Assessment of infant position and timing of stylet removal to improve lumbar puncture success in neonates (NeoCLEAR): an open-label, 2 × 2 factorial, randomised, controlled trial

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Summary

Background Newborn infants are the highest-risk age group for bacterial meningitis. Lumbar punctures are therefore frequently performed in neonates, but success rates are low (50–60%). In Neonatal Champagne Lumbar punctures Every time–A Randomised Controlled Trial (NeoCLEAR), we sought to optimise infant lumbar puncture by evaluating two modifications to traditional technique: sitting position versus lying down and early stylet removal (stylet removal after transecting the subcutaneous tissue) versus late stylet removal.

Methods NeoCLEAR was an open-label, 2×2 factorial, randomised, controlled trial, conducted in 21 UK neonatal and maternity units. Infants requiring lumbar puncture at 27⁺⁰ to 44⁺⁰ weeks corrected gestational age and weighing 1000 g or more were randomly assigned (1:1:1:1) to sitting position and early stylet removal, sitting position and late stylet removal, lying position and early stylet removal, or lying position and late stylet removal using a 24/7, web-based, secure, central randomisation system. Block randomisation was stratified within site by corrected gestational age (27⁺⁰ to 31⁺⁶ weeks, 32⁺⁰ to 36⁺⁶ weeks, 37⁺⁰ to 40⁺⁶ weeks, or 41⁺⁰ to 44⁺⁰ weeks), using variable block sizes of four and eight with equal frequency. Laboratory staff were masked to allocation. The primary outcome was successful first lumbar puncture, defined as obtaining a cerebrospinal fluid sample with a red blood cell count of less than 10 000 cells per µL. The primary and secondary (including safety) outcomes were analysed by the groups to which infants were assigned regardless of deviation from the protocol or allocation received, but with exclusion of infants who were withdrawn before data collection or who did not undergo lumbar puncture (modified intention-to-treat analysis). This study is registered with ISRCTN, ISRCTN14040914.

Findings Between Aug 3, 2018, and Aug 31, 2020, 1082 infants were randomly assigned to sitting (n=546) or lying (n=536), and early (n=549) or late (n=533) stylet removal. 1076 infants were followed-up until discharge and included in the modified intention-to-treat analysis. 961 (89%) infants were term, and 936 (87%) were younger than 3 days. Successful first lumbar puncture was more frequently observed in sitting than in lying position (346 [63·7%] of 543 *vs* 307 [57·6%] of 533; adjusted risk ratio 1·10 [95% CI 1·01 to 1·21], p=0·029; number needed to treat=16). Timing of stylet removal had no discernible effect on the primary outcome (338 [62·0%] of 545 infants in the early stylet removal group and 315 [59·3%] of 531 in the late stylet removal group had a successful first lumbar puncture; adjusted risk ratio 1·04 [95% CI 0·94–1·15], p=0·45). Sitting was associated with fewer desaturations than was lying (median lowest oxygen saturations during first lumbar puncture 93% [IQR 89–96] *vs* 90% [85–94]; median difference 3·0% [2·1–3·9], p<0·0001). One infant from the sitting plus late stylet removal group developed a scrotal haematoma 2 days after lumbar puncture, which was deemed to be possibly related to lumbar puncture.

Interpretation NeoCLEAR is the largest trial investigating paediatric lumbar puncture so far. Success rates were improved when sitting rather than lying. Sitting lumbar puncture is safe, cost neutral, and well tolerated. We predominantly recruited term neonates younger than 3 days; other populations warrant further study. Neonatal lumbar puncture is commonly performed worldwide; these results therefore strongly support the widespread adoption of sitting technique for neonatal lumbar puncture.

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Introduction

The neonatal period carries the highest risk of bacterial meningitis (about 0·3 per 1000 births), which is associated with substantial mortality (about a 10% case-fatality rate) and neurological morbidity (20–50%).[1–3] Meningitis is diagnosed by analysis of cerebrospinal fluid (CSF), obtained via lumbar puncture. Lumbar punctures are frequently performed in infants because of the non-specific features of meningitis in this age group.[4] However, published success rates for neonatal lumbar puncture are only 50–60%,[5,6] compared with 78–87% in older children.[7,8] Unsuccessful lumbar punctures include

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Research in context

Evidence before this study

The technique used for infant lumbar puncture has remained essentially unchanged since its first description in 1891. Published success rates for neonatal lumbar puncture are low at 50–60%. We searched for studies describing interventions or modifiable factors that had the potential to improve lumbar puncture success rates using MEDLINE and Embase from inception to Aug 1, 2017, using the terms "lumbar puncture" AND ("sitting" OR "stylet") AND ("neonat*" OR "newborn*" OR "infant*"). Spinal ultrasound studies conducted in neonates held in different positions show that sitting position might have anatomical advantages over lying position. However, success rates for lumbar puncture were not consistently higher for infants held in sitting position (than with lying down) in observational studies or in one small randomised controlled trial. The technique early stylet removal, first described in case series in 1971, aims to reduce the chance of a blood-stained sample by avoiding passing the needle tip into the veins beyond the subarachnoid space. This technique was reported to be associated with increased lumbar puncture success in two observational studies. However, these observational studies (and those describing sitting versus lying position) were all retrospective, with significant risk of bias due to confounding factors that might affect whether sitting versus lying position and early versus late stylet removal was chosen, such as operator experience, or infant size or age. There were no safety concerns with sitting position or early stylet removal in previous studies.

Added value of this study

Neonatal Champagne Lumbar punctures Every time–A Randomised Controlled Trial (NeoCLEAR) is the first adequately powered randomised controlled trial investigating methods to improve lumbar puncture success in the paediatric population, having recruited 1082 infants from 21 UK centres. A 2 × 2 factorial design allowed simultaneous comparison of sitting versus lying position, and early versus late stylet removal. Our results demonstrate clear evidence of benefit for sitting position: babies allocated to sitting had significantly higher rates of successful lumbar puncture than those allocated to lying position, with a number needed to treat of 16. Sitting was also associated with better cardiorespiratory stability (fewer with oxygen desaturations and bradycardias), and less infant struggling. There was no evidence of a significant difference between early and late stylet removal for any outcomes.

