Elsevier Editorial System(tm) for The Lancet
Manuscript Draft

Manuscript Number: THELANCET-D-15-06879R1

Title: Neurodevelopmental outcome at 2 years of age after general and awake-regional anaesthesia in infancy: a randomised controlled trial

Article Type: Article (Randomised Controlled Trial)

Corresponding Author: Dr. Andrew J Davidson, MD

Corresponding Author's Institution: Murdoch Childrens Research Institute, Royal Children's Hospital and University of Melbourne

First Author: Andrew J Davidson, MD

Order of Authors: Andrew J Davidson, MD; Nicola Disma, MD; Jurgen C de Graaff, PhD; Davinia E Withington, BM; Liam Dorris, DClinPsy; Graham Bell, MBChB; Robyn Stargatt, PhD; David C Bellinger, PhD; Tibor Schuster, PhD; Sarah J Arnup, MBiostat; Pollyanna Hardy, MSc; Rodney W Hunt, PhD; Michael J Takagi, PhD; Gaia Giribaldi, MD; Penelope L Hartmann, BPsych (Hons); Ida Salvo, MD; Neil S Morton, MD; Britta S von Ungern-Sternberg, MD; Bruno Guido Locatelli, MD; Niall C Wilton, MBBS; Anne Lynn, MD; Joss J Thomas, MD; David Polaner, MD; Oliver Bagshaw, FRCA; Peter Szmuk, MD; Anthony R Absalom, MBChB; Geoff Frawley, MBBS; Charles Berde, MD; Gillian D Ormond, MSc; Jacki Marmor, MEd; Mary Ellen McCann, MD

Abstract: ABSTRACT
Background:
There is pre-clinical evidence that general anaesthetics affect brain development. There is mixed evidence from cohort studies that young children exposed to anaesthesia may have an increased risk of poorer neurodevelopmental outcome. This trial aims to determine if GA in infancy has any impact on neurodevelopmental outcome. The primary outcome for the trial is neurodevelopmental outcome at 5 years of age. The secondary outcome is neurodevelopmental outcome at two years of age and is reported here.

Methods:
We performed an international assessor-masked randomised controlled equivalence trial in infants less than 60 weeks post-menstrual age, born at greater than 26 weeks gestational age having inguinal herniorrhaphy. Infants were excluded if they had existing risk factors for neurologic injury. Infants were randomly assigned to awake-regional (RA) or sevoflurane-based general anaesthesia (GA). Web-based randomisation was performed in blocks of two or four and stratified by site and gestational age at birth. The outcome for analysis was the composite cognitive score of the Bayley Scales of Infant and Toddler Development, Third Edition. The analysis was as-per-protocol adjusted for gestational age at birth. A difference in means of five points (1/3 SD) was predefined as the clinical equivalence margin. The trial was registered at ANZCTR, ACTRN12606000441516 and ClinicalTrials.gov, NCT00756600.

Findings:
Between February 2007, and January 2013, 363 infants were randomised to RA and 359 to GA. Outcome data were available for 238 in the RA and 294

in the GA arms. The median duration of anaesthesia in the GA arm was 54 minutes. For the cognitive composite score there was equivalence in means between arms (RA-GA: +0·169, 95% CI -2·30 to +2·64).

Interpretation:
For this secondary outcome we found no evidence that just under an hour of sevoflurane anaesthesia in infancy increases the risk of adverse neurodevelopmental outcome at two years of age compared to RA.

Funding:
Australia National Health and Medical Research Council (NHMRC), Health Technologies Assessment- National Institute for Health Research UK, The National Institute of Health, The Food and Drug Administration, The Australian and New Zealand College of Anaesthetists, The Murdoch Childrens Research Institute, The Canadian Institute of Health Research, Canadian Anesthesiologists' Society, Pfizer Canada Inc, Italian Ministry of Heath, Fonds NutsOhra grant

Manuscript: THELANCET-D-15-06879, Neurodevelopmental outcome at 2 years of age after general and awake-regional anaesthesia in infancy: a randomised controlled trial

Thank you for the opportunity to respond to the editorial and reviewer comments. In light of these comments we hope we have substantially improved the manuscript.

The reviewers have noted several key issues.

Firstly, it was noted that the paper presents results for a secondary outcome and thus should inherently be regarded with caution. We acknowledge this and have changed the paper accordingly. However the protocol did specifically indicate that we would do these analyses and the tight confidence interval suggests it is unlikely to be underpowered.

Secondly, the reviewers wished us to tone down our recommendations given the inherent limitations of the study (the most significant being that it is a two year assessment which is less sensitive than a five year assessment). We agree that this is appropriate and we are now more circumspect in our conclusions and recommendations. However we wish to point out that the evidence available before the trial is of very poor quality. Societies are recommending caution when anaesthetising children, parents are showing concern and clinicians are changing practice even though the evidence is extremely weak. It is in this context that our results should be interpreted. We agree that the 5 year outcome data will be important; however we have found very strong evidence for equivalence using the best measure available at 2 years of age. It may not be definitive, but it is almost certainly substantially better than that which is currently guiding practice. Indeed this is a perfect example of the need to perform large prospective randomised trials.

As an example of the interest in our trial, The Smarttots executive (a partnership between the FDA and the International Anesthesia Research Society to address the issue of neurotoxicity) just contacted me indicating they will withhold releasing their updated consensus statement (which had taken them over a year to finally agree on) given the imminent availability of our results.

Lastly the question of longer exposure cannot be answered in this trial. We acknowledge this and we have been careful not to suggest that this trial answers the question of whether or not anaesthetics are neurotoxic; however the results are probably relevant to more than half the young children receiving anaesthesia

Detailed responses as follows:

EDITORS' COMMENTS

1. Please ensure that your manuscript is written up according to CONSORT guidelines and submit a completed CONSORT checklist with your revised manuscript.

Done (attached)

2. Please ensure that your abstract is written up according to CONSORT for Abstracts (eg, includes method of randomisation). For more details, see The Lancet DOI:10.1016/S0140-6736(07)61835-2.

Done, apologies for this somewhat obvious oversight (page 4)

3. As your study is a non-inferiority trial, please present just the per-protocol analysis in the Abstract (both per-protocol and intention-to-treat analyses can be provided in the text).

Done (page 4)

4. Please make clear in the text that you are presenting a secondary endpoint of the main trial.

We have now made this clearer in the text and abstract (page 4, 6, 9, 13, 17)

5. Please confirm that all neurodevelopmental outcomes at 2 years are presented in your paper.

All 2 year neurodevelopmental outcomes are reported in this paper. We have made this clearer in the paper. (page 6)

6. Please include a section in your discussion about limitations.

We have now grouped and indicated all limitations (page 15-17)

7. At present there are too many non-text items for our layout. I would be grateful if you could move figures 1, 4, 7, and 8 to a web appendix. Please also consider also placing tables 5, 10, and 11 in the appendix.

We have moved tables 1, 4, 7, 8, 10 and 11 to a web appendix

8. Please move the Research in Context panel to the start of the Discussion section and include a sentence in the text to link to this. Please could you then renumber the references so that reference 1 is the first paper mentioned in your Introduction. Please note that references cited in panels should be numbered in the order in which they appear in the context of where the panel is cited. (So for numbering purposes the figure/table is considered as part of the text.)

Done

9. We normally require signed statements from all people mentioned in the Acknowledgments section, giving their permission to be mentioned in your paper. Who are the people in the Acknowledgments section? If they are investigators, perhaps they could be moved out of the Acknowledgments section and placed after the members of the GAS trial group.

Those in the acknowledgment section are people that contributed to the running of the study but do not qualify for authors ship or investigator status. We can seek their signed permission if that is required.

10. I note that you report funding from the National Institute of Health Research (among others). Please check whether you will need to comply with their open access policies.

As far as we can gather the NIH requests "*The Director of the National Institutes of Health ("NIH") shall require in the current fiscal year and thereafter that all investigators funded by the NIH submit or have submitted for them to the National Library of Medicine's PubMed Central an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication: Provided, that the NIH shall implement the public access policy in a manner consistent with copyright law.*"

Reviewer #1 (statistician):

Major comments:

1. *Power of this study. There is no mention of power calculation for this study in terms of primary endpoint of the composite cognitive score at 2 year for this sub-study. The GAS study was designed and powered as an equivalence study in terms of WPPSI-III Full Scale IQ Score at 5 year but was not powered to evaluate the equivalence in terms of the composite cognitive score at 2 year. As a result, the results from this report are exploratory in nature and should be interpreted with caution.*

The reviewer is correct that the trial was powered for the primary outcome in terms of the WPPSI-III Full Scale IQ Score at 5 year. We did not perform a specific *a priori* power analysis to address secondary outcomes.

The reviewer raises two relevant issues that often occur in unplanned investigations such as retrospective studies and post-hoc comparisons within prospective trials. However, we believe that the comparative assessments of the 2 year GAS study data as presented in our article were neither explanatory nor 'underpowered'.

The composite cognitive score of the Bayley was clearly pre-specified in the protocol as a secondary outcome and there was a specific secondary hypothesis listed in the protocol – as such we believe it is not appropriate to describe the analysis as exploratory. The secondary analyses were planned for several reasons: 1) due to the recognition that there was growing concern over the issue of neurotoxicity and existing evidence to guide practice was inherently limited, and while the two year assessment was not definitive, it would still provide higher quality evidence than that which existed to date; 2) due to concerns over the feasibility of maintaining the cohort for the longer term follow-up; 3) to support overall understanding of the primary research question of the trial: consistency of the primary (long-term) and secondary (short-term) outcome analysis results is strongly desired to maximize evidence either for or against the principal research question.

The Bayley has similar psychometric properties to the WPPSI-III with a mean of 100 and SD of 15. The 95% CI are indeed well within a third of a standard deviation which we regarded *a priori* as the pre-specified margins of equivalence. Since this secondary outcome analyses led to a rejection of the null-hypothesis of 'no equivalence', a lack of statistical power is technically not indicated.

However we do acknowledge that the Bayley was indeed a secondary outcome, that there was no *a priori* power analysis for this outcome and thus we have now clearly indicated in the paper that the results should be regarded with caution for these reasons.

(page 13, 16)

2. *Abstract. The conclusion "Fifty-four minutes of exposure to a GA in infancy does not increase the risk of adverse neurodevelopmental outcome at two years of age compared to RA." is not supported by the data since this is an exploratory study and not designed and powered to provide the definitive answer to the research question. Same problems in "Research in context" and Discussion.*

We have altered the text to reflect this point. (page 4, 13-14, 17)

3. Statistical analysis. Table 11 reports the adjusted risk ratio by gestational age at birth but it is not clear what method was used to calculate the adjusted risk ratio.

We have used generalized linear models for a binomial distributed response variable employing a log link (binomial log-linear regression)

(page 10)

4. Discussion. The following limitation should be mentioned: this two-year sub-study is not powered in terms of the composite cognitive score at 2 year and therefore is an exploratory study and its results should not be interpreted as a definitive trial.

We have altered the text accordingly (see above).(page 4, 13-14, 17)

Minor comments:

1. *Table 1 is less important and should perhaps be provided as a supplementary table.*

Moved to appendix

2. Table 4 could be treated as a supplementary table.

Moved to appendix

3. Table 5 looks messy. Summary statistics could be expressed as "n,mean(SD)" such as "238,9.7(2.8)".

Changed as suggested (page 27)

4. Table 10 and 11. These two tables could be combined into a single table.

Moved to appendix

Reviewer #2: This is a well designed study that addresses a timely and important topic. Although the study suffers from the short duration of exposure and high rate of failure among the RA group the results are nonetheless appear valid and are important to both researchers in the area and most to parents. The short exposure time of 54 minutes significantly reduces the impact of the study as single brief exposures have been shown in both preclinical and retrospective cohort studies to be unlikely to result in an effect. Thus the fact that GA and RA were shown to be equivalent are not surprising and unfortunately reduces the impact of an otherwise well designed study.

We acknowledge a failure rate in the RA group. (pages 15-16) Fortunately the near identical results in the APP and ITT analysis indicate that this failure rate does not reduce the veracity of the results, as suggested by the reviewer. The median exposure is just under an hour. Although data are scarce, our internal audits suggest this represents about half the anaesthetics given to infants (note that these audits are in tertiary children's hospitals which are biased toward more complex cases and slower surgery due to teaching). Thus the results are likely to be relevant to over 50% of anaesthetics administered to infants. In preclinical studies most injury is indeed seen after longer exposures (although as the reviewer points out below, this is not a universal finding). (page 15) The translation from animal to human is sufficiently unclear that it is not possible to specify any particular duration of exposure as critical.
The reviewer is incorrect in saying that single brief exposures have not been associated with altered neurodevelopmental outcome in human cohort studies. DiMaggio et al and Ing et al both found an association with a single exposure and outcome. In the Di Maggio studies the exposure was brief. Other studies have found an association only after multiple exposures – very likely due to confounding as children that require multiple anaesthetics are far more likely to have other co-morbidity. All these cohort studies have very significant limitations in terms of confounding, sub-optimal and insensitive outcome measures. Thus we believe it is not correct to say these cohort studies indicate it was unlikely that we would see an effect in the trial.

The authors correctly point out that the primary outcome measure (Bayley III)is a relatively crude measure of ultimate cognitive performance leaving open the possibility that equivalence in this setting is a function of a non-discriminatory outcome rather than an absence of effect. That said the outcome is nonetheless suggestive if not definitive and will provide reassurance to both parents and providers of anesthesia care to young children as well as help guide regulators such has the FDA.

We have acknowledged in the discussion that the Bayley has this significant limitation. However, albeit not perfect, there is indeed some correlation between Bayley and longer term neurodevelopmental outcome. Our finding of considerable precision around an estimate of almost zero significantly reduces the chance that we are missing a great effect. However we emphasise this in the text and have toned down our conclusions as suggested. (page 16)

1. It is noted that masking was consistently observed among those performing the Bayley assessment. Can the authors be more specific than "mostly" in the manuscript text.

We have indicated the exact degree of masking. (page 11)

2. The study population is heavily skewed toward males as would be expected based on procedure and age. The authors fail to mention this as a limitation of the study. Is it possible that there is a gender difference in susceptibility to the effects of anesthetics on the developing brain.

Yes, this is an important point that should have addressed. Reporting of gender effects in children with acquired brain injury is extremely variable with differences in findings most probably due to differences in the outcome of interest and timing of injury. Sexual dimorphism has been identified in neonates (1) and in general the difference between the

sexes is thought to be the sequence of development of the various brain regions (2). The gender effect from a neurotoxic brain injury may be on differentially developed brain regions at the time of injury and the cognitive functions they subserve. Sexual dimorphism may then result in different cognitive and behavioural outcomes in injuries that occur at a specific age according to gender. There is a well recognized gender bias towards males being more vulnerable to neurodevelopmental disorders and some preclinical studies indeed have found a greater effect in male animals (3). Given that this sample was predominantly male, it would be expected that if there was a significant gender effect it would be identifiable in this analyses. We have added to the discussion to highlight these important points. (pages 16-17)

1 Rebecca C. Knickmeyer, Jiaping Wang, Hongtu Zhu, et al. Impact of Sex and Gonadal Steroids on Neonatal Brain Structure. *Cereb. Cortex* 2014; 24 (10): 2721-2731

2. Lenroot RK, Gogtay N, Greenstein DK, et al. Sexual dimorphism of brain developmental trajectories during childhood and adolescence. *Neuroimage*. 2007; 36(4): 1065-73.

2. Lee B, Chan J, Kraeva E, Petersen K & Sall J. Isoflurane exposure in newborn rats induces long-term cognitive dysfunction in males but not females. *Neuropharmacology* 2014; 83: 9-17

3. 15-18% of children enrolled had anesthetic exposure subsequent to the inguinal hernia repair. Among those in the RA group these exposures constitute a crossover into the GA arm. Am I correct and should this be discussed as a limitation that may tend to bias toward equivalence.

Yes, this could bias toward equivalence, as could many other post randomisation random confounding events. As the incidence of subsequent anaesthetic was well balanced between arms we do not feel this is important and we do not feel we need to highlight this in the discussion.

Reviewer #3: To the Authors:

The reported data is clearly novel. The reported mc RCT is still ongoing and, and before launched 6 years ago, based on a well-vetted study protocol. While today study aims and methods could be debated intensely, this reviewer believes the reported data are important for the field although the follow-up interval reflected in this data set is short and answers to the secondary, and not to the primary hypothesis of the study design.

We have added to the text to highlight that this is indeed a secondary outcome
(pages 4, 6, 9, 13, 17)

The most important critique targets the strong conclusions that are drawn from the presented data set with respect to current clinical practice in pediatric medicine. In contrast, I believe that the data from this study is rather limited and inconclusive at this point and therefore the conclusions should be tuned down significantly. This is my reasoning:

We have toned down our conclusions as suggested. We do however believe it is important to emphasize that by being a prospective randomised trial this study provides far higher quality evidence than any previously published studies. Clinical recommendations to date are based

on extremely poor evidence – all the cohort studies have multiple very significant limitations, and the translation of the preclinical data is still hotly disputed. Thus while our trial is not definitive, when assessing the evidence in its entirety it must be regarded as far stronger than any of the existing evidence. Thus we believe that while the trial does not answer the question definitively, the trial provides the strongest evidence to date that an hour of general anaesthesia does not pose significant neurodevelopmental risk to a child. The logical implication to this is that brief general anaesthesia should not be avoided in this age group; however we are happy to leave other commentators to decide if this conclusion should be drawn or not.

(pages 4, 13-14, 17

1) *The data reports results from assessments of children at 2 years of age. However, many cognitive and behavioral abilities have not developed at that age and only assessments at a later age would be able to rule in or out any adverse effects with at some higher certainty, many of them having significant impact on socio-emotional and intellectual well-being of human life. - For instance, language formation, logical reasoning and executive functions are very rudimentary at age 2, and retrospective clinical studies suggest deficits in just these functions. Moreover, based on the early (2-year) assessment here and the rather late assessments (often at school age) used in relevant retrospective clinical studies, the presented data cannot be used to confirm or refute the data reported from that body of literature. The study's stated primary hypothesis (outcome) is that the 5-year assessments are equivalent between groups (and even that is a relatively short follow-up design but pragmatic given the difficulties in follow-up). The authors should therefore refrain from drawing any strong conclusions at this point.*

See above, text toned down accordingly.

(page 16 and Pages 4, 13-14, 17)

2) *Similarly, the assessment tools available for children in this age range allow the assessment of a very limited spectrum of neurocognitive and behavior functions, and a key portion of the presented data set is even based on parent self-reporting (Bayley-III components; all of MacArthur-Bates Inventory). Also, specific tests of the sensorium have not been applied (e.g. test of visual acuity or hearing) while primate studies suggest robust structural injury, e.g. in occipital cortex following infant anesthesia exposure.*

We agree detailed tests of vision are impossible in this age group. Pre-clinical studies have shown a very wide distribution of injury and not just in the occipital cortex. Added to text as limitation. (page 14)

3) *While following a multicenter design with many contributing sites across several Western industrialized nations, the data is based on a rather small and selected group of patients undergoing one specific surgical intervention. One example is the very high rate of caesarian section as the mode of delivery [about 50% across the two groups]) in this study sample.*

We disagree that this significantly erodes the veracity of the study. By choosing a standardised procedure this increases our capacity to detect an effect of the anaesthetic per se. A standardised anaesthetic and procedure reduces the variability and chance of random confounding that would be introduced by varying techniques or surgical procedures.

4) Another issue is that more than 80% of the study subjects are of male gender.

See above (pages 16-17)

5) The exposure to general anesthesia brief (mean of 54 min) and the invasiveness of the surgical intervention was relatively limited (herniorraphy).

See above. The exposure was brief, but probably relevant to more than half the cases performed. (pages 15-16)

6) No nitrous oxide was allowed in this study, as it is clinical practice in many institutions. However, experimental data in rodents and non-human primates have shown that while moderate doses of individual volatile anesthetics may not have an injurious potential in a specific setting, their combination with a standard dose of nitrous oxide caused widespread structural injury in the developing brains.

We respectfully disagree with the reviewer. The use of nitrous oxide is rapidly declining in clinical practice. Very few of the pre-clinical studies used nitrous oxide. We are not aware of any non-human primate studies that used nitrous oxide.

7) No other drugs were allowed in the GA group ('SEVOFLURANE only'), as it would be typical practice in almost all institutions worldwide. Infants would be co-exposed either to barbiturates or propofol, to muscle relaxants, to opioids and many other drugs.

We believe that by limiting the exposure to one agent (and an agent that clearly has an effect in pre-clinical studies) we have been able to provide a more credible and cleaner result. The impact of opioids is unclear. There is no evidence that muscle relaxants have any impact. Barbiturates are rarely if ever used in this age group. Propofol is likely to have an effect similar to sevoflurane as it acts on similar receptor populations.

We have added to the discussion about the use of other agents (page 17)

Based on the above, the authors should therefore refrain from drawing any strong conclusions at this point and rather discuss the above aspects carefully as limitations of the presented set of data. This reviewer requests that any strong statement indicating conclusions about the safety of 'general anesthesia' in neonates should be removed from the manuscript (including from the abstract and the 'research context').

See above (pages 4, 13-14, 17)

Another aspect that should be addressed throughout the manuscript is the confusing language regarding the 'primary outcome' (the study outline clearly states that the primary outcome for the GAS study is the 5-year neurobehavioral assessment results). In contrast, the

2-year outcome is stated as a 'secondary outcome'. Also, there is no hypothesis stated in the manuscript; rather the authors formulate an 'aim' at the end of the introduction. These problems should be resolved throughout the manuscript: in the introduction; methods, results and particularly in the discussion since the hypothesis (primary; secondary) should guide / and limit the interpretation of the data.

