic and other baseline characteristics

The baseline patient characteristics at trial entry are presented in *Table 1*, and patient characteristics at first LP are presented in *Table 2*. Characteristics were well balanced throughout the four groups. Most patients were term-born infants and most had a working weight of ≥ 2500 g. We saw a slight male predominance, which was consistent between groups. The indications for LP were as per modified NICE guidance, i.e. predominantly exclusion of meningitis, suspected either because of patient and maternal history, or laboratory parameters [i.e. raised C-reactive protein (CRP)].[12]

TABLE 1 Infant (parent) characteristics at trial entry

Characteristic	Position	
	Sitting (*N* = 543)	Lying (*N* = 533)
CGA at trial entry (weeks^{+days}), *n* (%)		
27^{+0} to 31^{+6}	11 (2.0)	11 (2.1)
32^{+0} to 36^{+6}	46 (8.5)	47 (8.8)
37^{+0} to 40^{+6}	299 (55.1)	295 (55.3)
≥ 41^{+0}	187 (34.4)	180 (33.8)
Median (IQR)	40 (39–41)	40 (39–41)
Gestational age at birth (weeks^{+days}), *n* (%)		
< 27^{+0}	1 (0.2)	6 (1.1)
27^{+0} to 31^{+6}	12 (2.2)	20 (3.8)
32^{+0} to 36^{+6}	48 (8.8)	39 (7.3)
37^{+0} to 40^{+6}	329 (60.6)	322 (60.4)
≥ 41^{+0}	153 (28.2)	146 (27.4)
Median (IQR)	40 (39–41)	40 (39–41)
Age (days)		
Median (IQR)	1 (1–2)	2 (1–2)
Range (minimum–maximum)	0–76	0–91
≥ 3 days, *n* (%)	70 (12.9)	70 (13.1)
Birthweight (*g*), median (IQR)	3500 (3110–3910)	3529 (3150–3895)
Missing, *n*	0	1
Working weight (g) at trial entry		
Median (IQR)	3500 (3110–3910)	3530 (3155–3890)
1000–2499, *n* (%)	55 (10.1)	50 (9.4)

TABLE 1 Infant (parent) characteristics at trial entry (*continued*)

Characteristic	Position	
	Sitting (*N* = 543)	Lying (*N* = 533)
2500–3500, *n* (%)	217 (40.0)	207 (38.8)
≥ 3501, *n* (%)	271 (49.9)	276 (51.8)
Infant sex, *n* (%)		
Male	325 (59.9)	336 (63.0)
Female	218 (40.1)	197 (37.0)
One of a multiple pregnancy, *n* (%)	5 (0.9)	13 (2.4)
Receiving antibiotics, *n* (%)	505 (93.0)	489 (91.7)
Any previous LPs, *n* (%)	2 (0.4)	3 (0.6)
Days since last LP, median (IQR)	22 (15–29)	22 (19–23)
CSF from last LP sent to laboratory, *n* (%)	2 (100)	3 (100)
RBC from last LP ($\times 10^6$/l), median (IQR)	230 (0–460)	435 (0–870)
Missing	0	1
WBC from last LP ($\times 10^6$/l), median (IQR)	3 (0–5)	1 (0–2)
Missing	0	1
Primary indication for current LP (not mutually exclusive), n (%)		
Risk factor for sepsis	201 (37.0)	203 (38.2)
Clinical signs of sepsis	137 (25.2)	145 (27.3)
Abnormal WBC count/morphology	8 (1.5)	8 (1.5)
Raised CRP	466 (85.8)	444 (83.5)
Specific signs of meningitis/encephalitis	12 (2.2)	8 (1.5)
Neurometabolic investigation	4 (0.7)	3 (0.6)
Therapeutic (raised intracranial pressure)	0	0
Recent failed LP	0	0
Positive blood culture	3 (0.6)	3 (0.6)
Other	4 (0.7)	6 (1.1)
Missing	0	1
Parental anxiety score (STAI-S)		
n	95	102
Mean (SD)	50.2 (13.0)	48.0 (14.4)
Recruiting centre, *n* (%)		
Birmingham Heartlands Hospital, Birmingham, UK	16 (2.9)	14 (2.6)
Royal Berkshire Hospital, Reading, UK	12 (2.2)	12 (2.3)
John Radcliffe Hospital, Oxford, UK	81 (14.9)	82 (15.4)
Bradford Royal Infirmary, Bradford, UK	21 (3.9)	17 (3.2)
Colchester General Hospital, Colchester, UK	11 (2.0)	14 (2.6)
Derriford Hospital, Plymouth, UK	15 (2.8)	13 (2.4)
Gloucestershire Royal Hospital, Gloucester, UK	13 (2.4)	12 (2.3)

continued

TABLE 1 Infant (parent) characteristics at trial entry (*continued*)

Characteristic	Position	
	Sitting (*N* = 543)	Lying (*N* = 533)
Great Western Hospital, Swindon, UK	8 (1.5)	12 (2.3)
Leicester Royal Infirmary, Leicester, UK	59 (10.9)	58 (10.9)
Medway Maritime Hospital, Kent, UK	63 (11.6)	63 (11.8)
Norfolk and Norwich University Hospital, Norfolk, UK	49 (9.0)	48 (9.0)
Northampton General Hospital, Northampton, UK	36 (6.6)	38 (7.1)
Princess Anne Hospital, Southampton, UK	20 (3.7)	16 (3.0)
Royal Devon and Exeter Hospital, Exeter, UK	13 (2.4)	14 (2.6)
Royal Hampshire County Hospital, Winchester, UK	6 (1.1)	5 (0.9)
Royal Oldham Hospital, Oldham, UK	24 (4.4)	22 (4.1)
Southmead Hospital, Bristol, UK	53 (9.8)	55 (10.3)
St Michael's Hospital, Bristol, UK	21 (3.9)	22 (4.1)
St Peter's Hospital, Chertsey, UK	7 (1.3)	7 (1.3)
Stoke Mandeville Hospital, Aylesbury, UK	12 (2.2)	8 (1.5)
Basingstoke and North Hampshire Hospital, Basingstoke, UK	3 (0.6)	1 (0.2)

TABLE 2 Clinical characteristics at first LP

Characteristic	Position	
	Sitting (*N* = 543)	Lying (*N* = 533)
Type of sedation and analgesia received (not mutually exclusive), *n* (%)		
None	24 (4.4)	19 (3.6)
Non-nutritive sucking	231 (42.7)	199 (37.3)
Oral sucrose/dextrose/glucose	443 (81.9)	458 (85.9)
Milk	13 (2.4)	9 (1.7)
Topical local anaesthetic	269 (49.7)	261 (49.0)
Paracetamol	0	1 (0.2)
NSAID	0	0
Opiate	3 (0.6)	7 (1.3)
Chloral hydrate	2 (0.4)	0
Midazolam	0	0
Phenobarbitone/phenytoin	1 (0.2)	2 (0.4)
Missing	2	0
Respiratory status immediately before LP, *n* (%)		
Self-ventilating in air	466 (85.8)	448 (84.1)
Low-flow oxygen (< 2 l/minute)	13 (2.4)	16 (3.0)
High-flow oxygen (≥ 2 l/minute)	57 (10.5)	59 (11.1)
CPAP/BiPAP	7 (1.3)	10 (1.9)

TABLE 2 Clinical characteristics at first LP (*continued*)

Characteristic	Position	
	Sitting (*N* = 543)	Lying (*N* = 533)
Previous diagnosis of intraventricular haemorrhage, *n* (%)	2 (1.0)	5 (2.5)
Not scanned	334	336
Grade of intraventricular haemorrhage at latest scan, *n* (%)		
I	2 (100.0)	2 (40.0)
II	0	2 (40.0)
III	0	1 (20.0)
IV	0	0
Coagulopathy treatment within last 24 hours, *n* (%)	4 (0.7)	5 (0.9)
Confirmed or probable infection (not mutually exclusive), *n* (%)		
Necrotising enterocolitis	1 (0.2)	0
Pneumonia	28 (5.2)	35 (6.6)
Sepsis	301 (55.4)	301 (56.5)
Blood culture positive	20 (3.7)	19 (3.6)
Urine infection	0	0
Line infection	0	0
Other localised infection	0	2 (0.4)
Other	1 (0.2)	3 (0.6)
Missing	0	0
Results of latest blood test		
Platelets (× 10^9/l), mean (SD)	249.8 (70.5)	249.6 (72.6)
Missing, *n*	28	21
WBC count (× 10^9/l), median (IQR)	15 (11–20)	15 (11–20)
Missing, *n*	25	21
Neutrophils (× 10^9/l), mean (SD)	10.7 (5.7)	10.1 (5.5)
Missing, *n*	34	34
RBC count (× 10^{12}/l), median (IQR)	5 (5–6)	5 (5–6)
Missing, *n*	29	25
CRP (mg/l), median (IQR)	39 (25–59)	40 (24–60)
Missing, *n*	4	3

BiPAP, bilevel positive airway pressure; CPAP, continuous positive airway pressure; NSAID, non-steroidal anti-inflammatory drug.

Outcomes

A total of 1079 participants had a first LP, of whom 166 (15.4%) had a second (each of these LP 'procedures' involved one or more 'attempts') (*Figure 2*). Nine participants were withdrawn during the trial, but in only one case was consent withdrawn before data collection for the primary outcome. Three infants did not undergo LP, and for two infants the consent form was missing. Overall, six infants were excluded post randomisation, leaving 1076 infants for the final (modified intention-to-treat) analysis. All infants were followed up until discharge.

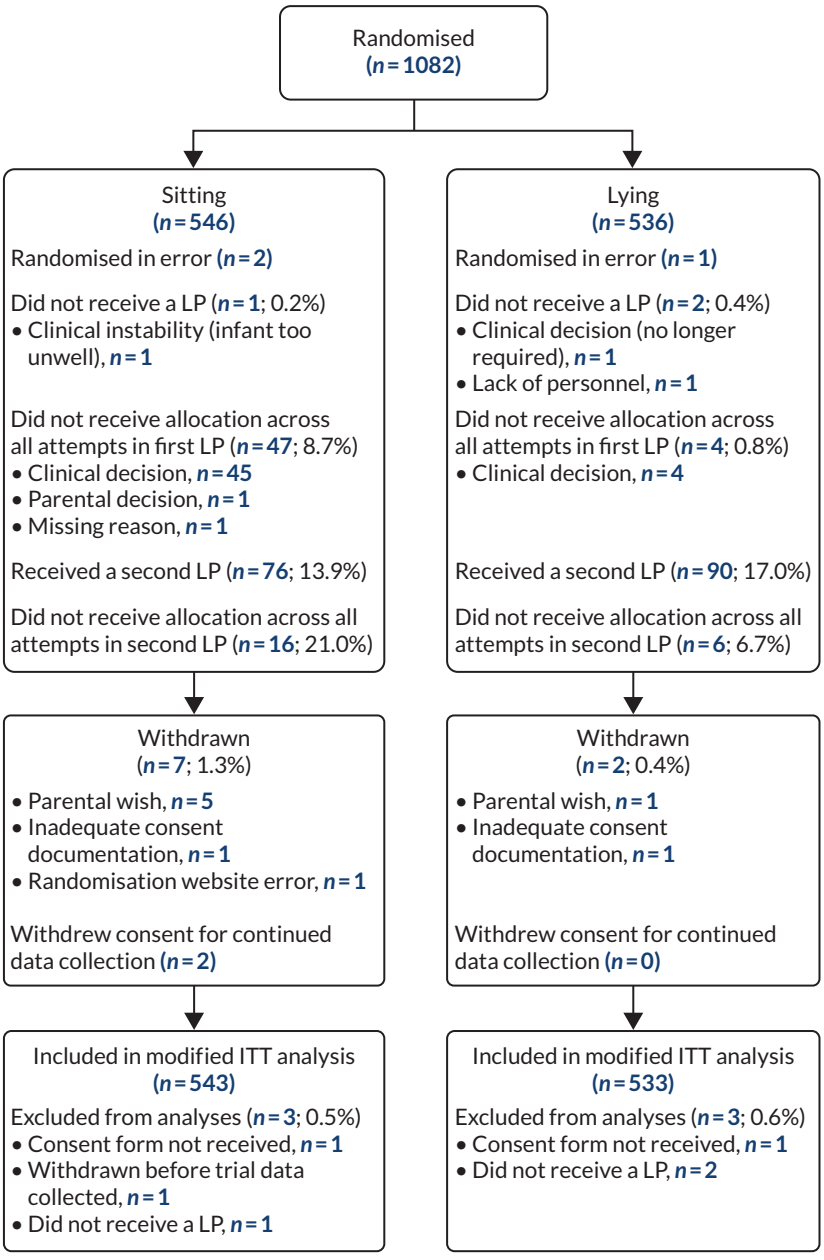

FIGURE 2 Flow of participants by principal comparison: sitting position vs. lying position. ITT, intention to treat.

Part A: principal comparison – sitting position compared with lying position

Primary outcome: sitting position compared with lying position

The primary outcome – a successful first LP – was achieved in 346 of 543 (63.7%) infants in the sitting arm and 307 of 533 (57.6%) infants in the lying arm [aRR 1.10 (95% CI 1.01 to 1.21); $p = 0.029$; adjusted absolute risk difference 6.1% (95% CI 0.7% to 11.4%); adjusted number needed to treat (NNT) 16 (95% CI 9 to 134)] (*Table 3*).

Secondary clinical outcomes: sitting position compared with lying position

Infants allocated to sitting position were less likely than infants allocated to lying position to show moderate or severe struggling at the time of needle insertion [169/541 (31.2%) vs. 202/527 (38.4%), aRR 0.82 (95% CI 0.71 to 0.94)]. Other secondary outcome data did not reach statistical significance, but predominantly favoured the sitting position (*Table 4*). Further untested secondary outcome data, including CSF appearance and number of procedures performed, are detailed in *Table 5*.

Looking at diagnoses based on CSF results from the first and second LPs (and any culture/PCR results), infants who were sitting were more likely than infants who were lying to be diagnosed as 'negative' for meningitis [396/537 (73.7%) vs. 359/521 (68.9%)]. Infants who were lying were more likely than infants who were sitting to be diagnosed with 'uninterpretable CSF', no sample obtained or CSF not possible to analyse, usually due to a blood-contaminated or clotted sample [139/521 (26.7%) vs. 114/537 (21.2%)].

Economic analysis per intervention group: sitting position compared with lying position

Economic analysis was carried out with a focus on resource consumption, as calculated for the individual trial procedures. The median duration of antibiotics and length of stay were not significantly different when comparing sitting and lying groups [median 5 (IQR 4–6) days in each arm] (*Table 6*).

Safety outcomes: sitting position compared with lying position

Four (0.3%) of 1241 first or second LPs, two in each arm, were abandoned because of cardiovascular deterioration. Lowest SpO_2 during the first LP averaged a median of 93% (IQR 89–96%) in the sitting position and 90% (IQR 85–94%) when in the lying position (adjusted Med D 3.0, 95% CI 2.1 to 3.9; $p < 0.001$). Three of 1075 (0.3%) infants required increased respiratory support within 1 hour of their first LP (sitting, $n = 1$; lying, $n = 2$; not significantly different). To explore the clinical implication of this, we analysed (post hoc) the proportion of infants whose lowest SpO_2 fell below 80% during the first LP, and this was 35 of 532 (6.6%) infants during sitting LPs and 72 of 508 (14.2%) infants during lying LPs (*Table 7*).

TABLE 3 Primary outcome

Outcome	Position		Relative risk			Absolute risk difference	
	Sitting ($N = 543$), n (%)	Lying ($N = 533$), n (%)	Unadjusted RR (95% CI)	aRR[a] (95% CI)	Adjusted p-value	Unadjusted risk difference (%) (95% CI)	Adjusted[a] risk difference (%) (95% CI)
CSF obtained and RBC count < 10,000/mm^3 on first LP procedure (any attempt)	346 (63.7)	307 (57.6)	1.11 (1.00 to 1.22)	1.10 (1.01 to 1.21)	0.029	6.12 (0.29 to 11.95)	6.08 (0.74 to 11.41)

a Adjusted for timing of stylet removal allocation, gestational age at randomisation and centre (as a random effect).

TABLE 4 Secondary clinical outcomes (tested)

Outcome	Position		Unadjusted effect estimate (95% CI)	Adjusted[a] effect estimate (95% CI)	Adjusted *p*-value	Unadjusted risk difference (%) (95% CI)	Adjusted[a] risk difference (%) (95% CI)
	Sitting (*N* = 543)	Lying (*N* = 533)					
Appearance of clearest sample on first procedure (any attempt),[b] *n* (%)			RR 1.05 (0.98 to 1.12)	RR 1.05 (0.99 to 1.11)	0.125	3.57 (−1.38 to 8.52)	3.61 (−1.03 to 8.26)
Clear CSF	270 (49.7)	233 (43.7)					
Blood-stained CSF	163 (30.0)	173 (32.5)					
Pure-blood/clotted CSF	85 (15.7)	100 (18.8)					
No CSF sample obtained	25 (4.6)	27 (5.1)					
CSF obtained with any RBC on first procedure (any attempt), *n* (%)	390 (71.8)	357 (67.0)	RR 1.07 (0.99 to 1.16)	RR 1.07 (0.98 to 1.17)	0.136	4.84 (−0.66 to 10.34)	4.82 (−1.53 to 11.17)
CSF obtained with WBC count not requiring correction[c] on first procedure (any attempt), *n* (%)	356 (65.7)	322 (60.4)	RR 1.09 (0.99 to 1.19)	RR 1.09 (0.99 to 1.19)	0.066	5.27 (−0.49 to 11.03)	5.25 (−0.41 to 10.92)
Missing, *n*	1	0					
Final clinical diagnosis at discharge [from LP(s)],[d] *n* (%)			RR 1.02 (0.96 to 1.09)	RR 1.02 (0.94 to 1.11)	0.612	1.68 (−3.28 to 6.65)	1.72 (−4.95 to 8.39)
Definite/probable meningitis	7 (1.3)	9 (1.7)					
Possible meningitis or equivocal CSF result	12 (2.2)	11 (2.1)					
Negative CSF result	424 (79.0)	408 (77.3)					
Uninterpretable CSF result (e.g. very high RBC or clotted CSF)	31 (5.8)	36 (6.8)					
No CSF obtained	63 (11.7)	64 (12.1)					

Outcome	Position		Unadjusted effect estimate (95% CI)	Adjusted[a] effect estimate (95% CI)	Adjusted *p*-value	Unadjusted risk difference (%) (95% CI)	Adjusted[a] risk difference (%) (95% CI)
	Sitting (*N* = 543)	Lying (*N* = 533)					
Other clinical reason for LP, *n*	6	5					
From clearest CSF sample							
WBC count (× 10^6/l), *n*	429	389					
Median (IQR)	3 (1–6)	2 (0–6)	Med D 1.0 (0.3 to 1.7)	Med D 0.1 (−0.7 to 0.9)	0.786		
RBC count (× 10^6/l), *n*	428	392					
Median (IQR)	380 (26–2370)	240 (12–2680)	Med D 142.0 (−74.1 to 358.1)	Med D 145.9 (−124.3 to 416.1)	0.289		
Corrected[e] WBC count (× 10^6/l), *n*	429	389					
Median (IQR)	1 (0–4)	1 (0–4)	Med D 0.0 (−0.7 to 0.7)	Med D 0.0 (−0.5 to 0.5)	1.000		
PMN (× 10^6/l), *n*	256	221					
Median (IQR)	0 (0–1)	0 (0–1)	Med D 0.0 (0.0 to 0.0)	Med D 0.0 (−0.1 to 0.1)	1.000		
Lymphocytes (× 10^6/l), *n*	259	228					
Median (IQR)	0 (0–4)	0 (0–3)	Med D 0.0 (−0.4 to 0.4)		1.000		
Total number of procedures performed,[f] *n* (%)			RR 0.86 (0.68 to 1.11)	RR 0.86 (0.68 to 1.09)	0.215	−2.77 (−7.46 to 1.92)	−2.68 (−6.99 to 1.62)
One	447 (82.3)	424 (79.5)					
Two	83 (15.3)	82 (15.4)					
Three or more	13 (2.4)	27 (5.1)					
Total number of attempts performed,[f] *n* (%)			RR 0.99 (0.88 to 1.13)	RR 1.00 (0.87 to 1.16)	0.995	−0.24 (−6.22 to 5.73)	−0.39 (−7.56 to 6.77)
One	282 (51.9)	275 (51.7)					
Two	131 (24.1)	111 (20.9)					
Three or more	130 (23.9)	146 (27.4)					
Missing	0	1					

continued

TABLE 4 Secondary clinical outcomes (tested) (*continued*)

Outcome	Position		Unadjusted effect estimate (95% CI)	Adjusted[a] effect estimate (95% CI)	Adjusted *p*-value	Unadjusted risk difference (%) (95% CI)	Adjusted[a] risk difference (%) (95% CI)
	Sitting (*N* = 543)	Lying (*N* = 533)					
Time (minutes) taken to complete first procedure, from start of cleaning skin to removing needle at end of all attempts, median (IQR)	8(5–12)	8 (5–13)	Med D 0.0 (−1.0 to 1.0)	Med D 0.0 (−0.8 to 0.9)	0.935		
Range (minimum to maximum)	0–55	0–37					
Missing	8	12					
Level of infant struggling movement on first attempt of first procedure,[g] *n* (%)			RR 0.81 (0.69 to 0.96)	RR 0.82 (0.71 to 0.94)	0.006	−7.09 (−12.79 to −1.39)	
None	125 (23.1)	85 (16.1)					
Mild	247 (45.7)	240 (45.5)					
Moderate	129 (23.8)	159 (30.2)					
Severe	40 (7.4)	43 (8.2)					
Missing	2	6					

a Adjusted for timing of stylet removal allocation, gestational age at randomisation and centre (as a random effect).
b Clear CSF/blood-stained CSF vs. pure-blood CSF/clotted CSF/no sample obtained.
c WBC count < 20 regardless of the RBC count, or RBC count < 500.
d Negative vs. definite/probable/possible/equivocal/uninterpretable/no CSF.
e If the RBC count is ≥ 500, then the WBC count will be reduced by 1 for every 500 RBCs to give a 'corrected' WBC count.
f One vs. more than one.
g None/mild vs. moderate/severe.