Implications of all the available evidence

For newborn infants requiring lumbar puncture, sitting position has now been demonstrated to be more successful in obtaining an interpretable cerebrospinal fluid sample, compared with lying position. Furthermore, sitting lumbar puncture appears to be safer, and better tolerated. This is a cost-neutral intervention, which practitioners can learn with brief training. Since neonatal lumbar puncture is an essential procedure globally, the results of this trial should be disseminated widely, and sitting position should be adopted as the standard neonatal practice. Our results also indicate that robust trials comparing different lumbar puncture techniques are warranted in older infants and children, and in less mature neonates, to improve lumbar puncture success rates further.

those with heavily blood-stained CSF or failure to obtain any CSF.[9] These scenarios often lead to repeated attempts or cautious management with prolonged courses of intravenous antimicrobial agents—often requiring 14–21 days of inpatient care—because the clinical team cannot exclude a diagnosis of meningitis.[9,10] Extended-spectrum antibiotic use is associated with a range of complications in infants, including antimicrobial resistance.[11,12] Consequently, interventions to improve infant lumbar puncture success rates should allow more accurate diagnosis of meningitis, prevent repeated lumbar punctures, and reduce unnecessary antibiotic use and hospitalisation, saving resources.[9,11]

Despite persistently low success rates for neonatal lumbar puncture, there has been little modification to the traditional lumbar puncture technique, pioneered by Quincke in 1891.[13,14] Modifications investigated so far include sitting position[15–20] and early stylet removal.[7,8] Sitting has anatomical advantages of an increased interspinous distance[17,18,21] and width of the CSF space.[17,22] However, evidence regarding its effect on lumbar puncture success has been inconclusive.[15,18–20] The only published systematic review concluded: "A large-scale prospective clinical trial directly addressing LP [lumbar puncture] success and safety in different positions would clarify the need to change current practice".[23]

Early stylet removal involves stylet removal after transecting the skin and subcutaneous tissue, before slowly advancing the needle tip into the CSF. In infants, a loss of resistance on entering the CSF is often indistinguishable, and a blood-stained sample might be obtained if the needle inadvertently punctures the anterior internal vertebral venous plexus, which lies beyond the subarachnoid space, impairing CSF interpretation. For infants in an emergency room setting, early stylet removal was associated with increased lumbar puncture success in two observational studies.[7,8]

Neonatal Champagne Lumbar punctures Every time–A Randomised Controlled Trial (NeoCLEAR) was designed to establish the optimal lumbar puncture technique in newborn infants in terms of the effects of infant position (sitting *vs* lying) and timing of stylet removal (early *vs* late) on lumbar puncture success, as well as other short-term clinical, safety, and health-care resource outcomes. An efficient 2 × 2 factorial design was

used to allow simultaneous evaluation of the two techniques, predicated on no plausible reason to expect an interaction—ie, a differential effect of one intervention dependent on the presence or absence of the other.

Methods

Study design and participants

NeoCLEAR was an open-label, 2×2 full factorial, randomised, controlled trial with an internal pilot according to the published protocol.[24] The trial was done in 21 UK centres providing newborn care (appendix p 2). Infants requiring a lumbar puncture at a corrected gestational age of 27⁺⁰ to 44⁺⁰ weeks, with a weight of 1000 g or more, were eligible. Infants were ineligible if they had already had a lumbar puncture for the same indication, were on ventilation, were unable to be held in sitting position, or if sitting was deemed to be difficult or unsafe.

The National Perinatal Epidemiology Unit Clinical Trials Unit, University of Oxford (UK), coordinated the trial. Ethics approval was received from the NHS Health Research Authority. Parents and public representatives were involved in study design, funding applications, and preparing study materials. Parents gave written informed consent before randomisation. The protocol is available online.

Randomisation and masking

Infants were randomly assigned (1:1:1:1) using a 24/7, web-based, secure, central randomisation system (to conceal allocations) into four groups: sitting position and early stylet removal; sitting position and late stylet removal; lying position and early stylet removal; or lying position and late stylet removal. Block randomisation was stratified within site by corrected gestational age (27⁺⁰ to 31⁺⁶ weeks, 32⁺⁰ to 36⁺⁶ weeks, 37⁺⁰ to 40⁺⁶ weeks, or 41⁺⁰ to 44⁺⁰ weeks), using variable block sizes of four and eight with equal frequency. An independent statistician generated the randomisation schedule, a senior trial programmer wrote the randomisation program, and both the schedule and program were independently validated. Masking of practitioners and parents was not possible, but the primary outcome was based on laboratory tests performed by technicians who were masked to allocation.

Procedures

Staff were trained in all four techniques, as detailed in online training videos and the published protocol.[24] Training intended to standardise needle type and analgesia was advised. A procedure was defined as a single practitioner performing a lumbar puncture. An attempt was defined as the needle passing through the skin. It was recommended that each procedure include no more than two attempts. If a second lumbar puncture was required, the same allocated technique was followed. The need for any further lumbar punctures and the techniques used were determined by local clinical teams. Data were collected at recruitment, at the first lumbar puncture, second lumbar puncture (if performed), and at discharge. Fidelity was monitored by each procedure being recorded contemporaneously by the team present, usually comprising at least three members. This included reporting when the allocated technique was not adhered to (appendix p 11).

Outcomes

The primary outcome was the proportion of infants with a successful first lumbar puncture, defined as obtaining a CSF sample with a red blood cell count of less than 10 000 cells per µL. This definition has been used in similar studies,[25,26] because interpretation of white blood cell count becomes less reliable above this threshold. Secondary outcomes were short-term clinical measures (alternative red blood cell thresholds, CSF appearance, white and red blood cell counts, number of procedures and attempts per infant, proportions with different CSF-based diagnoses, time taken, and infant movement); health-care resource use (duration of antibiotics and length of stay); and safety metrics (cardiorespiratory stability and adverse event reporting; appendix pp 12–15). We planned to assess parental anxiety using a standardised questionnaire, but this analysis was stopped after the pilot phase because of low completion rates. Infant movement was a subjective assessment agreed by the clinicians present, based on a 4-point scale used in a previous study,[26] with the following guidance statements: no struggling (baby completely still), mild struggling (some movement, easily able to hold baby in position), moderate struggling (moderate movement but able to hold); and severe struggling (significant movement).