Changed. The hypothesis as stated in the published protocol is in the text. (pages 4, 6, 9, 17)

- *Abstract-background: instead of 'changes' please consider 'brain injury' or 'structural alterations'*

We do not agree that we should make this change. Many researchers do not consider the changes to reflect "injury" and some changes seen (for example to mitochondria or micro-tubules) are not structural.

- *Abstract: please clarify confusing language about primary and secondary outcomes (see above)*

Done (page 4)

- *Abstract: when talking about GA it should be clearly stated that this equals 'Sevoflurane-only anesthesia'*

We have made this clearer (page 4 and elsewhere through text)

- *Abstract: please indicate each funding entity (organized according to according to the monetary support)*

Done (page 5)

- *Research context: please mention the significant limitation of the data set (see the above summary)*

Done (pages 12-13)

- *Research context: please remove all strong statements related to clinical practice as the presented data have profound limitations and cannot rule in or out injurious potential of the 'sevoflurane-only' general anesthesia, and in particular not that of any "general anesthesia' using drug combinations, other anesthetics, longer durations, etc. Independent of that, only longer exposure-to-assessment intervals can advice valid risk assessments, even for this single-drug anesthetic.*

See above, emphasis toned down (page 13-14)

- *Introduction: please differentiate between behavioral and cognitive outcomes and organize specific references accordingly. Most experimental studies have assessed cognitive and not behavioral outcomes.*

Agree and changed (page 5)

- *Please consider adding 'later in life' after 'neurologic injury'*

We do not wish to make this change as the change would suggest the neurologic injury occurs later in life; which is incorrect.

- *Introduction: please state the hypothesis for this report; differentiate between primary and secondary hypothesis for this study.*

The hypothesis is stated in the statistical analysis section. The aims are clearer now. (page 9)

- *Methods: when and at what intervals were blood glucose levels measured; please indicate details.*

The blood glucose was measure intra-op. We have added this detail. We do not think any greater precision in timing is necessary. (page 8)

- *Please clearly indicate that the 2-year assessments where testing the secondary hypothesis in this trial.*

Done (pages 4, 6, 9, 17)

- *Please provide detail regarding the 'brief physical and neurological examination".*

. The physical examination included anthropometric measurements such as length, weight, arm and head circumference. The neurologic examination included cranial nerve examination, posture assessment and the muscle strength, tone and reflexes of the upper and lower extremities to confirm any diagnosis of CP. (page 8)

- *Statistical analysis: Again, please clarify confusing language about hypothesis; primary and secondary outcomes; etc. Please clearly state that the evaluation of the 5-year outcome is paramount and planned.*

Done (page 16)

- *Results: please indicate clearly that the data from the MacArthur-Bates scores reflect results of parent self-reporting.*

We have indicated this already in the methods and discussion (pages 8, 17)

- *Results: the ability to assess the sensorium (e.g. visual acuity) is very limited at age 2; please clearly indicate that.*

Now specifically mentioned in discussion (page 14)

- *Discussion: if 'GA' is stated it should be termed "sevoflurane-only GA"*

We have defined GA as sevoflurane only GA (pages 4, 5 and throughout text)

- *Discussion (considerations about the fit between the results of previous cohort studies with preclinical animal data): Duration of exposure; type, dose and combination of drugs used; follow-up period.*

This has been addressed elsewhere in the discussion (pages 14-15)

- *Discussion: based on the young age of the assessed children, the presented data are (still) very limited in their ability to help determining the human risk of anesthesia in infancy; this should be clearly stated and carefully discussed (see the above).*

This has been clearly stated. (page 16)

- *Discussion: there is preclinical data demonstrating that short exposures (e.g. 1 hr) to volatile anesthetics are in fact injurious in mice pups (Johnson SA & Olney JW 2008).*

Agree, however most data suggest longer exposure is more injurious. (pages 15-16)

- *Discussion: it should be stated clearly that on average up to 50% of all children receive general anesthetics for longer than 60 min. This is an important limitation of the design of this particular RCT. AT the same time it is obviously tremendously important studying shorter exposure times. This should be carefully discussed.*

We have stated this. (page 15-16)

- *Discussion: it should be clearly addressed that the vast majority of infants and children in clinical practice are exposed to a number of different anesthetics and adjuncts when undergoing 'general anesthesia'; all of which may be not only be additive in their effects but may 'unmask' toxic effects of each other (see above). It needs to be clearly stated that the 'GA' group in this study in fact represents a "sevoflurane-only" technique, an approach that is rather rare in clinical practice.*

We disagree that this is a rather rare technique. We have added that other agents may have an impact on any possible neurotoxicity, however we do this reluctantly as this discussion is speculative and goes beyond the scope of the paper. (page 17)

- *Discussion: there is also strong evidence from experimental studies (mice to non-human primates) that single exposures can produce adverse functional outcomes in young animals.*

Agree, as pointed out above

- *Conclusions: please tone down the conclusions as suggested throughout the above review.*

Done (pages 4, 13-14, 17)

- *References: please provide precise citations for pending 2015 publications.*

Added (page 32)

- *Table 2: please revise the line item "End tital sevoflurane concentration". This reviewer assumes that the numbers in brackets reflect the respective 'MAC'. Please clarify.*

No, it is end tidal concentration and not MAC

- *Table 3: can the authors please clarify the types of 'chronic illnesses' that are affecting ~15% of the included patients.*

There were a variety of chronic illnesses. We do not believe it adds to the paper to list them all. We believe it is sufficient to indicate that the incidence is balanced.

Reviewer #4: Summary:

1) Interpretation: I think that "Fifty-four minutes of exposure" is a very odd way to begin the conclusion as it is only a mean. Something like general anesthesia administration for about an hour (or precisely state that "a mean duration of 54 minutes of GA")may be a better way. Also, since this was clearly a sevoflurane based GA (and not all forms of GA in general), that needs to be specified as well.

Agree, changed (Page 4, 13, 17)

Methods:

1) *Page 7, para 3: Under exclusion criteria, what do the authors mean by "any social factor"?*

This implied that we thought it very unlikely that they would return – e.g. the parents were itinerant or moving. (page 7)

2) Page 8, para 3: Were opioids administered postoperatively? How was postoperative pain managed and was postoperative pain assessed?

Opioids were very infrequently given (see paper on apnoea). Pain was managed with acetaminophen (paracetamol) and caudal or regional local anaesthesia block. (page 8)

3) Was socio-economic status of caregivers assessed? If so, how was it precisely done? In one of the references quoted by the authors (Ref 39) validating the Bayley scale, the controls were matched for socio-economic status to "increase the strength of the study". Was anything attempted in this study?

We measure maternal education. This has been found to be a better predictor of outcome than any other measures of socioeconomic status.

Results:

1) *There is clearly a male preponderance in this study (herniae being more common in males). Did the authors do any analysis of each sex to see if there was any difference between the groups.*

No we performed no post hoc analysis to look at gender. We have addressed the gender issue as a limitation. (page 16-17)

2) *Table 4: Data of n (% of non-existing data) is not very clear. For example, where caregivers knew the arm, I cannot understand the difference between the GA Arm APP and the GA Arm ITT (106 vs 91). I think that this needs to be explained clearly.*

Thank you for spotting this transcription error. (appendix)

3) *If socio-economic status of caregivers was obtained, please provide the data.*

See above, we report the maternal education.

4) Please provide data of postoperative pain assessment and management if available. As everyone recalls, the increasing role of the use of general anesthesia in neonates and infants evolved from data showing the detrimental effects of insufficient analgesia.

We do not agree that this requires reporting as this operation is not associated with significant pain.

Discussion:

1) *Page 14, para 2: "Thus the results ... anaesthetic techniques." It is important to emphasize "for a single anaesthetic" in this sentence (even though the authors have mentioned this as a limitation elsewhere in the discussion).*

We have removed this sentence.

2) Page 15, para 2: Again "54 minutes of anesthesia" sounds odd. What about 55 minutes of anesthesia?

Agree, changed to “just under an hour” (Page 4, 13, 17)

Yours sincerely

Andrew Davidson

On behalf of all authors and the GAS consortium

Neurodevelopmental outcome at two years of age after general and awake-regional anaesthesia in infancy: a randomised controlled trial

Authors: and The GAS Consortium

1. Andrew J. Davidson MD 1,2,3
2. Nicola Disma MD 4
3. Jurgen C. de Graaff PhD 5
4. Davinia E. Withington BM 6,7
5. Liam Dorris DClinPsy 8,9
6. Graham Bell MBChB 10
7. Robyn Stargatt PhD 11,12
8. David C. Bellinger PhD 13,14,15
9. Tibor Schuster PhD 16
10. Sarah J. Arnup MBiostat 16
11. Pollyanna Hardy MSc 17
12. Rodney W. Hunt PhD 3,18,19
13. Michael J. Takagi PhD 1,12
14. Gaia Giribaldi MD 4
15. Penelope L. Hartmann BPsych(Hons) 1
16. Ida Salvo MD 20
17. Neil S. Morton MD 10,21
18. Britta S von Ungern Sternberg PhD 22,23
19. Bruno Guido Locatelli MD 24
20. Niall Wilton MBBS 25
21. Anne Lynn MD 26
22. Joss J. Thomas MD 27
23. David Polaner MD 28
24. Oliver Bagshaw FRCA 29
25. Peter Szmuk MD 30
26. Anthony R. Absalom MBChB 31
27. Geoff Frawley MBBS 1,2
28. Charles Berde MD 32
29. Gillian D Ormond MSc 1

30. Jacki Marmor MEd 13
31. Mary Ellen McCann 32

Institutions

1. Anaesthesia and Pain Management Research Group, Murdoch Childrens Research Institute, Melbourne, Australia
2. Department of Anaesthesia and Pain Management, The Royal Children's Hospital, Melbourne, Australia
3. Department of Paediatrics, University of Melbourne, Melbourne, Australia
4. Department of Anesthesia, Istituto Giannina Gaslini, Genoa, Italy
5. Department of Anaesthesia, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands
6. Department of Anesthesia, Montreal Children's Hospital, Montreal, Canada
7. Department of Anesthesia, McGill University, Montreal, Canada
8. Paediatric Neurosciences Research Group, Fraser of Allander Unit, Royal Hospital for Children, Glasgow, Scotland, UK.
9. Mental Health and Wellbeing, University of Glasgow, Glasgow, Scotland, UK
10. Department of Anaesthesia, Royal Hospital for Children, Glasgow, Scotland, UK
11. School of Psychological Science, La Trobe University, Victoria, Australia
12. Child Neuropsychology, Murdoch Childrens Research Institute, Melbourne, Australia
13. Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA
14. Department of Psychiatry, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA
15. Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
16. Clinical Epidemiology and Biostatistics Unit, Murdoch Childrens Research Institute, Melbourne, Australia
17. National Perinatal Epidemiology Unit, Clinical Trials Unit, University of Oxford, Oxford, UK
18. Department of Neonatal Medicine, The Royal Children's Hospital, Melbourne, Australia
19. Neonatal Research Group, Murdoch Childrens Research Institute, Melbourne, Australia
20. Department of Anesthesiology & Paediatric Intensive Care, Ospedale Pediatrico 'Vittore Buzzi', Milan, Italy

21. University of Glasgow, Glasgow, Scotland, UK
22. School of Medicine and Pharmacology, The University of Western Australia, Perth, Australia
23. Department of Anaesthesia and Pain Management, Princess Margaret Hospital for Children, Perth, Australia
24. Department of Anaesthesia, Ospedale Papa Giovanni XXIII, Bergamo, Italy
25. Department of Paediatric Anaesthesia and Operating Rooms, Starship Children's Hospital, Auckland District Health Board, Auckland, New Zealand.
26. Department of Anesthesiology, University of Washington, Seattle, Washington, USA
27. Department of Anesthesia, University of Minnesota, Minneapolis, Minnesota, USA
28. Children's Hospital Colorado and University of Colorado School of Medicine, Department of Anesthesiology, Aurora, Colorado, USA
29. Department of Anaesthesia, Birmingham Children's Hospital, Birmingham, UK
30. Children's Medical Centre Dallas, Department of Anesthesiology, Dallas, Texas, USA
31. Department of Anesthesiology, University Medical Center Groningen, Groningen University, Groningen, The Netherlands
32. Department of Anesthesiology, Perioperative and Pain Medicine, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA

Corresponding Author:

Andrew J. Davidson, Anaesthesia and Pain Management Research Group, Murdoch Childrens Research Institute, The Royal Children's Hospital, Flemington Road, Parkville, Victoria, 3052. Australia. Email Address: andrew.davidson@rch.org.au Phone Number: +61 (0)3 9345 4008

Summary (word count 301)

Background:

There is pre-clinical evidence that general anaesthetics affect brain development. There is mixed evidence from cohort studies that young children exposed to anaesthesia may have an increased risk of poorer neurodevelopmental outcome. This trial aims to determine if GA in infancy has any impact on neurodevelopmental outcome. The primary outcome for the trial is neurodevelopmental outcome at 5 years of age. The secondary outcome is neurodevelopmental outcome at two years of age and is reported here.

Methods:

We performed an international assessor-masked randomised controlled equivalence trial in infants less than 60 weeks post-menstrual age, born at greater than 26 weeks gestational age having inguinal herniorrhaphy. Infants were excluded if they had existing risk factors for neurologic injury. Infants were randomly assigned to awake-regional (RA) or sevoflurane-based general anaesthesia (GA). Web-based randomisation was performed in blocks of two or four and stratified by site and gestational age at birth. The outcome for analysis was the composite cognitive score of the Bayley Scales of Infant and Toddler Development, Third Edition. The analysis was as-per-protocol adjusted for gestational age at birth. A difference in means of five points (1/3 SD) was predefined as the clinical equivalence margin. The trial was registered at ANZCTR, ACTRN12606000441516 and ClinicalTrials.gov, NCT00756600.

Findings:

Between February 2007, and January 2013, 363 infants were randomised to RA and 359 to GA. Outcome data were available for 238 in the RA and 294 in the GA arms. The median duration of anaesthesia in the GA arm was 54 minutes. For the cognitive composite score there was equivalence in means between arms (RA-GA: +0·169, 95% CI -2·30 to +2·64).

Interpretation:

For this secondary outcome we found no evidence that just under an hour of sevoflurane anaesthesia in infancy increases the risk of adverse neurodevelopmental outcome at two years of age compared to RA.

Funding**:**
Australia National Health and Medical Research Council (NHMRC), Health Technologies Assessment- National Institute for Health Research UK, The National Institute of Health, The Food and Drug Administration, The Australian and New Zealand College of Anaesthetists, The Murdoch Childrens Research Institute, The Canadian Institute of Health Research, Canadian Anesthesiologists' Society, Pfizer Canada Inc, Italian Ministry of Heath, Fonds NutsOhra grant

Introduction:

There is considerable preclinical evidence describing how GA agents alter brain development in young animals.[1] This includes accelerated apoptosis and a variety of other changes including changes to dendritic morphology.[2-5] There is also evidence that exposure to GA in young animals is associated with long term cognitive and behavioural changes.[3, 6, 7] These effects have been described in a variety of species including non-human primates.[7-10] The changes are seen with several different GA agents, are greater with longer exposure and less severe in older animals.[2, 8] The clinical significance of these findings is unknown and hotly debated.[11-14]

In humans there is conflicting evidence for an association between exposure to anaesthesia in early childhood and adverse long term neurodevelopmental outcome; however confounding limits any assumption of causality.[15-30] Young children that receive anaesthesia are inevitably having surgery or an investigative procedure. Added risk of poor neurodevelopmental outcome may be due to the underlying pathology, co-morbidity or other peri-operative risk factors.

These results have prompted recommendations to consider delaying surgery in infancy and there have been several calls for more research to address this important issue.[12, 13, 31] Given the large number of potential confounding factors, a randomised trial is the best study design to determine if anaesthesia exposure in early childhood causes long term neurodevelopmental changes. Fortuitously there are two established anaesthetic techniques for inguinal herniorrhaphy in infancy; RA and sevoflurane based GA. We therefore undertook a randomised controlled trial comparing neurodevelopmental outcome in children who were randomly assigned to receive RA or sevoflurane based GA for inguinal herniorrhaphy in early infancy: the General Anaesthesia compared to Spinal anaesthesia (GAS) trial. The aim

of the trial is to determine if GA does not increase the risk of adverse neurodevelopmental outcome. The primary outcome for the overall trial will be the Wechsler Preschool and Primary Scale of Intelligence Third Edition (WPPSI-III) Full Scale Intelligence Quotient score at five years of age. As a secondary outcome we also planned *a priori* to assess neurodevelopmental outcome at 2 years of age. In this paper we report all secondary outcomes at two years of age. Data from the trial relating to post- anaesthesia apnoea and success of regional block have been published elsewhere.[32, 33]

Methods:

Study design:

The GAS trial is a prospective, observer blind, international multi-site, randomised, controlled, equivalence trial examining RA versus GA in infants undergoing inguinal herniorrhaphy. The trial was performed at 28 hospitals in Australia, Italy, The USA, The UK, Canada, The Netherlands and New Zealand. Institutional Review Board or Human Research Ethics Committee approval was obtained at each site and written consent obtained from the child's parents or guardians. The protocol has been previously published at http://www.thelancet.com/protocol-reviews/09PRT-9078

Participants:

Eligibility criteria included infants up to 60 weeks' postmenstrual age scheduled for unilateral or bilateral inguinal herniorraphy born at greater than 26 weeks gestation. Exclusion criteria included any contraindication for either anaesthetic technique, a history of congenital heart disease requiring surgery or pharmacotherapy, mechanical ventilation immediately prior to surgery, known chromosomal abnormalities or other known acquired or congenital abnormalities which might affect neurodevelopment, previous exposure to volatile GA or benzodiazepines as a neonate or in the third trimester *in utero*, any known neurologic injury such as cystic peri-ventricular leukomalacia or grade three or four intra-ventricular haemorrhage (IVH), any social or geographic factor that may make follow up difficult (such as planned house move, homelessness, no telephone communication available), or having a primary language at home in a region where neurodevelopmental tests are not available in that language. Eligible infants were identified from operating room schedules or at pre-

admission clinics and recruited in the clinic or in the preadmission areas of the operating floor.

Randomisation and Masking:

A 24-hour web-based randomisation service was managed by The Data Management & Analysis Centre, Department of Public Health, University of Adelaide, South Australia. Participants were randomised with a 1:1 allocation ratio to either GA or RA. Randomisation was performed in blocks of two or four and stratified by site and gestational age at birth: 26 to 29 weeks and six days, 30 to 36 weeks and six days and 37 weeks or more. The anaesthetist was aware of group allocation. Parents were not informed of the group allocation but were told if they asked. The psychologists and paediatricians performing the assessment were masked to group allocation. Once their assessment was completed they were asked to indicate if they were aware of group allocation.

Procedures:

The RA group received either an awake-spinal anaesthetic, an awake-caudal anaesthetic, or a combined spinal-caudal anaesthetic according to institutional protocols. Spinal anaesthesia was performed with 0·2 ml/kg 0·5% isobaric bupivacaine with a minimum volume of 0·5ml. Due to unavailability of isobaric bupivacaine at some sites other agents were used (in the US, 0·13ml/kg of hyperbaric 0·75% bupivacaine and in the UK 0·2 ml/kg 0·5% levobupivacaine). Caudal anaesthesia was performed with up to a total dose of 2·5 $mg.kg^{-1}$ of 0·25% bupivacaine. In the UK 0·25% levobupivacaine was used. In the US if surgery was likely to take greater than one hour, some patients were given a loading dose of 3% chloroprocaine (1ml/kg in divided doses of no more than 0·25ml/kg per 15 seconds) via a caudal cannula and then an infusion of 1-2 ml/kg/hr. Ilioinguinal and field blocks could also be done. The total dose of bupivacaine did not exceed 2·5 mg/kg. In the RA group oral sucrose was used to settle the child if required and all other forms of sedation avoided. If the RA was ineffective then a GA was performed with sevoflurane, and if the child became unsettled intra-operatively sevoflurane was administered to supplement the RA. Both were regarded as protocol violations.

The GA group received sevoflurane for induction and maintenance in an air/oxygen mix. The concentration of sevoflurane was left to the discretion of the anaesthetist, as was choice of airway device, ventilation technique and use of any neuromuscular blocking agents. No

opioid or nitrous oxide was allowed. A caudal, ilioinguinal-iliohypogastric and/or field block with bupivacaine could be performed in both groups to provide post-operative analgesia. Oral or intra venous acetaminophen could also be given. Heart rate, blood pressure, oxygen saturation and (where applicable) expired sevoflurane concentrations were recorded every five minutes.

Serum glucose was measured after anesthetic induction. There were rescue protocols for hypoglycaemia, hypotension and hypoxemia. If the blood pressure fell >20% below baseline an intravenous bolus fluid was administered and vasoactive drugs given if deemed necessary. Hypoglycaemia (blood sugar <3·0mmol/L) was treated with a bolus of 5ml/kg of 10% dextrose. Oxygen by face mask in the RA arm and an increased FiO_2 in the GA arm was used at the discretion of the anaesthetist to maintain arterial oxygen saturation > 95%.