TABLE 5 Untested clinical outcomes

Outcome	Position	
	Sitting (*N* = 543), *n* (%)	Lying (*N* = 533), *n* (%)
Appearance		
Appearance of CSF on first attempt of first procedure		
Clear CSF	205 (37.8)	186 (35.0)
Blood-stained CSF	95 (17.5)	102 (19.2)
Pure-blood CSF or clotted CSF	140 (25.8)	147 (27.6)
No CSF sample obtained	102 (18.8)	97 (18.2)
Missing	1	1
Appearance of clearest sample from first or second procedure		
Clear CSF	289 (53.2)	246 (46.2)
Blood-stained CSF	188 (34.6)	203 (38.1)
Pure-blood CSF or clotted CSF	58 (10.7)	68 (12.8)
No sample obtained	8 (1.5)	16 (3.0)
Procedures		
Number of attempts in first procedure		
One	295 (54.3)	287 (53.9)
Two	191 (35.2)	183 (34.4)
Three or more	57 (10.5)	62 (11.7)
Missing	0	1
Had second procedure	75	89
Number of attempts in second procedure		
One	39 (52.0)	45 (50.6)
Two	33 (44.0)	32 (36.0)
Three or more	3 (4.0)	12 (13.5)
Missing	1	1
Success		
CSF obtained and any RBC count		
First attempt of first procedure	277 (51.0)	258 (48.4)
Any attempt of first procedure	390 (71.8)	357 (67.0)
First or second procedure	429 (79.0)	395 (74.1)
CSF obtained and RBC count of < 500/mm^3		
First attempt of first procedure	163 (30.0)	168 (31.5)
Any attempt of first procedure	214 (39.4)	216 (40.5)
First or second procedure	227 (41.8)	229 (43.0)

continued

TABLE 5 Untested clinical outcomes (*continued*)

Outcome	Position	
	Sitting (*N* = 543), *n* (%)	Lying (*N* = 533), *n* (%)
CSF obtained and RBC count < 5000/mm^3		
First attempt of first procedure	240 (44.2)	222 (41.7)
Any attempt of first procedure	328 (60.4)	289 (54.2)
First or second procedure	353 (65.0)	312 (58.5)
CSF obtained and RBC count < 10,000/mm^3		
First attempt of first procedure	250 (46.0)	235 (44.1)
Any attempt of first procedure	346 (63.7)	307 (57.6)
First or second procedure	374 (68.9)	335 (62.9)
CSF obtained and RBC count < 25,000/mm^3		
First attempt of first procedure	265 (48.8)	247 (46.3)
Any attempt of first procedure	369 (68.0)	337 (63.2)
First or second procedure	401 (73.8)	368 (69.0)
CSF obtained with WBC count not requiring correction		
First attempt of first procedure	257 (47.3)	239 (44.8)
Any attempt of first procedure	356 (65.6)	322 (60.4)
First or second procedure	388 (71.5)	356 (66.8)
Diagnoses of meningitis via CSF from first or second procedure		
Positive: either diagnosed by WBC criteria (A) or by culture/PCR (B)	22 (4.1)	21 (4.0)
(A) WBC count (or corrected WBC) of ≥ 20 from clearest CSF	21 (3.9)	21 (4.0)
(B) Any true-positive culture or PCR from first or second LP	2 (0.4)	0
Equivocal: does not have a positive diagnosis and either has isolated raised PMNs (C) or isolated organisms on Gram stain (D)	5 (0.9)	2 (0.4)
(C) In clearest CSF a RBC count of < 500, a WBC count of < 20 and PMN > 2	3 (0.6)	0
(D) Organism found on any Gram stain (first or second LP) and clearest CSF has a WBC count (or corrected WBC) of < 20 or no RBC count was possible	2 (0.4)	2 (0.4)
Negative: does not have positive or equivocal diagnosis and has negative microscopy (E) or is missing PMN but would otherwise be equivocal (F)	396 (73.7)	359 (68.9)
(E) In clearest CSF a WBC count (or corrected WBC) of < 20	321 (59.8)	274 (52.6)
(F) Equivocal diagnosis except for missing PMN	80 (14.9)	88 (16.9)
Uninterpretable: either no sample obtained (G) or no cell count possible (H)	114 (21.2)	139 (26.7)
(G) No sample obtained on any attempt in first or second LP	8 (1.5)	15 (2.9)
(H) CSF obtained but clotted/insufficient and so either not sent to laboratory or not analysed, or no RBC count possible from clearest CSF	106 (19.7)	124 (23.8)
Other clinical reason for LP	6	5
Unknown	0	7

TABLE 6 Resource consumption

Resource use	Position		Unadjusted Med D (95% CI)	Adjusted[a] Med D (95% CI)	Adjusted *p*-value
	Sitting (*N* = 543)	Lying (*N* = 533)			
Received antibiotics during trial, *n*	530	521			
Duration (days) of antibiotic course from trial entry to discharge home, median (IQR)	5 (4–6)	5 (4–6)	0.0 (–0.2 to 0.2)	0.0 (–0.2 to 0.2)	1.000
Range (minimum to maximum)	1–24	0–25			
Missing, *n*	1	2			
Surviving infants, *n*	541	533			
Length of stay (days) in hospital (in surviving infants) from trial entry until discharge home, median (IQR)	5 (4–7)	5 (4–7)	0.0 (–0.3 to 0.3)	–0.1 (–0.5 to 0.3)	0.585
Range (minimum to maximum)	1–158	1–371			

a Adjusted for timing of stylet removal allocation, gestational age at randomisation and centre.

A depiction of the safety data on changes in SpO_2 and HR during either lying or sitting LPs can be found in the *Appendix 9*, *Figures 7* and *8*, and a depiction of the safety data on changes in SpO_2 and HR during either ESR and LSR for LPs can be found in *Appendix 9*, *Figures 9* and *10*.

Compliance with allocated technique: sitting position compared with lying position

In 47 of 543 (8.7%) first LPs in infants allocated to the sitting position, at least one attempt involved switching to the lying position. [For comparison, an attempt in the sitting position was carried out in only 4/533 (0.8%) infants allocated to the lying position.] The decision to change position was usually made by a clinician (45/47 LPs), and mostly on the second (22/247) or third (24/57) attempt at LP. The sitting allocation was followed even less often in the case of the second LP: in 16 of 76 (22.5%) infants allocated to the sitting arm, at least one attempt to carry out the second LP was made in the lying position. [For comparison, an attempt in the sitting position was carried out in 6/90 (7.0%) infants allocated to the lying position.] There were no obvious differences in the characteristics at baseline of infants in whom the allocated position was adhered to and those in whom it was not (*Table 8*). *Appendix 9* shows the baseline characteristics of infants in whom non-adherence to position allocation occurred.

Subgroup analyses of the primary outcome: sitting position compared with lying position

The prespecified subgroup analysis of the primary outcome overall confirms the statistically and clinically significant advantages of performing neonatal LP in the sitting position (success rate 63.7%) over the lying position (success rate 57.6%).

The effect of sitting position was consistent across all subgroups of CGA and weight. The benefit of sitting was less clear in the small subgroup of infants enrolled at ≥ 3 days old. As expected, this subgroup of infants had a lower gestational age at birth and a lower birthweight and, therefore, may have been more likely to require respiratory support at the time of LP. It is also reassuring that the results for infants with a working weight of < 2500 g and a CGA of 27^{+0} to 36^{+6} weeks were consistent with the overall findings (*Figure 3*).

TABLE 7 Safety outcomes

Outcome	Position		Unadjusted effect measure (95% CI)	Adjusted[a] effect measure (95% CI)	Adjusted *p*-value	Unadjusted risk difference (%) (95% CI)
	Sitting (*N* = 543)	Lying (*N* = 533)				
Procedure abandoned because of cardiovascular deterioration (first procedure), *n* (%)	2 (0.4)	1 (0.2)	RR 1.96 (0.18 to 21.55)	RR 1.95 (0.17 to 22.08)	0.588	0.18 (−0.45 to 0.81)
Missing, *n*	0	1				
Procedure abandoned because of cardiovascular deterioration (second procedure), *n/N* (%)	0/76 (0.0)	1/90 (1.1)				
Infant's lowest SpO_2 (%) (first procedure), median (IQR)	93 (89–96)	90 (85–94)	Med D 3.0 (2.1 to 3.9)	Med D 3.0 (2.1 to 3.9)	< 0.001	
Missing, *n*	11	25				
Infant's lowest HR (b.p.m.) (first procedure), mean (SD)	129.5 (19.9)	127.0 (21.5)	MD 2.5 (−0.1 to 5.0)	MD 2.5 (0.6 to 4.4)	0.011	
Missing, *n*	20	32				
Infant's highest HR (b.p.m.) (first procedure), mean (SD)	163.7 (21.7)	163.6 (21.9)	MD 0.1 (−2.6 to 2.8)	MD 0.1 (−2.1 to 2.4)	0.897	
Missing, *n*	18	32				
Respiratory deterioration post LP (requirement for escalating respiratory support within 1 hour of LP) (first procedure), *n* (%)	1 (0.2)	2 (0.4)	RR 0.49 (0.04 to 5.39)	RR 0.49 (0.04 to 5.71)	0.567	−0.19 (−0.82 to 0.44)
Missing, *n*	0	1				
Respiratory deterioration post LP (requirement for escalating respiratory support within 1 hour of LP) (second procedure), *n/N* (%)	0/76 (0.0)	0/90 (0.0)				

a Adjusted for timing of stylet removal allocation, gestational age at randomisation and centre.

TABLE 8 Compliance with allocated intervention in first or second procedure

Procedure	Position	
	Sitting	Lying
First LP only		
N	543	533
Time from randomisation to first LP (hours), median (IQR)	0.9 (0.5–1.8)	0.8 (0.4–1.5)
≥ 12 hours, *n* (%)	9 (1.7)	11 (2.1)
Missing, *n*	13	12
At least one attempt at LP in which the allocated technique was not adhered to, *n* (%)	47 (8.7)	4 (0.8)
Clinician decision	45 (97.8)	4 (100.0)
Parental decision	1 (2.2)	0 (0.0)
Unintentional use of alternative technique	0	0
Missing	1	0
Attempt in which the allocated technique was not adhered to, *n/N* (%) (not mutually exclusive)		
First	7/543 (1.3)	1/532 (0.2)
Second	22/247 (8.9)	0/245 (0.0)
Third	24/57 (42.1)	3/62 (4.8)
Total number of attempts, *n*	848	844
Number of attempts in which the allocated technique was not adhered to, *n* (%)	53 (6.3)	4 (0.5)
Missing	0	1
Second LP only		
N	76	90
At least one attempt at LP in which the allocated technique was not adhered to, *n* (%)	16 (22.5)	6 (7.0)
Clinician decision	14 (93.3)	6 (100.0)
Parental decision	1 (6.7)	0 (0.0)
Missing	1	0
Attempt in which the allocated technique was not adhered to, *n/N* (%) (not mutually exclusive)		
First	9/71 (12.7)	2/86 (2.3)
Second	10/34 (29.4)	2/42 (4.8)
Third	2/3 (66.7)	3/11 (27.3)
Total number of attempts, *n*	114	145
Number of attempts in which the allocated technique was not adhered to,[a] *n* (%)	21 (18.4)	7 (4.8)
Missing	5	4

a As a proportion of all attempts.

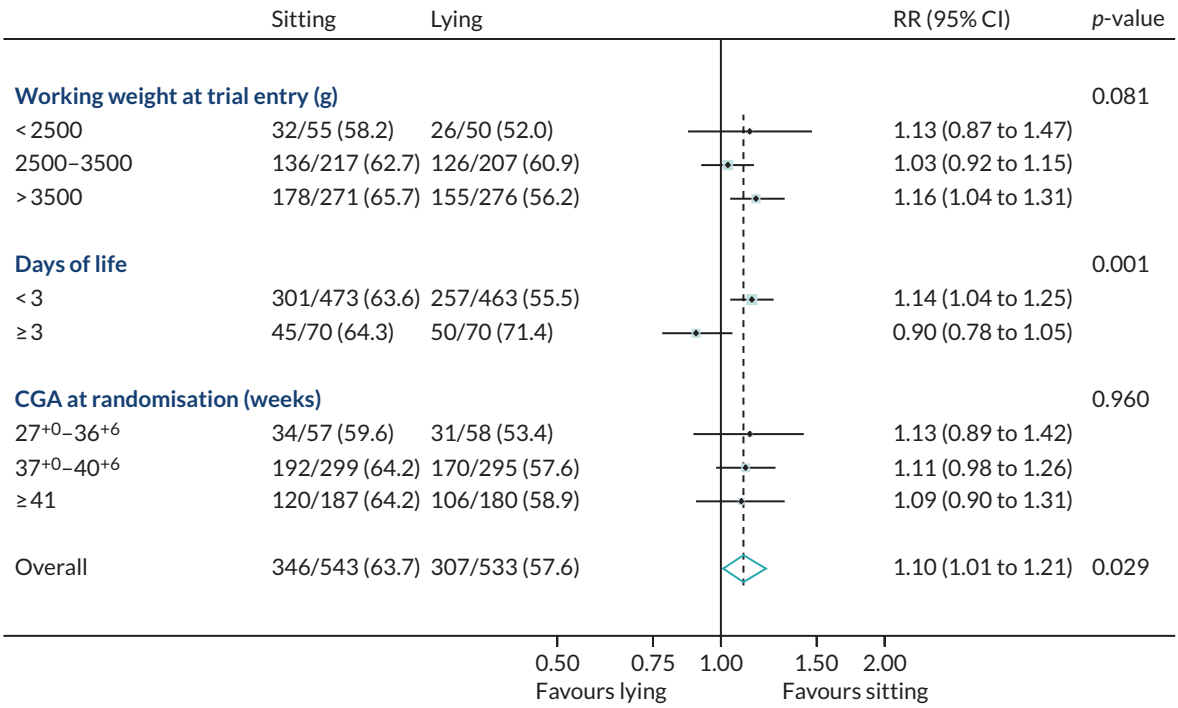

FIGURE 3 Subgroup analysis forest plot: principal comparison – sitting position vs. lying position. Adjusted for timing of stylet removal allocation, gestational age at randomisation and centre (as a random effect).

Part B: principal comparison – early stylet removal compared with late stylet removal

The flow chart of participants by principal comparison for the interventions with ESR compared with LSR is shown in *Figure 4*. The infants' characteristics at trial entry (*Table 9*) and at first LP were comparable, with no relevant differences between the groups (*Table 10*).

Primary outcome: early stylet removal compared with late stylet removal

As shown in *Table 11*, for the 1076 studied infants, the primary outcome was achieved in 338 of 545 (62.0%) infants following ESR and in 315 of 531 (59.3%) infants following LSR. There was no significant difference between the groups (aRR 1.04, 95% CI 0.94 to 1.15; adjusted *p*-value = 0.45) (see *Table 11*).

Secondary outcomes: early stylet removal compared with late stylet removal

There were no obvious differences between the groups in any of the tested secondary outcomes (*Table 12*) or in untested outcomes (*Table 13*).

Economic analysis per intervention group: early stylet removal compared with late stylet removal

In the limited economic analysis, there was no difference in resource consumption between groups (*Table 14*) or by allocation per recruiting site (see *Appendix 8*).

Safety: early stylet removal compared with late stylet removal

The first procedure was abandoned in one patient in the ESR group and in two patients in the LSR group. The second procedure was abandoned in one patient in the LSR group (*Table 15*). The difference was non-significant. In the 1076 studied infants, there were no statistically relevant differences in any of the safety outcomes, including no differences in lowest SpO_2, lowest HR or highest HR between the two arms (*Table 15*).

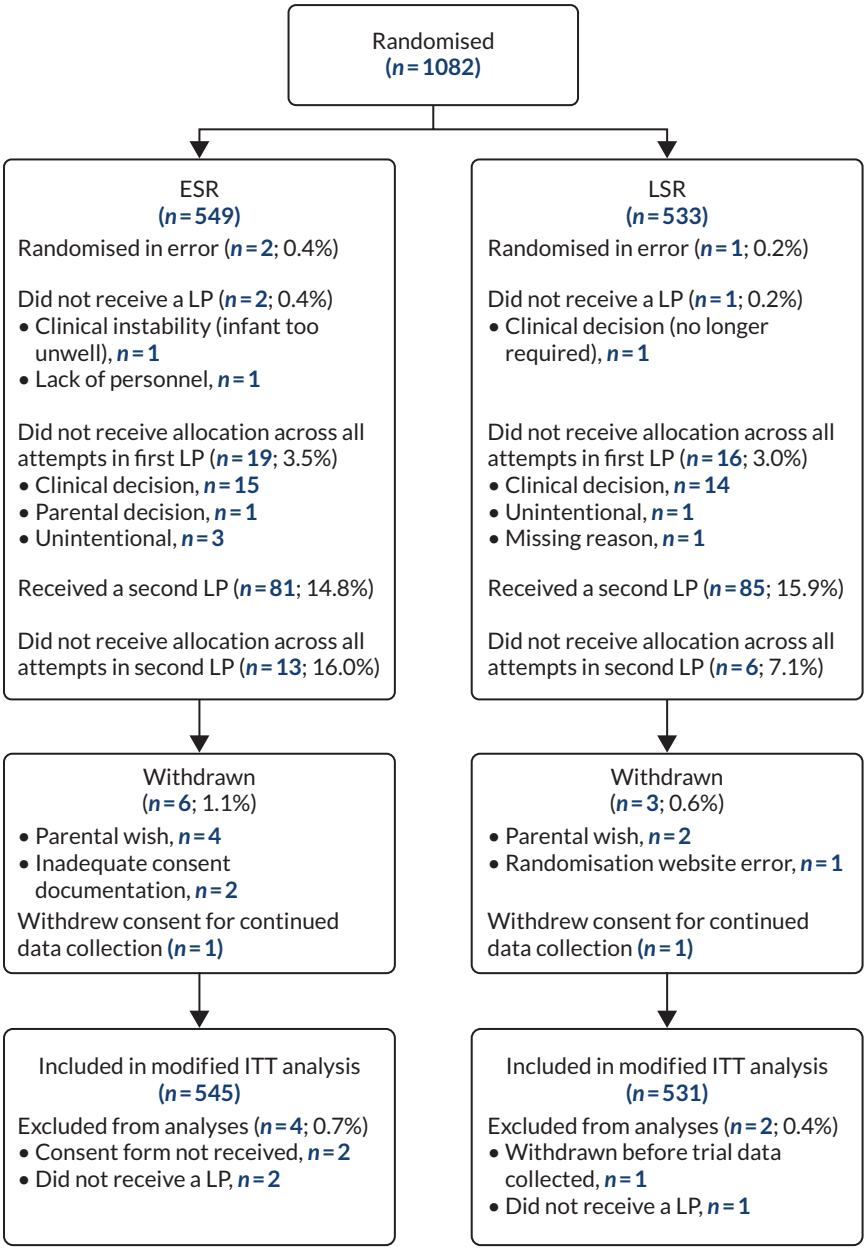

FIGURE 4 Flow of participants by principal comparison: ESR vs. LSR. ITT, intention to treat.