Statistical analysis

NeoCLEAR was designed to detect a 10% absolute difference in the primary outcome (estimated comparator event rate 59%),[24,25] with 90% power and a 5% two-sided significance level, and assuming (based on expert opinion and the absence of external evidence) no interaction between infant position and timing of stylet removal. 483 infants were required for each group of each principal comparison (sitting *vs* lying and early *vs* late stylet removal). Allowing for 5% attrition, the recruitment target was 1020 infants.

Analyses (factorial) were prespecified in the statistical analysis plan (appendix p 18), which was approved before data lock. Primary and secondary outcomes were analysed by the groups to which infants were assigned regardless of deviation from the protocol or allocation received, but excluding infants who were withdrawn before data collection or who did not undergo lumbar puncture (modified intention-to-treat analysis).[24] In a 2×2 analysis to assess infant positioning, we compared the two groups allocated to sitting position (sitting plus early stylet removal and sitting plus late stylet removal) with the two lying groups (lying plus early stylet removal and lying

See Online for appendix

For the **protocol** see https://www.npeu.ox.ac.uk/assets/downloads/neoclear/protocol/NeoCLEAR_Protocol_V70_13JUL2020_signed_-_for_publishing.pdf

For the **training videos** see https://npeu.ox.ac.uk/neoclear

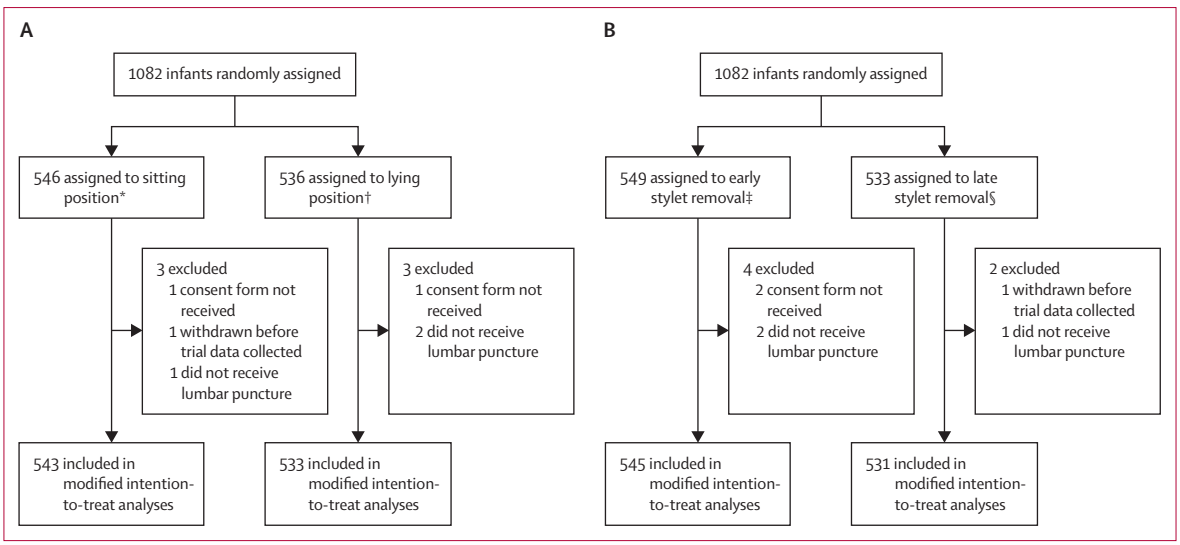

Figure 1: **Trial profile**
(A) Sitting versus lying position. (B) Early versus late stylet removal. *Sitting group: two infants were randomised in error and one did not receive a lumbar puncture because of clinical instability (infant too unwell); seven were withdrawn from the trial (five because of parental wish, one because of inadequate consent documentation, and one because of an error on the randomisation website), and consent for continued data collection was withdrawn for two of these infants. †Lying group: one infant was randomised in error, and two did not receive a lumbar puncture (one no longer required a lumbar puncture and one because of lack of personnel); two infants were withdrawn (one because of parental wish and one because of inadequate consent documentation), but none had consent withdrawn for continued data collection. ‡Early stylet removal group: two infants were randomised in error, and two did not receive a lumbar puncture (one because of clinical instability and one because of lack of personnel); six infants were withdrawn (four because of parental wish and two because of inadequate consent documentation), and consent for continued data collection was withdrawn for one of these infants. §Late stylet removal group: one infant was randomised in error, and one did not receive a lumbar puncture because it was no longer required; three infants were withdrawn from the trial (two because of parental wish and one because of an error on the randomisation website), and consent for continued data collection was withdrawn for one of these infants. As prespecified, not all deviations from protocol resulted in exclusion from the analysis, including those randomised in error, those who were withdrawn after data collection, and those for whom the allocation was not followed (appendix p 11).

plus late stylet removal). To assess the timing of stylet removal, we compared the two early stylet removal groups (whether sitting or lying) with the two late stylet removal groups (whether sitting or lying). Handling of the primary estimands is summarised in the appendix (p 3). We calculated risk ratios with 95% CIs for the primary outcome and all other dichotomous outcomes, the mean differences with 95% CIs for normally distributed continuous outcomes, and the median differences with 95% CIs for skewed continuous outcomes, plus the absolute risk differences with 95% CIs for (tested) dichotomous clinical outcomes. We compared groups using regression analysis, adjusting for centre and corrected gestational age, and for the allocation to the other intervention (ie, the position comparison was adjusted for the stylet removal allocation, and vice versa). We estimated adjusted risk ratios using log-binomial regression. We used linear regression for normally distributed outcomes and quantile regression for skewed continuous outcomes. We did prespecified subgroup analyses for the primary outcome for working weight, day of life, and corrected gestational age at trial entry, based on the statistical test for interaction. Two-sided p values of 0·05 or less were considered to be significant. To mitigate multiple testing, inference was restricted to certain prespecified (tested) outcomes. A masked interim analysis was reviewed by an independent Data Monitoring Committee (DMC) after 624 infants had been randomly assigned, but no formal statistical stopping guidelines were used. We also did a complementary descriptive multi-arm analysis (for each of the four randomised groups) for the primary outcome, other tested outcomes, and baseline characteristics. We investigated the effect modification between sitting versus lying position and the timing of stylet removal using the statistical test for interaction, while acknowledging that the trial was not powered to detect an interaction. In post-hoc analyses we examined the number of infants who had desaturations less than 80%, or bradycardic episodes less than 100 bpm, during first lumbar puncture. We also compared the baseline characteristics of infants younger than 3 days old with those aged 3 days or older. Analyses were done with Stata (version 15).