Two Year Assessments:

Assessments were performed within two months either side of two years of age (corrected for prematurity). The assessment took approximately two hours to complete. A trained psychologist administered the Bayley-III. [34] The Bayley-III has cognitive, language and motor scales. The cognitive scale includes tasks assessing attention, memory, sensorimotor development, exploration, concept formation, and simple problem solving. The language scale assesses expressive and receptive skills, and the motor scale assesses fine and gross motor skills. Parents completed the Bayley-III Social-Emotional and Adaptive Behavior Questionnaires and the MacArthur-Bates Communicative Development Inventory: Words and Sentences (MacArthur-Bates).[35] The MacArthur-Bates is a parent informant measure that assesses expressive language in children aged 16-30 months of age. Demographic data, family history, and medical history were also noted, and a brief physical and neurological examination was performed. The physical examination included anthropometric measurements such as length, weight, arm and head circumference. The neurologic examination included cranial nerve examination, posture assessment and the muscle strength, tone and reflexes of the upper and lower extremities.

All study data were sent to the Murdoch Childrens Research Institute in Melbourne, Australia. All forms were checked for data quality by trained research assistants and double checked by a research assistant who was not involved in the primary data collection or entry.

An independent data safety monitoring committee met at six monthly intervals during recruitment. Summary data by allocation were presented to the committee. There were no formal interim analyses of neurodevelopmental outcome.

Statistical Analysis:

The main outcome for the analysis at 2 years of age was pre-specified to be the composite cognitive score of the Bayley-III. The hypothesis (as stated in the protocol) was that the composite cognitive score of the Bayley-III measured at two years of age in infants who are anaesthetised for inguinal herniorraphy is equivalent when using GA compared with RA. The components of the Bayley-III are reported as scaled scores and as composite scores. The five composite scores (Cognitive, Language, Motor, Adaptive Behaviour, and Social-Emotional Scales) are standardised to have a mean of 100 and a SD of 15 in the reference population. The sub-scales (e.g., fine motor scale) are reported as scaled scores, with a mean of 10 and a SD of 3. The other secondary outcomes for this analysis are the language, motor, social-emotional, and adaptive behaviour scores from the Bayley-III and the age-adjusted Vocabulary Production Score from the MacArthur-Bates. Published normative scores were used at all sites with forms and instructions translated locally. Diagnosis of cerebral palsy was another pre-specified secondary outcome

Since this is an equivalence study, the outcome was analysed on an APP basis to ensure a conservative estimate in the direction of non-equivalence. Equivalence was defined *a-priori* if the 95% confidence interval of the difference in means lies within -five and +five points. ITT analyses were also planned. Analyses were adjusted for categories of gestational age at birth (182-209 days; 210-258 days; ≥259 days)

The sample size was based on the primary outcome for the GAS trial; the five year follow up WPPSI-III Full Scale IQ score. Assuming an expected difference of one standardised score point, and a 90% chance that a 95% confidence interval will exclude a difference of more than five points (the largest difference acceptable to demonstrate equivalence), the trial would need 598 infants in total. Enrolling approximately 720 participants would allow for 10% loss to follow-up and 10% with a major protocol violation.

Multiple imputation using chained equations was used to impute missing outcome data in the analysis of all outcomes.[36] The following pre-specified variables were used as predictor

variables within the imputation approach: anaesthesia group, country, gender, gestational age at birth, standardized z-score for birth weight, mother received antenatal steroids, mother diagnosed with chorioamnionitis, IVH, maternal age, maternal education, rescue glucose given intra-venously, need for fluid bolus for hypotension, vasoactive drugs given for hypotension, duration of surgery, dose of sevoflurane (concentration x hours), significant post-operative apnoea, corrected age at assessment, any more anaesthetic exposures since the inguinal herniorraphy, any malformations, any chronic illness, any prescribed medication for two months or longer, total length of any readmission to hospital, any interventions for neurodevelopmental problems, diagnosis of cerebral palsy, any other neurological abnormality.

For the purpose of sensitivity analysis, effect estimates were computed using best and worst case imputation scenarios. Furthermore, effect estimates and confidence intervals based on inverse probability of censoring weighting were reported.[37]

Risk ratios with 95% confidence intervals were reported for the proportion of individuals that fall below one and two SDs of the composite cognitive score. Risk ratios were generated using generalized linear models for a binomial distributed response variable employing a log link (binomial log-linear regression). These analyses were not pre-specified in the study protocol (*post hoc* analyses). All analyses were carried out in Stata 13 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

The GAS trial is registered in Australia and New Zealand at ANZCTR: ID# ACTRN12606000441516 first registered on 16th October 2006; in the United States (US) at ClinicalTrials.gov: ID#: NCT00756600 first registered on 18th September 2008; and in the United Kingdom (UK) at UK Clinical Research Network (UKCRN) ID#: 6635 (ISRCTN ID#: 12437565; MREC No: 07/S0709/20).

Role of the Funding Source:
The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had access to the data, and AJD, GO and Suzette Sheppard were responsible for submitting the manuscript. AJD made the final decision to submit the paper for publication.

Results

Seven hundred twenty-two infants were recruited into the trial between February 9, 2007, and January 31, 2013 from 28 centres in Australia, the US, the UK, Italy, the Netherlands, Canada and New Zealand (appendix). There were two mis-randomisations and one withdrawal of consent leaving 361 in the ITT analysis in the RA arm and 358 in the GA arm. Table 1 summarises demographic data for each arm at baseline and table 2 summarises demographic data at two years. There were 74 protocol violations in the RA arm (five due to surgery being cancelled and 69 receiving some sevoflurane or other GA) and two violations in the GA arm (surgery cancelled).

Follow up was from March 5, 2009 to March 6, 2015. Forty-seven families were lost to follow up in the GA arm and 52 in the RA arm. Of those lost to follow up some reason for non-attendance was gained in 19 and in only one case was non-attendance due to developmental delay (this child was in the RA arm). Of those that attended for assessment, the cognitive scale of the Bayley-III was completed by 292 in the RA arm and 295 in the GA arm (Figure 1). Very few children were unable to complete the Bayley-III due to developmental delay or other recognised reasons for cognitive impairment. In 97% of cases the psychologist and paediatrician were unaware of group allocation at the time of assessment. (appendix)

The Bayley-III Cognitive, Language, Motor, Social-Emotional and Adaptive Behaviour scores, and the MacArthur-Bates data are summarised for each group in table 3.

For the Cognitive Composite score there was evidence for equivalence in means between RA and GA arms in both the APP and the ITT analyses using multiple imputation to account for missing outcome data (RA-GA: +0·169, 95% CI -2·30 to +2·64; and RA-GA: +0·256, 95% CI -2·06 to +2·57 for APP and ITT respectively). These results were consistent with the findings of the complete case analyses (RA-GA: +0·458, 95% CI -2·02 to +2·94; and RA-GA: +0·430, 95% CI -1·90 to +2·76, for APP and ITT respectively). There was also evidence for equivalence between arms in the Composite Motor scores, Composite Language scores and the Composite Adaptive Behavior scores (Table 4). The results were consistent in both APP and ITT analyses, and when using complete case and multiple imputation. With mean differences of one and two score points (multiple imputation and complete case analysis for APP/ITT) and upper 95% confidence interval limits exceeding the pre-specified five point

equivalence margin, evidence for equivalence with regard to the Social-Emotional Composite scale of the Bayley-III was not compelling. There was no evidence for a difference between groups in MacArthur-Bates scores (Table 4).

The results of the inverse probability weighting and worst case imputation scenarios for missing data are presented in the appendix. The worst case scenario results represent theoretical boundaries to what extent the actual effect estimates could have been affected by selective dropout. However, both multiple imputation analysis as well as inverse probability weighting demonstrated consistent robustness of the study findings with regard to data missingness.

Overall a low number of children had a diagnosis of cerebral palsy, hearing or visual impairment or specific behavioural diagnoses such as autistic spectrum disorder (ASD) (table 5). The event rate was too low for any meaningful comparative analysis. There was no evidence for a difference between arms in the proportion of children one or two SDs below the age mean on the cognitive composite score (appendix).

Details of adverse events during and immediately after anaesthesia have been reported in the earlier publication.[32]

Research in context

Evidence before this study

Medline and Cochrane controlled trial register were searched (search last done 18th September 2015) for original research and meta-analyses describing the association between anaesthesia exposure in early life and neurodevelopmental outcome. Combinations of search terms “anesthesia”, and “child development”, or “learning disorders” were used. The search revealed no randomised trials but several cohort studies. There have been numerous reviews that have concluded that there is an association between anaesthesia in childhood and neurodevelopmental outcome.[19, 31] There have been two meta-analyses that have found evidence for an association between anaesthesia in children and a range of neurodevelopmental outcomes.[16, 30] All reviews and meta-analyses acknowledge the weaknesses of the cohort studies; including strong likelihood of confounding, bias, heterogeneous populations at times of exposure, and heterogeneous outcome measures, some

of which are poorly defined or insensitive. All conclude that causation cannot be established or excluded.

Added value of this study

We report a secondary outcome from the first randomised controlled trial assessing the impact of general anaesthesia in infancy on neurodevelopmental outcome. Using the best measure of neurodevelopment available for assessing a two year old child, strong evidence for equivalence between awake-regional and just under an hour of general anaesthesia was found. However it should be noted that this was an analysis of a secondary outcome with the primary outcome planned at five years of age, and given the limited sensitivity of developmental assessment at two years of age, this trial does not provide the definitive answer.

Implications of all the available evidence

Although there are some limitations that should be noted when interpreting the trial, the randomised prospective design adds significantly to the weight that should be given to the results compared to the mixed results found in previous cohort studies. It should however be emphasised that reassessment at an older age is necessary before definitive conclusions can be drawn. The trial does not rule out the possibility that longer, or multiple exposures to anaesthesia in early childhood may cause neurodevelopmental changes. Further research is needed to address these questions.

Discussion

In this trial we found strong evidence for equivalence between RA and GA in infancy in terms of neurodevelopmental outcome at two years of age. Equivalence was demonstrated in multiple domains of neurodevelopmental assessment and the 95% confidence intervals fell within a third of a SD; well inside our pre-defined boundaries of clinical equivalence.

There are no previous randomised trials examining the effect of anaesthesia in infancy on long term neurodevelopmental outcomes (see research in context panel). Previous cohort studies have found mixed results.[19] Some studies have found an association between

exposure to anaesthesia in early childhood and increased risk of poor neurodevelopmental outcome.[16-18, 20-24, 28] Although this association fits with preclinical animal data, it may also be explained by the confounding effects of surgery, pathology or co-morbidity. Conversely some cohort studies have found no evidence for an association.[25-27] These studies have limited ability to rule out a link between anaesthesia and neurodevelopmental outcome due to a reliance on outcome measures, such as school grade, which may not detect subtle effects, or their broad inclusion criteria include children exposed to anaesthesia at an older age where the risk may be less. The heterogeneity of the cohort studies also make it difficult to analyse the effects of duration of exposure, type of anaesthetic drugs used, doses or combination of drugs used. The above limitations inherently limit the capacity for cohort studies to determine the link between exposure to anaesthesia and neurodevelopmental outcome. These limitations highlight the importance of methodologically robust and adequately powered trials such as this trial.[31]

In this analysis we chose the cognitive scale of the Bayley-III as the main outcome of interest. Changes seen in preclinical studies tend to be diffusely distributed over several brain regions. Such diffuse changes are most likely to have an impact on general cognition. Of note, there was also no evidence for a difference in any of the other Bayley-III domains.

Two recent studies have found that while children exposed to anaesthesia had similar school grades, those exposed had an increased risk of not sitting the tests.[26, 28] This raises the possibility that a sub-population of exposed children may have significant neurodevelopmental delay. To investigate this possibility we compared the proportion of children in each arm that scored two standard deviations below the age mean on the composite cognitive score. There was no evidence for a difference; however given the limited power of this analysis, equivalence cannot be assumed. We have also reported the number of children with the diagnosis of ASD, cerebral palsy and visual or hearing defects. This trial was not powered to detect differences in these diagnoses or events, and as expected we found a low event rate in both arms. It should also be noted that at two years of age it is difficult to accurately diagnose the presence of disorders such as ASD, or to accurate assess vision and hearing, and it is possible some children may still have undiagnosed neurologic or neurobehavioural disorders.

Most pre-clinical studies suggest that prolonged exposure to GA is required before injury is seen, usually at least two to three hours.[8] However changes have been seen with one hour of exposure.[38] In this trial the median sevoflurane exposure was 54 minutes in the GA arm and hence the results are consistent with the majority of pre-clinical data. The trial is an important adjunct to these data as translating doses and exposures from animal to humans is uncertain, and it is possible that shorter duration of exposure may still have clinically relevant effects that cannot be detected in animal models.

In human cohorts some studies have found an association with a single short exposure. [17, 39] Others studies have only found an association after longer or multiple exposures.[22] This study that found there was no increase in learning disabilities in infants and toddlers exposed to two or less hours of GA.[22] This study revealed that anaesthetic exposure was less than 90 minutes in 61% of the exposed patients and less than two hours in 85% of the exposed patients highlighting that the vast majority of anaesthetics in young children are of fairly brief duration. An internal audit of anaesthetic duration in infants at Boston Children's Hospital revealed that 53% of anaesthetics done in babies less than12 months of age were less than two hours duration. Thus, as far as duration of exposure, it is likely our results are pertinent to approximately half the anaesthetics delivered to infants.

The finding of equivalence after short exposure does not rule out the possibility that longer exposure to anaesthetics may have an effect on neurodevelopment. Further trials are required before any assumptions can be made about the impact of prolonged anaesthesia exposure in infancy.

Some studies have also found a stronger association between multiple anaesthesia exposures and adverse outcome than with single exposure.[20, 30] It is possible this reflects a greater effect of confounding; inevitably children having multiple procedures are more likely to have significant conditions or chronic disease. Our trial cannot address the possible increased toxicity with multiple exposures.

There are a number of limitations to our trial. RA inevitably has a failure rate. As this was an equivalence trial we took the APP analysis to be the most conservative analysis – assuming that treatment failure would bias toward no difference. Given the possibly contentious nature of this assumption, we planned *a priori* to perform a secondary ITT analysis. There were no

measureable differences between APP and ITT analyses, implying no bias was introduced by treatment failure. In this study there was a loss to follow up of almost 14%. This, along with RA failure lead to an appreciable amount of missing data, however both the multiple imputation analysis and the inverse probability weighting demonstrated consistent robustness of the findings.

Another limitation is that while the Bayley-IIII is a well validated assessment tool of current development, early neurobehavioural assessment of children is not a perfect predictor of long term outcome due to the considerable variability in developmental timing in young children. Whilst the Bayley-III has been shown to have a stronger correlation with IQ at age five years than earlier versions of the test, it was not designed to assess a broad range of cognitive functions. Cognitive skills emerge and differentiate over childhood and a more detailed neuropsychological assessment is required at a later date to identify mild or circumscribed deficits in cognitive functions as executive skills and memory.[40 41] It is thus important that the children be reassessed later in their development to confirm the results and to more thoroughly examine multiple domains of cognition. Children in this trial are undergoing assessment at five years of age and the results should be known after 2018.

It is important to note that this manuscript reports the results of a secondary outcome. The primary outcome is planned at 5 years, for the reason mentioned above. This analysis of the secondary outcome was pre-specified in the study protocol, however the study was not specifically powered for the secondary outcome and thus it should be interpreted with caution and not regarded as definitive. The analysis of the secondary outcome was planned due to the recognition that there was growing concern over the issue of neurotoxicity and existing evidence to guide practice was inherently limited, and while the two year assessment was not definitive, it would still provide higher quality evidence than that which existed to date. The two year assessment was also planned due to concerns over the feasibility of maintaining the cohort for the longer term follow-up.

In this study over 80% of participants were male. It is well recognised that gender can have an impact on recovery from brain injury. The effect is variable and depends on the nature of the injury and outcome measured, though generally greater effects are seen in males and indeed the neurotoxic effect of anaesthesia on rodents has been shown to be greater in

males.[42] Thus the finding of equivalence in our trial with a preponderance of males makes it unlikely that equivalence would not also be demonstrated in females.

In this trial sevoflurane was used without other general anaesthetics. We chose a sevoflurane only anaesthetic as this reflects common practice for anaesthesia for inguinal herniorrhaphy, and the preclinical effects of sevoflurane have been clearly described. There are some preclinical studies that suggest combinations of general anaesthetics may be more injurious and thus our trial cannot shed light on the possibility that an effect may be seen if other agents are added. [3]

Lastly it should also be noted that the MacArthur-Bates is dependent on parental report and hence may be open to bias. In addition the standardisation data is of varying degrees of validation across different languages.

In conclusion, this trial found strong evidence that exposure of just under an hour to a sevoflurane GA in infancy does not increase the risk of adverse neurodevelopmental outcome at two years of age. While not definitive, this is the strongest clinical evidence to date that just under an hour of sevoflurane GA in infancy does not result in significant neurotoxicity.

(word count 4493)

Authors' contributions:

AJD was involved in study design and concept, conduct, data coordination, contribution to the statistical analysis plan, data interpretation, writing and coordinating drafts of the manuscript and revising it critically and approving the version to be published. ND was involved in study design and conduct, data acquisition and coordination, data interpretation and revising the manuscript critically. JCdeG was involved in the coordination and supervision of data collection, data analyses and interpretation, contribution to the statistical analysis plan, revised the manuscript and approved the final manuscript as submitted. DEW was involved in study design and conduct, data acquisition and coordination, data interpretation and revising the manuscript critically. LD contributed to protocol development, data collection, statistical plan, statistical analysis, data interpretation and writing of the manuscript. GB was involved in study conduct, data coordination and writing and reviewing the manuscript. RS was the lead neuropsychologist and along with DCB and RWH was

involved in study design, concept, conduct, data interpretation and critically revising the manuscript. TS and SJA were involved in interim analyses, contribution to the statistical analysis plan, data interpretation, and revising the manuscript critically. PH was involved in study design, study conduct, interim analyses, contribution to the statistical analysis plan, data interpretation and editing of the manuscript. MJT contributed to the statistical analysis, data interpretation, and manuscript preparation. GG and PLH were involved in study conduct, data acquisition, data interpretation and revising the manuscript critically. IS, BSvonUS, BGL, NW, AL, JTT, DP, OB, PS, ARA and JM were involved in study conduct, data acquisition and coordination and revising the manuscript critically. NSM and MEMcC was involved in study design, concept and conduct, data coordination, data interpretation, writing the manuscript and revising it critically. GF and CB was involved in study design and concept, study conduct, data acquisition, contribution to data interpretation and revising the manuscript critically. GDO was involved in study conduct, data acquisition and coordination, contribution to the statistical analysis plan and revising the manuscript.

Declaration of Interests:

We declare no competing interests.

Disclaimer:

The views expressed in this publication are those of the author(s) and not necessarily those of the MRC, NHS, NIHR or the Department of Health UK.