Compliance with allocated technique: early stylet removal compared with late stylet removal

The allocated technique was not adhered to in 35 of 1076 (3.3%) first LPs, with similar numbers in each arm. For the second LP, 13 of 81 infants allocated to ESR received at least one attempt without ESR, compared with 6 of 85 infants allocated to LSR for whom the stylet was withdrawn early on at least one attempt; however, denominators were small, and this was an untested outcome (*Table 16*). *Appendix 8* shows allocation per recruiting site, and *Appendix 9* shows the baseline characteristics for non-adherence to timing of stylet removal allocation, which were similar for infants undergoing non-adherent and adherent LPs.

Subgroup analyses: early stylet removal compared with late stylet removal

The effect of timing of stylet removal on the proportion of infants in whom the first LP was successful was consistent across working weight at trial entry, CGA at randomisation and day of life at time of enrolment, showing no significant difference in LP success in each subgroup.

TABLE 9 Infant (parent) characteristics at trial entry: ESR vs. LSR

Characteristic	Stylet removal allocation	
	ESR (*N* = 545)	LSR (*N* = 531)
CGA at trial entry (weeks^{+days}), *n* (%)		
27^{+0} to 31^{+6}	10 (1.8)	12 (2.3)
32^{+0} to 36^{+6}	49 (9.0)	44 (8.3)
37^{+0} to 40^{+6}	297 (54.5)	297 (55.9)
≥ 41^{+0}	189 (34.7)	178 (33.5)
Median (IQR)	40 (39–41)	40 (39–41)
Gestational age at birth (weeks^{+days}), *n* (%)		
< 27^{+0}	5 (0.9)	2 (0.4)
27^{+0} to 31^{+6}	16 (2.9)	16 (3.0)
32^{+0} to 36^{+6}	45 (8.3)	42 (7.9)
37^{+0} to 40^{+6}	327 (60.0)	324 (61.0)
≥ 41^{+0}	152 (27.9)	147 (27.7)
Median (IQR)	40 (39–41)	40 (39–41)
Age (days), median (IQR)	2 (1–2)	1 (1–2)
Range (minimum to maximum)	0–91	0–50
≥ 3 days, *n* (%)	74 (13.6)	66 (12.4)
Birthweight (g), median (IQR)	3518 (3133–3895)	3510 (3140–3910)
Missing, *n*	1	0
Working weight (g) at trial entry		
Median (IQR)	3520 (3130–3890)	3510 (3155–3910)
1000–2499, *n* (%)	57 (10.5)	48 (9.0)
2500–3500, *n* (%)	207 (38.0)	217 (40.9)
≥ 3501, *n* (%)	281 (51.6)	266 (50.1)
Infant sex, *n* (%)		
Male	336 (61.7)	325 (61.2)
Female	209 (38.3)	206 (38.8)
One of a multiple pregnancy, *n* (%)	12 (2.2)	6 (1.1)
Receiving antibiotics, *n* (%)	503 (92.3)	491 (92.5)
Any previous LPs, *n* (%)	4 (0.7)	1 (0.2)
Days since last LP, median (IQR)	21 (17–26)	22 (22–22)
CSF from last LP sent to laboratory, *n* (%)	4 (100.0)	1 (100.0)
RBC from last LP (× 10^6/l), median (IQR)	230 (0–665)	
Missing, *n*	0	1
WBC from last LP (× 10^6/l), median (IQR)	1 (0–4)	
Missing, *n*	0	1

TABLE 9 Infant (parent) characteristics at trial entry: ESR vs. LSR (*continued*)

Characteristic	Stylet removal allocation	
	ESR (*N* = 545)	LSR (*N* = 531)
Primary indication for current LP (not mutually exclusive), *n* (%)		
Risk factor for sepsis	196 (36.0)	208 (39.2)
Clinical signs of sepsis	147 (27.0)	135 (25.5)
Abnormal WBC count/morphology	9 (1.7)	7 (1.3)
Raised CRP	457 (83.9)	453 (85.5)
Specific signs of meningitis/encephalitis	11 (2.0)	9 (1.7)
Neurometabolic investigation	4 (0.7)	3 (0.6)
Therapeutic (raised intracranial pressure)	0	0
Recent failed LP	0	0
Positive blood culture	1 (0.2)	5 (0.9)
Other	6 (1.1)	4 (0.8)
Missing	0	1
Parental anxiety score (STAI-S), *n*		
n	94	103
Mean (SD)	49.0 (13.8)	49.1 (13.7)
Recruiting centre, *n* (%)		
Birmingham Heartlands Hospital	15 (2.8)	15 (2.8)
Royal Berkshire Hospital	11 (2.0)	13 (2.4)
John Radcliffe Hospital	83 (15.2)	80 (15.1)
Bradford Royal Infirmary	19 (3.5)	19 (3.6)
Colchester General Hospital	12 (2.2)	13 (2.4)
Derriford Hospital	16 (2.9)	12 (2.3)
Gloucestershire Royal Hospital	13 (2.4)	12 (2.3)
Great Western Hospital	10 (1.8)	10 (1.9)
Leicester Royal Infirmary	60 (11.0)	57 (10.7)
Medway Maritime Hospital	63 (11.6)	63 (11.9)
Norfolk and Norwich University Hospital	47 (8.6)	50 (9.4)
Northampton General Hospital	38 (7.0)	36 (6.8)
Princess Anne Hospital	18 (3.3)	18 (3.4)
Royal Devon and Exeter Hospital	14 (2.6)	13 (2.4)
Royal Hampshire County Hospital	6 (1.1)	5 (0.9)
Royal Oldham Hospital	24 (4.4)	22 (4.1)
Southmead Hospital	56 (10.3)	52 (9.8)
St Michael's Hospital	22 (4.0)	21 (4.0)
St Peter's Hospital	6 (1.1)	8 (1.5)
Stoke Mandeville Hospital	10 (1.8)	10 (1.9)
Basingstoke and North Hampshire Hospital	2 (0.4)	2 (0.4)

TABLE 10 Clinical characteristics at first LP

Characteristic	Stylet removal allocation	
	ESR (*N* = 545)	LSR (*N* = 531)
Type of sedation and analgesia received (not mutually exclusive), *n* (%)		
None	24 (4.4)	19 (3.6)
Non-nutritive sucking	212 (39.0)	218 (41.1)
Oral sucrose/dextrose/glucose	464 (85.3)	437 (82.5)
Milk	12 (2.2)	10 (1.9)
Topical local anaesthetic	267 (49.1)	263 (49.6)
Paracetamol	1 (0.2)	0
NSAID	0	0
Opiate	7 (1.3)	3 (0.6)
Chloral hydrate	1 (0.2)	1 (0.2)
Midazolam	0	0
Phenobarbitone/phenytoin	2 (0.4)	1 (0.2)
Missing	1	1
Respiratory status immediately before LP, *n* (%)		
Self-ventilating in air	459 (84.2)	455 (85.7)
Low-flow oxygen (< 2 l/minute)	16 (2.9)	13 (2.4)
High-flow oxygen (≥ 2 l/minute)	59 (10.8)	57 (10.7)
CPAP/BiPAP	11 (2.0)	6 (1.1)
Previous diagnosis of intraventricular haemorrhage, *n* (%)	5 (2.5)	2 (1.0)
Not scanned	346	324
Grade of intraventricular haemorrhage at latest scan, *n* (%)		
I	3 (60.0)	1 (50.0)
II	1 (20.0)	1 (50.0)
III	1 (20.0)	0
IV	0	0
Coagulopathy treatment within last 24 hours, *n* (%)	4 (0.7)	5 (0.9)
Confirmed or probable infection (not mutually exclusive), *n* (%)		
Necrotising enterocolitis	0	1 (0.2)
Pneumonia	33 (6.1)	30 (5.6)
Sepsis	310 (56.9)	292 (55.0)
Blood culture positive	16 (2.9)	23 (4.3)
Urine infection	0	0
Line infection	0	0
Other localised infection	0	2 (0.4)
Other	0	4 (0.8)
Missing	0	0

TABLE 10 Clinical characteristics at first LP (*continued*)

Characteristic	Stylet removal allocation	
	ESR (*N* = 545)	LSR (*N* = 531)
Results of latest blood test		
Platelets (× 10^9/l), mean (SD)	250.9 (71.5)	248.5 (71.6)
Missing, *n*	24	25
WBC count (× 10^9/l), median (IQR)	15 (11–20)	16 (11–20)
Missing, *n*	24	22
Neutrophils (× 10^9/l), mean (SD)	10.4 (5.6)	10.4 (5.6)
Missing, *n*	33	35
RBC count (× 10^{12}/l), median (IQR)	5 (5–6)	5 (5–6)
Missing, *n*	28	26
CRP (mg/l), median (IQR)	39 (24–58)	40 (25–61)
Missing, *n*	3	4

BiPAP, bilevel positive airway pressure; CPAP, continuous positive airway pressure; NSAID, non-steroidal anti-inflammatory drug.

TABLE 11 Primary outcome: ESR vs. LSR

Outcome	Stylet removal allocation		Relative risk			Absolute risk difference	
	ESR (*N* = 531)	LSR (*N* = 545)	Unadjusted RR (95% CI)	Adjusted[a] RR (95% CI)	Adjusted *p*-value	Unadjusted risk difference (%) (95% CI)	Adjusted[a] risk difference (%) (95% CI)
CSF obtained and RBC count < 10,000/mm^3 on first LP procedure (any attempt), *n* (%)	338 (62.0)	315 (59.3)	1.05 (0.95 to 1.15)	1.04 (0.94 to 1.15)	0.447	2.70 (–3.14 to 8.53)	2.57 (–3.38 to 8.52)

a Adjusted for position allocation, gestational age at randomisation and centre (as a random effect).

Part C: multiarm analysis – sitting position plus early stylet removal, sitting position plus late stylet removal, lying position plus early stylet removal and lying position plus late stylet removal

Figure 5 illustrates the multiarm analysis flow of participants by randomised group. *Table 17* summarises the parent and infant characteristics at trial entry. *Table 18* summarises the infants' characteristics at first LP. The groups were well matched, and no significant interaction between infant position and timing of stylet removal was detected ($p = 0.136$) (*Table 19*). Multiarm baseline characteristics and analyses did not reveal any new obvious differences (only those previously described for sitting position vs. lying position).

Secondary outcomes: multiarm analysis

The secondary outcomes in the multiarm analysis are shown in *Table 20*. There were no differences between the groups (only those previously described for sitting vs. lying position).

TABLE 12 Secondary clinical outcomes: ESR vs. LSR (tested)

Outcome	Stylet removal allocation		Unadjusted effect estimate (95% CI)	Adjusted[a] effect estimate (95% CI)	Adjusted *p*-value	Unadjusted risk difference (%) (95% CI)	Adjusted[a] risk difference (%) (95% CI)
	ESR (*N* = 545)	LSR (*N* = 531)					
Appearance of clearest sample on first procedure (any attempt),[b] *n* (%)			RR 1.01 (0.95 to 1.08)	RR 1.01 (0.95 to 1.08)	0.742	1.13 (−3.82 to 6.08)	0.93 (−4.05 to 5.91)
Clear CSF	259 (47.5)	244 (46.0)					
Blood-stained CSF	169 (31.0)	167 (31.5)					
Pure-blood/clotted CSF	90 (16.5)	95 (17.9)					
No CSF sample obtained	27 (5.0)	25 (4.7)					
CSF obtained with any RBC on first procedure (any attempt), *n* (%)	383 (70.3)	364 (68.5)	RR 1.03 (0.95 to 1.11)	RR 1.02 (0.95 to 1.09)	0.580	1.73 (−3.78 to 7.23)	1.54 (−3.38 to 6.46)
CSF obtained with WBC count not requiring correction[c] on first procedure (any attempt), *n* (%)	349 (64.2)	329 (62.0)	RR 1.04 (0.94 to 1.13)	RR 1.03 (0.95 to 1.12)	0.452	2.20 (−3.57 to 7.97)	2.16 (−3.21 to 7.52)
Missing, *n*	1	0					
Final clinical diagnosis at discharge [from LP(s)],[d] *n* (%)			RR 0.99 (0.93 to 1.06)	RR 0.99 (0.95 to 1.04)	0.794	−0.53 (−5.50 to 4.43)	−0.47 (−3.78 to 2.85)
Definite/probable meningitis	9 (1.7)	7 (1.3)					
Possible meningitis or equivocal CSF result	13 (2.4)	10 (1.9)					
Negative CSF result	422 (77.9)	410 (78.4)					
Uninterpretable CSF result (e.g. very high RBC count or clotted CSF)	33 (6.1)	34 (6.5)					
No CSF obtained	65 (12.0)	62 (11.9)					

Outcome	Stylet removal allocation		Unadjusted effect estimate (95% CI)	Adjusted[a] effect estimate (95% CI)	Adjusted *p*-value	Unadjusted risk difference (%) (95% CI)	Adjusted[a] risk difference (%) (95% CI)
	ESR (*N* = 545)	LSR (*N* = 531)					
Other clinical reason for LP, *n*	3	8					
From clearest CSF sample							
WBC count (× 106/l), *n*	419	399					
Median (IQR)	2 (1–6)	2 (0–6)	Med D 0.0 (−0.7 to 0.7)	Med D 0.3 (−0.5 to 1.0)	0.474		
RBC count (× 106/l), *n*	421	399					
Median (IQR)	270 (14–2340)	301 (25–3070)	Med D −31.0 (−254.9 to 192.9)	Med D 32.2 (−237.7 to 302.0)	0.815		
Corrected[e] WBC count (× 106/l), *n*	419	399					
Median (IQR)	1 (0–4)	1 (0–4)	Med D 0.0 (−0.7 to 0.7)	Med D 0.0 (−0.5 to 0.5)	1.000		
PMN (× 106/l), *n*	231	246					
Median (IQR)	0 (0–1)	0 (0–0)	Med D 0.0 (0.0 to 0.0)	Med D 0.0 (−0.1 to 0.1)	1.000		
Lymphocytes (× 106/l), *n*	236	251					
Median (IQR)	0 (0–3)	0 (0–3)	Med D 0.0 (−0.2 to 0.2)		1.000		
Total number of procedures performed,[f] *n* (%)			RR 0.93 (0.73 to 1.19)	RR 0.92 (0.77 to 1.11)	0.380	−1.43 (−6.12 to 3.27)	−1.12 (−4.71 to 2.46)
One	445 (81.7)	426 (80.2)					
Two	81 (14.9)	84 (15.8)					
Three or more	19 (3.5)	21 (4.0)					
Total number of attempts performed,[f] *n* (%)			RR 1.01 (0.90 to 1.15)	RR 1.01 (0.92 to 1.12)	0.808	0.70 (−5.28 to 6.67)	0.58 (−4.35 to 5.51)
One	280 (51.5)	277 (52.2)					
Two	127 (23.3)	115 (21.7)					
Three or more	137 (25.2)	139 (26.2)					
Missing	1	0					

continued

TABLE 12 Secondary clinical outcomes: ESR vs. LSR (tested) (*continued*)

Outcome	Stylet removal allocation		Unadjusted effect estimate (95% CI)	Adjusted[a] effect estimate (95% CI)	Adjusted *p*-value	Unadjusted risk difference (%) (95% CI)	Adjusted[a] risk difference (%) (95% CI)
	ESR (*N* = 545)	LSR (*N* = 531)					
Time (minutes) taken to complete first procedure, from start of cleaning skin to removing needle at end of all attempts, median (IQR)	8(5–13)	8 (5–13)	Med D 0.0 (−1.0 to 1.0)	Med D −0.1 (−1.0 to 0.7)	0.752		
Range (minimum to maximum)	0–37	0–55					
Missing, *n*	11	9					
Level of infant struggling movement on first attempt of first procedure,[g] *n* (%)			RR 1.02 (0.87 to 1.21)	RR 1.01 (0.87 to 1.18)	0.886	0.79 (−4.92 to 6.50)	
None	101 (18.8)	109 (20.6)					
Mild	248 (46.1)	239 (45.1)					
Moderate	148 (27.5)	140 (26.4)					
Severe	41 (7.6)	42 (7.9)					
Missing	7	1					

a Adjusted for position allocation, gestational age at randomisation and centre (as a random effect).
b Clear CSF/blood-stained CSF vs. pure-blood CSF/clotted CSF/no CSF sample obtained.
c A WBC count of < 20 regardless of the RBC count, or a RBC count of < 500.
d Negative vs. definite/probable/possible/equivocal/uninterpretable/no CSF.
e If the RBC count is ≥ 500, then the WBC count will be reduced by 1 for every 500 RBCs to give a 'corrected' WBC count.
f One vs. more than one.
g None/mild vs. moderate/severe.

TABLE 13 Untested clinical outcomes: ESR vs. LSR

Outcome	Stylet removal allocation	
	ESR (*N* = 545), *n* (%)	LSR (*N* = 531), *n* (%)
Appearance		
Appearance of CSF on first attempt of first procedure, *n* (%)		
Clear CSF	199 (36.6)	192 (36.2)
Blood-stained CSF	102 (18.8)	95 (17.9)
Pure-blood CSF or clotted CSF	142 (26.1)	145 (27.4)
No CSF sample obtained	101 (18.6)	98 (18.5)
Missing	1	1
Appearance of clearest sample from first or second procedure, *n* (%)		
Clear CSF	275 (50.5)	260 (49.0)
Blood-stained CSF	196 (36.0)	195 (36.7)
Pure-blood CSF or clotted CSF	60 (11.0)	66 (12.4)
No CSF sample obtained	14 (2.6)	10 (1.9)
Procedures		
Number of attempts in first procedure, *n* (%)		
One	293 (53.9)	289 (54.4)
Two	190 (34.9)	184 (34.7)
Three or more	61 (11.2)	58 (10.9)
Missing	1	0
Had second procedure, *n*	81	83
Number of attempts in second procedure, *n* (%)		
One	46 (56.8)	38 (45.8)
Two	29 (35.8)	36 (43.4)
Three or more	6 (7.4)	9 (10.8)
Missing	0	2
Success		
CSF obtained and any RBC count		
First attempt of first procedure	272 (49.9)	263 (49.5)
Any attempt of first procedure	383 (70.3)	364 (68.5)
First or second procedure	423 (77.6)	401 (75.5)
CSF obtained and RBC count < 500/mm^3		
First attempt of first procedure	169 (31.0)	162 (30.5)
Any attempt of first procedure	222 (40.7)	208 (39.2)
First or second procedure	234 (42.9)	222 (41.8)

continued

TABLE 13 Untested clinical outcomes: ESR vs. LSR (*continued*)

Outcome	Stylet removal allocation	
	ESR (*N* = 545), *n* (%)	LSR (*N* = 531), *n* (%)
CSF obtained and RBC count < 5000/mm^3		
First attempt of first procedure	238 (43.7)	224 (42.2)
Any attempt of first procedure	319 (58.5)	298 (56.1)
First or second procedure	345 (63.3)	320 (60.3)
CSF obtained and RBC count < 10,000/mm^3		
First attempt of first procedure	250 (45.9)	235 (44.3)
Any attempt of first procedure	338 (62.0)	315 (59.3)
First or second procedure	369 (67.7)	340 (64.0)
CSF obtained and RBC count < 25,000/mm^3		
First attempt of first procedure	261 (47.9)	251 (47.3)
Any attempt of first procedure	360 (66.1)	346 (65.2)
First or second procedure	394 (72.3)	375 (70.6)
CSF obtained with WBC count not requiring correction		
First attempt of first procedure	252 (46.2)	244 (46.0)
Any attempt of first procedure	349 (64.0)	329 (62.0)
First or second procedure	384 (70.5)	360 (67.8)
Diagnoses of meningitis via CSF from first or second procedure		
Positive: either diagnosed by WBC criteria (A) or by culture/PCR (B)	25 (4.7)	18 (3.5)
(A) WBC count (or corrected WBC) of ≥ 20 from clearest CSF	24 (4.5)	18 (3.5)
(B) Any true-positive culture or PCR from first or second LP	2 (0.4)	0
Equivocal: does not have a positive diagnosis and either has isolated raised PMNs (C) or isolated organisms on Gram stain (D)	4 (0.7)	3 (0.6)
(C) In clearest CSF a RBC count of < 500, a WBC count of < 20 and PMN > 2	1 (0.2)	2 (0.4)
(D) Organism found on any Gram stain (first or second LP) and clearest CSF has a WBC count (or corrected WBC) of < 20 or no RBC count was possible	3 (0.6)	1 (0.2)
Negative: does not have positive or equivocal diagnosis and has negative microscopy (E) or is missing PMN but would otherwise be equivocal (F)	384 (71.5)	371 (71.2)
(E) In clearest CSF a WBC count (or corrected WBC) of < 20	291 (54.2)	304 (58.3)
(F) Equivocal diagnosis except for missing PMN	95 (17.7)	73 (14.0)
Uninterpretable: either no sample obtained (G) or no cell count possible (H)	124 (23.1)	129 (24.8)
(G) No sample obtained on any attempt in first or second LP	14 (2.6)	9 (1.7)
(H) CSF obtained but clotted/insufficient and so either not sent to laboratory or not analysed, or no RBC count possible from clearest CSF	110 (20.5)	120 (23.0)
Other clinical reason for LP	3	8
Unknown	5	2

TABLE 14 Resource consumption

Resource use	Stylet removal allocation		Unadjusted Med D (95% CI)	Adjusted[a] Med D (95% CI)	Adjusted *p*-value
	ESR (*N* = 545)	LSR (*N* = 531)			
Received antibiotics during trial, *n*	533	518			
Duration (days) of antibiotic course from trial entry to discharge home, median (IQR)	5 (4–6)	5 (4–6)	0.0 (–0.2 to 0.2)	0.0 (–0.2 to 0.2)	1.000
Range (minimum to maximum)	1–25	0–19			
Missing, *n*	0	3			
Surviving infants, *n*	544	530			
Length of stay (days) in hospital (in surviving infants) from trial entry until discharge home, median (IQR)	5 (4–7)	5 (4–7)	0.0 (–0.3 to 0.3)	0.0 (–0.4 to 0.4)	1.000
Range (minimum to maximum)	1–371	1–119			

a Adjusted for position allocation, gestational age at randomisation and centre.