This study is registered with ISRCTN, ISRCTN14040914.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit for publication.

Results

Between Aug 3, 2018, and Aug 31, 2020, 1082 infants were randomly assigned to sitting (n=546) versus lying (n=536) position, and early (n=549) versus late (n=533)

	Comparison 1		Comparison 2	
	Sitting group (n=543)	Lying group (n=533)	Early stylet removal group (n=545)	Late stylet removal group (n=531)
Infant characteristics at randomisation				
Corrected gestational age, weeks	40 (39–41)	40 (39–41)	40 (39–41)	40 (39–41)
27⁺⁰ to 31⁺⁶	11 (2·0%)	11 (2·1%)	10 (1·8%)	12 (2·3%)
32⁺⁰ to 36⁺⁶	46 (8·5%)	47 (8·8%)	49 (9·0%)	44 (8·3%)
37⁺⁰ to 40⁺⁶	299 (55·1%)	295 (55·3%)	297 (54·5%)	297 (55·9%)
41⁺⁰ to 44⁺⁰	187 (34·4%)	180 (33·8%)	189 (34·7%)	178 (33·5%)
Age, days	1 (1–2)	2 (1–2)	2 (1–2)	1 (1–2)
<3	473 (87·1%)	463 (86·9%)	471 (86·4%)	465 (87·6%)
≥3	70 (12·9%)	70 (13·1%)	74 (13·6%)	66 (12·4%)
Working weight, g	3500 (3110–3910)	3530 (3155–3890)	3520 (3130–3890)	3510 (3155–3910)
1000 to <2500	55 (10·1%)	50 (9·4%)	57 (10·5%)	48 (9·0%)
2500 to 3500	217 (40·0%)	207 (38·8%)	207 (38·0%)	217 (40·9%)
>3500	271 (49·9%)	276 (51·8%)	281 (51·6%)	266 (50·1%)
Sex				
Male	325 (59·9%)	336 (63·0%)	336 (61·7%)	325 (61·2%)
Female	218 (40·1%)	197 (37·0%)	209 (38·3%)	206 (38·8%)
Any previous lumbar punctures	2 (0·4%)	3 (0·6%)	4 (0·7%)	1 (0·2%)
Primary indication for current lumbar puncture (not mutually exclusive)				
Risk factor for sepsis	201 (37·0%)	203 (38·2%)	196 (36·0%)	208 (39·2%)
Clinical signs of sepsis	137 (25·2%)	145 (27·3%)	147 (27·0%)	135 (25·5%)
Raised C-reactive protein	466 (85·8%)	444 (83·5%)	457 (83·9%)	453 (85·5%)
Other*	26 (4·8%)	25 (4·7%)	26 (4·8%)	25 (4·7%)
Clinical characteristics at first lumbar puncture				
Type of sedation and analgesia received (not mutually exclusive)				
None	24 (4·4%)	19 (3·6%)	24 (4·4%)	19 (3·6%)
Non-nutritive sucking	231 (42·7%)	199 (37·3%)	212 (39·0%)	218 (41·1%)
Oral sucrose or dextrose	443 (81·9%)	458 (85·9%)	464 (85·3%)	437 (82·5%)
Topical local anaesthetic	269 (49·7%)	261 (49·0%)	267 (49·1%)	263 (49·6%)
Other†	19 (3·5%)	16 (3·0%)	21 (3·9%)	14 (2·6%)
Respiratory status immediately before lumbar puncture				
Self-ventilating in air	466 (85·8%)	448 (84·1%)	459 (84·2%)	455 (85·7%)
Low-flow oxygen (<2L/min)	13 (2·4%)	16 (3·0%)	16 (2·9%)	13 (2·4%)
High-flow oxygen (≥2L/min)	57 (10·5%)	59 (11·1%)	59 (10·8%)	57 (10·7%)
Continuous or bi-level positive air pressure	7 (1·3%)	10 (1·9%)	11 (2·0%)	6 (1·1%)
Previous diagnosis of intra-ventricular haemorrhage	2 (1·0%)	5 (2·5%)	5 (2·5%)	2 (1·0%)
Not scanned	334	336	346	324
Coagulopathy treatment within past 24 h	4 (0·7%)	5 (0·9%)	4 (0·7%)	5 (0·9%)

Data are median (IQR), n (%), or n. *Other indications included abnormal blood white cell count or morphology, specific signs of meningitis or encephalitis, neurometabolic investigations, and positive blood cultures (appendix p 4). †Other sedation or analgesia included milk, paracetamol, opioids, chloral, and phenobarbitone or phenytoin (appendix p 5).

Table 1: **Baseline characteristics**

stylet removal (figure 1). 1079 infants had a first lumbar puncture; 166 (15·4%) had a second lumbar puncture (each of these lumbar puncture procedures involved one or more attempts). Nine infants were withdrawn from follow-up during the trial, but for eight of them consent was only withdrawn after data collection for the primary outcome, so they were not excluded from this analysis. One infant had consent withdrawn before data collection for the primary outcome, three infants did not receive a lumbar puncture, and two had missing consent forms, so that six in total were excluded, leaving 1076 for the final analysis (figure 1). All infants who were included in the final analysis were followed-up until discharge. Baseline characteristics are shown in table 1 and the appendix (pp 4–5). At the time of trial entry, 961 (89%) were term by corrected gestational age, and 936 (87%) were younger than 3 days.