GAS Trial Group

Trial Steering Committee

Prof Paul Myles (chair); Members – AJD, MEMcC, NSM, ND, DEW, JCdeG, GF, RWH, DCB, CB, Prof Andy Wolf, Prof Neil McIntosh, Prof John Carlin, Prof Kate Leslie,

Data Monitoring Committee

Dr Jonathan de Lima (chair), Prof Greg Hammer, Prof David Field, Prof Val Gebski, Prof Dick Tibboel

GAS Consortium

Australia:

Andrew J. Davidson and Geoff Frawley (Department of Anaesthesia and Pain Management, Murdoch Childrens Research Institute and The Royal Children's Hospital and University of Melbourne, Melbourne, Australia); Pollyanna Hardy (National Perinatal Epidemiology Unit, Clinical Trials Unit, University of Oxford, Oxford, UK); Sarah J. Arnup, Tibor Schuster and Katherine Lee (Clinical Epidemiology and Biostatistics Unit, Murdoch Childrens Research Institute, Melbourne, Australia); Rodney W. Hunt (Department of Neonatal Medicine, The Royal Children's Hospital and Murdoch Childrens Research Institute and University of Melbourne, Melbourne, Australia); Robyn Stargatt (School of Psychological Science, La Trobe University and Child Neuropsychology, Murdoch Childrens Research Institute, Melbourne, Australia); Suzette Sheppard, Gillian D. Ormond, Penelope L. Hartmann, Michael J. Takagi, Stephanie Malarbi and Melissa Doyle (Department of Anaesthesia and Pain Management, Murdoch Childrens Research Institute, Melbourne, Australia); Philip Ragg (Department of Anaesthesia and Pain Management, The Royal Children's Hospital, Melbourne, Australia); Marie Backstrom (Department of Anaesthesia, Monash Medical Centre, Melbourne, Australia); David Costi (Paediatric Anaesthesia, Women's and Children's Hospital, Adelaide, Australia); Britta S. von Ungern-Sternberg (Department of Anaesthesia and Pain Management, Princess Margaret Hospital for Children and The University of Western Australia, Perth, Australia)

New Zealand:

Niall C. Wilton, and Graham Knottenbelt (Department of Paediatric Anaesthesia and Operating Rooms, Starship Children's Hospital, Auckland, New Zealand)

Italy:

Nicola Disma, Giovanni Montobbio, Leila Mameli, Pietro Tuo, and Gaia Girbaldi (Department of Anesthesia, Istituto Giannina Gaslini, Genoa, Italy); Alessio Pini Prato (Department of Pediatric Surgery, Istituto Giannina Gaslini, Genoa, Italy); Girolamo Mattioli (DINOGMI University of Genoa, Genoa, Italy); Andrea Wolfler, Francesca Izzo, and Ida Salvo (Department of Anesthesiology & Paediatric Intensive Care, Ospedale Pediatrico 'Vittore Buzzi', Milan, Italy); Valter Sonzogni, Bruno Guido Locatelli, and Magda Khotcholava (Department of Anaesthesia, Ospedale Papa Giovanni XXIII, Bergamo, Italy)

The Netherlands:

Jurgen C. de Graaff, Jose T.D.G van Gool, Sandra C. Numan, D. Judy S. van Veldhuijzen and Cor J. Kalkman (Department of Anesthesiology, Wilhelmina Children's Hospital,

University Medical Centre Utrecht, Utrecht, The Netherlands); Anneloes L. van Baar and Leonie J.P. Steenis (Utrecht Centre for Child and Adolescent Studies. Utrecht University**,** Utrecht, The Netherlands); J.H.M. Hagenaars, Anthony R. Absalom, Frouckje M. Hoekstra, and Martin Volkers (Department of Anesthesiology, University Medical Center Groningen, Groningen University, Groningen, The Netherlands)

Canada:

Davinia Withington (Department of Anesthesia, Montreal Children's Hospital and McGill University, Montreal, Canada); Koto Furue (Département d'Anesthésie, Centre Hospitalier Universitaire Sainte-Justine, Montreal, Canada); Josee Gaudreault (Montreal Children's Hospital, Montreal, Canada)

USA:

Mary Ellen McCann, Charles Berde, Sulpicio Soriano, Vanessa Young, Navil Sethna, Pete Kovatsis, and Joseph Cravero (Department of Anesthesiology, Perioperative and Pain Medicine, Children's Hospital Boston, Boston, USA); David Bellinger and Jacki Marmor (Department of Neurology, Children's Hospital Boston, Boston, USA); Anne Lynn, Iskra Ivanova, Agnes Hunyady, and Shilpa Verma (University of Washington, Seattle Children's Hospital, Department of Anesthesia and Pain Medicine, Seattle, USA); David Polaner (Children's Hospital Colorado and University of Colorado, Department of Anesthesiology, Colorado, USA); Joss Thomas, Martin Mueller, and Denisa Haret (The University of Iowa Hospital, Department of Anesthesia, Iowa, USA); Peter Szmuk, Jeffery Steiner, and Brian Kravitz (Children's Medical Centre Dallas, Department of Anesthesiology, University of Texas Southwestern Medical Center, Dallas and Children's Medical Center at Dallas and Outcome Research Consortium, Texas, USA); Suresh Surantham (Department of Pediatric Anesthesiology, Ann & Robert H Lurie Children's Hospital of Chicago, Northwestern University, Illinois, USA); Stephen Hays (Pediatric Anesthesia, Monroe Carell Jr. Children's Hospital at Vanderbilt, Tennessee, USA); Andreas Taenzer (Dartmouth-Hitchcock Medical Center, Department of Anesthesiology, New Hampshire, USA); Lynne Maxwell (Department of Anesthesiology and Critical Care Children's Hospital of Philadelphia, Philadelphia, USA); Robert K. Williams (Anesthesia and Pediatrics, College of Medicine, University of Vermont, Vermont Children's Hospital, Vermont, USA)

UK:

Neil S. Morton (University of Glasgow and Department of Anaesthesia, Royal Hospital for Sick Children, Glasgow, Scotland, UK); Graham Bell (Department of Anaesthesia, Royal

Hospital for Sick Children, Glasgow, Scotland, UK); Liam Dorris and Claire Adey (Paediatric Neurosciences Research Group, Fraser of Allander Unit, Royal Hospital for Children, Glasgow, Scotland, UK); Jaycee Pownall (Institute of Health and Wellbeing, University of Glasgow, Glasgow, Scotland, UK); Oliver Bagshaw (Anaesthetic Department, Birmingham Children's Hospital, Birmingham, UK); Anthony Chisakuta (Department of Anaesthetics, Royal Belfast Hospital for Sick Children, Belfast, Northern Ireland, UK); Ayman Eissa (Anaesthetic Department, Sheffield Children's Hospital, Sheffield, UK); Peter Stoddart (Bristol Royal Hospital for Children, Bristol, UK);Annette Davis (Alder hey Childrens' NHS Foundation Trust, Liverpool, UK)

Acknowledgements:

Mark Fagjman (Department of Anaesthesia, Monash Medical Centre, Melbourne, Australia); Daniela Tronconi (Department of Anesthesia, Istituto Giannina Gaslini, Genoa, Italy); David C. van der Zee (Department of Pediatric Surgery, Wilhelmina Children's Hospital, University Medical Centre Utrecht, The Netherlands); Jan Hulscher (Department of Surgery, University Medical Center Groningen, Groningen University, Groningen, The Netherlands); Michael Rivkin (Department of Neurology, Children's Hospital Boston, Boston, USA); Michelle Sadler-Greever (University of Washington, Seattle Children's Hospital, Department of Anesthesia and Pain Medicine, Seattle, USA); Debra Faulk (Children's Hospital Colorado and University of Colorado School of Medicine, Department of Anesthesiology, Colorado, USA); Greta Wilkening (Neurosciences Institute, Children's Hospital Colorado, Colorado, USA); Edward Goldson (Department of Pediatrics, Division of Developmental-Behavioral Pediatrics, Children's Hospital Colorado, Colorado, USA). Danai Udomtecha andSarah Titler (The University of Iowa Hospital, Department of Anesthesia, Iowa, USA); Roxana Ploski and Alan Farrow-Gilespie (Children's Medical Centre Dallas, Department of Anesthesiology, University of Texas Southwestern Medical Center, Dallas and Children's Medical Center at Dallas and Outcome Research Consortium, Texas, USA); Timothy Cooper (Division of Developmental Medicine & the Center for Child Development, Monroe Carell Jr. Children's Hospital at Vanderbilt, Tennessee, USA); Elizabeth Card (Perioperative Clinical Research Institute, Vanderbilt University Medical Center, Tennessee, USA); Wendy Boardman (Dartmouth-Hitchcock Medical Center, Department of Anesthesiology, New Hampshire, USA);Theodora K. Goebel (Department of Anesthesiology and Critical Care Children's Hospital of Philadelphia, Philadelphia, USA)

Funding

All hospitals and centres were generously supported by departmental funding. In addition to this funding, specific grants received for this study are as follows:

Australia: The Australian National Health & Medical Research Council (Project Grants #1002906 and 491226); Australian and New Zealand College of Anaesthetists (Project Grant #11/021 and 07/012); The Murdoch Childrens Research Institute. This study was supported by the Victorian Government's Operational Infrastructure Support Program.

US: National Institute of Health (Project Grant # NIH 1-R01 HD06 1136-01A1); Food and Drug Administration (Project Grant #FDA-SOL-08-SAFEKIDS-Clin 002-Project 2)

Italy: Italian Ministry of Heath, Young Researchers Grant (Project Grant #167/GR-2009-1476067)

Netherlands: Fonds NutsOhra grant #1305-144 and Vrienden WKZ 16.13.038

Canada: Canadian Institute of Health Research (Project Grant #MCT-98031), Canadian Anesthesiologists' Society, Pfizer Canada Inc.

UK: Health Technologies Assessment- National Institute for Health Research UK (Project Grant # 07/01/05)

Title Figure 1: Trial profile

Table 1: Descriptive statistics of birth, pregnancy and peri-anaesthesia data

	RA Arm APP (N=287)	**GA Arm APP (N=356)**	**RA Arm ITT (N=361)**	**GA Arm ITT (N=358)**
Baseline demographics				
Gender, Male	232 (81%)	304 (85%)	294 (82%)	306 (86%)
Chronological age at surgery (days)	68·9 (31)	71·1 (32)	70·1 (32)	71·0 (32)
Post menstrual age at surgery (days)	317·2 (32)	319·7 (32)	318·3 (33)	319·5 (32)
Weight of child at surgery (kg)	4·2 (1·1)	4·3 (1·1)	4·2 (1·1)	4·3 (1·1)
Pregnancy and birth details				
Mean (SD) Post menstrual age at birth (days)	248·2 (29)	248·6 (27)	248.3 (29)	248.6 (27)
Prematurity (Born < 37 weeks gestation)	160 (56%)	195 (55%)	198 (55%)	196 (55%)
Birth Weight (kg)	2·3 (0·9)	2·3 (0·9)	2·4 (0·9)	2·3 (0·9)
Z score for birth weight	-0.68 (1·3)	0.69 (1·3)	-0.66 (1·2)	-0.69 (1·3)
Median (IQR) Apgar score at 1 minute	9 (7-9)	8·5 (7-9)	9 (7-9)	9 (7-9)
Median (IQR) Apgar score at 5 minutes	9 (9-10)	9 (9-10)	9 (9-10)	9 (9-10)
One of a multiple pregnancy	52 (18%)	61 (17%)	62 (17%)	62 (17%)
Mother received partial course antenatal steroids	16 (6%)	19 (5%)	20 (6%)	19 (5%)
Mother received complete course antenatal steroids	95 (33%)	98 (28%)	114 (32%)	98 (28%)
Mother diagnosed with chorioamnionitis	10 (4%)	12 (3%)	11 (3%)	12 (3%)
Prolonged rupture of the membranes (>24 hours)	28 (10%)	34 (10%)	32 (9%)	34 (10%)
Mother diagnosed with pre-eclampsia	50 (17%)	68 (19%)	60 (17%)	68 (19%)
Sepsis during pregnancy	36 (13%)	50 (14%)	43 (12%)	50 (14%)
Mode of delivery of birth				
Cephalic vaginal	135 (47%)	157 (44%)	169 (47%)	157 (44%)
Breech vaginal	1 (<1%)	6 (2%)	3 (1%)	6 (2%)
Compound vaginal	2 (1%)	4 (1%)	3 (1%)	4 (1%)
Caesarean section	149 (52%)	189 (53%)	185 (51%)	191 (53%)
Caesarean section and mother went into labour	42 (15%)	58 (16%)	52 (14%)	59 (16%)
Mother exposed to nitrous oxide during delivery	48 (18%)	62 (18%)	61 (18%)	62 (18%)
IVH	7 (2%)	6 (2%)	8 (2%)	6 (2%)
IVH Grade 1	5 (2%)	6 (2%)	5 (2%)	6 (2%)
IVH Grade 2	2 (1%)	0	2 (1%)	0
Retinopathy of prematurity	17 (9%)	16 (6%)	30 (8%)	16 (6%)
Hearing defects detected by perinatal screening	7 (3%)	10 (3%)	8 (3%)	10 (3%)
PDA diagnosed	23 (8%)	21 (6%)	27 (8%)	21 (6%)
PDA never treated	9 (3%)	9 (3%)	11 (3%)	9 (3%)
PDA treated with non-steroidal anti-inflammatory drugs	14 (5%)	10 (3%)	16 (4%)	10 (3%)
Familial Demographics:				
Primary language(s) only spoken*	252 (88%)	305 (86%)	311 (86%)	307 (86%)
Maternal Age at Birth >21	273 (96%)	339 (95%)	339 (95%)	341 (95%)
Family structure two caregivers together, at birth	261 (91%)	324 (91%)	328 (91%)	326 (91%)
Maternal education				
Completed tertiary studies	150 (52%)	171 (48%)	181 (51%)	171 (48%)
Continuing tertiary studies	50 (17%)	67 (19%)	68 (19%)	67 (19%)
Completed year 11 or 12	62 (22%)	83 (23%)	77 (22%)	84 (24%)
Did not complete year 11	25 (9%)	33 (9%)	32 (9%)	34 (10%)
Anaesthesia Details:				
Median (IQR)Blood glucose level (mmol/L)	5·4 (4·7-6·1)	5·5 (4·8-6·4)	5·4 (4·7-6·2)	5·5 (4·8-6·4)

Rescue glucose given IV	2 (1%)	4 (1%)	2 (1%)	4 (1%)
Haemoglobin (g/100 ml)	10·3 (2·1)	10·2 (2·0)	10·3 (2·1)	10·2 (2·0)
Need for fluid bolus for hypotension	15 (5%)	59 (17%)	21 (6%)	59 (17%)
Vasoactive drugs given (including atropine)	4 (1%)	17 (5%)	6 (2%)	17 (5%)
Median (IQR)Duration of surgery (mins)	26·0 (19·0-35·0)	28·0 (20·0-40·0)	28·0 (20·0-38·0)	28·0 (20·0-40·0)
Median (IQR) Duration of sevoflurane exposure (mins)	NA	54.0 (41.0-70.0)	42.0 (31.0-62.5)**	54.0 (41.0-70.0)
End tidal sevoflurane concentration (%)	NA	2·6 (0·7)	2·3 (0·8)**	2·6 (0·7)
Total concentration x hours per	NA	2·6 (1·1)	1·9 (1·0)**	2·6 (1·1)
Any significant apnoea to 12hrs postop***	6 (2%)	15 (4%)	10 (3%)	15 (4%)

Data are n(% of non-missing data) or Mean (SD), unless otherwise stated. APP= As Per Protocol; GA= General Anaesthesia; ITT= Intention to treat; IV= Intra-venously; IVH= Intra ventricular haemorrhage; IQR= Interquartile Range; PDA = Patent ductus arteriosus; RA= Awake Regional Anaesthesia.

* The primary language spoken at home, is the primary language in each country that the Bayley was conducted e.g in Italy it was conducted in Italian

** For those cases that received sevoflurane

*** significant apnoea defined as a pause in breathing for more than 15 seconds or more than 10 seconds if associated with oxygen saturation less than 80% or bradycardia (20% decrease in heart rate)

Table 2: 2 year descriptive statistics demographic data

	RA Arm APP (N=287)	**GA Arm APP (N=356)**	**RA Arm ITT (N=361)**	**GA Arm ITT (N=358)**
Assessment details				
Location of two year assessment at hospital	204 (96%)	240 (94%)	250 (95%)	241 (94%)
Family demographics at two years				
Paid employment is main family income	222 (90%)	267 (88%)	274 (90%)	268 (88%)
Family structure, two caregivers living together	226 (91%)	274 (90%)	277 (90%)	275 (90%)
Number of children at home				
1	88 (36%)	118 (39%)	115 (37%)	118 (39%)
2	109 (44%)	120 (40%)	131 (43%)	121 (40%)
3	37 (15%)	43 (14%)	45 (14%)	43 (14%)
>3	14 (6%)	22 (7%)	17 (6%)	22 (7%)
Birth order				
1	123 (50%)	161 (53%)	154 (50%)	161 (53%)
2	87 (35%)	90 (30%)	107 (35%)	91 (30%)
>2	37 (15%)	52 (17%)	46 (15%)	52 (17%)
Corrected age at assessment (weeks)	108·9 (13·0)	108 (9·8)	108·7 (12·5)	108 (9·8)
Events since original anaesthesia				
Number of hospitalisations since inguinal herniorrhaphy operation				
0	172 (69%)	206 (68%)	210 (68%)	207 (68%)
1	51 (20%)	64 (21%)	69 (22%)	64 (21%)
2	14 (6%)	18 (6%)	16 (5%)	18 (6%)
>2	6 (2%)	8 (3%)	8 (3%)	8 (3%)
Number of anaesthetics since inguinal herniorrhaphy operation				
1	34 (14%)	36 (12%)	42 (14%)	36 (12%)
2	5 (2%)	6 (2%)	6 (2%)	6 (2%)
>2	4 (2%)	4 (1%)	4 (1%)	4 (1%)
Child had a head injury that involved the loss of consciousness	7 (3%)	4 (1%)	7 (2%)	4 (1%)
Child has an acquired brain injury	1 (0%)	1 (0%)	1 (0%)	1 (0%)
Child has any malformations				
Cardiac	0	4 (1%)	0	4 (1%)
Central Nervous System	3 (1%)	1 (<1%)	3 (1%)	1 (<1%)
Genitourinary	6 (2%)	4 (1%)	8 (3%)	4 (1%)
Genetic condition	1 (<1%)	0	1 (<1%)	0
Respiratory	0	1 (<1%)	0	1 (<1%)
Skeletal	4 (2%)	11 (4%)	4 (1%)	11 (4%)
Cleft lip/palate	1 (<1%)	0	1 (<1%)	0
Craniofacial	2 (1%)	0	2 (1%)	0
Child has any chronic illness	42 (17%)	43 (14%)	50 (16%)	43 (14%)
Child had any prescribed medication for two months or longer	43 (17%)	50 (16%)	93 (17%)	59 (19%)
Child had febrile seizures following the hernia repair	8 (3%)	9 (3%)	10 (3%)	9 (3%)

Child had other seizures following the hernia repair	1 (<1%)	4 (1%)	1 (<1%)	4 (1%)
The child has had an intervention for neurodevelopmental issues since the inguinal herniorrhaphy operation	46 (19%)	55 (18%)	54 (18%)	55 (18%)
Speech Therapy	22 (9%)	27 (9%)	28 (9%)	27 (9%)
Physiotherapy	22 (9%)	27 (9%)	26 (8%)	27 (9%)
Occupational Therapy	9 (4%)	12 (4%)	12 (4%)	12 (4%)
Psychology	1(<1%)	6 (2%)	1 (<1%)	6 (2%)
Developmental medicine/early intervention	8 (3%)	7 (2%)	9 (3%)	7 (2%)
Child attends play group/child care on a regular basis	147 (60%)	177 (58%)	186 (61%)	178 (58%)
Physical examination				
Height (cm)	86·6 (5·5)	86·9 (4·9)	86·4 (5·2)	86·9 (4·9)
Weight (kg)	12·6 (2·0)	12·6 (1·9)	12·6 (2·0)	12·6 (1·9)
Head circumference (cm)	49·1 (2·1)	48·8 (2·2)	49·0 (2·0)	48·8 (2·2)
Arm circumference (cm)	16·4 (2·0)	16·1 (1·8)	16·4 (2·0)	16·1 (1·8)

Data are n(% of non-missing data) or Mean (SD), unless otherwise stated. APP= As Per Protocol; GA=General Anaesthesia; ITT= Intention to treat; RA= Awake Regional Anaesthesia.

Table 3: Descriptive statistics Bayley-III and Macarthur-Bates Scores by group

	RA Arm APP	GA Arm APP	RA Arm ITT	GA Arm ITT
Cognitive				
Cognitive, Scaled Score	238, 9·7 (2·8)	294, 9·6 (2·9)	292, 9·7 (2·8)	295, 9·6 (2·9)
Cognitive, Composite Score	238, 98·6 (14·2)	294, 98·2 (14·7)	292, 98·6 (14·2)	295, 98·2 (14·6)
Language				
Receptive Language, Scaled Score	236, 8·7 (2·9)	285, 8·6 (2·9)	287, 8·8 (2·9)	286, 8·6 (2·9)
Expressive Language Scaled Score	235, 9·3 (2·9)	290, 9·3 (3·0)	287, 9·4 (2·9)	291, 9·3 (3·0)
Language, Composite Score	235, 94·6 (15·4)	285, 94·0 (15·6)	286, 94·9 (15·5)	286, 94·0 (15·6)
Motor				
Fine Motor, Scaled Score	234, 10·5 (2·7)	287, 10·4 (2·7)	287, 10·6 (2·8)	288, 10·4 (2·7)
Gross Motor, Scaled Score	234, 8·8 (2·4)	279, 8·7 (2·6)	285, 8·9 (2·5)	280, 8·7 (2·6)
Motor, Composite Score	232, 98·3 (13·2)	274, 97·9 (13·4)	283, 98·9 (13·5)	275, 97·8 (13·4)
Social Emotional				
Social Emotional, Scaled Score	218, 9·5 (3·8)	267, 9·1 (3·7)	267, 9·5 (3·8)	268, 9·1 (3·7)
Social Emotional, Composite Score	218, 97·4 (19·0)	267, 95·4 (18·3)	267, 97·4 (19·2)	268, 95·4 (18·3)
Adaptive Behaviour				
Communication Scaled Score	233, 9·7 (2·9)	291, 9·6 (2·9)	288, 9·8 (2·9)	292, 9·6 (2·9)
Community Use Scaled Score	233, 9·8 (2·8)	291, 9·9 (2·7)	288, 9·9 (2·8)	292, 9·8 (2·7)
Functional Pre-Academics Scaled Score	233, 9·0 (3·0)	291, 9·2 (2·9)	288, 9·1 (3·0)	292, 9·2 (2·9)
Home Living Scaled Score	233, 9·9 (2·8)	291, 10·1 (2·7)	288, 9·9 (2·9)	292, 10·1 (2·7)
Health and Safety Scaled Score	233, 9·0 (2·8)	291, 9·3 (2·7)	288, 9·0 (2·9)	292, 9·3 (2·7)
Leisure Scaled Score	233, 9·4 (3·0)	291, 9·9 (2·8)	288, 9·5 (3·1)	292, 9·9 (2·8)
Self-Care Scaled Score	233, 6·8 (2·6)	291, 6·6 (2·5)	288, 6·8 (2·6)	292, 6·6 (2·5)
Self-Direction Scaled Score	233, 9·7 (3·2)	291, 10·0 (3·2)	288, 9·8 (3·2)	292, 10·0 (3·2)
Social Scaled Score	233, 9·3 (2·9)	291, 9·5 (2·8)	288, 9·4 (2·9)	292, 9·5 (2·8)
Motor Scaled Score	233, 9·8 (3·2)	291, 10·0 (2·9)	288, 9·9 (3·3)	292, 10·0 (2·9)
Adaptive Behaviour Composite Score	233, 93·1 (15·6)	291, 94·3 (14·7)	288, 93·4 (16·1)	292, 94·3 (14·7)
MacArthur Bates Percentile Score	195, 32·4 (27·9)	247, 34·7 (28·7)	240, 33·6 (28·0)	247, 34·7 (28·7)

Data as n, mean (SD). APP= As Per Protocol; GA= General Anaesthesia; ITT= Intention to treat; RA= Awake-Regional Anaesthesia.