Economic analysis: multiarm analysis

Our economic assessment was also assessed in the multiarm analysis. As shown in *Table 21*, there were no obvious differences in resource consumption between the groups in terms of number of infants receiving antibiotics, duration of antibiotic use, duration of antibiotic therapy up to discharge from hospital or length of stay.

Safety outcomes: multiarm analysis

When analysed in the multiarm analysis, there were no differences in safety outcomes between groups (*Table 22*).

Multiarm analysis: compliance with allocated intervention

There were no obvious differences in adherence to treatment allocation, other than those already described for the sitting and lying comparison (*Table 23*). Sample sizes were small for second LPs.

Serious adverse events

Four SAEs were reported during the trial (*Table 24*). Three were deemed unrelated to LP. One infant, from the sitting plus LSR group, developed a scrotal haematoma 2 days after LP. The infant did not undergo further investigations to identify a cause for this, and so a relationship with the LP could not be ruled out. Therefore, this event was deemed ‘possibly related’.

TABLE 15 Safety outcomes

Outcome	Stylet removal allocation		Unadjusted effect measure (95% CI)	Adjusted[a] effect measure (95% CI)	Adjusted *p*-value	Unadjusted risk difference (%) (95% CI)
	ESR (*N* = 545)	LSR (*N* = 531)				
Procedure abandoned because of cardiovascular deterioration (first procedure), *n* (%)	1 (0.2)	2 (0.4)	RR 0.49 (0.04 to 5.37)	RR 0.49 (0.04 to 5.53)	0.564	−0.19 (−0.83 to 0.44)
Missing, *n*	1	0				
Procedure abandoned because of cardiovascular deterioration (second procedure), *n/N* (%)	0/81 (0.0)	1/85 (1.2)				
Infant's lowest SpO_2 (%) (first procedure), median (IQR)	92 (86–95)	92 (87–95)	Med D 0.0 (−1.0 to 1.0)	Med D 0.0 (−0.9 to 0.9)	1.000	
Missing, *n*	23	13				
Infant's lowest HR (b.p.m.) (first procedure), mean (SD)	128.1 (21.0)	128.4 (20.4)	MD −0.3 (−2.9 to 2.2)	MD −0.3 (−2.3 to 1.7)	0.754	
Missing, *n*	34	18				
Infant's highest HR (b.p.m.) (first procedure), mean (SD)	163.9 (21.6)	163.4 (22.0)	MD 0.5 (−2.2 to 3.2)	MD 0.5 (−1.9 to 2.9)	0.673	
Missing, *n*	31	19				
Respiratory deterioration post LP (requirement for escalating respiratory support within 1 hour of LP) (first procedure), *n* (%)	1 (0.2)	2 (0.4)	RR 0.49 (0.04 to 5.37)	RR 0.49 (0.04 to 5.63)	0.564	−0.19 (−0.83 to 0.44)
Missing, *n*	1	0				
Respiratory deterioration post LP (requirement for escalating respiratory support within 1 hour of LP) (second procedure), *n/N* (%)	0/81 (0.0)	0/85 (0.0)				

a Adjusted for position allocation, gestational age at randomisation and centre.

TABLE 16 Compliance with allocated intervention in first or second procedure

Procedure	Stylet removal allocation	
	ESR	LSR
First LP only		
N	545	531
Time (hours) from randomisation to first LP, median (IQR)	0.8 (0.4–1.6)	0.9 (0.4–1.7)
≥ 12 hours, *n* (%)	10 (1.9)	10 (1.9)
Missing, *n*	14	11
At least one attempt at LP in which the allocated technique was not adhered to (any attempt), *n* (%)	19 (3.5)	16 (3.0)
Clinician decision	15 (78.9)	14 (93.3)
Parental decision	1 (5.3)	0 (0.0)
Unintentional use of alternative technique	3 (15.8)	1 (6.7)
Missing	0	1
Attempt in which the allocated technique was not adhered to, *n/N* (%) (not mutually exclusive)		
First	1/543 (0.2)	2/530 (0.4)
Second	8/250 (3.2)	8/241 (3.3)
Third	11/61 (18.0)	8/58 (13.8)
Total number of attempts, *n*	861	831
Number of attempts in which the allocated technique was not adhered to, *n* (%)	20 (2.3)	18 (2.2)
Missing	2	1
Second LP only		
N	81	85
At least one attempt at LP in which the allocated technique was not adhered to (any attempt), *n* (%)	13 (16.7)	6 (7.6)
Clinician decision	10 (90.9)	5 (83.3)
Parental decision	1 (9.1)	1 (16.7)
Unintentional use of alternative technique	0	0
Missing	2	0
Attempt in which the allocated technique was not adhered to, *n/N* (%) (not mutually exclusive)		
First	6/78 (7.7)	3/79 (3.8)
Second	7/34 (20.6)	3/42 (7.1)
Third	3/6 (50.0)	1/8 (12.5)
Total number of attempts, *n*	122	137
Attempts at LP in which the allocated technique was not adhered to (any attempt),[a] *n* (%)	16 (13.1)	7 (5.1)
Missing	3	6

a As a proportion of all attempts.

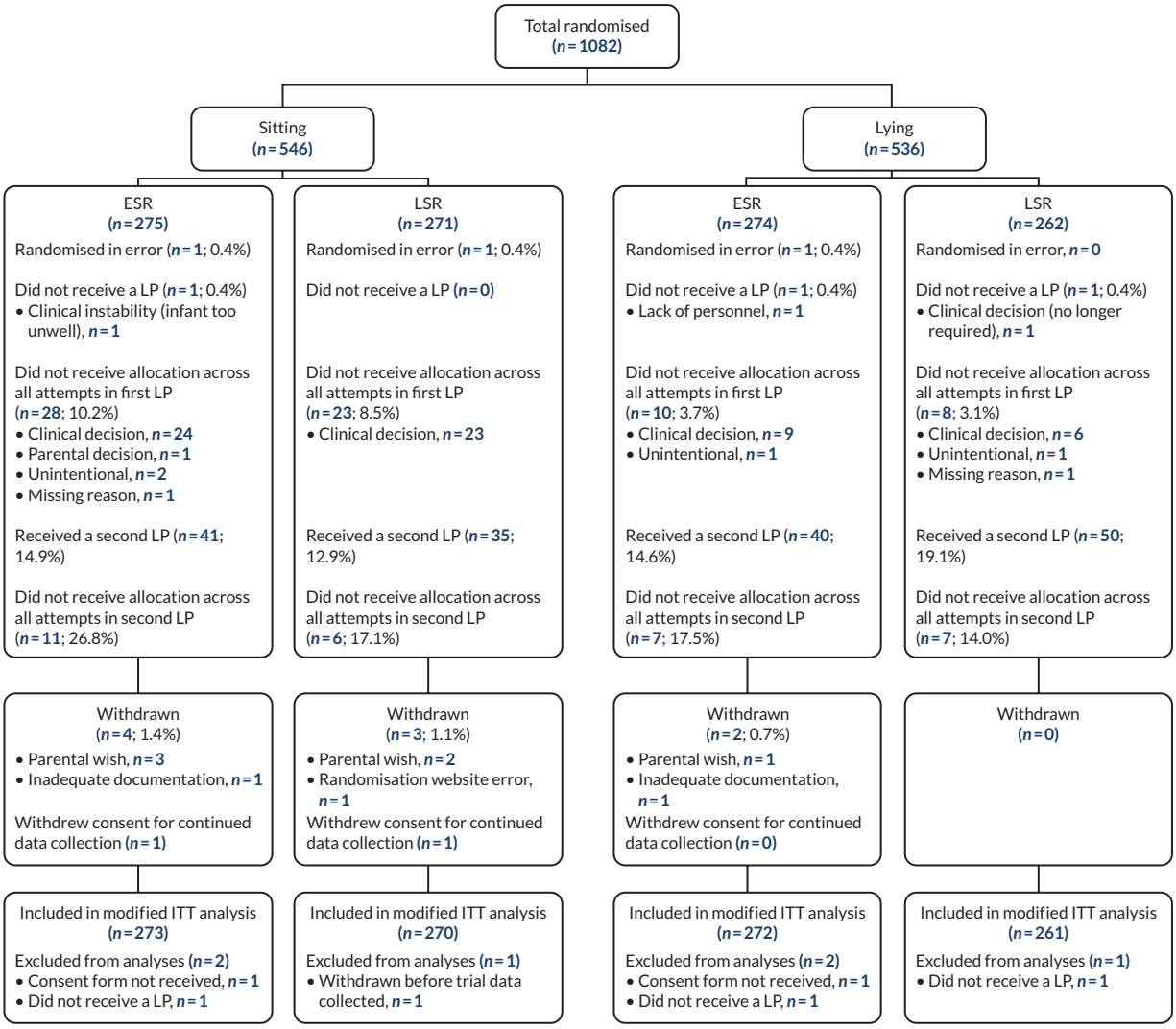

FIGURE 5 Flow of participants by randomised group: multiarm analysis. ITT, intention to treat.

TABLE 17 Infant (parent) characteristics at trial entry: multiarm analysis

Characteristic	Group			
	Sitting plus ESR (*N* = 273)	Sitting plus LSR (*N* = 270)	Lying plus ESR (*N* = 272)	Lying plus LSR (*N* = 261)
CGA at trial entry (weeks^{+days}), *n* (%)				
27^{+0} to 31^{+6}	4 (1.5)	7 (2.6)	6 (2.2)	5 (1.9)
32^{+0} to 36^{+6}	23 (8.4)	23 (8.5)	26 (9.6)	21 (8.0)
37^{+0} to 40^{+6}	149 (54.6)	150 (55.6)	148 (54.4)	147 (56.3)
≥ 41^{+0}	97 (35.5)	90 (33.3)	92 (33.8)	88 (33.7)
Median (IQR)	40 (39–41)	40 (39–41)	40 (39–41)	40 (39–41)
Gestational age at birth (weeks^{+days}), *n* (%)				
< 27^{+0}	1 (0.4)	0	4 (1.5)	2 (0.8)
27^{+0} to 31^{+6}	5 (1.8)	7 (2.6)	11 (4.0)	9 (3.4)
32^{+0} to 36^{+6}	22 (8.1)	26 (9.6)	23 (8.5)	16 (6.1)
37^{+0} to 40^{+6}	164 (60.1)	165 (61.1)	163 (59.9)	159 (60.9)
≥ 41^{+0}	81 (29.7)	72 (26.7)	71 (26.1)	75 (28.7)
Median (IQR)	40 (39–41)	40 (39–41)	40 (39–41)	40 (39–41)

TABLE 17 Infant (parent) characteristics at trial entry: multiarm analysis (*continued*)

Characteristic	Group			
	Sitting plus ESR (*N* = 273)	Sitting plus LSR (*N* = 270)	Lying plus ESR (*N* = 272)	Lying plus LSR (*N* = 261)
Age (days), median (IQR)	1 (1–2)	1 (1–2)	2 (1–2)	2 (1–2)
Range (minimum to maximum)	(0–76)	(0–35)	(0–91)	(0–50)
≥ 3 days, *n* (%)	31 (11.4)	39 (14.4)	43 (15.8)	27 (10.3)
Birthweight (g), median (IQR)	3502 (3185–3870)	3498 (3020–3925)	3530 (3055–3905)	3524 (3200–3885)
Missing	0	0	1	0
Working weight (g) at trial entry, median (IQR)	3510 (3185–3870)	3498 (3020–3925)	3534 (3048–3903)	3520 (3200–3885)
1000–2499, *n* (%)	24 (8.8)	31 (11.5)	33 (12.1)	17 (6.5)
2500–3500, *n* (%)	111 (40.7)	106 (39.3)	96 (35.3)	111 (42.5)
≥ 3501, *n* (%)	138 (50.5)	133 (49.3)	143 (52.6)	133 (51.0)
Infant sex, *n* (%)				
Male	165 (60.4)	160 (59.3)	171 (62.9)	165 (63.2)
Female	108 (39.6)	110 (40.7)	101 (37.1)	96 (36.8)
One of a multiple pregnancy, *n* (%)	4 (1.5)	1 (0.4)	8 (2.9)	5 (1.9)
Receiving antibiotics, *n* (%)	255 (93.4)	250 (92.6)	248 (91.2)	241 (92.3)
Any previous LPs, *n* (%)	2 (0.7)	0	2 (0.7)	1 (0.4)
Days since last LP, median (IQR)	22 (15–29)		21 (19–23)	22 (22–22)
CSF from last LP sent to laboratory, *n* (%)	2 (100.0)		2 (100.0)	1 (100.0)
RBC from last LP (× 10^6/l), median (IQR)	230 (0–460)		435 (0–870)	
Missing, *n*	0		0	1
WBC from last LP (× 10^6/l), median (IQR)	3 (0–5)		1 (0–2)	
Missing, *n*	0		0	1
Primary indication for current LP (not mutually exclusive), *n* (%)				
Risk factor for sepsis	99 (36.3)	102 (37.8)	97 (35.7)	106 (40.8)
Clinical signs of sepsis	70 (25.6)	67 (24.8)	77 (28.3)	68 (26.2)
Abnormal WBC count/ morphology	6 (2.2)	2 (0.7)	3 (1.1)	5 (1.9)
Raised CRP	232 (85.0)	234 (86.7)	225 (82.7)	219 (84.2)
Specific signs of meningitis/ encephalitis	6 (2.2)	6 (2.2)	5 (1.8)	3 (1.2)
Neurometabolic investigation	2 (0.7)	2 (0.7)	2 (0.7)	1 (0.4)
Therapeutic (raised intracranial pressure)	0	0	0	0
Recent failed LP	0	0	0	0
Positive blood culture	0	3 (1.1)	1 (0.4)	2 (0.8)
Other	4 (1.5)	0	2 (0.7)	4 (1.5)
Missing	0	0	0	1
Parental anxiety score (STAI-S)				
n	46	49	48	54
Mean (SD)	51.6 (11.8)	48.8 (14.0)	46.5 (15.2)	49.3 (13.6)

TABLE 18 Clinical characteristics at first LP: multiarm analysis

Characteristic	Group			
	Sitting plus ESR (*N* = 273)	Sitting plus LSR (*N* = 270)	Lying plus ESR (*N* = 272)	Lying plus LSR (*N* = 261)
Type of sedation and analgesia received (not mutually exclusive), *n* (%)				
None	13 (4.8)	11 (4.1)	11 (4.0)	8 (3.1)
Non-nutritive sucking	113 (41.5)	118 (43.9)	99 (36.4)	100 (38.3)
Oral sucrose/dextrose/glucose	227 (83.5)	216 (80.3)	237 (87.1)	221 (84.7)
Milk	9 (3.3)	4 (1.5)	3 (1.1)	6 (2.3)
Topical local anaesthetic	139 (51.1)	130 (48.3)	128 (47.1)	133 (51.0)
Paracetamol	0	0	1 (0.4)	0
NSAID	0	0	0	0
Opiate	2 (0.7)	1 (0.4)	5 (1.8)	2 (0.8)
Chloral hydrate	1 (0.4)	1 (0.4)	0	0
Midazolam	0	0	0	0
Phenobarbitone/phenytoin	0	1 (0.4)	2 (0.7)	0
Missing	1	1	0	0
Respiratory status immediately before LP, *n* (%)				
Self-ventilating in air	237 (86.8)	229 (84.8)	222 (81.6)	226 (86.6)
Low-flow oxygen (< 2 l/minute)	7 (2.6)	6 (2.2)	9 (3.3)	7 (2.7)
High-flow oxygen (≥ 2 l/minute)	24 (8.8)	33 (12.2)	35 (12.9)	24 (9.2)
CPAP/BiPAP	5 (1.8)	2 (0.7)	6 (2.2)	4 (1.5)
Previous diagnosis of intraventricular haemorrhage, *n* (%)	1 (1.0)	1 (0.9)	4 (4.1)	1 (1.0)
Not scanned	171	163	175	161
Grade of intraventricular haemorrhage at latest scan, *n* (%)				
I	1 (100.0)	1 (100.0)	2 (50.0)	0
II	0	0	1 (25.0)	1 (100.0)
III	0	0	1 (25.0)	0
IV	0	0	0	0
Coagulopathy treatment within last 24 hours, *n* (%)	2 (0.7)	2 (0.7)	2 (0.7)	3 (1.1)
Confirmed or probable infection (not mutually exclusive), *n* (%)				
Necrotising enterocolitis	0	1 (0.4)	0	0
Pneumonia	13 (4.8)	15 (5.6)	20 (7.4)	15 (5.7)
Sepsis	151 (55.3)	150 (55.6)	159 (58.5)	142 (54.4)
Blood culture positive	10 (3.7)	10 (3.7)	6 (2.2)	13 (5.0)
Urine infection	0	0	0	0
Line infection	0	0	0	0
Other localised infection	0	0	0	2 (0.8)
Other	0	1 (0.4)	0	3 (1.1)
Missing	0	0	0	0
Results of latest blood test				
Platelets (× 10^9/l), mean (SD)	251.7 (70.1)	247.8 (71.1)	250.0 (73.0)	249.2 (72.2)
Missing, *n*	11	17	13	8
WBC count (× 10^9/l), median (IQR)	15 (11–21)	15 (11–20)	15 (10–19)	16 (11–21)

TABLE 18 Clinical characteristics at first LP: multiarm analysis (*continued*)

Characteristic	Group			
	Sitting plus ESR (*N* = 273)	Sitting plus LSR (*N* = 270)	Lying plus ESR (*N* = 272)	Lying plus LSR (*N* = 261)
Missing, *n*	11	14	13	8
Neutrophils (× 10^9/l), mean (SD)	10.9 (5.6)	10.4 (5.8)	9.8 (5.6)	10.4 (5.3)
Missing, *n*	14	20	19	15
RBC count (× 10^{12}/l), median (IQR)	5 (5–6)	5 (5–6)	5 (5–6)	5 (5–6)
Missing, *n*	12	17	16	9
CRP (mg/l), median (IQR)	39 (25–56)	40 (25–61)	39 (24–60)	40 (24–62)
Missing, *n*	1	3	2	1

BiPAP, bilevel positive airway pressure; CPAP, continuous positive airway pressure; NSAID, non-steroidal anti-inflammatory drug.