For the first principal comparison, sitting versus lying position, there was evidence of a significant difference in favour of sitting for the primary outcome: a successful first lumbar puncture was achieved in 346 (63·7%) of 543 infants in the sitting group and 307 (57·6%) of 533 infants in the lying group (adjusted risk ratio 1·10 [95% CI 1·01–1·21], p=0·029; table 2; adjusted absolute risk difference 6·1% [95% CI 0·7–11·4], number needed to treat=16; appendix p 6).

Infants allocated to sitting position were less likely to show moderate-to-severe struggling at needle insertion (169 [31·2%] of 541 *vs* 202 [38·3%] of 527, adjusted risk

	Sitting group (n=543)	Lying group (n=533)	Adjusted risk ratio* (95% CI)	p value	Early stylet removal group (n=545)	Late stylet removal group (n=531)	Adjusted risk ratio* (95% CI)	p value
Primary outcome								
CSF obtained on first lumbar puncture† with red blood cell count <10 000 cells per µL	346 (63·7%)	307 (57·6%)	1·10 (1·01–1·21)	0·029	338 (62·0%)	315 (59·3%)	1·04 (0·94–1·15)	0·45
Secondary clinical outcomes (tested)‡								
Total number of procedures§	..	..	0·86 (0·68–1·09)	..	..	..	0·92 (0·77–1·11)	..
One	447 (82·3%)	424 (79·5%)	..	..	445 (81·7%)	426 (80·2%)	..	..
Two	83 (15·3%)	82 (15·4%)	..	..	81 (14·9%)	84 (15·8%)	..	..
Three or more	13 (2·4%)	27 (5·1%)	..	..	19 (3·5%)	21 (4·0%)	..	..
Total number of attempts§	..	..	1·00 (0·87–1·16)	..	..	..	1·01 (0·92–1·12)	..
One	282 (51·9%)	275 (51·7%)	..	..	280 (51·5%)	277 (52·2%)	..	..
Two	131 (24·1%)	111 (20·9%)	..	..	127 (23·3%)	115 (21·7%)	..	..
Three or more	130 (23·9%)	146 (27·4%)	..	..	137 (25·2%)	139 (26·2%)	..	..
Missing	0	1	..	..	1	0	..	..
Level of struggling movement¶	..	..	0·82 (0·71–0·94)	..	..	..	1·01 (0·87–1·18)	..
None	125 (23·1%)	85 (16·1%)	..	..	101 (18·8%)	109 (20·6%)	..	..
Mild	247 (45·7%)	240 (45·5%)	..	..	248 (46·1%)	239 (45·1%)	..	..
Moderate	129 (23·8%)	159 (30·2%)	..	..	148 (27·5%)	140 (26·4%)	..	..
Severe	40 (7·4%)	43 (8·2%)	..	..	41 (7·6%)	42 (7·9%)	..	..
Missing	2	6	..	..	7	1	..	..
Appearance of clearest|| sample (first lumbar puncture)†**	..	..	1·05 (0·99–1·11)	..	..	..	1·01 (0·95–1·08)	..
Clear CSF	270 (49·7%)	233 (43·7%)	..	..	259 (47·5%)	244 (46·0%)	..	..
Blood-stained CSF	163 (30·0%)	173 (32·5%)	..	..	169 (31·0%)	167 (31·5%)	..	..
Pure blood or clotted	85 (15·7%)	100 (18·8%)	..	..	90 (16·5%)	95 (17·9%)	..	..
No sample obtained	25 (4·6%)	27 (5·1%)	..	..	27 (5·0%)	25 (4·7%)	..	..
CSF obtained (first lumbar puncture)† with any red blood cell count	390 (71·8%)	357 (67·0%)	1·07 (0·98–1·17)	..	383 (70·3%)	364 (68·5%)	1·02 (0·95–1·09)	..
CSF obtained (first lumbar puncture)† not requiring white blood cell correction††	356 (65·7%)	322 (60·4%)	1·09 (0·99–1·19)	..	349 (64·2%)	329 (62·0%)	1·03 (0·95–1·12)	..
Missing	1	0	..	..	1	0	..	..
Final clinical diagnosis at discharge‡‡	..	..	1·02 (0·94–1·11)	..	..	..	0·99 (0·95–1·04)	..
Definite or probable meningitis	7 (1·3%)	9 (1·7%)	..	..	9 (1·7%)	7 (1·3%)	..	..
Possible meningitis or equivocal CSF result	12 (2·2%)	11 (2·1%)	..	..	13 (2·4%)	10 (1·9%)	..	..
Negative CSF result	424 (79·0%)	408 (77·3%)	..	..	422 (77·9%)	410 (78·4%)	..	..
Uninterpretable CSF result§§	31 (5·8%)	36 (6·8%)	..	..	33 (6·1%)	34 (6·5%)	..	..
No CSF obtained	63 (11·7%)	64 (12·1%)	..	..	65 (12·0%)	62 (11·9%)	..	..
Other clinical reason for lumbar puncture	6	5	..	..	3	8	..	..

Data are n (%) unless otherwise stated. CSF=cerebrospinal fluid. *Adjusted for other allocation, gestational age at randomisation, and centre. †From any attempt in first procedure. ‡Further secondary outcomes (tested and untested) are in the appendix (pp 7–15). §Adjusted risk ratio for one versus more than one. ¶Adjusted risk ratio for none or mild versus moderate or severe level of struggling movement on first attempt of first procedure. ||Clearest defined as getting a sample (rather than none); in a sample, clear CSF was better than blood-stained CSF, which was better than pure blood or clotted sample; CSF sent to the laboratory rather than not sent; if the laboratory was able to do microscopy (not clotted, and reporting red and white cell blood counts) rather than not; if the laboratory was able to do microscopy, then the lower the red blood cell count, the better. **Adjusted risk ratio for clear CSF or blood stained versus pure blood, clotted, or no sample obtained from any attempt on first procedure. ††White blood cell count of less than 20 per µL whatever the red blood cell count, or a red blood cell count of less than 500 per µL. ‡‡Adjusted risk ratio for negative versus definite meningitis, probable meningitis, possible meningitis, equivocal CSF result, uninterpretable CSF sample, or no CSF, in relation to indication for lumbar puncture. §§Usually blood-clotted CSF sample, or very high red blood cell count.