Table 4: Between group comparisons in Bayley-III and MacArthur-Bates scores

Scale		***Δ: RA - GA**	**SE Δ**	**95% CI for Δ: RA - GA**	
Cognitive composite score	APP multiple imputation	0·169	1·26	-2·30	2·64
	APP complete case	0·458	1·26	-2·02	2·94
	ITT multiple imputation	0·256	1·18	-2·06	2·58
	ITT complete case	0·430	1·19	-1·90	2·76
Language composite score	APP multiple imputation	1·146	1·39	-1·59	3·88
	APP complete case	0·628	1·37	-2·07	3·32
	ITT multiple imputation	1·454	1·32	-1·14	4·05
	ITT complete case	0·942	1·30	-1·61	3·49
Motor composite score	APP multiple imputation	0·598	1·20	-1·77	2·97
	APP complete case	0·410	1·19	-1·92	2·74
	ITT multiple imputation	0·143	1·13	-1·08	3·37
	ITT complete case	1·031	1·14	-1·20	3·26
Social emotional composite score	APP multiple imputation	1·005	2·09	-3·12	5·13
	APP complete case	2·012	1·70	-1·32	5·35
	ITT multiple imputation	1·183	2·03	-2·82	5·19
	ITT complete case	2·015	1·62	-1·17	5·20
Adaptive behaviour composite score	APP multiple imputation	-0·893	1·34	-3·52	1·73
	APP complete case	-1·223	1·33	-3·83	1.38
	ITT multiple imputation	-0·502	1·28	-3·03	2·02
	ITT complete case	-0·830	1·28	-3·34	1·68
MacArthur Bates Percentile Score	APP multiple imputation	-1·811	3·06	-7·85	4·23
	APP complete case	-2·359	2·71	-7·69	2·98
	ITT multiple imputation	-0·544	2·87	-6·20	5·11
	ITT complete case	-1·113	2·57	-6·17	3·94

*Adjusted for gestational age at birth. APP= As Per Protocol; GA= General Anaesthesia; ITT= Intention to treat; RA= Awake Regional Anaesthesia.

Table 5: 2 year non-psychometric outcome data

	RA Arm APP (N=287)	**GA Arm APP (N=356)**	**RA Arm ITT (N=361)**	**GA Arm ITT (N=358)**
Child has a hearing defect				
Conductive	9 (3%)	6 (2%)	9 (2%)	6 (2%)
Sensorineural	0	3 (1%)	1 (<1%)	3 (1%)
Child has a hearing aid	1 (8%)	3 (25%)	2 (15%)	3 (23%)
Child is legally blind (<6/60 in both eyes)	1 (2%)	0	1 (2%)	0
Child has Cerebral Palsy	1 (<1%)	4 (1%)	1 (0%)	4 (1%)
The child has Autism Spectrum Disorder	2 (1%)	0	2 (1%)	0

Data are n (% of non-missing data), unless otherwise stated. APP= As Per Protocol; GA= General Anaesthesia; ITT= Intention to treat; RA= Regional Anaesthesia.

References

1. Jevtovic-Todorovic V, Absalom AR, Blomgren K, Brambrink A, Crosby G, Culley DJ, et al. Anaesthetic neurotoxicity and neuroplasticity: an expert group report and statement based on the BJA Salzburg Seminar. British journal of anaesthesia. 2013; **111**(2): 143-51.
2. Briner A, Nikonenko I, De Roo M, Dayer A, Muller D, Vutskits L. Developmental Stage-dependent persistent impact of propofol anesthesia on dendritic spines in the rat medial prefrontal cortex. Anesthesiology. 2011; **115**(2): 282-93.
3. Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, et al. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci. 2003; **23**(3): 876-82.
4. Sanchez V, Feinstein SD, Lunardi N, Joksovic PM, Boscolo A, Todorovic SM, et al. General Anesthesia Causes Long-term Impairment of Mitochondrial Morphogenesis and Synaptic Transmission in Developing Rat Brain. Anesthesiology. 2011; **115**(5): 992-1002.
5. Istaphanous GK, Ward CG, Nan X, Hughes EA, McCann JC, McAuliffe JJ, et al. Characterization and quantification of isoflurane-induced developmental apoptotic cell death in mouse cerebral cortex. Anesthesia and analgesia. 2013; **116**(4): 845-54.
6. Paule MG, Li M, Allen RR, Liu F, Zou X, Hotchkiss C, et al. Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys. Neurotoxicol Teratol. 2011; **33**(2): 220-30.
7. Raper J, Alvarado MC, Murphy KL, Baxter MG. Multiple Anesthetic Exposure in Infant Monkeys Alters Emotional Reactivity to an Acute Stressor. Anesthesiology. 2015.
8. Slikker W, Jr., Zou X, Hotchkiss CE, Divine RL, Sadovova N, Twaddle NC, et al. Ketamine-induced neuronal cell death in the perinatal rhesus monkey. Toxicol Sci. 2007; **98**(1): 145-58.
9. Brambrink AM, Back SA, Riddle A, Gong X, Moravec MD, Dissen GA, et al. Isoflurane-induced apoptosis of oligodendrocytes in the neonatal primate brain. Annals of neurology. 2012; **72**(4): 525-35.
10. Brambrink AM, Evers AS, Avidan MS, Farber NB, Smith DJ, Zhang X, et al. Isoflurane-induced neuroapoptosis in the neonatal rhesus macaque brain. Anesthesiology. 2010; **112**(4): 834-41.
11. Hansen TG. Anesthesia-related neurotoxicity and the developing animal brain is not a significant problem in children. Paediatric anaesthesia. 2015; **25**(1): 65-72.
12. Psaty BM, Platt R, Altman RB. Neurotoxicity of generic anesthesia agents in infants and children: an orphan research question in search of a sponsor. Jama. 2015; **313**(15): 1515-6.
13. Rappaport BA, Suresh S, Hertz S, Evers AS, Orser BA. Anesthetic neurotoxicity--clinical implications of animal models. The New England journal of medicine. 2015; **372**(9): 796-7.
14. Crosby G, Davis PJ. General anesthesia in infancy is associated with learning disabilities-or not. Anesthesia and analgesia. 2013; **117**(6): 1270-2.
15. Davidson AJ. Anesthesia and neurotoxicity to the developing brain: the clinical relevance. Paediatric anaesthesia. 2011; **21**(7): 716-21.
16. DiMaggio C, Sun LS, Ing C, Li G. Pediatric anesthesia and neurodevelopmental impairments: a Bayesian meta-analysis. Journal of neurosurgical anesthesiology. 2012; **24**(4): 376-81.
17. Ing C, DiMaggio C, Whitehouse A, Hegarty MK, Brady J, von Ungern-Sternberg BS, et al. Long-term differences in language and cognitive function after childhood exposure to anesthesia. Pediatrics. 2012; **130**(3): e476-85.
18. Ing CH, DiMaggio CJ, Malacova E, Whitehouse AJ, Hegarty MK, Feng T, et al. Comparative analysis of outcome measures used in examining neurodevelopmental effects of early childhood anesthesia exposure. Anesthesiology. 2014; **120**(6): 1319-32.
19. Lei SY, Hache M, Loepke AW. Clinical research into anesthetic neurotoxicity: does anesthesia cause neurological abnormalities in humans? Journal of neurosurgical anesthesiology. 2014; **26**(4): 349-57.

20. Flick RP, Katusic SK, Colligan RC, Wilder RT, Voigt RG, Olson MD, et al. Cognitive and behavioral outcomes after early exposure to anesthesia and surgery. Pediatrics. 2011; **128**(5): e1053-61.
21. Sprung J, Flick RP, Katusic SK, Colligan RC, Barbaresi WJ, Bojanic K, et al. Attention-deficit/hyperactivity disorder after early exposure to procedures requiring general anesthesia. Mayo Clinic proceedings. 2012; **87**(2): 120-9.
22. Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, Mickelson C, et al. Early exposure to anesthesia and learning disabilities in a population-based birth cohort. Anesthesiology. 2009; **110**(4): 796-804.
23. DiMaggio C, Sun LS, Li G. Early childhood exposure to anesthesia and risk of developmental and behavioral disorders in a sibling birth cohort. Anesthesia and analgesia. 2011; **113**(5): 1143-51.
24. DiMaggio C, Sun LS, Kakavouli A, Byrne MW, Li G. A retrospective cohort study of the association of anesthesia and hernia repair surgery with behavioral and developmental disorders in young children. Journal of neurosurgical anesthesiology. 2009; **21**(4): 286-91.
25. Bartels M, Althoff RR, Boomsma DI. Anesthesia and cognitive performance in children: no evidence for a causal relationship. Twin Res Hum Genet. 2009; **12**(3): 246-53.
26. Hansen TG, Pedersen JK, Henneberg SW, Pedersen DA, Murray JC, Morton NS, et al. Academic performance in adolescence after inguinal hernia repair in infancy: a nationwide cohort study. Anesthesiology. 2011; **114**(5): 1076-85.
27. Hansen TG, Pedersen JK, Henneberg SW, Morton NS, Christensen K. Educational outcome in adolescence following pyloric stenosis repair before 3 months of age: a nationwide cohort study. Paediatric anaesthesia. 2013; **23**(10): 883-90.
28. Block RI, Thomas JJ, Bayman EO, Choi JY, Kimble KK, Todd MM. Are anesthesia and surgery during infancy associated with altered academic performance during childhood? Anesthesiology. 2012; **117**(3): 494-503.
29. Bong CL, Allen JC, Kim JT. The effects of exposure to general anesthesia in infancy on academic performance at age 12. Anesthesia and analgesia. 2013; **117**(6): 1419-28.
30. Wang X, Xu Z, Miao CH. Current clinical evidence on the effect of general anesthesia on neurodevelopment in children: an updated systematic review with meta-regression. PloS one. 2014; **9**(1): e85760.
31. Davidson AJ, Becke K, de Graaff J, Giribaldi G, Habre W, Hansen T, et al. Anesthesia and the developing brain: a way forward for clinical research. Paediatric anaesthesia. 2015; **25**(5): 447-52.
32. Davidson AJ, Morton NS, Arnup SJ, de Graaff JC, Disma N, Withington DE, et al. Apnea after Awake Regional and General Anesthesia in Infants: The General Anesthesia Compared to Spinal Anesthesia Study-Comparing Apnea and Neurodevelopmental Outcomes, A Randomized Controlled Trial. Anesthesiology. 2015; **123**(1): 38-54.
33. Frawley G, Bell G, Disma N, Withington DE, de Graaff JC, Morton NS, et al. Predictors of Failure of Awake Regional Anesthesia for Neonatal Hernia Repair: Data from the General Anesthesia Compared to Spinal Anesthesia Study-Comparing Apnea and Neurodevelopmental Outcomes. Anesthesiology. 2015; **123**(1): 55-65.
34. Bayley N. Bayley Scales of Infant and Toddler Development. 3rd ed. San Antonio: Harcourt Assessment Inc; 2006.
35. Fenson L, Marchman VA, Thal DJ, Dale PS, Reznic JS, Bates E. MacArthur-Bates Communicative Development Inventories: User's Guide and Technical Manual 2nd ed. Baltimore Brookes Publishing; 2007.
36. van Buuren S. Multiple imputation of discrete and continuous data by fully conditional specification. Statistical methods in medical research. 2007; **16**(3): 219-42.
37. Seaman SR, White IR. Review of inverse probability weighting for dealing with missing data. Statistical methods in medical research. 2013; **22**(3): 278-95.
38. Johnson SA, Young C, Olney JW. Isoflurane-induced neuroapoptosis in the developing brain of nonhypoglycemic mice. Journal of neurosurgical anesthesiology. 2008; **20**(1): 21-8.

39. Di Maggio C, Sun L, Kakavuoli A, Byrne M, Li G. A retrspective cohort study of the association of anesthesia and hernia repair surgery with behavioral and developmental disorders in young children. Journal of neurosurgical anesthesiology. 2009; **21**: 286-91.
40. Harris SR, Langkamp DL. Predictive value of the Bayley mental scale in the early detection of cognitive delays in high-risk infants. Journal of perinatology : official journal of the California Perinatal Association. 1994; **14**(4): 275-9.
41. Bode MM, D'Eugenio DB, Mettelman BB, Gross SJ. Predictive validity of the Bayley, Third Edition at 2 years for intelligence quotient at 4 years in preterm infants. Journal of developmental and behavioral pediatrics : JDBP. 2014; **35**(9): 570-5.
42. Lee BH, Chan JT, Kraeva E, Peterson K, Sall JW. Isoflurane exposure in newborn rats induces long-term cognitive dysfunction in males but not females. Neuropharmacology. 2014; **83**: 9-17.

Neurodevelopmental outcome at two years of age after general and awake-regional anaesthesia in infancy: a randomised controlled trial

Authors: and The GAS Consortium

1. Andrew J. Davidson MD 1,2,3
2. Nicola Disma MD 4
3. Jurgen C. de Graaff PhD 5
4. Davinia E. Withington BM 6,7
5. Liam Dorris DClinPsy 8,9
6. Graham Bell MBChB 10
7. Robyn Stargatt PhD 11,12
8. David C. Bellinger PhD 13,14,15
9. Tibor Schuster PhD 16
10. Sarah J. Arnup MBiostat 16
11. Pollyanna Hardy MSc 17
12. Rodney W. Hunt PhD 3,18,19
13. Michael J. Takagi PhD 1,12
14. Gaia Giribaldi MD 4
15. Penelope L. Hartmann BPsych(Hons) 1
16. Ida Salvo MD 20
17. Neil S. Morton MD 10,21
18. Britta S von Ungern Sternberg PhD 22,23
19. Bruno Guido Locatelli MD 24
20. Niall Wilton MBBS 25
21. Anne Lynn MD 26
22. Joss J. Thomas MD 27
23. David Polaner MD 28
24. Oliver Bagshaw FRCA 29
25. Peter Szmuk MD 30
26. Anthony R. Absalom MBChB 31
27. Geoff Frawley MBBS 1,2
28. Charles Berde MD 32
29. Gillian D Ormond MSc 1

30. Jacki Marmor MEd 13
31. Mary Ellen McCann 32

Institutions

1. Anaesthesia and Pain Management Research Group, Murdoch Childrens Research Institute, Melbourne, Australia
2. Department of Anaesthesia and Pain Management, The Royal Children's Hospital, Melbourne, Australia
3. Department of Paediatrics, University of Melbourne, Melbourne, Australia
4. Department of Anesthesia, Istituto Giannina Gaslini, Genoa, Italy
5. Department of Anaesthesia, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands
6. Department of Anesthesia, Montreal Children's Hospital, Montreal, Canada
7. Department of Anesthesia, McGill University, Montreal, Canada
8. Paediatric Neurosciences Research Group, Fraser of Allander Unit, Royal Hospital for Children, Glasgow, Scotland, UK.
9. Mental Health and Wellbeing, University of Glasgow, Glasgow, Scotland, UK
10. Department of Anaesthesia, Royal Hospital for Children, Glasgow, Scotland, UK
11. School of Psychological Science, La Trobe University, Victoria, Australia
12. Child Neuropsychology, Murdoch Childrens Research Institute, Melbourne, Australia
13. Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA
14. Department of Psychiatry, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA
15. Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
16. Clinical Epidemiology and Biostatistics Unit, Murdoch Childrens Research Institute, Melbourne, Australia
17. National Perinatal Epidemiology Unit, Clinical Trials Unit, University of Oxford, Oxford, UK
18. Department of Neonatal Medicine, The Royal Children's Hospital, Melbourne, Australia
19. Neonatal Research Group, Murdoch Childrens Research Institute, Melbourne, Australia
20. Department of Anesthesiology & Paediatric Intensive Care, Ospedale Pediatrico 'Vittore Buzzi', Milan, Italy

21. University of Glasgow, Glasgow, Scotland, UK
22. School of Medicine and Pharmacology, The University of Western Australia, Perth, Australia
23. Department of Anaesthesia and Pain Management, Princess Margaret Hospital for Children, Perth, Australia
24. Department of Anaesthesia, Ospedale Papa Giovanni XXIII, Bergamo, Italy
25. Department of Paediatric Anaesthesia and Operating Rooms, Starship Children's Hospital, Auckland District Health Board, Auckland, New Zealand.
26. Department of Anesthesiology, University of Washington, Seattle, Washington, USA
27. Department of Anesthesia, University of Minnesota, Minneapolis, Minnesota, USA
28. Children's Hospital Colorado and University of Colorado School of Medicine, Department of Anesthesiology, Aurora, Colorado, USA
29. Department of Anaesthesia, Birmingham Children's Hospital, Birmingham, UK
30. Children's Medical Centre Dallas, Department of Anesthesiology, Dallas, Texas, USA
31. Department of Anesthesiology, University Medical Center Groningen, Groningen University, Groningen, The Netherlands
32. Department of Anesthesiology, Perioperative and Pain Medicine, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA

Corresponding Author:

Andrew J. Davidson, Anaesthesia and Pain Management Research Group, Murdoch Childrens Research Institute, The Royal Children's Hospital, Flemington Road, Parkville, Victoria, 3052. Australia. Email Address: andrew.davidson@rch.org.au Phone Number: +61 (0)3 9345 4008

Summary

Background:

There is pre-clinical evidence that general anaesthetics ~~(GAs)~~ affect brain development. ~~The relevance to humans is unclear.~~ There is mixed evidence from cohort studies that young children exposed to anaesthesia may have an increased risk of poorer neurodevelopmental outcome. ~~It is unknown if any association is linked to the changes seen in the pre-clinical studies, or due to confounding peri-operative or patient factors.~~ This trial aims to determine if ~~brief~~ GA in infancy has any impact on neurodevelopmental outcome. The primary outcome for the trial is ~~the~~ neurodevelopmental outcome~~-~~ at 5 years of age. The secondary ~~neurodevelopmental~~ outcome is neurodevelopmental ~~Neurodevelopmental~~ outcome at two years of age and is reported here.

Methods:

We performed ~~An~~an international assessor-~~-~~masked randomised controlled equivalence trial in infants less than 60 weeks post-menstrual age, born at greater than 26 weeks gestational age having inguinal herniorrhaphy. Infants were excluded if they had existing risk factors for neurologic injury. ~~was conducted comparing neurodevelopmental outcome in 722 infants less than 60 weeks post-menstrual age~~ Infants were randomly assigned to ~~randomised to~~ awake-regional (RA) or sevoflurane-based general anaesthesia (GA). ~~for inguinal herniorrhaphy.~~ Web-based randomisation was performed in blocks of two or four and stratified by site and gestational age at birth. The ~~primary~~ outcome for ~~this~~ analysis was the composite cognitive score of the Bayley Scales of Infant and Toddler Development, Third Edition. ~~(Bayley-III). The composite scores in the reference population have a mean of 100 and standard deviation (SD) of 15. As-per-protocol (APP) and intention-to-treat (ITT) analyses were performed; both adjusted for gestational age at birth.~~ The ~~primary analysis population~~ analysis was as-per-protocol ~~APP~~ adjusted for gestational age at birth. A difference in means of five points (1/3 SD) was predefined as the clinical equivalence margin. The trial was registered at ANZCTR, ACTRN12606000441516 and ClinicalTrials.gov, NCT00756600.

Findings:

Between February 2007, and January 2013, 363 infants were randomised to RA and 359 to GA. Outcome data were available for ~~There were 361 and 359 children in the RA and GA~~

~~arms for ITT analysis and 287 and 356~~ 238 in the RA and 294 in the GA arms.~~for the APP.~~ The median duration of anaesthesia in the GA arm was 54 minutes. For the cognitive composite score there was equivalence in means between arms ~~in both the APP and ITT analyses~~ (RA-GA: +0·169, 95% CI -2·30 to +2·64~~; and RA-GA: +0·256, 95% CI -2·06 to +2·57 for APP and ITT respectively~~).

Interpretation:

For this secondary outcome we found no evidence that just under an hour of sevoflurane anaesthesia ~~Fifty-four minutes of exposure to a GA~~ in infancy increases ~~does not increase~~ the risk of adverse neurodevelopmental outcome at two years of age compared to RA.

Funding**:**

Australia National Health and Medical Research Council (NHMRC)~~ and others~~, Health~~.~~ Technologies Assessment- National Institute for Health Research UK, The National Institute of Health, The Food and Drug Administration, The Australian and New Zealand College of Anaesthetists, The Murdoch Childrens Research Institute ~~The National Institute of Health, The Food and Drug Administration~~, The ~~Italian Ministry of Heath, Fonds NutsOhra grant~~ Canadian Institute of Health Research, Canadian Anesthesiologists' Society, Pfizer Canada Inc, Italian Ministry of Heath, Fonds NutsOhra grant ~~Health Technologies Assessment- National Institute for Health Research UK~~

Introduction:

There is considerable preclinical evidence describing how GA agents alter brain development in young animals.[1] This includes accelerated apoptosis and a variety of other changes including changes to dendritic morphology.[2-5] There is also evidence that exposure to GA in young animals is associated with long term cognitive and behavioural changes.[3, 6, 7] These effects have been described in a variety of species including non-human primates.[7-10] The changes are seen with several different GA agents, are greater with longer exposure and less severe in older animals.[2, 8] The clinical significance of these findings is unknown and hotly debated.[11-14]

In humans there is conflicting evidence for an association between exposure to anaesthesia in early childhood and adverse long term neurodevelopmental outcome; however confounding

limits any assumption of causality.[15-30] Young children that receive anaesthesia are inevitably having surgery or an investigative procedure. Added risk of poor neurodevelopmental outcome ~~Neurologic injury~~ may be due to the underlying pathology, co-morbidity or other peri-operative risk factors.