TABLE 19 Primary outcome: multiarm analysis

Outcome	Group				
	Sitting plus ESR (*N* = 273)	Sitting plus LSR (*N* = 270)	Lying plus ESR (*N* = 272)	Lying plus LSR (*N* = 261)	Interaction *p*-value[a]
CSF obtained and RBC count < 10,000/mm^3 on first LP procedure (any attempt), *n* (%)	173 (63.4)	173 (64.1)	165 (60.7)	142 (54.4)	
RR (95% CI) compared with lying plus LSR group	1.16 (1.01 to 1.33)	1.19 (1.01 to 1.40)	1.12 (0.97 to 1.30)	1	0.136

a Wald test *p*-value for the interaction of position and stylet removal allocations in an adjusted binomial regression model.

TABLE 20 Secondary clinical outcomes: multiarm analysis

Outcome	Group			
	Sitting plus ESR (*N* = 273)	Sitting plus LSR (*N* = 270)	Lying plus ESR (*N* = 272)	Lying plus LSR (*N* = 261)
Appearance of clearest sample on first procedure (any attempt), *n* (%)				
Clear CSF	130 (47.6)	140 (51.9)	129 (47.4)	104 (39.8)
Blood-stained CSF	86 (31.5)	77 (28.5)	83 (30.5)	90 (34.5)
Pure-blood CSF/clotted CSF	43 (15.8)	42 (15.6)	47 (17.3)	53 (20.3)
No CSF sample obtained	14 (5.1)	11 (4.1)	13 (4.8)	14 (5.4)
CSF obtained with any RBC on first procedure (any attempt), *n* (%)	192 (70.3)	198 (73.3)	191 (70.2)	166 (63.6)
CSF obtained with WBC count not requiring correction[a] on first procedure (any attempt), *n* (%)	179 (65.8)	177 (65.6)	170 (62.5)	152 (58.2)
Missing	1	0	0	0
Final clinical diagnosis at discharge [from LP(s)], *n* (%)				
Definite/probable meningitis	3 (1.1)	4 (1.5)	6 (2.2)	3 (1.2)
Possible meningitis or equivocal CSF result	8 (2.9)	4 (1.5)	5 (1.9)	6 (2.3)

continued

TABLE 20 Secondary clinical outcomes: multiarm analysis (*continued*)

Outcome	Group			
	Sitting plus ESR (*N* = 273)	Sitting plus LSR (*N* = 270)	Lying plus ESR (*N* = 272)	Lying plus LSR (*N* = 261)
Negative CSF result	215 (79.0)	209 (78.9)	207 (76.7)	201 (77.9)
Uninterpretable CSF result (e.g. very high RBC or clotted CSF)	14 (5.1)	17 (6.4)	19 (7.0)	17 (6.6)
No CSF obtained	32 (11.8)	31 (11.7)	33 (12.2)	31 (12.0)
Other clinical reason for LP, *n*	1	5	2	3
From clearest CSF sample				
WBC count (× 10^6/l), *n*	214	215	205	184
Median (IQR)	3 (1–6)	3 (0–7)	2 (1–6)	2 (0–6)
RBC count (× 10^6/l), *n*	213	215	208	184
Median (IQR)	430 (19–2075)	317 (38–3200)	164 (11–2475)	266 (15–2825)
Corrected[b] WBC count (× 10^6/l), *n*	214	215	205	184
Median (IQR)	1 (0–4)	1 (0–4)	1 (0–4)	0 (0–3)
PMN (× 10^6/l), *n*	119	137	112	109
Median (IQR)	0 (0–1)	0 (0–0)	0 (0–1)	0 (0–1)
Lymphocytes (× 10^6/l), *n*	121	138	115	113
Median (IQR)	0 (0–4)	0 (0–4)	0 (0–3)	0 (0–3)
Total number of procedures performed, *n* (%)				
One	220 (80.6)	227 (84.1)	225 (82.7)	199 (76.2)
Two	45 (16.5)	38 (14.1)	36 (13.2)	46 (17.6)
Three or more	8 (2.9)	5 (1.9)	11 (4.0)	16 (6.1)
Total number of attempts performed, *n* (%)				
One	132 (48.4)	150 (55.6)	148 (54.6)	127 (48.7)
Two	71 (26.0)	60 (22.2)	56 (20.7)	55 (21.1)
Three or more	70 (25.6)	60 (22.2)	67 (24.7)	79 (30.3)
Missing	0	0	1	0
Time (minutes) taken to complete first procedure, from start of cleaning skin to removing needle at end of all attempts, median (IQR)	8 (5–13)	8 (5–12)	8 (5–12)	8 (5–13)
Range (minimum to maximum)	0–31	0–55	0–37	0–35
Missing	5	3	6	6
Level of infant struggling movement on first attempt of first procedure, *n* (%)				
None	55 (20.3)	70 (25.9)	46 (17.2)	39 (15.0)
Mild	126 (46.5)	121 (44.8)	122 (45.7)	118 (45.4)
Moderate	70 (25.8)	59 (21.9)	78 (29.2)	81 (31.2)
Severe	20 (7.4)	20 (7.4)	21 (7.9)	22 (8.5)
Missing	2	0	5	1

a A WBC count of < 20 regardless of the RBC count, or a RBC count of < 500.
b If the RBC count is ≥ 500, then the WBC count will be reduced by 1 for every 500 RBCs to give a 'corrected' WBC count.

TABLE 21 Resource consumption: multiarm analysis

Resource use	Group			
	Sitting plus ESR (*N* = 273)	Sitting plus LSR (*N* = 270)	Lying plus ESR (*N* = 272)	Lying plus LSR (*N* = 261)
Received antibiotics during trial, *n*	267	263	266	255
Duration (days) of antibiotic course from trial entry to discharge home, median (IQR)	5 (4–6)	5 (4–6)	5 (4–6)	5 (4–6)
Range (minimum to maximum)	1–24	1–15	1–25	0–19
Missing	0	1	0	2
Surviving infants, *n*	272	269	272	261
Length of stay (days) in hospital (in surviving infants) from trial entry until discharge home, median (IQR)	5 (4–7)	5 (4–7)	6 (4–7)	5 (4–7)
Range (minimum to maximum)	1–158	1–119	1–371	1–73

TABLE 22 Safety outcomes: multiarm analysis

Outcome	Group			
	Sitting plus ESR (*N* = 273)	Sitting plus LSR (*N* = 270)	Lying plus ESR (*N* = 272)	Lying plus LSR (*N* = 261)
Procedure abandoned because of cardiovascular deterioration (first procedure), *n* (%)	0 (0.0)	2 (0.7)	1 (0.4)	0 (0.0)
Missing, *n*	0	0	1	0
Infant's lowest SpO_2 (%) (first procedure), median (IQR)	93 (88–96)	92 (89–96)	89 (85–94)	90 (85–94)
Missing, *n*	8	3	15	10
Infant's lowest HR (b.p.m.) (first procedure), mean (SD)	129.7 (20.1)	129.3 (19.7)	126.5 (21.8)	127.6 (21.2)
Missing, *n*	13	7	21	11
Infant's highest HR (b.p.m.) (first procedure), mean (SD)	164.9 (20.5)	162.6 (22.8)	162.9 (22.7)	164.3 (21.1)
Missing, *n*	10	8	21	11
Respiratory deterioration post LP (requirement for escalating respiratory support within 1 hour of LP) (first procedure), *n* (%)	0 (0.0)	1 (0.4)	1 (0.4)	1 (0.4)
Missing, *n*	0	0	1	0

TABLE 23 Compliance with allocated intervention in first or second procedure

Procedure	Group			
	Sitting plus ESR	Sitting plus LSR	Lying plus ESR	Lying plus LSR
First LP only				
N	273	270	272	261
Time (hours) from randomisation to first LP, median (IQR)	0.8 (0.4–1.7)	1.0 (0.5–2.0)	0.8 (0.5–1.5)	0.8 (0.4–1.5)
≥ 12 hours, *n* (%)	5 (1.9)	4 (1.5)	5 (1.9)	6 (2.3)
Missing	7	6	7	5
At least one attempt at LP in which the allocated technique was not adhered to, *n* (%)	28 (10.3)	23 (8.5)	10 (3.7)	8 (3.1)
Clinician decision	24 (88.9)	23 (100.0)	9 (90.0)	6 (85.7)
Parental decision	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)
Unintentional use of alternative technique	2 (7.4)	0 (0.0)	1 (10.0)	1 (14.3)
Missing	1	0	0	1
Attempt in which the allocated technique was not adhered to, *n*/*N* (%) (not mutually exclusive)				
First	4/273 (1.5)	4/270 (1.5)	1/270 (0.4)	1/260 (0.4)
Second	13/134 (9.7)	11/113 (9.7)	2/116 (1.7)	2/128 (1.6)
Third	13/29 (44.8)	12/28 (42.9)	8/32 (25.0)	5/30 (16.7)
Total number of attempts, *n*	437	411	424	420
Number of attempts in which the allocated technique was not adhered to,[a] *n* (%)	30 (6.9)	27 (6.6)	11 (2.6)	8 (1.9)
Missing	0	0	2	1
Second LP only				
N	41	35	40	50
At least one attempt at LP in which the allocated technique was not adhered to (any attempt), *n* (%)	11 (28.2)	6 (18.8)	7 (17.9)	7 (14.9)
Clinician decision	9 (90.0)	5 (83.3)	6 (100.0)	7 (100.0)
Parental decision	1 (10.0)	1 (16.7)	0 (0.0)	0 (0.0)
Unintentional use of alternative technique	0	0	0	0
Missing	1	0	1	0
Attempt in which the allocated technique was not adhered to, *n*/*N* (%) (not mutually exclusive)				
First	7/39 (17.9)	3/32 (9.4)	3/39 (7.7)	2/47 (4.3)
Second	6/16 (37.5)	4/18 (22.2)	4/18 (22.2)	2/24 (8.3)
Third	2/3 (66.7)	0/0	2/3 (66.7)	4/8 (50.0)
Total number of attempts, *n*	61	53	61	84
Attempts at LP in which the allocated technique was not adhered to (any attempt), *n* (%)	15 (24.6)	7 (13.2)	9 (14.8)	8 (9.5)
Missing	2	3	1	3

a As a proportion of all attempts.

TABLE 24 Serious adverse events

SAE number	Allocation	Centre ID	Description	Severity	Related	Action taken	Outcome
1	Sitting plus ESR	18	Hypothyroidism diagnosed 10 days post LP from Guthrie card taken pre LP	Mild	Not related	None	Resolved
2	Sitting plus ESR	10	Infant born at 28 weeks' gestation and required LP on day 2 of life. Sudden desaturation on day 9. Sadly passed away despite extensive resuscitative efforts	Severe	Not related	N/A [intervention(s) stopped prior to the event starting]	Fatal
3	Sitting plus LSR	16	Infant born at 36 weeks. LP performed on day 1. Right scrotal haematoma noted on day 3. There was no description of these occurring post LP in the medical literature, but previously reported cases have been idiopathic, related to difficult delivery or due to asymptomatic intra-abdominal bleeding	Mild	Possibly	N/A [intervention(s) stopped prior to the event starting]	Unknown
4	Sitting plus ESR	16	Term infant admitted to neonatal unit for respiratory distress. Required LP on day 3. Abdominal distension and vomiting days 3–4. Pneumoperitoneum identified on day 7. Diagnosed with likely spontaneous intestinal perforation	Severe	Not related	N/A [intervention(s) stopped prior to the event starting]	Resolved with sequelae

ID, identification; N/A, not applicable.

Chapter 4 Economic evaluation

Introduction

In this brief chapter, we report the economic evaluation in regard to resource consumption, as part of the NeoCLEAR trial. The objective of the economic evaluation was to compare the relative cost-effectiveness of the two interventions in terms of the duration of intravenous antibiotic therapy and length of hospital stay.

Methods

We documented the number of infants receiving antibiotics during trial and the median (IQR) duration of antibiotic course from trial entry to discharge home (in days), and calculated the number (range) of missing data. Similarly, we documented the surviving infants and their median (IQR) length of stay in hospital from trial entry until discharge home (in days).

Outcomes, results and economic analysis

We did not find statistically significant differences in resource consumption between groups (see *Table 21*). Likewise, we did not find statistically significant differences for the number of infants receiving antibiotics during trial, the duration of antibiotic course from trial entry to discharge home (in days), survival rates, total length of stay in hospital or the number of infants with missing data.

Chapter 5 Discussion and conclusions

Summary of main findings

The NeoCLEAR trial is, to the best of our knowledge, the first adequately powered RCT comparing different LP techniques in newborns. Sitting position was superior to lying for achieving a successful first LP, with an absolute risk difference of 6.1% and a NNT of 16. CSF samples were more often interpretable, and sitting LP was better tolerated by participants, who were objectively less likely to have oxygen desaturations and dips in HR. Infants also showed reduced struggling in sitting position. The timing of stylet removal did not influence LP success.

To the best of our knowledge, this is the largest trial of neonatal LP technique worldwide. The demographic and clinical patient characteristics at baseline were well balanced across the four groups. The median age of patients was 40 weeks (term), with a median working weight of 3500 g. Eighty-seven per cent of infants were term born (i.e. with a CGA of 37–44 weeks). We observed a slight male predominance, which was equally balanced between groups. Similarly, failure to obtain any CSF was also equally distributed between groups. The sitting position resulted in significantly more successful LPs than the lying position. The advantageous effect of the sitting LP position was consistent across all subgroups of CGA and weight. The number of preterm infants was relatively small, especially the number with a CGA of below 32 weeks (n = 22, 2% of the total trial cohort). The NeoCLEAR trial showed that infants aged < 3 days significantly benefited from the sitting position, whereas this benefit was not demonstrated for the small subgroup of infants enrolled ≥ 3 days of life. As might be expected, babies in this subgroup had a lower gestational age at birth and a lower birthweight and were more likely to be on respiratory support.

Our results may, in part, be explained by the anatomical advantages of the sitting position, as described previously in neonates:[14,30,31] (1) the intervertebral spaces widen in the sitting position; (2) the CSF space increases at the lowest point of the spinal canal and close to the entry site of the needle; and (3) this position is more comfortable for the baby, as evidenced by the reduced struggling we observed.

The only other RCT[32] of the sitting position compared with the lying position involved 168 infants aged < 90 days in a paediatric emergency room setting. Success rates in this RCT[32] did not differ significantly depending on position (lying position, 63/82, 77%; sitting position, 61/85, 72%; difference 5.1%, 95% CI −8.2% to 18.3%).

The suggestion of potentially greater LP success rates with ESR was based on studies with non-styletted needles, which have reported 100% success rates,[21] and observational studies in which styletted needles were used and timing of stylet removal varied.[13,30] However, the use of unstyletted needles for LP is strongly discouraged, as it bears the risk of iatrogenic intraspinal epidermoid tumour formation.[4,22] As a safe alternative, the technique of ESR was introduced. In a prospective study that included infants aged < 12 weeks, Baxter *et al.*[13] found that ESR was associated with successful and non-traumatic LP. The NeoCLEAR trial did not replicate this apparent benefit of ESR. Likewise, we did not find a disadvantage of this approach. Therefore, we cannot advise for or against a specific timing of stylet removal. There was no statistically significant interaction between the position and stylet timing comparisons within our 2 × 2 factorial design.

Our safety analysis showed greater physiological stability (i.e. HR and SpO_2) for babies receiving LP in the sitting position than for babies receiving LP in the lying position, and this is similar to earlier reports by Gleason *et al.*[29] and Weisman *et al.*[28] Most other safety and secondary outcomes lacked clinical and statistical significance with respect to differences between the groups.

The very basic analysis of resource use showed no difference between techniques. Comprehensively evaluating this topic would require more in-depth study, possibly outside a pragmatic RCT like this. Although there was no difference in hospital days or antibiotic use in our study, it might, indeed, be speculated that because a sitting LP can be performed as quickly and at the same cost as a lying LP, and can result in a higher proportion of diagnostic LPs without evidence of adverse events, sitting LP might be cost-effective. More successful LP translates into less waste of professional time for repeated procedures, less opportunity for harm from additional procedures and greater parent/patient satisfaction.

The NeoCLEAR trial was designed with a 10% improvement in LP success rate as the primary outcome; however, the absolute increase was 6.1%, which is a clinically important finding. The reasons why the 10% margin was not reached should be carefully explored. This finding may, in part, be explained by a substantive level of non-adherence to the allocated procedure among infants randomised to the sitting position. We observed that non-adherence was 10-fold higher in this group than in infants allocated to the lying position. Our analysis shows that non-adherence was driven predominantly by practitioners, and not by patient characteristics. The phenomenon of non-adherence itself requires further analysis, potentially post hoc. However, contrary to a speculative perception held by practitioners (attitudes and opinions were not studied in our trial) that their defaulting to standard technique might improve the outcome of the LP, our results provide evidence that the lying position was not statistically significantly superior in any of the subgroup analyses. Moreover, the observation that sitting LP offers overall practical benefits (i.e. greater success rates, equally quick procedure, greater physiological stability), together with the final statistical analysis, cumulating in an impressive NNT of 16, convincingly demonstrates the superiority of sitting position for neonatal LP. Therefore, taking the other findings into consideration, we believe it seems reasonable to suggest adopting the sitting position as standard of care for 0- to 3-day-old term-born infants with body weights > 2.5 kg.

In conclusion, our results show that the success rate for LPs performed in the sitting position is 6.1% higher than for LPs performed in the lying position (NNT = 16). We found no difference in success rate between ESR and LSR. Compared with other modifications to LP technique (e.g. ultrasound guidance), sitting LP is cost-neutral and easy to learn. The results would be applicable in similar settings worldwide and should promote the sitting technique becoming the standard for neonatal lumbar puncture.

Strengths

The NeoCLEAR trial has several strengths. To the best of our knowledge, it is the largest RCT, to date, investigating two easily practised modifications of Quincke's traditional LP technique. The results are clear-cut, with the LP success rate 6.1% higher for the sitting position than for the lying position. This finding is in keeping with other studies,[40] using ultrasound to demonstrate that sitting position increases the lumbar interspinous distance,[40] which may explain why this position is more likely to be successful.

Limitations

Sitting LP was a novel concept for many practitioners, who were introduced to it for the first time during the preparatory training for the trial. The novelty factor and lack of familiarity with the technique (both for the assistant holding the baby and for the practitioner operating the needle) might explain why experienced practitioners were less compliant with sitting allocation on repeated procedures, irrespective of the training they had received at the start of the study. It might, therefore, be speculated that success rates could have been even higher if there had been more experience among the teams with sitting position LPs. This would indicate that there would be benefit in implementing education

and training in a sitting LP technique through medical education, from medical school and throughout paediatric training programmes, as well as for other healthcare providers who may be involved in performing a neonatal LP, such as advanced neonatal nurse practitioners.

The NeoCLEAR trial predominantly investigated term-born infants with suspected early-onset sepsis, and only a minority of infants were preterm or ≥ 3 days of life, which may limit the generalisability of our results for all infants. However, we did not find evidence that sitting position was less successful or less safe in infants with lower CGA or lower body weight, which is reassuring.

Blinding of practitioners was not possible, but the primary outcome was based on laboratory tests that were performed blinded to allocation.

The economic analysis was rudimentary, as cost outcomes were pragmatically measured by days spent in hospital and days of receiving antibiotic therapy. Although we found no differences in resource consumption between groups, a more detailed account of costs, including material costs and the cost in nurse and physician time, as well as the emotional expenditure on the part of the parents, seems warranted.

Implications for practice

We believe that the results of the NeoCLEAR trial should be universally applicable by paediatricians and neonatologists worldwide for the population of infants examined in our trial. The results would be applicable in similar settings worldwide and should promote the sitting technique becoming the standard for neonatal LP, especially for 0- to 3-day-old term-born infants with a body weight of > 2.5 kg.

Implications for research

As outlined in *Limitations*, the NeoCLEAR trial predominantly investigated term-born infants with suspected early-onset sepsis, and only a minority of infants were preterm or older than 3 days of life. Further trials should investigate the optimal position for LP in low and very low birthweight preterm infants, in ventilated patients and in cohorts of infants of all gestations with suspected late-onset sepsis. A detailed analysis of resource use and cost savings might also be desirable.

Further research could use ultrasound to investigate whether the anatomical benefits of sitting position relate to wider intervertebral spaces or to a greater lumbar CSF width. In addition, different age groups in infancy warrant further investigations, and the optimal time from sitting the infant up for LP to needle insertion requires further work. Finally, further study is required to answer the question of whether or not the advantages of sitting LP are also found in infants outside the neonatal period, including in infants presenting to emergency departments or children undergoing LP for neurological, oncologic or metabolic investigations.

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Independent Trial Steering Committee

The independent members of the TSC are listed in *Table 25*. These were Professor Paul Heath (chairperson), Dr Chris Gale (vice chairperson), Professor Richard Elmsley, Ms Marie Hubbard, Dr Julie Nelson (PPI) and Dr Bill Yoxall. The non-independent members are Associate Professor Charles Roehr and Professor Edmund Juszczak.