Table 2: **Primary and secondary clinical outcomes**

ratio 0·82 [95% CI 0·71–0·94]; table 2). There was no significant difference for most other secondary outcomes analysed (when tested), but the majority predominantly favoured sitting (table 2; appendix pp 6–7).

Considering diagnoses based on CSF results from the first and second lumbar punctures (and any culture or PCR results), infants who were sitting were more likely than those who were lying to be diagnosed as negative for meningitis (396 [73·7%] of 537 *vs* 359 [68·9%] of 521; appendix p 9); those who were lying were more likely than those who were sitting to be diagnosed with uninterpretable CSF (no sample obtained or CSF not possible to analyse, usually because of a heavily blood-contaminated, clotted sample; 139 [26·7%] of 521 *vs* 114 [21·2%] of 537; untested outcome). Resource outcomes were essentially identical for sitting and lying groups: median duration of antibiotics was 5 days (IQR 4–6), and median length of hospital stay was 5 days (4–7; appendix p 10).

In prespecified subgroup analyses, the effect of position on the proportion of infants with a successful first lumbar puncture was consistent across working weight and corrected gestational age at randomisation, but a differential effect was observed between infants enrolled when they were younger than 3 days compared with those who were 3 days or older (adjusted risk ratio 1·14 [95% CI 1·04–1·25] *vs* 0·90 [0·78–1·05]; p=0·0011; figure 2). In post-hoc analyses, the subgroup of infants aged at least 3 days old had a lower gestational age at birth and a lower birthweight, and were more likely to be on respiratory support (appendix p 17).

Adherence to allocated technique was lower in infants in the sitting group: in 47 (8·7%) of 543 first lumbar punctures allocated to the sitting position, at least one attempt involved switching to the lying position (*vs* four [0·8%] of 533 allocated to lying position, but switching to sitting; appendix p 11). This was usually a clinician decision (45 of 47 lumbar punctures) and mostly happened on the second (22 of 247) or third (24 of 57) attempt.

Four (0·3%) of 1241 first or second lumbar punctures, two per group, were abandoned because of cardio-respiratory deterioration (table 3; appendix p 12). Three (0·2%) of 1241 procedures required increased respiratory support within 1 h (one in the sitting group and two in the lying group).

Lowest oxygen saturations during first lumbar puncture had a median of 93% (IQR 89–96) in the sitting position and 90% (85–94) when lying (p<0·0001; table 3). We analysed (post-hoc) the clinical implication of this finding by examining the number of infants who had desaturations of less than 80% during first lumbar puncture (35 [6·6%] of 532 infants when sitting *vs* 72 [14·2%] of 508 when lying; appendix p 13). Fewer desaturations of less than 80% were found for both term and preterm infants in sitting position (appendix p 14).

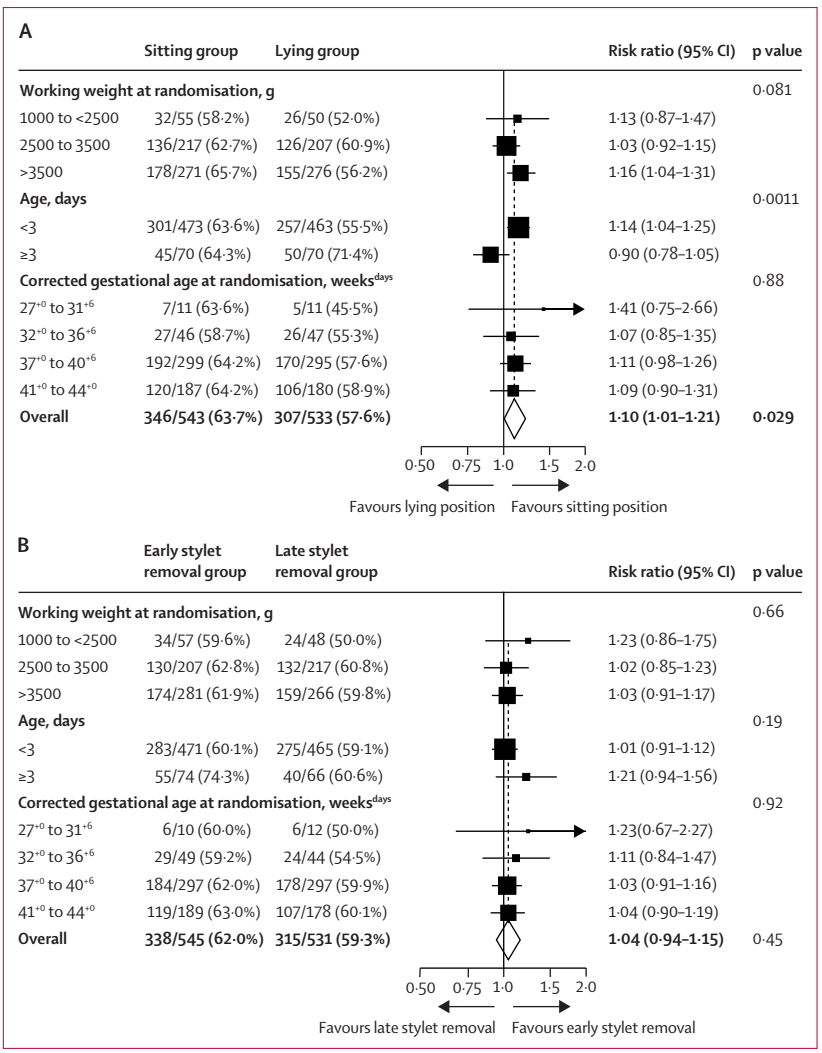

Figure 2: **Subgroup analyses of the primary outcome for sitting versus lying position (A) and early versus late stylet removal (B)**
The risk ratio is adjusted for corrected gestational age at randomisation, other allocation group, and centre. The size of the squares is proportional to the inverse of the variance.