These results have prompted recommendations to consider delaying surgery in infancy and there have been several calls for more research to address this important issue.[12, 13, 31] Given the large number of potential confounding factors, a randomised trial is the best study design to determine if anaesthesia exposure in early childhood causes long term neurodevelopmental changes. Fortuitously there are two established anaesthetic techniques for inguinal herniorrhaphy in infancy; RA and ~~volatile~~ sevoflurane based GA. We therefore undertook ~~A~~a randomised controlled trial ~~was performed~~ comparing neurodevelopmental outcome in children who were randomly assigned to receive RA or sevoflurane based GA for inguinal herniorrhaphy in early infancy: the General Anaesthesia compared to Spinal anaesthesia (GAS) trial. The aim of the trial is to determine if GA does not increase the risk of adverse neurodevelopmental outcome. The primary outcome for the overall trial will be the Wechsler Preschool and Primary Scale of Intelligence Third Edition (WPPSI-III) Full Scale Intelligence Quotient score at five years of age. As a secondary outcome we also planned *a priori* to assess neurodevelopmental outcome at 2 years of age. In this paper ~~the neurodevelopmental outcomes at two years of age are reported~~we report all secondary outcomes at two years of age. Data from the trial relating to post- anaesthesia apnoea and success of regional block have been published elsewhere.[32, 33]

Methods:

~~The GAS trial is registered in Australia and New Zealand at ANZCTR: ID# ACTRN12606000441516 first registered on 16th October 2006; in the United States (US) at ClinicalTrials.gov: ID#: NCT00756600 first registered on 18th September 2008; and in the United Kingdom (UK) at UK Clinical Research Network (UKCRN) ID#: 6635 (ISRCTN ID#: 12437565; MREC No: 07/S0709/20).~~ ~~The protocol has been previously published at http://www.thelancet.com/protocol-reviews/09PRT-9078~~

Study design~~and participants:~~

The GAS trial is a prospective, observer blind, international multi-site, randomised, controlled, equivalence trial examining RA versus GA in infants undergoing inguinal herniorrhaphy. The trial was performed at 28 hospitals in Australia, Italy, The USA, The UK, Canada, The Netherlands and New Zealand. Institutional Review Board or Human Research Ethics Committee approval was obtained at each site and written consent obtained from the child's parents or guardians. The protocol has been previously published at http://www.thelancet.com/protocol-reviews/09PRT-9078

Participants:

Eligibility criteria included infants up to 60 weeks' postmenstrual age ~~(PMA)~~ scheduled for unilateral or bilateral inguinal herniorraphy born at greater than 26 weeks gestation. Exclusion criteria included any contraindication for either anaesthetic technique, a history of congenital heart disease requiring surgery or pharmacotherapy, mechanical ventilation immediately prior to surgery, known chromosomal abnormalities or other known acquired or congenital abnormalities which might affect neurodevelopment, previous exposure to volatile GA or benzodiazepines as a neonate or in the third trimester *in utero*, any known neurologic injury such as cystic peri-ventricular leukomalacia or grade three or four intra-ventricular haemorrhage (IVH), any social or geographic factor that may make follow up difficult (such as planned house move, homelessness, no telephone communication available), or having a primary language at home in a region where neurodevelopmental tests are not available in that language. Eligible infants were identified from operating room schedules or at pre-admission clinics and recruited in the clinic or in the preadmission areas of the operating floor.

Randomisation and Masking:

A 24-hour web-based randomisation service was managed by The Data Management & Analysis Centre, Department of Public Health, University of Adelaide, South Australia. ~~Patients~~ Participants were randomised with a 1:1 allocation ratio to either GA or RA. Randomisation was performed in blocks of two or four and stratified by site and gestational age at birth: 26 to 29 weeks and six days, 30 to 36 weeks and six days and 37 weeks or more. The anaesthetist was aware of group allocation. Parents were not informed of the group allocation but were told if they asked. The psychologists and paediatricians performing the assessment were masked to group allocation. Once their assessment was completed they were asked to indicate if they were aware of group allocation.

Procedures:

The RA group received either an awake-spinal anaesthetic, an awake-caudal anaesthetic, or a combined spinal-caudal anaesthetic according to institutional protocols. Spinal anaesthesia was performed with 0·2 ml/kg 0·5% isobaric bupivacaine with a minimum volume of 0·5ml. Due to unavailability of isobaric bupivacaine at some sites other agents were used (in the US, 0·13ml/kg of hyperbaric 0·75% bupivacaine and in the UK 0·2 ml/kg 0·5% levobupivacaine). Caudal anaesthesia was performed with up to a total dose of 2·5 $mg.kg^{-1}$ of 0·25% bupivacaine. In the UK 0·25% levobupivacaine was used. In the US if surgery was likely to take greater than one hour, some patients were given a loading dose of 3% chloroprocaine (1ml/kg in divided doses of no more than 0·25ml/kg per 15 seconds) via a caudal cannula and then an infusion of 1-2 ml/kg/hr. Ilioinguinal and field blocks could also be done. The total dose of bupivacaine did not exceed 2·5 mg/kg. In the RA group oral sucrose was used to settle the child if required and all other forms of sedation avoided. If the RA was ineffective then a GA was performed with sevoflurane, and if the child became unsettled intra-operatively sevoflurane was administered to supplement the RA. Both were regarded as protocol violations.

The GA group received sevoflurane for induction and maintenance in an air/oxygen mix. The concentration of sevoflurane was left to the discretion of the anaesthetist, as was choice of airway device, ventilation technique and use of any neuromuscular blocking agents. No opioid or nitrous oxide was allowed. A caudal, ilioinguinal-iliohypogastric and/or field block with bupivacaine could be performed in both groups to provide post-operative analgesia. Oral or intra venous acetaminophen could also be given. Heart rate, blood pressure, oxygen

saturation and (where applicable) expired sevoflurane concentrations were recorded every five minutes.

Serum glucose was measured after anesthetic induction. There were rescue protocols for hypoglycaemia, hypotension and hypoxemia. If the blood pressure fell >20% below baseline an intravenous bolus fluid was administered and vasoactive drugs given if deemed necessary. Hypoglycaemia (blood sugar <3·0mmol/L) was treated with a bolus of 5ml/kg of 10% dextrose. Oxygen by face mask in the RA arm and an increased FiO_2 in the GA arm was used at the discretion of the anaesthetist to maintain arterial oxygen saturation > 95%.

Two Year Assessments:

Assessments were performed within two months either side of two years of age (corrected for prematurity). The assessment took approximately two hours to complete. A trained psychologist administered the Bayley-III. [34] The Bayley-III has cognitive, language and motor scales. The cognitive scale includes tasks assessing attention, memory, sensorimotor development, exploration, concept formation, and simple problem solving. The language scale assesses expressive and receptive skills, and the motor scale assesses fine and gross motor skills. Parents completed the Bayley-III Social-Emotional and Adaptive Behavior Questionnaires and the MacArthur-Bates Communicative Development Inventory: Words and Sentences (MacArthur-Bates).[35] The MacArthur-Bates is a parent informant measure that assesses expressive language in children aged 16-30 months of age. Demographic data, family history, and medical history were also noted, and a brief physical and neurological examination was performed. The physical examination included anthropometric measurements such as length, weight, arm and head circumference. The neurologic examination included cranial nerve examination, posture assessment and the muscle strength, tone and reflexes of the upper and lower extremities.

All study data were sent to the Murdoch Childrens Research Institute in Melbourne, Australia. All forms were checked for data quality by trained research assistants and double checked by a research assistant who was not involved in the primary data collection or entry.

An independent data safety monitoring committee met at six monthly intervals during recruitment. Summary data by allocation were presented to the committee. There were no formal interim analyses of neurodevelopmental outcome.

Statistical Analysis:

The ~~primary~~ main outcome for ~~this~~the ~~-~~analysis at 2 years of age was pre-specified to be the ~~was the~~ composite cognitive score of the Bayley-III. The hypothesis (as stated in the protocol) was that the composite cognitive score of the Bayley-III measured at two years of age in infants who are anaesthetised for inguinal herniorraphy is equivalent when using GA compared with RA. The components of the Bayley-III are reported as scaled scores and as composite scores. The five composite scores (Cognitive, Language, Motor, Adaptive Behaviour, and Social-Emotional Scales) are standardised to have a mean of 100 and a SD of 15 in the reference population. The sub-scales (e.g., fine motor scale) are reported as scaled scores, with a mean of 10 and a SD of 3. The other secondary outcomes for this analysis are the language, motor, social-emotional, and adaptive behaviour scores from the Bayley-III and the age-adjusted Vocabulary Production Score from the MacArthur-Bates. Published normative scores were used at all sites with forms and instructions translated locally. Diagnosis of cerebral palsy was another pre-specified secondary outcome

Since this is an equivalence study, the ~~primary~~ outcome was analysed on an APP basis to ensure a conservative estimate in the direction of non-equivalence. Equivalence was defined *a-priori* if the 95% confidence interval of the difference in means lies within -five and +five points. ITT analyses were also planned. Analyses were adjusted for categories of gestational age at birth (182-209 days; 210-258 days; ≥259 days)

The sample size was based on the primary outcome for the GAS trial; the five year follow up WPPSI-III Full Scale IQ score. Assuming an expected difference of one standardised score point, and a 90% chance that a 95% confidence interval will exclude a difference of more than five points (the largest difference acceptable to demonstrate equivalence), the trial would need 598 infants in total. Enrolling approximately 720 participants would allow for 10% loss to follow-up and 10% with a major protocol violation.

Multiple imputation using chained equations was used to impute missing outcome data in the analysis of ~~primary and secondary~~all outcomes.[36] The following pre-specified variables were used as predictor variables within the imputation approach: anaesthesia group, country, gender, gestational age at birth, standardized z-score for birth weight, mother received antenatal steroids, mother diagnosed with chorioamnionitis, IVH, maternal age, maternal

education, rescue glucose given intra-venously, need for fluid bolus for hypotension, vasoactive drugs given for hypotension, duration of surgery, dose of sevoflurane (concentration x hours), significant post-operative apnoea, corrected age at assessment, any more anaesthetic exposures since the inguinal herniorraphy, any malformations, any chronic illness, any prescribed medication for two months or longer, total length of any readmission to hospital, any interventions for neurodevelopmental problems, diagnosis of cerebral palsy, any other neurological abnormality.

For the purpose of sensitivity analysis, effect estimates were computed using best and worst case imputation scenarios. Furthermore, effect estimates and confidence intervals based on inverse probability of censoring weighting were reported.[37]

Risk ratios with 95% confidence intervals were reported for the proportion of individuals that fall below one and two SDs of the composite cognitive score. Risk ~~a~~ratios were generated using generalized linear models for a binomial distributed response variable employing a log link (binomial log-linear regression). These analyses were not pre-specified in the study protocol (*post hoc* analyses). All analyses were carried out in Stata 13 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

The GAS trial is registered in Australia and New Zealand at ANZCTR: ID# ACTRN12606000441516 first registered on 16th October 2006; in the United States (US) at ClinicalTrials.gov: ID#: NCT00756600 first registered on 18th September 2008; and in the United Kingdom (UK) at UK Clinical Research Network (UKCRN) ID#: 6635 (ISRCTN ID#: 12437565; MREC No: 07/S0709/20).

Role of the Funding Source:
The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had access to the data, and AJD, GO and Suzette Sheppard were responsible for submitting the manuscript. AJD made the final decision to submit the paper for publication.

Results

Seven hundred twenty-two infants were recruited into the trial between February 9, 2007, and January 31, 2013 from 28 centres in Australia, the US, the UK, Italy, the Netherlands,

Canada and New Zealand (~~table 1~~appendix). There were two mis-randomisations and one withdrawal of consent leaving 361 in the ITT analysis in the RA arm and 358 in the GA arm. Table ~~2~~ 1 summarises demographic data for each arm at baseline and table ~~3~~2 summarises demographic data at two years. There were 74 protocol violations in the RA arm (five due to surgery being cancelled and 69 receiving some sevoflurane or other GA) and two violations in the GA arm (surgery cancelled).

Follow up was from March 5, 2009 to March 6, 2015. Forty-seven families were lost to follow up in the GA arm and 52 in the RA arm. Of those lost to follow up some reason for non-attendance was gained in 19 and in only one case was non-attendance due to developmental delay (this child was in the RA arm). Of those that attended for assessment, the cognitive scale of the Bayley-III was completed by 292 in the RA arm and 295 in the GA arm (Figure 1). Very few children were unable to complete the Bayley-III due to developmental delay or other recognised reasons for cognitive impairment. In ~~most~~ 97% of cases the psychologist and paediatrician were unaware of group allocation at the time of assessment. (~~Table 4~~appendix)

The Bayley-III Cognitive, Language, Motor, Social-Emotional and Adaptive Behaviour scores, and the MacArthur-Bates data are summarised for each group in table ~~5~~3.

For the Cognitive Composite score there was evidence for equivalence in means between RA and GA arms in both the APP and the ITT analyses using multiple imputation to account for missing outcome data (RA-GA: +0·169, 95% CI -2·30 to +2·64; and RA-GA: +0·256, 95% CI -2·06 to +2·57 for APP and ITT respectively). These results were consistent with the findings of the complete case analyses (RA-GA: +0·458, 95% CI -2·02 to +2·94; and RA-GA: +0·430, 95% CI -1·90 to +2·76, for APP and ITT respectively). There was also evidence for equivalence between arms in the Composite Motor scores, Composite Language scores and the Composite Adaptive Behavior scores (Table ~~6~~4). The results were consistent in both APP and ITT analyses, and when using complete case and multiple imputation. With mean differences of one and two score points (multiple imputation and complete case analysis for APP/ITT) and upper 95% confidence interval limits exceeding the pre-specified five point equivalence margin, evidence for equivalence with regard to the Social-Emotional Composite scale of the Bayley-III was not compelling. There was no evidence for a difference between groups in MacArthur-Bates scores (Table ~~6~~4).

The results of the inverse probability weighting and worst case imputation scenarios for missing data are presented in ~~tables 7 and 8~~the appendix. The worst case scenario results represent theoretical boundaries to what extent the actual effect estimates could have been affected by selective dropout. However, both multiple imputation analysis as well as inverse probability weighting demonstrated consistent robustness of the study findings with regard to data missingness.

Overall a low number of children had a diagnosis of cerebral palsy, hearing or visual impairment or specific behavioural diagnoses such as autistic spectrum disorder (ASD) (table 5~~9~~). The event rate was too low for any meaningful comparative analysis. There was no evidence for a difference between arms in the proportion of children one or two SDs below ~~normal~~ the age mean on the cognitive composite score (~~tables 10 and 11~~appendix).

Details of adverse events during and immediately after anaesthesia have been reported in the earlier publication.[32]

Research in context

Evidence before this study

Medline and Cochrane controlled trial register were searched (search last done 18th September 2015) for original research and meta-analyses describing the association between anaesthesia exposure in early life and neurodevelopmental outcome. Combinations of search terms “anesthesia”, and “child development”, or “learning disorders” were used. The search revealed no randomised trials but several cohort studies. There have been numerous reviews that have concluded that there is an association between anaesthesia in childhood and neurodevelopmental outcome.[19, 31] There have been two meta-analyses that have found evidence for an association between anaesthesia in children and a range of neurodevelopmental outcomes.[16, 30] All reviews and meta-analyses acknowledge the weaknesses of the cohort studies; including strong likelihood of confounding, bias, heterogeneous populations at times of exposure, and heterogeneous outcome measures, some of which are poorly defined or insensitive. All conclude that causation cannot be established or excluded.

Added value of this study

We report a secondary outcome from the first randomised controlled trial assessing the impact of general anaesthesia in infancy on neurodevelopmental outcome. Using the best measure of neurodevelopment available for assessing a two year old child, strong evidence for equivalence between awake-regional and just under an hour of general anaesthesia was found. ~~Given the strengths of a prospective randomised trial design and a systematically administered outcome measure, this trial provides the strongest evidence to date that 54 minutes of GA exposure in infancy does not cause significant neurodevelopmental changes.~~ However it should be noted that this was an analysis of a secondary outcome with the primary outcome planned at five years of age, and given the limited sensitivity of developmental assessment at two years of age, this trial does not provide the definitive answer.

Implications of all the available evidence

Although there are some limitations that should be noted when interpreting the trial, the randomised prospective design adds significantly to the weight that should be given to the results compared to the mixed results found in previous cohort studies. It should however be emphasised that reassessment at an older age is necessary before definitive conclusions can be drawn. ~~The evidence suggest that for short procedures, general anaesthesia does not need to be avoided in young children.~~ The trial does not ~~however~~ rule out the possibility that longer, or multiple exposures to anaesthesia in early childhood may cause neurodevelopmental changes. Further research is needed to address these questions.

Discussion

In this trial we found strong evidence for equivalence between RA and GA in infancy in terms of neurodevelopmental outcome at two years of age. Equivalence was demonstrated in multiple domains of neurodevelopmental assessment and the 95% confidence intervals fell within a third of a SD; well inside our pre-defined boundaries of clinical equivalence.

There are no previous randomised trials examining the effect of anaesthesia in infancy on long term neurodevelopmental outcomes (see research in context panel). Previous cohort

studies have found mixed results.[19] Some studies have found an association between exposure to anaesthesia in early childhood and increased risk of poor neurodevelopmental outcome.[16-18, 20-24, 28] Although this association fits with preclinical animal data, it may also be explained by the confounding effects of surgery, pathology or co-morbidity. Conversely some cohort studies have found no evidence for an association.[25-27] These studies have limited ability to rule out a link between anaesthesia and neurodevelopmental outcome due to a reliance on outcome measures, such as school grade, which may not detect subtle effects, or their broad inclusion criteria include children exposed to anaesthesia at an older age where the risk may be less. The heterogeneity of the cohort studies also make it difficult to analyse the effects of duration of exposure, type of anaesthetic drugs used, doses or combination of drugs used. The above limitations inherently limit the capacity for cohort studies to determine the link between exposure to anaesthesia and neurodevelopmental outcome. These limitations highlight the importance of methodologically robust and adequately powered trials such as this trial.[31]

In this analysis we chose the cognitive scale of the Bayley-III as the ~~primary~~ main outcome of interest. Changes seen in preclinical studies tend to be diffusely distributed over several brain regions. Such diffuse changes are most likely to have an impact on general cognition. Of note, there was also no evidence for a difference in any of the other Bayley-III domains.

Two recent studies have found that while children exposed to anaesthesia had similar school grades, those exposed had an increased risk of not sitting the tests.[26, 28] This raises the possibility that a sub-population of exposed children may have significant neurodevelopmental delay. To investigate this possibility we compared the proportion of children in each arm that scored two standard deviations below the age mean on the composite cognitive score. There was no evidence for a difference; however given the limited power of this analysis, equivalence cannot be assumed. We have also reported the number of children with the diagnosis of ASD, cerebral palsy and visual or hearing defects. This trial was not powered to detect differences in these diagnoses or events, and as expected we found a low event rate in both arms. It should also be noted that at two years of age it is difficult to accurately diagnose the presence of disorders such as ASD, or to accurate assess vision and hearing, and it is possible some children may still have undiagnosed neurologic or neurobehavioural disorders.

Most ~~P~~pre-clinical studies suggest that prolonged exposure to GA is required before injury is seen, usually at least two to three hours.[8] However changes have been seen with one hour of exposure.[38] ~~This is particularly the case for accelerated apoptosis.~~ In this trial the median sevoflurane exposure was ~~around~~ 54 minutes in the GA arm and hence the results are consistent with the majority of pre-clinical data. The trial is~~, however,~~ an important adjunct to these data as translating doses and exposures from animal to humans is uncertain, and it is possible that shorter duration of exposure may still have clinically relevant effects that cannot be detected in animal models.

In human cohorts some studies have found an association with a single short exposure.[17, 39] Others studies have only found an association after longer or multiple exposures.[22] ~~Our findings are also consistent with at least one retrospective report~~This study that found there was no increase in learning disabilities in infants and toddlers exposed to two or less hours of GA.[22] This study revealed that anaesthetic exposure was less than 90 minutes in 61% of the exposed patients and less than two hours in 85% of the exposed patients highlighting that the vast majority of anaesthetics in young children are of fairly brief duration. An internal audit of anaesthetic duration in infants at Boston Children's Hospital revealed that 53% of anaesthetics done in babies less than~~'~~12 months of age ~~of~~ were less than two hours duration. Thus, as far as duration of exposure, it is likely our results are pertinent to approximately half the anaesthetics delivered to infants.

The finding of equivalence after short exposure does not rule out the possibility that longer exposure to anaesthetics may have an effect on neurodevelopment. Further trials are required before any assumptions can be made about the impact of prolonged anaesthesia exposure in infancy. ~~However the majority of anaesthetics in infancy are short. Thus the results suggest that practitioners can continue to provide volatile anaesthetic for the majority of surgeries and procedures in infancy, without exposing the child to the potential risk of delaying surgery or using less established anaesthetic techniques.~~

Some studies have also found a stronger association between multiple anaesthesia exposures and adverse outcome than with single exposure.[20, 30] It is possible this reflects a greater effect of confounding; inevitably children having multiple procedures are more likely to have significant conditions or chronic disease. Our trial cannot address the possible increased toxicity with multiple exposures.