The investigator ensured that this study was conducted in accordance with the principles of the Declaration of Helsinki.

Guidelines for good clinical practice

The investigator ensured that this study was conducted in accordance with relevant regulations and with good clinical practice.

Independent Data Monitoring Committee

The independent members of the DMC are listed in *Table 26*. These were Dr David Sweet (chairperson), Professor Kate Costeloe and Professor Siobhan Creanor.

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Charles C Roehr (https://orcid.org/0000-0001-7965-4637) (Consultant Neonatal Medicine and Clinical Director, NPEU) conceived the idea for the study and wrote the initial protocol. He was the chief investigator and was responsible for all aspects of the study, including preparation and submission of the grant application, securing funding, regulatory approvals, project management, data collection and preparation of the manuscript for publication, including drafting the final report.

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Data-sharing statement

All data requests should be submitted to the corresponding author for consideration. Access to data may be granted following review.

This work uses data provided by patients and collected by the NHS as part of their care and support. Using patient data is vital to improve health and care for everyone. There is huge potential to make better use of information from people's patient records, to understand more about disease, develop new treatments, monitor safety, and plan NHS services. Patient data should be kept safe and secure, to protect everyone's privacy, and it's important that there are safeguards to make sure that it is stored and used responsibly. Everyone should be able to find out about how patient data are used. #datasaveslives You can find out more about the background to this citation here: https://understandingpatientdata.org.uk/data-citation.

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Appendix 1 Details of members of the Trial Steering Committee

TABLE 25 Trial Steering Committee members

Membership	Name	Affiliation
Chairperson; independent	Professor Paul Heath	Professor of Paediatric Infectious Diseases, St George's, University of London, London
Vice chairperson; independent	Dr Christopher Gale	Reader in Neonatal Medicine, Imperial College London, London
Independent	Ms Marie Hubbard	Lead Neonatal Research Nurse, Deputy Research and Innovation Lead, Women's and Children's Clinical Management Group, Leicester Royal Infirmary, Leicester
Independent	Dr William Yoxall	Consultant Neonatologist, Liverpool Women's Hospital, Liverpool
Independent	Professor Richard Emsley	Professor of Medical Statistics and Trials Methodology, Biostatistics and Health Informatics Department, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London
Independent	Dr Julie Nelson	PPI representative, SSNAP Family Care Team volunteer, Buckinghamshire
Non-independent	Associate Professor Charles Roehr	Chief investigator Consultant Neonatologist, Oxford University Hospitals NHS Foundation Trust, Neonatal Unit, Oxford.
		Associate Professor, Department of Paediatrics, University of Oxford, Oxford
		Clinical Director, NPEU, Nuffield Department of Population Health, Oxford
Non-independent	Professor Ed Juszczak	Professor of Clinical Trials and Statistics in Medicine at Nottingham Clinical Trials Unit, University of Nottingham, Nottingham

Appendix 2 Details of members of the Data Monitoring Committee

TABLE 26 Data Monitoring Committee members

Membership	Name	Affiliation
Chair; independent	Dr David Sweet	Consultant Neonatologist, Royal Jubilee Maternity Hospital, Belfast
Independent	Professor Kate Costeloe	Professor of Paediatric Research, Queen Mary University, London
Independent	Professor Siobhan Creanor	Professor of Medical Statistics and Clinical Trials, University of Plymouth, Plymouth

Appendix 3 Participating neonatal centres

BOX 8 Participating neonatal centres

NeoCLEAR trial recruiting sites

- Basingstoke and North Hampshire Hospital, Basingstoke, UK
- Birmingham Heartlands Hospital, Birmingham, UK
- Bradford Royal Infirmary, Bradford, UK
- Colchester General Hospital, Colchester, UK
- Derriford Hospital, Plymouth, UK
- Gloucestershire Royal Hospital, Gloucester, UK
- Great Western Hospital, Swindon, UK
- John Radcliffe Hospital, Oxford, UK
- Leicester Royal Infirmary, Leicester, UK
- Medway Maritime Hospital
- Norfolk and Norwich University Hospital, Gillingham, UK
- Northampton General Hospital, Northampton, UK
- Princess Anne Hospital, Southampton, UK
- Royal Berkshire Hospital, Reading, UK
- Royal Devon and Exeter Hospital, Exeter, UK
- Royal Hampshire County Hospital, Winchester, UK
- Royal Oldham Hospital, Oldham, UK
- Southmead Hospital, Bristol, UK
- St Michael's Hospital, Bristol, UK
- St Peter's Hospital, Chertsey, UK
- Stoke Mandeville Hospital, Aylesbury, UK

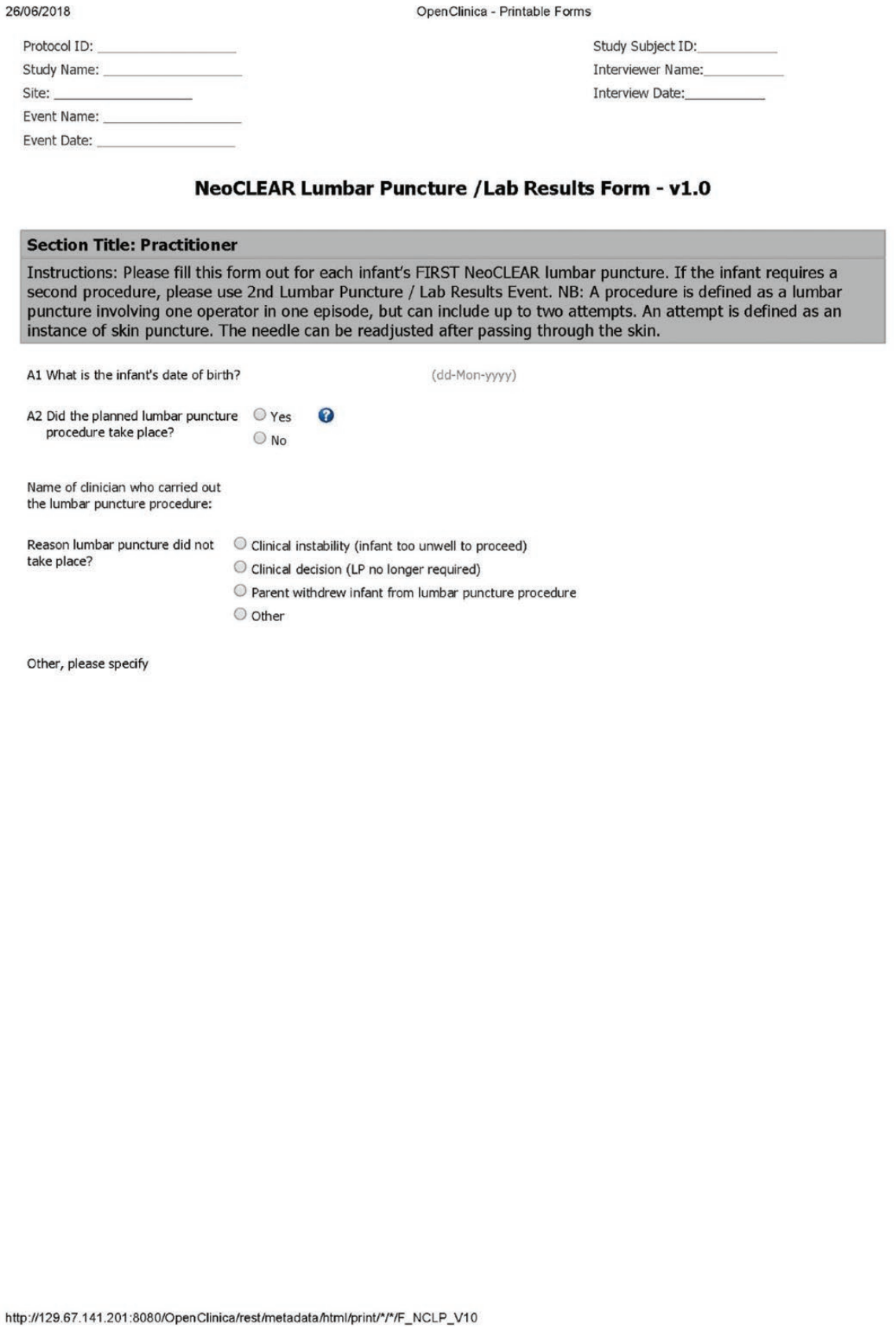
Appendix 4 Continuing care sites

BOX 9 Participating continuing care sites

NeoCLEAR trial continuing care sites

- Queen's Hospital, Burton upon Trent, UK.
- Royal Cornwall Hospital, Truro, UK.
- Royal Surrey County Hospital, Guildford, UK.
- William Harvey Hospital, Ashford, UK.
- The York Hospital, York, UK.

Appendix 5 Example electronic case report form



26/06/2018 OpenClinica - Printable Forms

Protocol ID: _____ Study Subject ID: _____
Study Name: _____ Interviewer Name: _____
Site: _____ Interview Date: _____
Event Name: _____
Event Date: _____

NeoCLEAR Lumbar Puncture /Lab Results Form - v1.0

Section Title: Practitioner

Instructions: Please fill this form out for each infant's FIRST NeoCLEAR lumbar puncture. If the infant requires a second procedure, please use 2nd Lumbar Puncture / Lab Results Event. NB: A procedure is defined as a lumbar puncture involving one operator in one episode, but can include up to two attempts. An attempt is defined as an instance of skin puncture. The needle can be readjusted after passing through the skin.

A1 What is the infant's date of birth? (dd-Mon-yyyy)

A2 Did the planned lumbar puncture procedure take place? ○ Yes ○ No

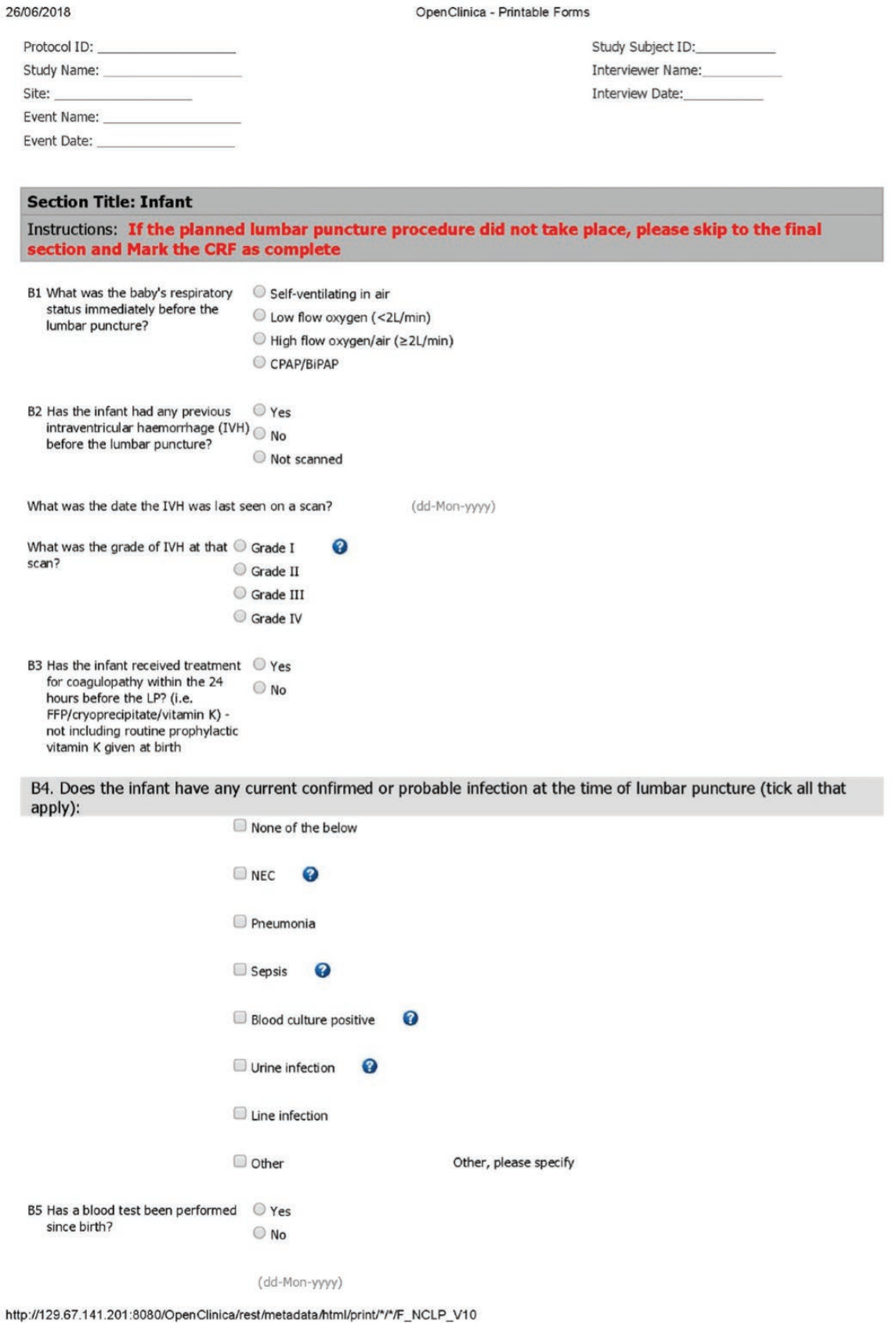
Name of clinician who carried out the lumbar puncture procedure:

Reason lumbar puncture did not take place?
○ Clinical instability (infant too unwell to proceed)
○ Clinical decision (LP no longer required)
○ Parent withdrew infant from lumbar puncture procedure
○ Other

Other, please specify

http://129.67.141.201:8080/OpenClinica/rest/metadata/html/print/*/*/F_NCLP_V10

FIGURE 6 Example eCRF. (*continued*)



26/06/2018 OpenClinica - Printable Forms

Protocol ID: _____ Study Subject ID:_____
Study Name: _____ Interviewer Name:_____
Site: _____ Interview Date:_____
Event Name: _____
Event Date: _____

Section Title: Infant

Instructions: **If the planned lumbar puncture procedure did not take place, please skip to the final section and Mark the CRF as complete**

B1 What was the baby's respiratory status immediately before the lumbar puncture?
- Self-ventilating in air
- Low flow oxygen (<2L/min)
- High flow oxygen/air (≥2L/min)
- CPAP/BiPAP

B2 Has the infant had any previous intraventricular haemorrhage (IVH) before the lumbar puncture?
- Yes
- No
- Not scanned

What was the date the IVH was last seen on a scan? (dd-Mon-yyyy)

What was the grade of IVH at that scan?
- Grade I
- Grade II
- Grade III
- Grade IV

B3 Has the infant received treatment for coagulopathy within the 24 hours before the LP? (i.e. FFP/cryoprecipitate/vitamin K) - not including routine prophylactic vitamin K given at birth
- Yes
- No

B4. Does the infant have any current confirmed or probable infection at the time of lumbar puncture (tick all that apply):
- None of the below
- NEC
- Pneumonia
- Sepsis
- Blood culture positive
- Urine infection
- Line infection
- Other — Other, please specify

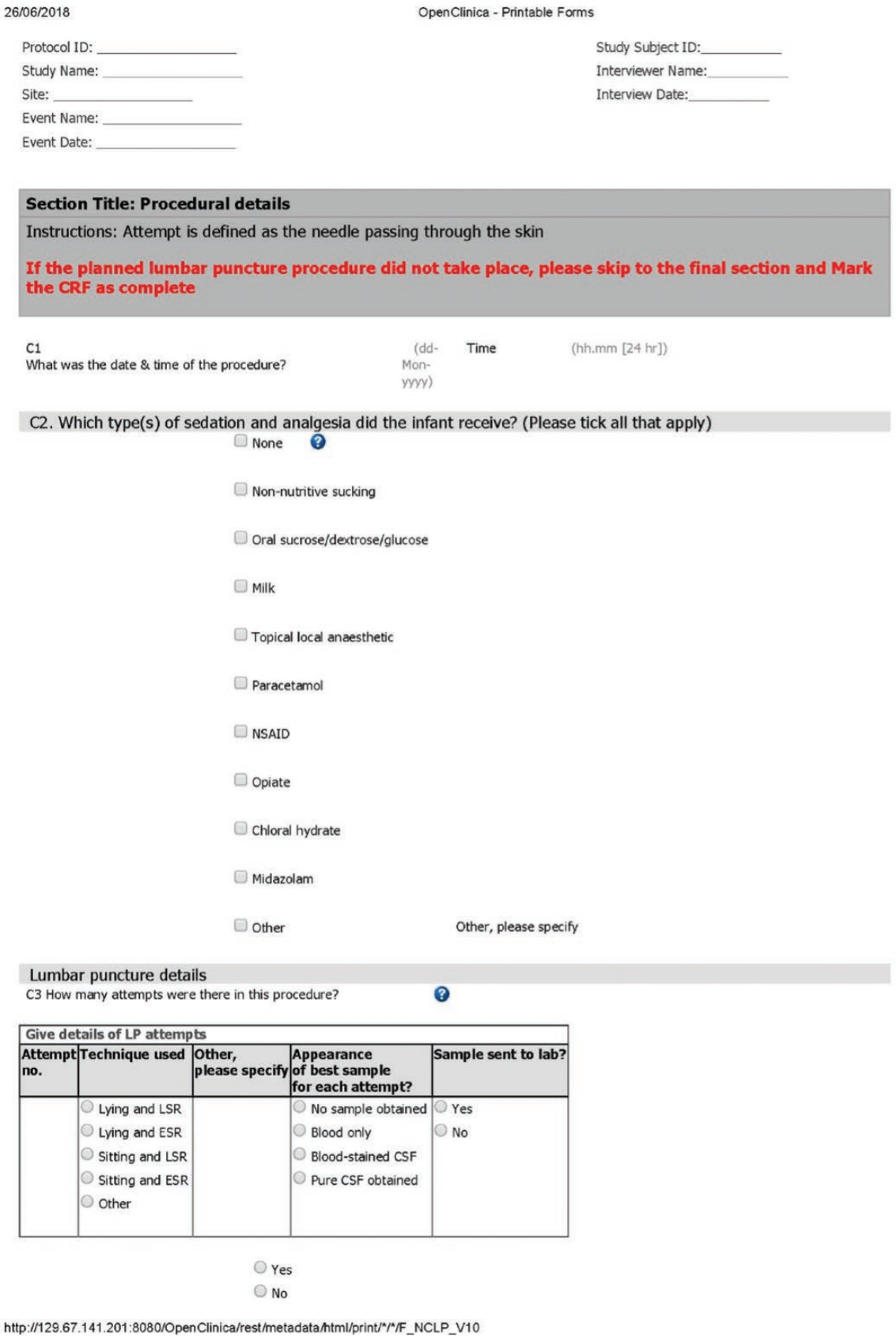
B5 Has a blood test been performed since birth?
- Yes
- No

(dd-Mon-yyyy)

http://129.67.141.201:8080/OpenClinica/rest/metadata/html/print/*/*/F_NCLP_V10

FIGURE 6 Example eCRF. (*continued*)

FIGURE 6 Example eCRF. (*continued*)



26/06/2018 OpenClinica - Printable Forms

Protocol ID: _____ Study Subject ID: _____
Study Name: _____ Interviewer Name: _____
Site: _____ Interview Date: _____
Event Name: _____
Event Date: _____

Section Title: Procedural details

Instructions: Attempt is defined as the needle passing through the skin

If the planned lumbar puncture procedure did not take place, please skip to the final section and Mark the CRF as complete

C1 What was the date & time of the procedure? (dd-Mon-yyyy) Time (hh.mm [24 hr])

C2. Which type(s) of sedation and analgesia did the infant receive? (Please tick all that apply)

- ☐ None
- ☐ Non-nutritive sucking
- ☐ Oral sucrose/dextrose/glucose
- ☐ Milk
- ☐ Topical local anaesthetic
- ☐ Paracetamol
- ☐ NSAID
- ☐ Opiate
- ☐ Chloral hydrate
- ☐ Midazolam
- ☐ Other — Other, please specify

Lumbar puncture details

C3 How many attempts were there in this procedure?