The mean lowest heart rate was significantly higher in sitting position (129·5 bpm [SD 19·9] *vs* 127·0 bpm [21·5]; adjusted mean difference 2·5 [95% CI 0·6–4·4], p=0·011; table 3). Similarly, we examined the clinical implication of this finding post-hoc: 26 (5·0%) of 523 infants in the sitting group had bradycardic episodes of less than 100 bpm compared with 44 (8·8%) of 501 infants in the lying group (appendix p 15).

For the second principal comparison, early versus late stylet removal, the primary outcome was observed in 338 (62·0%) of 545 infants following early stylet removal and 315 (59·3%) of 531 following late stylet removal: ie, no evidence of a difference (adjusted risk ratio 1·04 [95% CI 0·94–1·15], p=0·45; table 2). There was no evidence of any differences between these groups for any secondary outcomes (table 2; appendix pp 7–10).

	Sitting group (n=543)	Lying group (n=533)	Adjusted effect measure* (95% CI)	p value	Early stylet removal group (n=545)	Late stylet removal group (n=531)	Adjusted effect measure* (95% CI)	p value
Procedure abandoned because of cardiorespiratory deterioration (first lumbar puncture)	2 (0·4%)	1 (0·2%)	Risk ratio 1·96 (0·17 to 22·08)	0·59	1 (0·2%)	2 (0·4%)	Risk ratio 0·49 (0·04 to 5·53)	0·56
Missing	0	1	..	..	1	0	..	..
Procedure abandoned because of cardiorespiratory deterioration (second lumbar puncture)	0/76 (0·0%)	1/90 (1·1%)	..	..	0/81 (0·0%)	1/85 (1·2%)	..	..
Infant's lowest oxygen saturation (first lumbar puncture), %	93% (89 to 96)	90% (85 to 94)	Median difference 3·0% (2·1 to 3·9)	<0·0001	92% (86 to 95)	92% (87 to 95)	Median difference 0·0% (–0·9 to 0·9)	1·00
Missing	11	25	..	..	23	13	..	..
Infant's lowest heart rate (first lumbar puncture), bpm	129·5 (19·9)	127·0 (21·5)	Mean difference 2·5 (0·6 to 4·4)	0·011	128·1 (21·0)	128·4 (20·4)	Mean difference –0·3 (–2·3 to 1·7)	0·75
Missing	20	32	..	..	34	18	..	..
Infant's highest heart rate (first lumbar puncture), bpm	163·7 (21·7)	163·6 (21·9)	Mean difference 0·1 (–2·1 to 2·4)	0·90	163·9 (21·6)	163·4 (22·0)	Mean difference 0·5 (–1·9 to 2·9)	0·67
Missing	18	32	..	..	31	19	..	..
Respiratory deterioration after first lumbar puncture†	1 (0·2%)	2 (0·4%)	Risk ratio 0·49 (0·04 to 5·71)	0·57	1 (0·2%)	2 (0·4%)	Risk ratio 0·49 (0·04 to 5·63)	0·56
Missing	0	1	..	..	1	0	..	..
Respiratory deterioration after second lumbar puncture†	0/76 (0·0%)	0/90 (0·0%)	..	..	0/81 (0·0%)	0/85 (0·0%)	..	..

Data are n (%), n/N (%), median (IQR), or mean (SD), unless otherwise stated. Median difference indicates the use of quantile regression (skewed continuous outcomes). Mean difference indicates linear regression (normally distributed outcomes). *Adjusted for other allocation, gestational age at randomisation, and centre. †Requirement for escalating respiratory support within 1 h of lumbar puncture.

Table 3: **Safety outcomes**

In prespecified subgroup analyses, the absence of effect of timing of stylet removal on the proportion of infants with successful first lumbar puncture was consistent across working weight, corrected gestational age, and day of life at trial entry (figure 2). The allocated stylet technique was not adhered to in 19 (3·5%) of 545 first lumbar punctures in the early stylet removal group and 16 (3·0%) of 531 in the late stylet removal group (appendix p 11).

Four (0·3%) of 1241 procedures (two in each group), from the first or second lumbar puncture, were abandoned following cardiorespiratory deterioration (table 3; appendix p 12); three (0·2%) of 1241 procedures required increased respiratory support within 1 h (one with early and two with late stylet removal; no evidence of a difference). There was no evidence of a difference between early and late stylet removal for lowest oxygen saturations or lowest heart rate.

In a multi-arm analysis, comparing the four randomisation groups (sitting plus early stylet removal, sitting plus late stylet removal, lying plus early stylet removal, and lying plus late stylet removal), for the primary outcome, there was no evidence of an interaction between infant position and timing of stylet removal (p=0·14; appendix p 16). Lying with late stylet removal was least likely to be successful (142 [54·4%] of 261), followed by lying with early stylet removal (165 [60·7%] of 272). There was no evidence of a difference between sitting with early stylet removal (173 [63·4%] of 272) and sitting with late stylet removal (173 [64·1%] of 270). Multi-arm baseline characteristics and secondary outcome data did not reveal any additional findings (only those previously described for sitting *vs* lying position; data not shown).

Four serious adverse events (0·4% of infants) were reported: three were deemed to be unrelated to lumbar puncture; one infant from the sitting plus late stylet removal group developed a scrotal haematoma 2 days after lumbar puncture, which was deemed to be possibly related to lumbar puncture.

Discussion

For newborn infants needing lumbar puncture, sitting position was superior to lying for achieving a successful first lumbar puncture. The absolute difference in success rate between sitting and lying positions was 6·1%, which corresponds to a number needed to treat of 16. Sitting was also better tolerated in terms of infant comfort, oxygen saturations, and heart rate. Timing of stylet removal did not affect lumbar puncture success.