There are a number of limitations to our trial. RA inevitably has a failure rate. As this was an equivalence trial we took the APP analysis to be the most conservative analysis – assuming that treatment failure would bias toward no difference. Given the possibly contentious nature of this assumption, we planned *a priori* to perform a secondary ITT analysis. There were no measureable differences between APP and ITT analyses, implying no bias was introduced by treatment failure. In this study there was a loss to follow up of almost 14%. This, along with RA failure lead to an appreciable amount of missing data, however both the multiple imputation analysis and the inverse probability weighting demonstrated consistent robustness of the findings.

Another limitation is that while the Bayley-IIII is a well validated assessment tool of current development, early neurobehavioural assessment of children is not a perfect predictor of long term outcome due to the considerable variability in developmental timing in young children. Whilst the Bayley-III has been shown to have a stronger correlation with IQ at age five years than earlier versions of the test, it was not designed to assess a broad range of cognitive functions. Cognitive skills emerge and differentiate over childhood and a more detailed neuropsychological assessment is required at a later date to identify mild or circumscribed deficits in cognitive functions as executive skills and memory.[40 41] It is thus important that the children be reassessed later in their development to confirm the results and to more thoroughly examine multiple domains of cognition. Children in this trial are undergoing assessment at five years of age and the results should be known after 2018.

It is important to note that this manuscript reports the results of a secondary outcome. The primary outcome is planned at 5 years, for the reason mentioned above. This analysis of the secondary outcome was pre-specified in the study protocol, however the study was not specifically powered for the secondary outcome and thus it should be interpreted with caution and not regarded as definitive. The analysis of the secondary outcome was planned due to the recognition that there was growing concern over the issue of neurotoxicity and existing evidence to guide practice was inherently limited, and while the two year assessment was not definitive, it would still provide higher quality evidence than that which existed to date. The two year assessment was also planned due to concerns over the feasibility of maintaining the cohort for the longer term follow-up.

In this study over 80% of participants were male. It is well recognised that gender can have an impact on recovery from brain injury. The effect is variable and depends on the nature of the injury and outcome measured, though generally greater effects are seen in males and indeed the neurotoxic effect of anaesthesia on rodents has been shown to be greater in males.[42] Thus the finding of equivalence in our trial with a preponderance of males makes it unlikely that equivalence would not also be demonstrated in females.

In this trial sevoflurane was used without other general anaesthetics. We chose a sevoflurane only anaesthetic as this reflects common practice for anaesthesia for inguinal herniorrhaphy, and the preclinical effects of sevoflurane have been clearly described. There are some preclinical studies that suggest combinations of general anaesthetics may be more injurious and thus our trial cannot shed light on the possibility that an effect may be seen if other agents are added. [3]

Lastly it should also be noted that ~~the~~ ~~Lastly~~ the MacArthur-Bates is dependent on parental report and hence may be open to bias. In addition the standardisation data is of varying degrees of validation across different languages.

In conclusion, this trial found strong evidence that ~~brief~~ exposure of just under an hour to a sevoflurane GA in infancy does not increase the risk of adverse neurodevelopmental outcome at two years of age. While not definitive, ~~T~~this is the strongest clinical evidence to date that just under an hour ~~54 minutes~~ of sevoflurane GA in infancy does not result in significant neurotoxicity. ~~If required for clinical reasons, GA of less than 54 minutes duration does not need to be avoided in this age group.~~

Authors' contributions:

AJD was involved in study design and concept, conduct, data coordination, contribution to the statistical analysis plan, data interpretation, writing and coordinating drafts of the manuscript and revising it critically and approving the version to be published. ND was involved in study design and conduct, data acquisition and coordination, data interpretation and revising the manuscript critically. JCdeG was involved in the coordination and supervision of data collection, data analyses and interpretation, contribution to the statistical analysis plan, revised the manuscript and approved the final manuscript as submitted. DEW was involved in study design and conduct, data acquisition and coordination, data

interpretation and revising the manuscript critically. LD contributed to protocol development, data collection, statistical plan, statistical analysis, data interpretation and writing of the manuscript. GB was involved in study conduct, data coordination and writing and reviewing the manuscript. RS was the lead neuropsychologist and along with ~~,~~ DCB and RWH ~~were~~was involved in study design, concept, conduct, data interpretation and critically revising the manuscript. TS and SJA were involved in interim analyses, contribution to the statistical analysis plan, data interpretation, and revising the manuscript critically. PH was involved in study design, study conduct, interim analyses, contribution to the statistical analysis plan, data interpretation and editing of the manuscript. MJT contributed to the statistical analysis, data interpretation, and manuscript preparation. GG and PLH were involved in study conduct, data acquisition, data interpretation and revising the manuscript critically. IS, BSvonUS, BGL, NW, AL, JTT, DP, OB, PS, ARA and JM were involved in study conduct, data acquisition and coordination and revising the manuscript critically. NSM and MEMcC was involved in study design, concept and conduct, data coordination, data interpretation, writing the manuscript and revising it critically. GF and CB was involved in study design and concept, study conduct, data acquisition, contribution to data interpretation and revising the manuscript critically. GDO was involved in study conduct, data acquisition and coordination, contribution to the statistical analysis plan and revising the manuscript.

Declaration of Interests:

We declare no competing interests.

Disclaimer:

The views expressed in this publication are those of the author(s) and not necessarily those of the MRC, NHS, NIHR or the Department of Health UK.

GAS Trial Group

Trial Steering Committee

Prof Paul Myles (chair); Members – AJD, MEMcC, NSM, ND, DEW, JCdeG, GF, RWH, DCB, CB, ~~D~~Prof~~r~~ Andy Wolf, Prof Neil McIntosh, Prof John Carlin, Prof Kate Leslie, ~~Attendees- GDO, PH, SJA, RS, Ms Suzette Sheppard, Dr Katherine Lee.~~

Data Monitoring Committee

Dr Jonathan de Lima (chair), Prof Greg Hammer, Prof David Field, Prof Val Gebski, Prof Dick Tibboel

GAS Consortium

Australia:

Andrew J. Davidson and Geoff Frawley (Department of Anaesthesia and Pain Management, Murdoch Childrens Research Institute and The Royal Children's Hospital and University of Melbourne, Melbourne, Australia); Pollyanna Hardy (National Perinatal Epidemiology Unit, Clinical Trials Unit, University of Oxford, Oxford, UK); Sarah J. Arnup, Tibor Schuster and Katherine Lee (Clinical Epidemiology and Biostatistics Unit, Murdoch Childrens Research Institute, Melbourne, Australia); Rodney W. Hunt (Department of Neonatal Medicine, The Royal Children's Hospital and Murdoch Childrens Research Institute and University of Melbourne, Melbourne, Australia); Robyn Stargatt (School of Psychological Science, La Trobe University and Child Neuropsychology, Murdoch Childrens Research Institute, Melbourne, Australia); Suzette Sheppard, Gillian D. Ormond, Penelope L. Hartmann, Michael J. Takagi, Stephanie Malarbi and Melissa Doyle (Department of Anaesthesia and Pain Management, Murdoch Childrens Research Institute, Melbourne, Australia); Philip Ragg (Department of Anaesthesia and Pain Management, The Royal Children's Hospital, Melbourne, Australia); Marie Backstrom (Department of Anaesthesia, Monash Medical Centre, Melbourne, Australia); David Costi (Paediatric Anaesthesia, Women's and Children's Hospital, Adelaide, Australia); Britta S. von Ungern-Sternberg (Department of Anaesthesia and Pain Management, Princess Margaret Hospital for Children and The University of Western Australia, Perth, Australia)

New Zealand:

Niall C. Wilton, and Graham Knottenbelt (Department of Paediatric Anaesthesia and Operating Rooms, Starship Children's Hospital, Auckland, New Zealand)

Italy:

Nicola Disma, Giovanni Montobbio, Leila Mameli, Pietro Tuo, and Gaia Girbaldi (Department of Anesthesia, Istituto Giannina Gaslini, Genoa, Italy); Alessio Pini Prato (Department of Pediatric Surgery, Istituto Giannina Gaslini, Genoa, Italy); Girolamo Mattioli (DINOGMI University of Genoa, Genoa, Italy); Andrea Wolfler, Francesca Izzo, and Ida Salvo (Department of Anesthesiology & Paediatric Intensive Care, Ospedale Pediatrico 'Vittore Buzzi', Milan, Italy); Valter Sonzogni, Bruno Guido Locatelli, and

Magda Khotcholava (Department of Anaesthesia, Ospedale Papa Giovanni XXIII, Bergamo, Italy)

The Netherlands:

Jurgen C. de Graaff, Jose T.D.G van Gool, Sandra C. Numan, D. Judy S. van Veldhuijzen and Cor J. Kalkman (Department of Anesthesiology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, The Netherlands); Anneloes L. van Baar and Leonie J.P. Steenis (Utrecht Centre for Child and Adolescent Studies. Utrecht University**,** Utrecht, The Netherlands); J.H.M. Hagenaars, Anthony R. Absalom, Frouckje M. Hoekstra, and Martin Volkers (Department of Anesthesiology, University Medical Center Groningen, Groningen University, Groningen, The Netherlands)

Canada:

Davinia Withington (Department of Anesthesia, Montreal Children's Hospital and McGill University, Montreal, Canada); Koto Furue (Département d'Anesthésie, Centre Hospitalier Universitaire Sainte-Justine, Montreal, Canada); Josee Gaudreault (Montreal Children's Hospital, Montreal, Canada)

USA:

Mary Ellen McCann, Charles Berde, Sulpicio Soriano, Vanessa Young, Navil Sethna, Pete Kovatsis, and Joseph Cravero (Department of Anesthesiology, Perioperative and Pain Medicine, Children's Hospital Boston, Boston, USA); David Bellinger and Jacki Marmor (Department of Neurology, Children's Hospital Boston, Boston, USA); Anne Lynn, Iskra Ivanova, Agnes Hunyady, and Shilpa Verma (University of Washington, Seattle Children's Hospital, Department of Anesthesia and Pain Medicine, Seattle, USA); David Polaner (Children's Hospital Colorado and University of Colorado, Department of Anesthesiology, Colorado, USA); Joss Thomas, Martin Mueller, and Denisa Haret (The University of Iowa Hospital, Department of Anesthesia, Iowa, USA); Peter Szmuk, Jeffery Steiner, and Brian Kravitz (Children's Medical Centre Dallas, Department of Anesthesiology, University of Texas Southwestern Medical Center, Dallas and Children's Medical Center at Dallas and Outcome Research Consortium, Texas, USA); Suresh Surantham (Department of Pediatric Anesthesiology, Ann & Robert H Lurie Children's Hospital of Chicago, Northwestern University, Illinois, USA); Stephen Hays (Pediatric Anesthesia, Monroe Carell Jr. Children's Hospital at Vanderbilt, Tennessee, USA); Andreas Taenzer (Dartmouth-Hitchcock Medical Center, Department of Anesthesiology, New Hampshire, USA); Lynne Maxwell (Department of Anesthesiology and Critical Care Children's Hospital of Philadelphia, Philadelphia, USA); Robert K. Williams

(Anesthesia and Pediatrics, College of Medicine, University of Vermont, Vermont Children's Hospital, Vermont, USA)

UK:

Neil S. Morton (University of Glasgow and Department of Anaesthesia, Royal Hospital for Sick Children, Glasgow, Scotland, UK); Graham Bell (Department of Anaesthesia, Royal Hospital for Sick Children, Glasgow, Scotland, UK); Liam Dorris and Claire Adey (Paediatric Neurosciences Research Group, Fraser of Allander Unit, Royal Hospital for Children, Glasgow, Scotland, UK); Jaycee Pownall (Institute of Health and Wellbeing, University of Glasgow, Glasgow, Scotland, UK); Oliver Bagshaw (Anaesthetic Department, Birmingham Children's Hospital, Birmingham, UK); Anthony Chisakuta (Department of Anaesthetics, Royal Belfast Hospital for Sick Children, Belfast, Northern Ireland, UK); Ayman Eissa (Anaesthetic Department, Sheffield Children's Hospital, Sheffield, UK); Peter Stoddart (Bristol Royal Hospital for Children, Bristol, UK);Annette Davis (Alder hey Childrens' NHS Foundation Trust, Liverpool, UK)

Acknowledgements:

Mark Fagjman (Department of Anaesthesia, Monash Medical Centre, Melbourne, Australia); Daniela Tronconi (Department of Anesthesia, Istituto Giannina Gaslini, Genoa, Italy); David C. van der Zee (Department of Pediatric Surgery, Wilhelmina Children's Hospital, University Medical Centre Utrecht, The Netherlands); Jan Hulscher (Department of Surgery, University Medical Center Groningen, Groningen University, Groningen, The Netherlands); Michael Rivkin (Department of Neurology, Children's Hospital Boston, Boston, USA); Michelle Sadler-Greever (University of Washington, Seattle Children's Hospital, Department of Anesthesia and Pain Medicine, Seattle, USA); Debra Faulk (Children's Hospital Colorado and University of Colorado School of Medicine, Department of Anesthesiology, Colorado, USA); Greta Wilkening (Neurosciences Institute, Children's Hospital Colorado, Colorado, USA); Edward Goldson (Department of Pediatrics, Division of Developmental-Behavioral Pediatrics, Children's Hospital Colorado, Colorado, USA). Danai Udomtecha and~~,~~ Sarah Titler~~, and Martin Mueller~~ (The University of Iowa Hospital, Department of Anesthesia, Iowa, USA); Roxana Ploski and Alan Farrow-Gilespie (Children's Medical Centre Dallas, Department of Anesthesiology, University of Texas Southwestern Medical Center, Dallas and Children's Medical Center at Dallas and Outcome Research Consortium, Texas, USA); Timothy Cooper (Division of Developmental Medicine & the Center for Child Development, Monroe Carell Jr. Children's Hospital at Vanderbilt, Tennessee, USA); Elizabeth Card

(Perioperative Clinical Research Institute, Vanderbilt University Medical Center, Tennessee, USA); Wendy Boardman (Dartmouth-Hitchcock Medical Center, Department of Anesthesiology, New Hampshire, USA);Theodora K. Goebel (Department of Anesthesiology and Critical Care Children's Hospital of Philadelphia, Philadelphia, USA)

Funding

All hospitals and centres were generously supported by departmental funding. In addition to this funding, specific grants received for this study are as follows:

Australia: The Australian National Health & Medical Research Council (Project Grants #1002906 and 491226); Australian and New Zealand College of Anaesthetists (Project Grant #11/021 and 07/012); The Murdoch Childrens Research Institute. This study was supported by the Victorian Government's Operational Infrastructure Support Program.

US: National Institute of Health (Project Grant # NIH 1-R01 HD06 1136-01A1); Food and Drug Administration (Project Grant #FDA-SOL-08-SAFEKIDS-Clin 002-Project 2)

Italy: Italian Ministry of Heath, Young Researchers Grant (Project Grant #167/GR-2009-1476067)

Netherlands: Fonds NutsOhra grant #1305-144 and Vrienden WKZ 16.13.038

Canada: Canadian Institute of Health Research (Project Grant #MCT-98031), Canadian Anesthesiologists' Society, Pfizer Canada Inc.

UK: Health Technologies Assessment- National Institute for Health Research UK (Project Grant # 07/01/05)

Title Figure 1: Trial profile

Table ~~2~~1: Descriptive statistics of birth, pregnancy and peri-anaesthesia data

	RA Arm APP (N=287)	**GA Arm APP (N=356)**	**RA Arm ITT (N=361)**	**GA Arm ITT (N=358)**
Baseline demographics				
Gender, Male	232 (81%)	304 (85%)	294 (82%)	306 (86%)
Chronological age at surgery (days)	68·9 (31)	71·1 (32)	70·1 (32)	71·0 (32)
Post menstrual age at surgery (days)	317·2 (32)	319·7 (32)	318·3 (33)	319·5 (32)
Weight of child at surgery (kg)	4·2 (1·1)	4·3 (1·1)	4·2 (1·1)	4·3 (1·1)
Pregnancy and birth details				
Mean (SD) Post menstrual age at birth (days)	248·2 (29)	248·6 (27)	248.3 (29)	248.6 (27)
Prematurity (Born < 37 weeks gestation)	160 (56%)	195 (55%)	198 (55%)	196 (55%)
Birth Weight (kg)	2·3 (0·9)	2·3 (0·9)	2·4 (0·9)	2·3 (0·9)
Z score for birth weight	-0.68 (1·3)	0.69 (1·3)	-0.66 (1·2)	-0.69 (1·3)
Median (IQR) Apgar score at 1 minute	9 (7-9)	8·5 (7-9)	9 (7-9)	9 (7-9)
Median (IQR) Apgar score at 5 minutes	9 (9-10)	9 (9-10)	9 (9-10)	9 (9-10)
One of a multiple pregnancy	52 (18%)	61 (17%)	62 (17%)	62 (17%)
Mother received partial course antenatal steroids	16 (6%)	19 (5%)	20 (6%)	19 (5%)
Mother received complete course antenatal steroids	95 (33%)	98 (28%)	114 (32%)	98 (28%)
Mother diagnosed with chorioamnionitis	10 (4%)	12 (3%)	11 (3%)	12 (3%)
Prolonged rupture of the membranes (>24 hours)	28 (10%)	34 (10%)	32 (9%)	34 (10%)
Mother diagnosed with pre-eclampsia	50 (17%)	68 (19%)	60 (17%)	68 (19%)
Sepsis during pregnancy	36 (13%)	50 (14%)	43 (12%)	50 (14%)
Mode of delivery of birth				
Cephalic vaginal	135 (47%)	157 (44%)	169 (47%)	157 (44%)
Breech vaginal	1 (<1%)	6 (2%)	3 (1%)	6 (2%)
Compound vaginal	2 (1%)	4 (1%)	3 (1%)	4 (1%)
Caesarean section	149 (52%)	189 (53%)	185 (51%)	191 (53%)
Caesarean section and mother went into labour	42 (15%)	58 (16%)	52 (14%)	59 (16%)
Mother exposed to nitrous oxide during delivery	48 (18%)	62 (18%)	61 (18%)	62 (18%)
IVH	7 (2%)	6 (2%)	8 (2%)	6 (2%)
IVH Grade 1	5 (2%)	6 (2%)	5 (2%)	6 (2%)
IVH Grade 2	2 (1%)	0	2 (1%)	0
Retinopathy of prematurity	17 (9%)	16 (6%)	30 (8%)	16 (6%)
Hearing defects detected by perinatal screening	7 (3%)	10 (3%)	8 (3%)	10 (3%)
PDA diagnosed	23 (8%)	21 (6%)	27 (8%)	21 (6%)
PDA never treated	9 (3%)	9 (3%)	11 (3%)	9 (3%)
PDA treated with non-steroidal anti-inflammatory drugs	14 (5%)	10 (3%)	16 (4%)	10 (3%)
Familial Demographics:				
Primary language(s) only spoken*	252 (88%)	305 (86%)	311 (86%)	307 (86%)
Maternal Age at Birth >21	273 (96%)	339 (95%)	339 (95%)	341 (95%)
Family structure two caregivers together, at birth	261 (91%)	324 (91%)	328 (91%)	326 (91%)
Maternal education				
Completed tertiary studies	150 (52%)	171 (48%)	181 (51%)	171 (48%)
Continuing tertiary studies	50 (17%)	67 (19%)	68 (19%)	67 (19%)
Completed year 11 or 12	62 (22%)	83 (23%)	77 (22%)	84 (24%)
Did not complete year 11	25 (9%)	33 (9%)	32 (9%)	34 (10%)
Anaesthesia Details:				
Median (IQR)Blood glucose level (mmol/L)	5·4 (4·7-6·1)	5·5 (4·8-6·4)	5·4 (4·7-6·2)	5·5 (4·8-6·4)

Rescue glucose given IV	2 (1%)	4 (1%)	2 (1%)	4 (1%)
Haemoglobin (g/100 ml)	10·3 (2·1)	10·2 (2·0)	10·3 (2·1)	10·2 (2·0)
Need for fluid bolus for hypotension	15 (5%)	59 (17%)	21 (6%)	59 (17%)
Vasoactive drugs given (including atropine)	4 (1%)	17 (5%)	6 (2%)	17 (5%)
Median (IQR)Duration of surgery (mins)	26·0 (19·0-35·0)	28·0 (20·0-40·0)	28·0 (20·0-38·0)	28·0 (20·0-40·0)
Median (IQR) Duration of sevoflurane exposure (mins)	NA	54.0 (41.0-70.0)	42.0 (31.0-62.5)**	54.0 (41.0-70.0)
End tidal sevoflurane concentration (%)	NA	2·6 (0·7)	2·3 (0·8)**	2·6 (0·7)
Total concentration x hours per	NA	2·6 (1·1)	1·9 (1·0)**	2·6 (1·1)
Any significant apnoea to 12hrs postop***	6 (2%)	15 (4%)	10 (3%)	15 (4%)

Data are n(% of non-missing data) or Mean (SD), unless otherwise stated. APP= As Per Protocol; GA= General Anaesthesia; ITT= Intention to treat; IV= Intra-venously; IVH= Intra ventricular haemorrhage; IQR= Interquartile Range; PDA = Patent ductus arteriosus; RA= Awake Regional Anaesthesia.