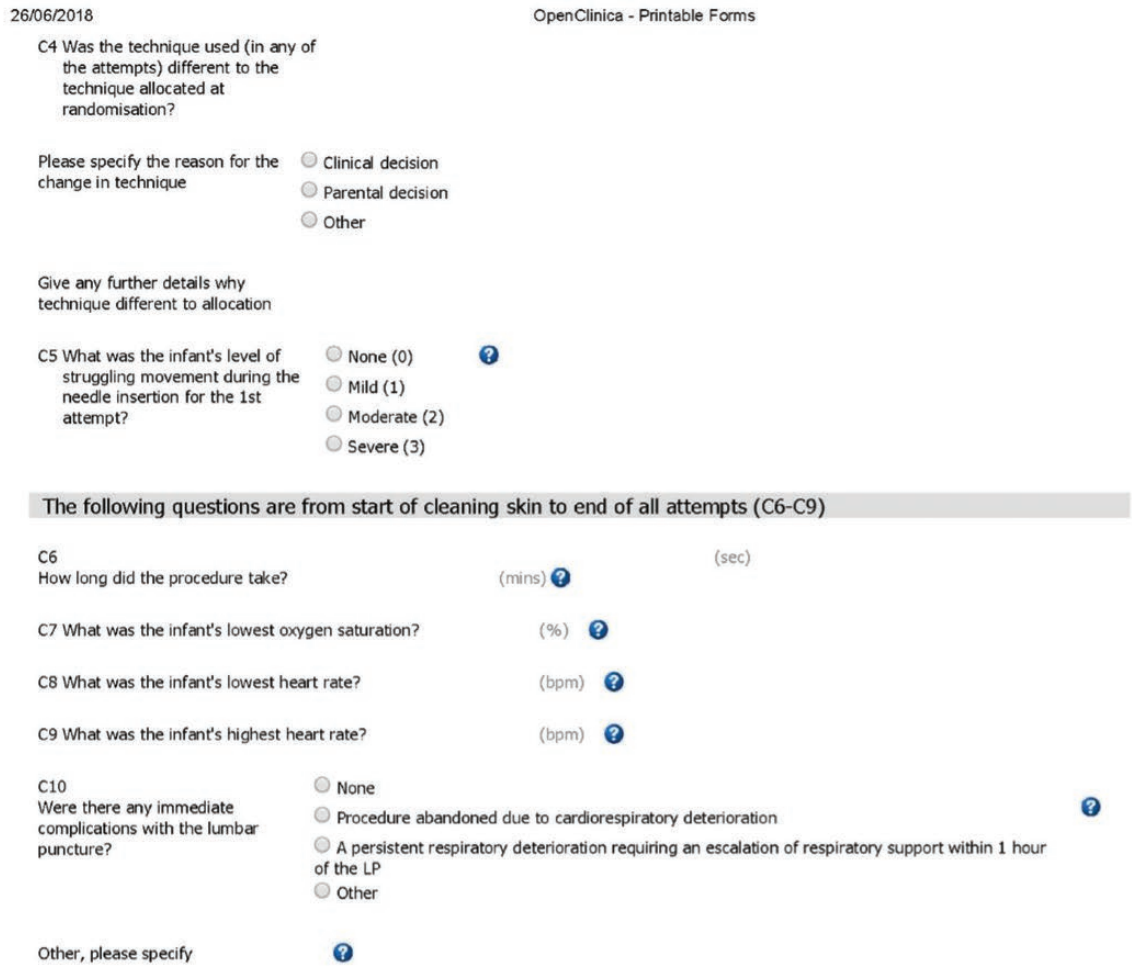
Give details of LP attempts

Attempt no.	Technique used	Other, please specify	Appearance of best sample for each attempt?	Sample sent to lab?
	○ Lying and LSR ○ Lying and ESR ○ Sitting and LSR ○ Sitting and ESR ○ Other		○ No sample obtained ○ Blood only ○ Blood-stained CSF ○ Pure CSF obtained	○ Yes ○ No

○ Yes
○ No

http://129.67.141.201:8080/OpenClinica/rest/metadata/html/print/*/*/F_NCLP_V10

FIGURE 6 Example eCRF. (*continued*)



26/06/2018 OpenClinica - Printable Forms

C4 Was the technique used (in any of the attempts) different to the technique allocated at randomisation?

Please specify the reason for the change in technique
- Clinical decision
- Parental decision
- Other

Give any further details why technique different to allocation

C5 What was the infant's level of struggling movement during the needle insertion for the 1st attempt?
- None (0)
- Mild (1)
- Moderate (2)
- Severe (3)

The following questions are from start of cleaning skin to end of all attempts (C6-C9)

C6
How long did the procedure take? (mins) (sec)

C7 What was the infant's lowest oxygen saturation? (%)

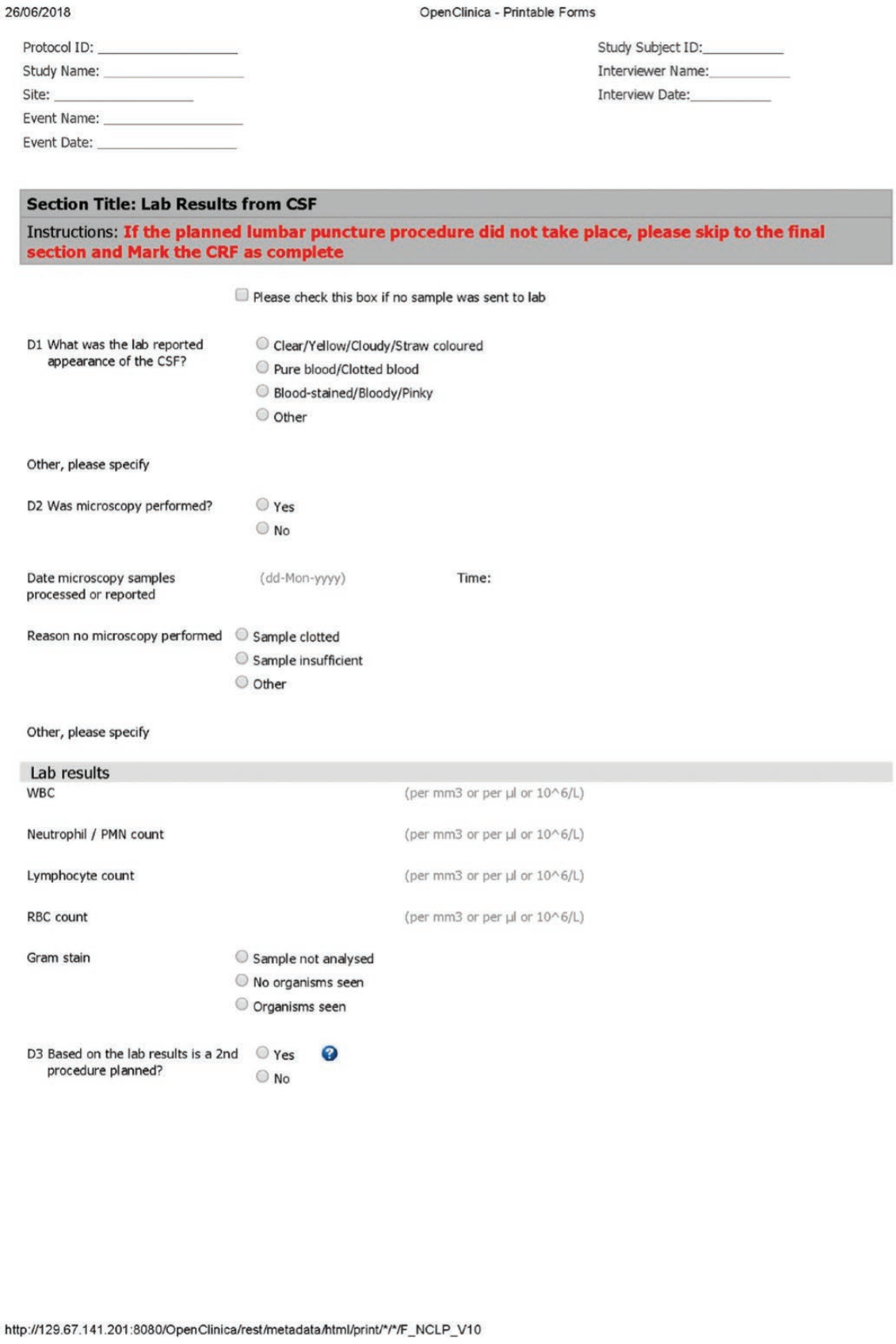
C8 What was the infant's lowest heart rate? (bpm)

C9 What was the infant's highest heart rate? (bpm)

C10
Were there any immediate complications with the lumbar puncture?
- None
- Procedure abandoned due to cardiorespiratory deterioration
- A persistent respiratory deterioration requiring an escalation of respiratory support within 1 hour of the LP
- Other

Other, please specify

FIGURE 6 Example eCRF. (*continued*)



26/06/2018 OpenClinica - Printable Forms

Protocol ID: _____ Study Subject ID: _____
Study Name: _____ Interviewer Name: _____
Site: _____ Interview Date: _____
Event Name: _____
Event Date: _____

Section Title: Lab Results from CSF

Instructions: **If the planned lumbar puncture procedure did not take place, please skip to the final section and Mark the CRF as complete**

☐ Please check this box if no sample was sent to lab

D1 What was the lab reported appearance of the CSF?
- ○ Clear/Yellow/Cloudy/Straw coloured
- ○ Pure blood/Clotted blood
- ○ Blood-stained/Bloody/Pinky
- ○ Other

Other, please specify

D2 Was microscopy performed?
- ○ Yes
- ○ No

Date microscopy samples processed or reported (dd-Mon-yyyy) Time:

Reason no microscopy performed
- ○ Sample clotted
- ○ Sample insufficient
- ○ Other

Other, please specify

Lab results

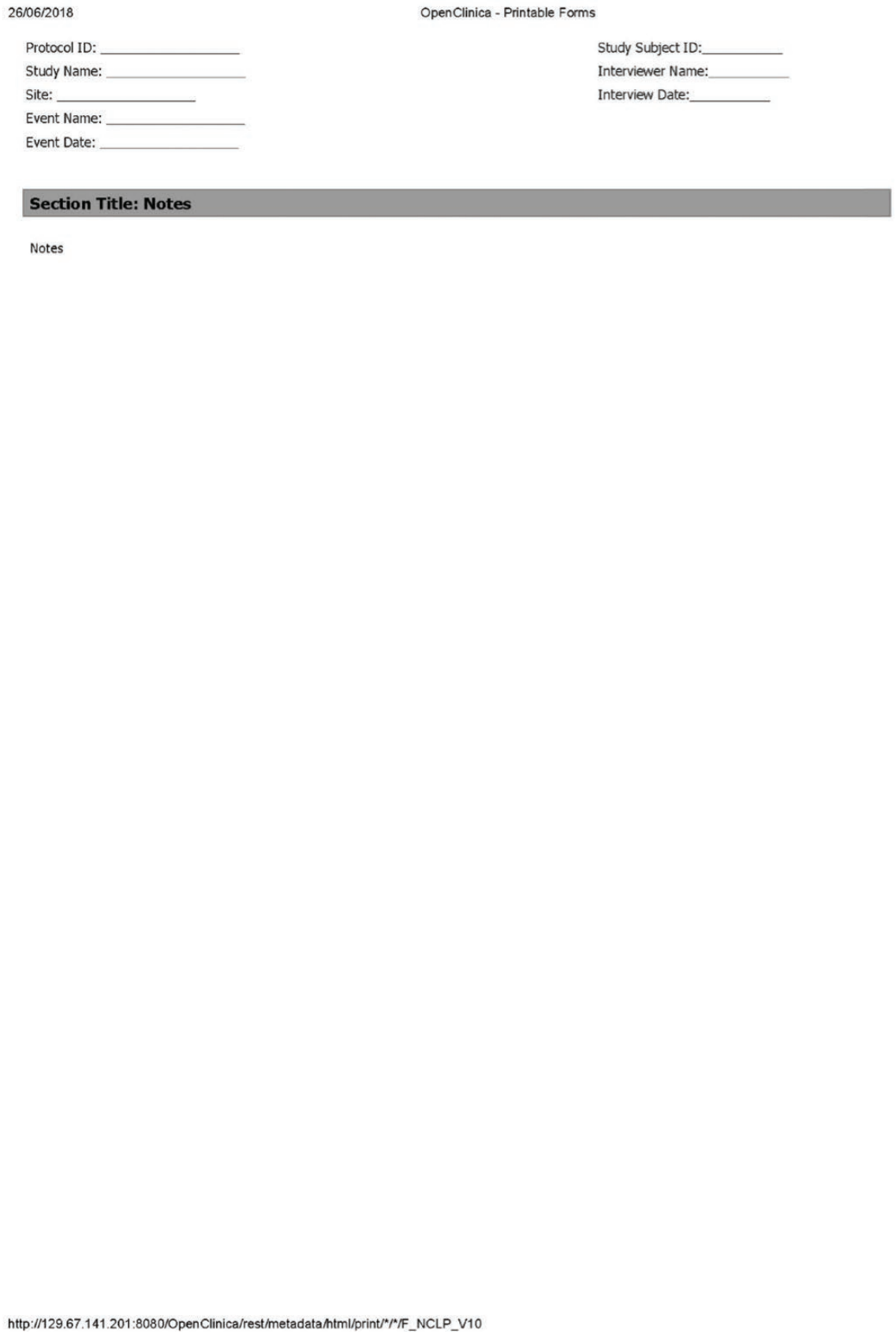
WBC	(per mm3 or per µl or 10^6/L)
Neutrophil / PMN count	(per mm3 or per µl or 10^6/L)
Lymphocyte count	(per mm3 or per µl or 10^6/L)
RBC count	(per mm3 or per µl or 10^6/L)

Gram stain
- ○ Sample not analysed
- ○ No organisms seen
- ○ Organisms seen

D3 Based on the lab results is a 2nd procedure planned?
- ○ Yes
- ○ No

http://129.67.141.201:8080/OpenClinica/rest/metadata/html/print/*/*/F_NCLP_V10

FIGURE 6 Example eCRF. (*continued*)


26/06/2018 OpenClinica - Printable Forms

Protocol ID: _____
Study Name: _____
Site: _____
Event Name: _____
Event Date: _____

Study Subject ID:_____
Interviewer Name:_____
Interview Date:_____

Section Title: Notes

Notes

http://129.67.141.201:8080/OpenClinica/rest/metadata/html/print/*/*/F_NCLP_V10

FIGURE 6 Example eCRF.

Appendix 6 Data collection forms

TABLE 27 Participating neonatal centres

Data collection form	Description
Entry form (eCRF)	Completed before and during randomisation, collecting data on baseline characteristics and eligibility/exclusion criteria
LP/laboratory results form (eCRF)	Completed for each infant's first NeoCLEAR LP
Second LP/laboratory results form (eCRF)	Used to collect details about second LP procedures only
Outcome form (eCRF)	Collection of outcome data. Completed for each infant at discharge or after death
Transfer/discharge/death form (eCRF)	Completed when the infant is transferred to another hospital, is discharged home or if the infant died
Withdrawal form (eCRF)	Completed when a parent or clinician decided to withdraw an infant from the trial
SAE report form	Completed for all reportable SAEs, as defined in the trial protocol
Incident and deviation	Completed in the event of any deviation from the trial protocol, other trial-specific procedures, good clinical practice or other regulations and legislation
Parent (STAI-S) questionnaire	Collected for the pilot phase: parental anxiety (STAI-S questionnaire)

Copies of NeoCLEAR data collection forms are available at URL: www.npeu.ox.ac.uk/neoclear/clinicians/data-collection-forms (accessed 16 June 2022).

Appendix 7 Summary of changes to the study protocol

TABLE 28 Changes to the study protocol

Amendment	Date of REC favourable opinion	Document name	Description
Amend 1: AM01	N/A	First Lumbar Puncture/ Lab Results Log Version 1.0, 17-Sep-2018	• To replace First Lumbar Puncture Form Version 3.0, dated 10 July 2018
Amend 1: AM01	N/A	Second Lumbar Puncture/ Lab Results Log Version 1.0, 17-Sep-2018	• To replace Second Lumbar Puncture Form Version 3.0, dated 10 July 2018
Amend 2: AM02	N/A	Guidance Sheet 1: Eligibility V2 05NOV2018	• To replace Guidance Sheet 1: Eligibility V1 25JUN2018
Amend 2: AM02	N/A	Guidance Sheet 2: Parental Consent	• To replace Guidance Sheet 2: Parental Consent V1 25JUN2018
Amend 2: AM02	N/A	Guidance Sheet 4: LP Procedure	• To replace Guidance Sheet 4: LP Procedure V1 25JUN2018
Amend 2: AM02	N/A	Guidance Sheet 5: Documentation	• To replace Guidance Sheet 5: Documentation V1 25JUN2018
Amend 2: AM02	N/A	Guidance Sheet 8: Withdrawal	• To replace Guidance Sheet 8: Withdrawal V1 25JUN2018
Amend 2: AM02	N/A	Consent Checklist	• To replace Consent Checklist V1 25JUN2018
Amend 3: AM03	N/A	Outcomes form – NCOT V2.0	• To replace V1 of the Outcomes form 03JAN2019
Amend 4: AM04	10 January 2019	Parent Poster V1 28/11/2018	• For display in patient-facing areas of recruiting sites to introduce the study to parents of potential participants. Viewing of the poster is not required for consent, which will continue to use the current approved parent information leaflet and consent form
Amend 4: AM04	10 January 2019	Cot Cards 28/11/2018	• For display on the participant's cot to aid the participant's clinical care team by informing or reminding them of their patient's participation in the NeoCLEAR trial
Amend 4: AM04	N/A	Consent Form Guide V1 23/01/2019	• Consent form guide printed onto clipboards for clinicians to use as prompt to ensure good clinical practice-compliant completion of consent form
Amend 5: AM05	4 July 2019	Consent Form V2.0	• To replace Consent Form V1.0, removing sections 6 and 7, which related to parent questionnaire, and amending section 3 to enable fathers/partners with legal parental responsibility to consent to their child's participation in the NeoCLEAR trial

continued

TABLE 28 Changes to the study protocol (*continued*)

Amendment	Date of REC favourable opinion	Document name	Description
Amend 5: AM05	4 July 2019	Parent Information Leaflet V3.0	• To replace PIL V2.0, removing reference to parent questionnaire
Amend 5: AM05	4 July 2019	Protocol V4.0	• To replace Protocol V3.0, clarifying discontinuation of parent questionnaire post pilot and correcting planned end of recruitment date to 29 February 2020
Amend 5: AM05	N/A	Guidance Sheet 2: Parental Consent V3.0	• To replace Guidance Sheet 2 V2.0, removing reference to parent questionnaire and clarifying who can legally give consent to their child's participation in the NeoCLEAR trial
Amend 5: AM05	N/A	Guidance Sheet 4: LP Procedure V3.0	• To replace Guidance Sheet 4 V2.0, removing reference to parent questionnaire
Amend 5: AM05	N/A	Guidance Sheet 5: Documentation V3.0	• To replace Guidance Sheet 5 V2.0, removing reference to parent questionnaire
Amend 5: AM05	N/A	Patient Flowchart V3.0	• To replace Patient Flowchart V2.0, removing reference to parent questionnaire and clarifying process for rare cases when first procedure is successful but a second procedure is still required
Amend 5: AM05	N/A	Consent Checklist V3.0	• To replace Consent Checklist V2.0, removing reference to parent questionnaire
Amend 6: AM06	13 January 2019	Protocol V6.0	• Replaces Protocol V4.0, with changes made to contents list and study flow chart; synopsis (clarification of wording within secondary objectives and outcome measures); glossary/definitions for objectives and outcome measures; study procedures; statistics and analysis; and references
Amend 6: AM06	13 January 2019	NeoCLEAR Parent and Family Certificate V1.0	• Parent and Family Certificate to hand to parents following consent to participate, providing a positive keepsake and information about where to access trial results
Amend 6: AM06	N/A	Training Poster	• For use in staff rooms to advertise upcoming NeoCLEAR trial training sessions
Amend 6: AM06	N/A	Version Control V9.0	• For site file
Amend 7	N/A	N/A	• Site specific: addition of recruiting site (Buckingham Healthcare NHS Trust)
Amend 8	N/A	N/A	• Site specific to Basingstoke and North Hampshire Hospital: addition of co-investigator at Basingstoke and North Hampshire Hospital
Amend 9	N/A	N/A	• Temporary halt to recruitment to the NeoCLEAR trial due to COVID-19, until further notice
Amend 10	N/A	N/A	• Following the previous temporary halt to recruitment due to COVID-19, re-opening of selected sites, working with research and development departments, PIs and site staff to ensure appropriate safety and capacity

TABLE 28 Changes to the study protocol (*continued*)

Amendment	Date of REC favourable opinion	Document name	Description
Amend 11	N/A	N/A	• Oxford University Hospitals NHS Foundation Trust specific: in cases where COVID-19-related visitor policies result in parents/ guardians not being physically present in the hospital until after their infant's LP is undertaken, study introduction and consent to be completed via telephone and documented in notes, and physical consent form to be signed next time the parent can visit the hospital in person • Category C exemption due to COVID-19
Amend 12	N/A	N/A	• In cases where COVID-19-related visitor policies result in parents/guardians not being physically present in the hospital until after their infant's LP is undertaken, study introduction and consent to be completed via telephone and documented in notes, and physical consent form to be signed next time the parent can visit the hospital in person • Category C exemption due to COVID-19
Amend 13	N/A	NeoCLEAR Protocol V7.0 13JUL2020	• Recruitment period and overall study award period extended. Updates throughout to reflect target recruitment of 1020 infants as minimum rather than absolute total

N/A, not applicable; REC, Research Ethics Committee.