Our results could be explained by the anatomical advantages of sitting position, which include an increased interspinous distance and a wider subarachnoid space as shown in ultrasound-based studies,[17,18,21,22] or by the reduced infant struggling we observed. One previous small randomised controlled trial involved 168 infants aged 90 days or younger in a paediatric emergency department setting, where success rates (defined as a red blood cell count of <10 000 cells per µL on the first or second attempt) were broadly similar between lying (63 [77%] of 82) and sitting (61 [72%] of 85; risk

difference 5·1% [95% CI −8·2 to 18·3]).[20] Observational studies in similar settings reported inconsistent results.[7,19,27,28] A recent small randomised controlled trial of neonates weighing less than 2500 g reported improved success rates with prone positioning, although the technique was deemed to be unsuitable for infants who are term or normal weight, which is the predominant population requiring lumbar puncture in the newborn period.[29]

Early stylet removal was originally introduced to replicate the higher success rates reported with non-styletted needles,[30,31] while attempting to avoid their association with iatrogenic intraspinal tumour formation.[31,32] In subsequent prospective cohort studies of infants aged 3 months or younger from two different emergency departments, early stylet removal was associated with increased success rates.[7,8] However, NeoCLEAR is the first randomised controlled trial to systematically investigate early stylet removal and demonstrates no evidence of a benefit (or harm) in a neonatal population. We therefore cannot advise for or against early or late stylet removal in neonates.

Our safety analyses showed greater physiological stability (saturations and heart rate) for sitting lumbar puncture, corroborating previous observations.[15,16] The median of the lowest recorded saturations was 3% higher in the sitting group than in the lying group; in post-hoc analyses, 6·6% of infants in sitting position had oxygen levels below 80%, compared with 14·2% in lying position, with an even wider difference for more premature infants (appendix p 14). Similarly, the mean lowest heart rate was 2·5 bpm higher in the sitting group, which corresponded to fewer infants having bradycardic episodes below 100 bpm in the sitting position (5·0%) compared with lying (8·8%).

Results for other secondary outcomes fell short of conventional levels of statistical significance, including no differences observed for the prespecified health-care resource use outcomes. Only one serious adverse event was deemed to be possibly related to a trial procedure: a scrotal haematoma. Scrotal haematomas can occur spontaneously, and alternative causes could not be identified because the infant did not undergo further aetiological investigations.

NeoCLEAR is the first adequately powered randomised controlled trial examining lumbar puncture technique in newborn infants, and the largest one investigating sitting versus lying position and early versus late stylet removal in any population. We investigated techniques that are cost neutral and easily learned: practitioners had one simulator session plus access to training videos. Our results show a significantly higher success rate for sitting lumbar punctures, on average, throughout all corrected gestational ages (>27 weeks) and all weight subgroups (>1000 g). Although the average number of procedures, length of hospital stay, and duration of antibiotics were not significantly different between the sitting and lying groups, an individual baby having a successful (rather than unsuccessful) lumbar puncture (about one in 16 according to the number needed to treat) would be very unlikely to have a repeat procedure and more likely to have their antibiotics stopped and be discharged sooner. This finding has implications for infant care and parental satisfaction (in terms of repeat procedures, and length of hospital stay) as well as antimicrobial stewardship.

Limitations include many practitioners being previously unfamiliar with sitting technique. This might have led to more practitioners switching from sitting allocation to lying position following initially unsuccessful attempts. We speculate that success rates would have been even higher if there had been more experience of performing lumbar punctures in sitting position, and if fewer practitioners had switched position. Our data for infants younger than 32 weeks are limited by small numbers in this gestational age group; however, success rates and safety metrics were favourable for infants of all corrected gestational ages. Furthermore, as we excluded infants on ventilation and those younger than 27 or older than 44 weeks corrected gestational age, our results might not apply to these populations. We also found evidence of a differential treatment effect in relation to chronological age at recruitment (infants aged <3 days or ≥3 days). However, it should be noted that infants 3 days or older were a relatively small subgroup (140 [13%] of 1076) in whom there was no significant treatment effect, and there were no safety concerns with sitting position. Finally, we acknowledge that it was impossible for practitioners to be masked to allocation in this study, which might have affected certain secondary outcomes, but the primary outcome was based on CSF analysis by laboratory technicians, who were masked. Research should now examine sitting technique (as well as other techniques such as prone positioning and early stylet removal) in infants of lower gestational ages, older infants, and children.

In conclusion, our results show that sitting position is superior to lying for neonatal lumbar puncture success, with no evidence of a difference between early and late stylet removal. Sitting position is cost neutral, safe, well tolerated, and easy to learn. These results are applicable in similar settings worldwide and should prompt a change in practice towards sitting technique as standard for neonatal lumbar puncture.

Contributors

ASJM and CCR conceived the idea for the study and wrote the initial protocol, with clinical and scientific contributions from MS, JY, EA, LL, and EJ. CCR was the chief investigator responsible for all aspects of the study, including preparation and submission of the grant application, securing funding and regulatory approvals, project management, data collection, and preparation of the manuscript for publication. AS and RW helped to design and refine the study, and develop the protocol. JLB, LL, PH and EJ provided study design, and statistical and methodological expertise, and RW contributed logistical aspects of the trial. JLB, LL, and PH had access to the data which was primarily analysed by JLB and verified by LL and PH. All authors approved the final version of the manuscript.

Declaration of interests
JLB, LL, EJ, PH, and MS report receipt of funding from the UK National Institute for Health and Care Research, outside the submitted work. MS reports grants from GlaxoSmithKline, Pfizer, Merck, Symvivo, Sanofi Pasteur, Seqirus, and VBI Vaccines, outside the submitted work. All funds have been paid to his institute, with no personal payments. EJ was a member of the NIHR-HTA General Board from 2016 to 2017, and the NIHR-HTA Commissioning Board from 2013 to 2016, but was not involved in decisions about funding this trial. PH is a member of the NIHR HTA Funding Committee (Commissioning Board), but was not involved in decisions about funding this trial. CCR reports speaker's bureau fees from Chiesi Pharmaceuticals Italy, outside the submitted work. All other authors declare no competing interests.

Data sharing
The National Perinatal Epidemiology Unit (NPEU) is committed to sharing of data with the research community. Data will be shared in accordance with the NPEU Data Sharing Policy. Requests for access to the data will be considered by the NPEU Data Sharing Committee. Access to anonymised data can be requested from general@npeu.ox.ac.uk. The trial protocol and statistical analysis plan and other study documents are also available through this route.

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