Appendix 8 Group allocation per recruiting site

TABLE 29 Group allocation per recruiting site

Recruiting centre	Group			
	Sitting plus ESR (*N* = 273), *n* (%)	Sitting plus LSR (*N* = 270), *n* (%)	Lying plus ESR (*N* = 272), *n* (%)	Lying plus LSR (*N* = 261), *n* (%)
Birmingham Heartlands Hospital	8 (2.9)	8 (3.0)	7 (2.6)	7 (2.7)
Royal Berkshire Hospital	5 (1.8)	7 (2.6)	6 (2.2)	6 (2.3)
John Radcliffe Hospital	41 (15.0)	40 (14.8)	42 (15.4)	40 (15.3)
Bradford Royal Infirmary	11 (4.0)	10 (3.7)	8 (2.9)	9 (3.4)
Colchester General Hospital	5 (1.8)	6 (2.2)	7 (2.6)	7 (2.7)
Derriford Hospital	9 (3.3)	6 (2.2)	7 (2.6)	6 (2.3)
Gloucestershire Royal Hospital	7 (2.6)	6 (2.2)	6 (2.2)	6 (2.3)
Great Western Hospital	4 (1.5)	4 (1.5)	6 (2.2)	6 (2.3)
Leicester Royal Infirmary	29 (10.6)	30 (11.1)	31 (11.4)	27 (10.3)
Medway Maritime Hospital	32 (11.7)	31 (11.5)	31 (11.4)	32 (12.3)
Norfolk and Norwich University Hospital	24 (8.8)	25 (9.3)	23 (8.5)	25 (9.6)
Northampton General Hospital	18 (6.6)	18 (6.7)	20 (7.4)	18 (6.9)
Princess Anne Hospital	10 (3.7)	10 (3.7)	8 (2.9)	8 (3.1)
Royal Devon and Exeter Hospital	6 (2.2)	7 (2.6)	8 (2.9)	6 (2.3)
Royal Hampshire County Hospital	3 (1.1)	3 (1.1)	3 (1.1)	2 (0.8)
Royal Oldham Hospital	12 (4.4)	12 (4.4)	12 (4.4)	10 (3.8)
Southmead Hospital	28 (10.3)	25 (9.3)	28 (10.3)	27 (10.3)
St Michael's Hospital	10 (3.7)	11 (4.1)	12 (4.4)	10 (3.8)
St Peter's Hospital	3 (1.1)	4 (1.5)	3 (1.1)	4 (1.5)
Stoke Mandeville Hospital	6 (2.2)	6 (2.2)	4 (1.5)	4 (1.5)
Basingstoke and North Hampshire Hospital	2 (0.7)	1 (0.4)	0	1 (0.4)

Appendix 9 Post hoc exploratory analyses

TABLE 30 Baseline characteristics by non-adherence to position allocation (any attempt in first LP)

Characteristic	Non-adherence (*N* = 51)	Adherence (*N* = 1024)
CGA at trial entry (weeks^{+days}), *n* (%)		
27^{+0} to 31^{+6}	0	22 (2.1)
32^{+0} to 36^{+6}	4 (7.8)	89 (8.7)
37^{+0} to 40^{+6}	27 (52.9)	566 (55.3)
≥ 41^{+0}	20 (39.2)	347 (33.9)
Median (IQR)	41 (39–41)	40 (39–41)
Gestational age at birth (weeks^{+days}), *n* (%)		
< 27^{+0}	0	7 (0.7)
27^{+0} to 31^{+6}	1 (2.0)	31 (3.0)
32^{+0} to 36^{+6}	3 (5.9)	84 (8.2)
37^{+0} to 40^{+6}	33 (64.7)	617 (60.3)
≥ 41^{+0}	14 (27.5)	285 (27.8)
Median (IQR)	41 (39–41)	40 (39–41)
Age (days)		
Median (IQR)	2 (1–2)	1 (1–2)
≥ 3 days, *n* (%)	6 (11.8)	133 (13.0)
Birthweight (g)		
Median (IQR)	3646 (3200–4095)	3510 (3130–3890)
Missing, *n*	0	1
Working weight (g) at trial entry, *n* (%)		
1000–2499	4 (7.8)	101 (9.9)
2500–3500	20 (39.2)	404 (39.5)
≥ 3501	27 (52.9)	519 (50.7)
Median (IQR)	3646 (3200–4095)	3510 (3133–3890)

TABLE 31 Baseline characteristics by non-adherence to stylet removal allocation (any attempt in first LP)

Characteristic	Non-adherence (*N* = 51)	Adherence (*N* = 1024)
CGA at trial entry (weeks^{+days}), *n* (%)		
27^{+0} to 31^{+6}	0	22 (2.1)
32^{+0} to 36^{+6}	2 (5.7)	91 (8.8)
37^{+0} to 40^{+6}	21 (60.0)	570 (54.9)
≥ 41^{+0}	12 (34.3)	355 (34.2)
Median (IQR)	41 (39–41)	40 (39–41)
Gestational age at birth (weeks^{+days}), *n* (%)		
< 27^{+0}	0	7 (0.7)
27^{+0} to 31^{+6}	1 (2.9)	31 (3.0)
32^{+0} to 36^{+6}	1 (2.9)	86 (8.3)
37^{+0} to 40^{+6}	24 (68.6)	624 (60.1)
≥ 41^{+0}	9 (25.7)	290 (27.9)
Median (IQR)	40 (39–41)	41 (39–41)
Age (days)		
Median (IQR)	1 (1–2)	1 (1–2)
≥ 3 days, n (%)	2 (5.7)	137 (13.2)
Birthweight (g)		
Median (IQR)	3695 (3214–4100)	3510 (3120–3900)
Missing, *n*	0	1
Working weight (g) at trial entry, *n* (%)		
1000–2499	2 (5.7)	103 (9.9)
2500–3500	12 (34.3)	412 (39.7)
≥ 3501	21 (60.0)	523 (50.4)
Median (IQR)	3695 (3214–4100)	3510 (3130–3900)

TABLE 32 Characteristics at baseline/first LP by age at randomisation

Characteristic	Age at randomisation	
	≥ 3 days (*N* = 140)	< 3 days (*N* = 936)
CGA at trial entry (weeks^{+days}), *n* (%)		
27^{+0} to 31^{+6}	14 (10.0)	8 (0.9)
32^{+0} to 36^{+6}	26 (18.6)	67 (7.2)
37^{+0} to 40^{+6}	65 (46.4)	529 (56.5)
≥ 41^{+0}	35 (25.0)	332 (35.5)
Median (IQR)	40 (36–41)	40 (39–41)
Gestational age at birth (weeks^{+days}), *n* (%)		
< 27^{+0}	7 (5.0)	0
27^{+0} to 31^{+6}	23 (16.4)	9 (0.1)
32^{+0} to 36^{+6}	17 (12.1)	70 (7.5)
37^{+0} to 40^{+6}	74 (52.9)	577 (61.6)
≥ 41^{+0}	19 (13.6)	280 (29.9)
Median (IQR)	39 (34–40)	40 (39–41)
Birthweight (g)		
Median (IQR)	3200 (19653645)	3549 (3214–3920)
Missing, *n*	1	0
Working weight (g) at trial entry, *n* (%)		
1000–2499	39 (27.9)	66 (7.1)
2500–3500	53 (37.9)	317 (39.6)
≥ 3501	48 (34.3)	499 (53.3)
Median (IQR)	3178 (2170–3643)	3549 (3215–3920)
Respiratory status immediately before LP, *n* (%)		
Self-ventilating in air	93 (66.4)	821 (87.7)
Low flow oxygen (< 2 l/minute)	6 (4.3)	23 (2.5)
High flow oxygen (≥ 2 l/minute)	34 (24.3)	82 (8.8)
CPAP/BiPAP	7 (5.0)	10 (1.1)

BiPAP, bilevel positive airway pressure; CPAP, continuous positive airway pressure.

TABLE 33 Infant's lowest SpO_2 < 80% at first LP by position allocation

CGA at trial entry (weeks^{+days})	Position	
	Sitting (*N* = 543)	Lying (*N* = 533)
27^{+0} to 36^{+6}, *n*/*N* (%)	4/57 (7.0)	13/57 (22.8)
Median lowest SpO_2 (%) (IQR)	93 (89–96)	87 (82–92)
≥ 37^{+0}, *n*/*N* (%)	31/475 (6.5)	59/451 (13.1)
Median lowest SpO_2 (%) (IQR)	93 (89–96)	90 (85–94)

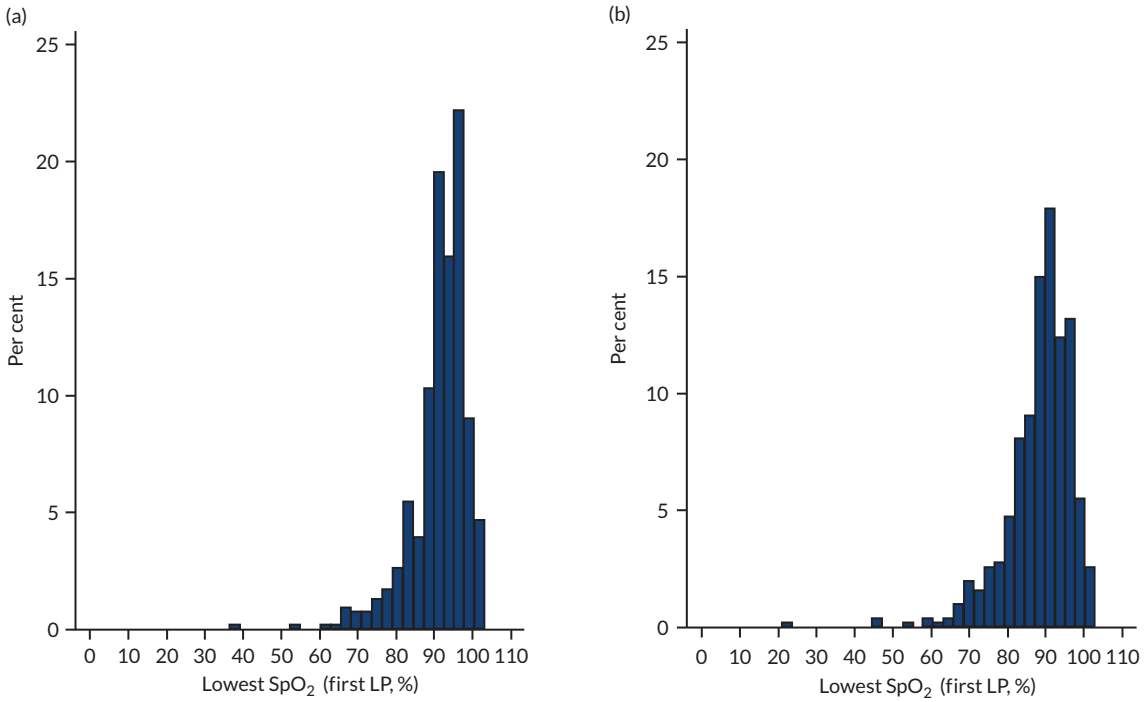


FIGURE 7 Infant's lowest SpO_2 (%) at first LP by position allocation. (a) Sitting; and (b) lying. The lowest SpO_2 during the first procedure was < 80% in 35 of 532 (6.6%) and 72 of 508 (14.2%) infants in the sitting and lying arms, respectively.

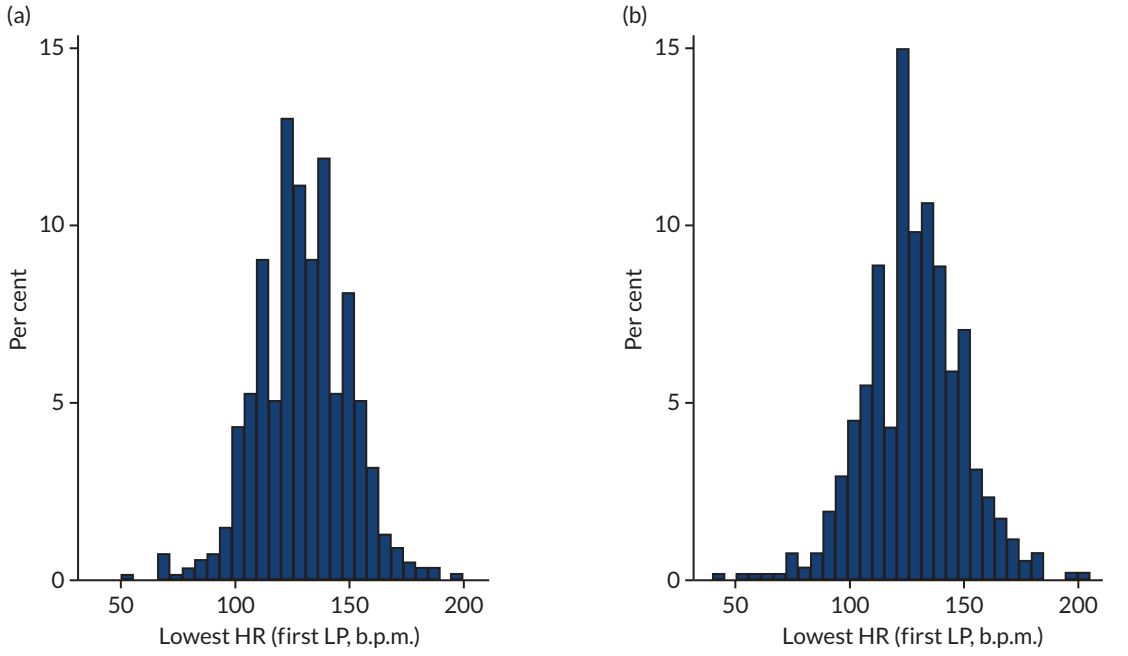


FIGURE 8 Infant's lowest HR (b.p.m.) at first LP by position allocation. (a) Sitting; and (b) lying. The lowest HR during the first procedure was < 100 b.p.m. in 26 of 523 (5.0%) and 44 of 501 (8.8%) infants in the sitting and lying arms, respectively.

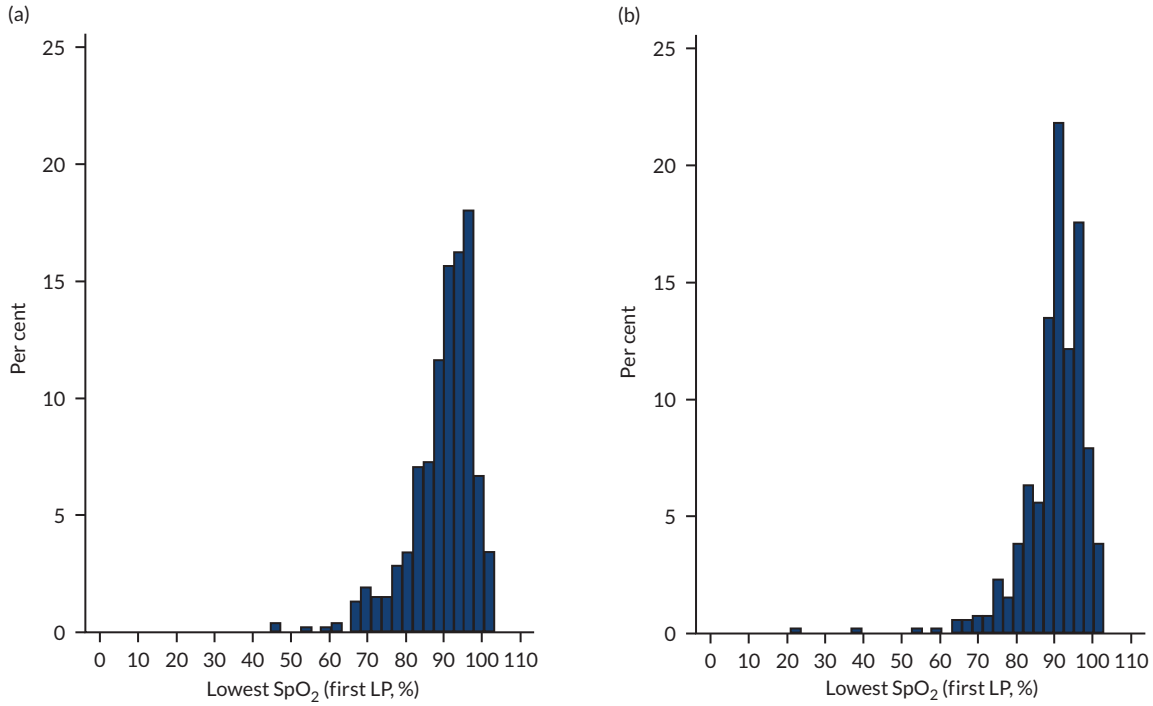


FIGURE 9 Infant's lowest SpO_2 (%) at first LP by stylet removal allocation. (a) ESR; and (b) LSR. The lowest SpO_2 during the first procedure was < 80% in 61 of 522 (11.7%) and 46 of 518 (8.9%) infants in the ESR and LSR removal arms, respectively.

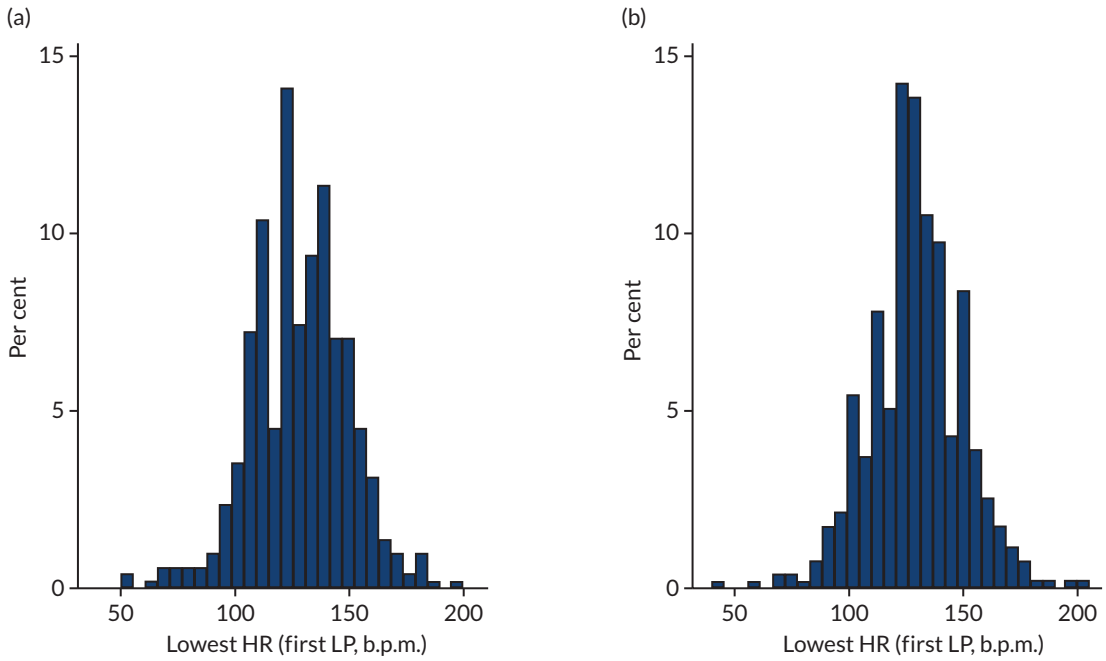


FIGURE 10 Infant's lowest HR (b.p.m.) at first LP by stylet removal allocation. (a) ESR; and (b) LSR. The lowest HR during the first procedure was < 100 b.p.m. in 35 of 511 (6.8%) and 35 of 513 (6.8%) infants in the ESR and LSR removal arms, respectively.

EME
HSDR
HTA
PGfAR
PHR

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