* The primary language spoken at home, is the primary language in each country that the Bayley was conducted e.g in Italy it was conducted in Italian

** For those cases that received sevoflurane

*** significant apnoea defined as a pause in breathing for more than 15 seconds or more than 10 seconds if associated with oxygen saturation less than 80% or bradycardia (20% decrease in heart rate)

Table ~~3~~2: 2 year descriptive statistics demographic data

	RA Arm APP (N=287)	GA Arm APP (N=356)	RA Arm ITT (N=361)	GA Arm ITT (N=358)
Assessment details				
Location of two year assessment at hospital	204 (96%)	240 (94%)	250 (95%)	241 (94%)
Family demographics at two years				
Paid employment is main family income	222 (90%)	267 (88%)	274 (90%)	268 (88%)
Family structure, two caregivers living together	226 (91%)	274 (90%)	277 (90%)	275 (90%)
Number of children at home				
1	88 (36%)	118 (39%)	115 (37%)	118 (39%)
2	109 (44%)	120 (40%)	131 (43%)	121 (40%)
3	37 (15%)	43 (14%)	45 (14%)	43 (14%)
>3	14 (6%)	22 (7%)	17 (6%)	22 (7%)
Birth order				
1	123 (50%)	161 (53%)	154 (50%)	161 (53%)
2	87 (35%)	90 (30%)	107 (35%)	91 (30%)
>2	37 (15%)	52 (17%)	46 (15%)	52 (17%)
Corrected age at assessment (weeks)	108·9 (13·0)	108 (9·8)	108·7 (12·5)	108 (9·8)
Events since original anaesthesia				
Number of hospitalisations since inguinal herniorrhaphy operation				
0	172 (69%)	206 (68%)	210 (68%)	207 (68%)
1	51 (20%)	64 (21%)	69 (22%)	64 (21%)
2	14 (6%)	18 (6%)	16 (5%)	18 (6%)
>2	6 (2%)	8 (3%)	8 (3%)	8 (3%)
Number of anaesthetics since inguinal herniorrhaphy operation				
1	34 (14%)	36 (12%)	42 (14%)	36 (12%)
2	5 (2%)	6 (2%)	6 (2%)	6 (2%)
>2	4 (2%)	4 (1%)	4 (1%)	4 (1%)
Child had a head injury that involved the loss of consciousness	7 (3%)	4 (1%)	7 (2%)	4 (1%)
Child has an acquired brain injury	1 (0%)	1 (0%)	1 (0%)	1 (0%)
Child has any malformations				
Cardiac	0	4 (1%)	0	4 (1%)
Central Nervous System	3 (1%)	1 (<1%)	3 (1%)	1 (<1%)
Genitourinary	6 (2%)	4 (1%)	8 (3%)	4 (1%)
Genetic condition	1 (<1%)	0	1 (<1%)	0
Respiratory	0	1 (<1%)	0	1 (<1%)
Skeletal	4 (2%)	11 (4%)	4 (1%)	11 (4%)
Cleft lip/palate	1 (<1%)	0	1 (<1%)	0
Craniofacial	2 (1%)	0	2 (1%)	0
Child has any chronic illness	42 (17%)	43 (14%)	50 (16%)	43 (14%)
Child had any prescribed medication for two months or longer	43 (17%)	50 (16%)	93 (17%)	59 (19%)
Child had febrile seizures following the hernia repair	8 (3%)	9 (3%)	10 (3%)	9 (3%)

Child had other seizures following the hernia repair	1 (<1%)	4 (1%)	1 (<1%)	4 (1%)
The child has had an intervention for neurodevelopmental issues since the inguinal herniorrhaphy operation	46 (19%)	55 (18%)	54 (18%)	55 (18%)
Speech Therapy	22 (9%)	27 (9%)	28 (9%)	27 (9%)
Physiotherapy	22 (9%)	27 (9%)	26 (8%)	27 (9%)
Occupational Therapy	9 (4%)	12 (4%)	12 (4%)	12 (4%)
Psychology	1(<1%)	6 (2%)	1 (<1%)	6 (2%)
Developmental medicine/early intervention	8 (3%)	7 (2%)	9 (3%)	7 (2%)
Child attends play group/child care on a regular basis	147 (60%)	177 (58%)	186 (61%)	178 (58%)
Physical examination				
Height (cm)	86·6 (5·5)	86·9 (4·9)	86·4 (5·2)	86·9 (4·9)
Weight (kg)	12·6 (2·0)	12·6 (1·9)	12·6 (2·0)	12·6 (1·9)
Head circumference (cm)	49·1 (2·1)	48·8 (2·2)	49·0 (2·0)	48·8 (2·2)
Arm circumference (cm)	16·4 (2·0)	16·1 (1·8)	16·4 (2·0)	16·1 (1·8)

Data are n(% of non-missing data) or Mean (SD), unless otherwise stated. APP= As Per Protocol; GA=General Anaesthesia; ITT= Intention to treat; RA= Awake Regional Anaesthesia.

Table ~~5~~3: Descriptive statistics Bayley-III and Macarthur-Bates Scores by group

	RA Arm APP	GA Arm APP	RA Arm ITT	GA Arm ITT
Cognitive				
Cognitive, Scaled Score	238, 9·7 (2·8)	294, 9·6 (2·9)	292, 9·7 (2·8)	295, 9·6 (2·9)
Cognitive, Composite Score	238, 98·6 (14·2)	294, 98·2 (14·7)	292, 98·6 (14·2)	295, 98·2 (14·6)
Language				
Receptive Language, Scaled Score	236, 8·7 (2·9)	285, 8·6 (2·9)	287, 8·8 (2·9)	286, 8·6 (2·9)
Expressive Language Scaled Score	235, 9·3 (2·9)	290, 9·3 (3·0)	287, 9·4 (2·9)	291, 9·3 (3·0)
Language, Composite Score	235, 94·6 (15·4)	285, 94·0 (15·6)	286, 94·9 (15·5)	286, 94·0 (15·6)
Motor				
Fine Motor, Scaled Score	234, 10·5 (2·7)	287, 10·4 (2·7)	287, 10·6 (2·8)	288, 10·4 (2·7)
Gross Motor, Scaled Score	234, 8·8 (2·4)	279, 8·7 (2·6)	285, 8·9 (2·5)	280, 8·7 (2·6)
Motor, Composite Score	232, 98·3 (13·2)	274, 97·9 (13·4)	283, 98·9 (13·5)	275, 97·8 (13·4)
Social Emotional				
Social Emotional, Scaled Score	218, 9·5 (3·8)	267, 9·1 (3·7)	267, 9·5 (3·8)	268, 9·1 (3·7)
Social Emotional, Composite Score	218, 97·4 (19·0)	267, 95·4 (18·3)	267, 97·4 (19·2)	268, 95·4 (18·3)
Adaptive Behaviour				
Communication Scaled Score	233, 9·7 (2·9)	291, 9·6 (2·9)	288, 9·8 (2·9)	292, 9·6 (2·9)
Community Use Scaled Score	233, 9·8 (2·8)	291, 9·9 (2·7)	288, 9·9 (2·8)	292, 9·8 (2·7)
Functional Pre-Academics Scaled Score	233, 9·0 (3·0)	291, 9·2 (2·9)	288, 9·1 (3·0)	292, 9·2 (2·9)
Home Living Scaled Score	233, 9·9 (2·8)	291, 10·1 (2·7)	288, 9·9 (2·9)	292, 10·1 (2·7)
Health and Safety Scaled Score	233, 9·0 (2·8)	291, 9·3 (2·7)	288, 9·0 (2·9)	292, 9·3 (2·7)
Leisure Scaled Score	233, 9·4 (3·0)	291, 9·9 (2·8)	288, 9·5 (3·1)	292, 9·9 (2·8)
Self-Care Scaled Score	233, 6·8 (2·6)	291, 6·6 (2·5)	288, 6·8 (2·6)	292, 6·6 (2·5)
Self-Direction Scaled Score	233, 9·7 (3·2)	291, 10·0 (3·2)	288, 9·8 (3·2)	292, 10·0 (3·2)
Social Scaled Score	233, 9·3 (2·9)	291, 9·5 (2·8)	288, 9·4 (2·9)	292, 9·5 (2·8)
Motor Scaled Score	233, 9·8 (3·2)	291, 10·0 (2·9)	288, 9·9 (3·3)	292, 10·0 (2·9)
Adaptive Behaviour Composite Score	233, 93·1 (15·6)	291, 94·3 (14·7)	288, 93·4 (16·1)	292, 94·3 (14·7)
MacArthur Bates Percentile Score	195, 32·4 (27·9)	247, 34·7 (28·7)	240, 33·6 (28·0)	247, 34·7 (28·7)

Data as n, mean (SD). APP= As Per Protocol; GA= General Anaesthesia; ITT= Intention to treat; RA= Awake-Regional Anaesthesia.

Table ~~6~~4: Between group comparisons in Bayley-III and MacArthur-Bates scores

Scale		***Δ: RA - GA**	**SE Δ**	**95% CI for Δ: RA - GA**	
Cognitive composite score	APP multiple imputation	0·169	1·26	-2·30	2·64
	APP complete case	0·458	1·26	-2·02	2·94
	ITT multiple imputation	0·256	1·18	-2·06	2·58
	ITT complete case	0·430	1·19	-1·90	2·76
Language composite score	APP multiple imputation	1·146	1·39	-1·59	3·88
	APP complete case	0·628	1·37	-2·07	3·32
	ITT multiple imputation	1·454	1·32	-1·14	4·05
	ITT complete case	0·942	1·30	-1·61	3·49
Motor composite score	APP multiple imputation	0·598	1·20	-1·77	2·97
	APP complete case	0·410	1·19	-1·92	2·74
	ITT multiple imputation	0·143	1·13	-1·08	3·37
	ITT complete case	1·031	1·14	-1·20	3·26
Social emotional composite score	APP multiple imputation	1·005	2·09	-3·12	5·13
	APP complete case	2·012	1·70	-1·32	5·35
	ITT multiple imputation	1·183	2·03	-2·82	5·19
	ITT complete case	2·015	1·62	-1·17	5·20
Adaptive behaviour composite score	APP multiple imputation	-0·893	1·34	-3·52	1·73
	APP complete case	-1·223	1·33	-3·83	1.38
	ITT multiple imputation	-0·502	1·28	-3·03	2·02
	ITT complete case	-0·830	1·28	-3·34	1·68
MacArthur Bates Percentile Score	APP multiple imputation	-1·811	3·06	-7·85	4·23
	APP complete case	-2·359	2·71	-7·69	2·98
	ITT multiple imputation	-0·544	2·87	-6·20	5·11
	ITT complete case	-1·113	2·57	-6·17	3·94

*Adjusted for gestational age at birth. APP= As Per Protocol; GA= General Anaesthesia; ITT= Intention to treat; RA= Awake Regional Anaesthesia.

Table ~~9~~5: 2 year non-psychometric outcome data

	RA Arm APP (N=287)	**GA Arm APP (N=356)**	**RA Arm ITT (N=361)**	**GA Arm ITT (N=358)**
Child has a hearing defect				
Conductive	9 (3%)	6 (2%)	9 (2%)	6 (2%)
Sensorineural	0	3 (1%)	1 (<1%)	3 (1%)
Child has a hearing aid	1 (8%)	3 (25%)	2 (15%)	3 (23%)
Child is legally blind (<6/60 in both eyes)	1 (2%)	0	1 (2%)	0
Child has Cerebral Palsy	1 (<1%)	4 (1%)	1 (0%)	4 (1%)
The child has Autism Spectrum Disorder	2 (1%)	0	2 (1%)	0

Data are n (% of non-missing data), unless otherwise stated. APP= As Per Protocol; GA= General Anaesthesia; ITT= Intention to treat; RA= Regional Anaesthesia.

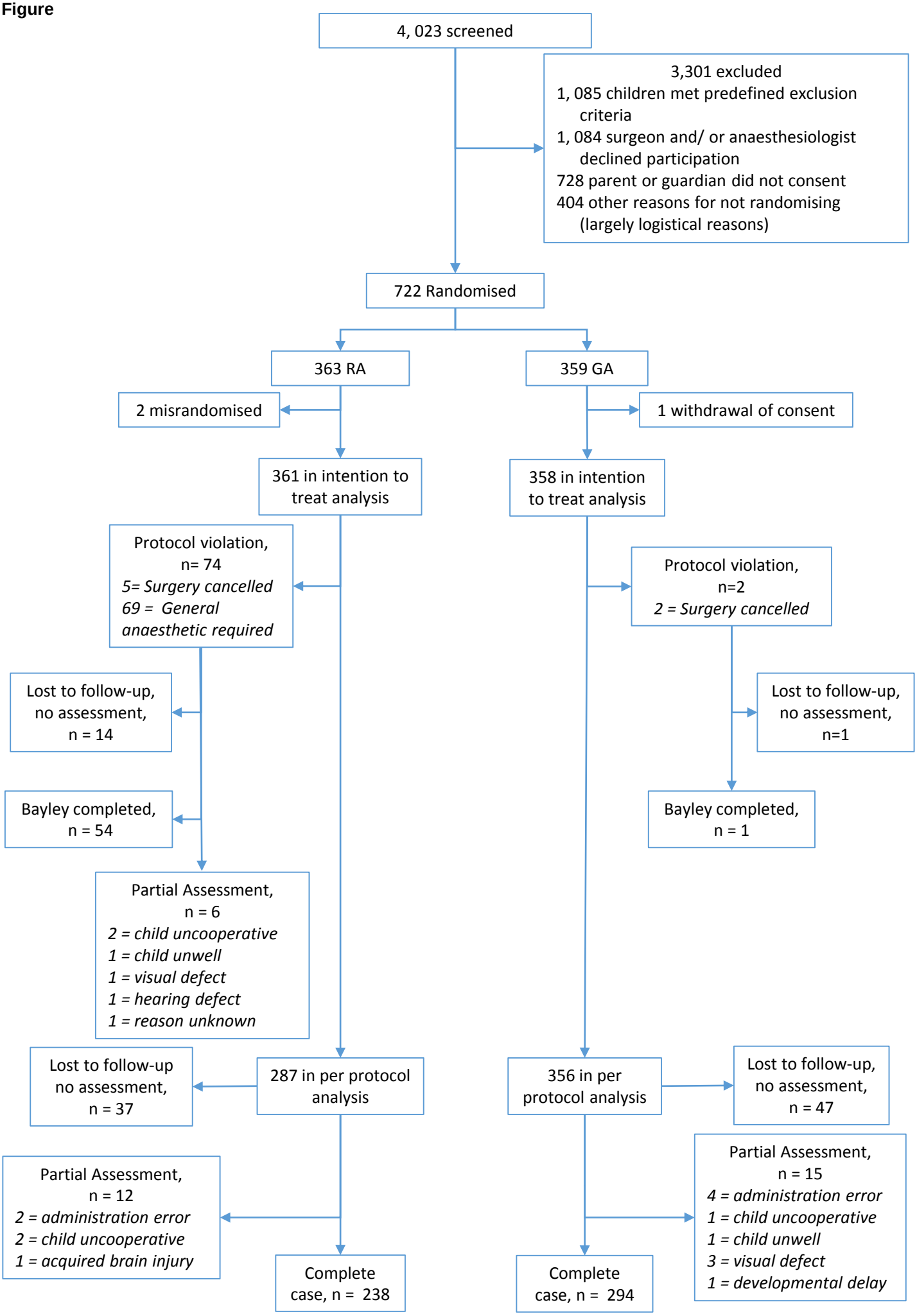
References

1. Jevtovic-Todorovic V, Absalom AR, Blomgren K, Brambrink A, Crosby G, Culley DJ, et al. Anaesthetic neurotoxicity and neuroplasticity: an expert group report and statement based on the BJA Salzburg Seminar. British journal of anaesthesia. 2013; **111**(2): 143-51.
2. Briner A, Nikonenko I, De Roo M, Dayer A, Muller D, Vutskits L. Developmental Stage-dependent persistent impact of propofol anesthesia on dendritic spines in the rat medial prefrontal cortex. Anesthesiology. 2011; **115**(2): 282-93.
3. Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, et al. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci. 2003; **23**(3): 876-82.
4. Sanchez V, Feinstein SD, Lunardi N, Joksovic PM, Boscolo A, Todorovic SM, et al. General Anesthesia Causes Long-term Impairment of Mitochondrial Morphogenesis and Synaptic Transmission in Developing Rat Brain. Anesthesiology. 2011; **115**(5): 992-1002.
5. Istaphanous GK, Ward CG, Nan X, Hughes EA, McCann JC, McAuliffe JJ, et al. Characterization and quantification of isoflurane-induced developmental apoptotic cell death in mouse cerebral cortex. Anesthesia and analgesia. 2013; **116**(4): 845-54.
6. Paule MG, Li M, Allen RR, Liu F, Zou X, Hotchkiss C, et al. Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys. Neurotoxicol Teratol. 2011; **33**(2): 220-30.
7. Raper J, Alvarado MC, Murphy KL, Baxter MG. Multiple Anesthetic Exposure in Infant Monkeys Alters Emotional Reactivity to an Acute Stressor. Anesthesiology. 2015.
8. Slikker W, Jr., Zou X, Hotchkiss CE, Divine RL, Sadovova N, Twaddle NC, et al. Ketamine-induced neuronal cell death in the perinatal rhesus monkey. Toxicol Sci. 2007; **98**(1): 145-58.
9. Brambrink AM, Back SA, Riddle A, Gong X, Moravec MD, Dissen GA, et al. Isoflurane-induced apoptosis of oligodendrocytes in the neonatal primate brain. Annals of neurology. 2012; **72**(4): 525-35.
10. Brambrink AM, Evers AS, Avidan MS, Farber NB, Smith DJ, Zhang X, et al. Isoflurane-induced neuroapoptosis in the neonatal rhesus macaque brain. Anesthesiology. 2010; **112**(4): 834-41.
11. Hansen TG. Anesthesia-related neurotoxicity and the developing animal brain is not a significant problem in children. Paediatric anaesthesia. 2015; **25**(1): 65-72.
12. Psaty BM, Platt R, Altman RB. Neurotoxicity of generic anesthesia agents in infants and children: an orphan research question in search of a sponsor. Jama. 2015; **313**(15): 1515-6.
13. Rappaport BA, Suresh S, Hertz S, Evers AS, Orser BA. Anesthetic neurotoxicity--clinical implications of animal models. The New England journal of medicine. 2015; **372**(9): 796-7.
14. Crosby G, Davis PJ. General anesthesia in infancy is associated with learning disabilities-or not. Anesthesia and analgesia. 2013; **117**(6): 1270-2.
15. Davidson AJ. Anesthesia and neurotoxicity to the developing brain: the clinical relevance. Paediatric anaesthesia. 2011; **21**(7): 716-21.
16. DiMaggio C, Sun LS, Ing C, Li G. Pediatric anesthesia and neurodevelopmental impairments: a Bayesian meta-analysis. Journal of neurosurgical anesthesiology. 2012; **24**(4): 376-81.
17. Ing C, DiMaggio C, Whitehouse A, Hegarty MK, Brady J, von Ungern-Sternberg BS, et al. Long-term differences in language and cognitive function after childhood exposure to anesthesia. Pediatrics. 2012; **130**(3): e476-85.
18. Ing CH, DiMaggio CJ, Malacova E, Whitehouse AJ, Hegarty MK, Feng T, et al. Comparative analysis of outcome measures used in examining neurodevelopmental effects of early childhood anesthesia exposure. Anesthesiology. 2014; **120**(6): 1319-32.
19. Lei SY, Hache M, Loepke AW. Clinical research into anesthetic neurotoxicity: does anesthesia cause neurological abnormalities in humans? Journal of neurosurgical anesthesiology. 2014; **26**(4): 349-57.

20. Flick RP, Katusic SK, Colligan RC, Wilder RT, Voigt RG, Olson MD, et al. Cognitive and behavioral outcomes after early exposure to anesthesia and surgery. Pediatrics. 2011; **128**(5): e1053-61.
21. Sprung J, Flick RP, Katusic SK, Colligan RC, Barbaresi WJ, Bojanic K, et al. Attention-deficit/hyperactivity disorder after early exposure to procedures requiring general anesthesia. Mayo Clinic proceedings. 2012; **87**(2): 120-9.
22. Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, Mickelson C, et al. Early exposure to anesthesia and learning disabilities in a population-based birth cohort. Anesthesiology. 2009; **110**(4): 796-804.
23. DiMaggio C, Sun LS, Li G. Early childhood exposure to anesthesia and risk of developmental and behavioral disorders in a sibling birth cohort. Anesthesia and analgesia. 2011; **113**(5): 1143-51.
24. DiMaggio C, Sun LS, Kakavouli A, Byrne MW, Li G. A retrospective cohort study of the association of anesthesia and hernia repair surgery with behavioral and developmental disorders in young children. Journal of neurosurgical anesthesiology. 2009; **21**(4): 286-91.
25. Bartels M, Althoff RR, Boomsma DI. Anesthesia and cognitive performance in children: no evidence for a causal relationship. Twin Res Hum Genet. 2009; **12**(3): 246-53.
26. Hansen TG, Pedersen JK, Henneberg SW, Pedersen DA, Murray JC, Morton NS, et al. Academic performance in adolescence after inguinal hernia repair in infancy: a nationwide cohort study. Anesthesiology. 2011; **114**(5): 1076-85.
27. Hansen TG, Pedersen JK, Henneberg SW, Morton NS, Christensen K. Educational outcome in adolescence following pyloric stenosis repair before 3 months of age: a nationwide cohort study. Paediatric anaesthesia. 2013; **23**(10): 883-90.
28. Block RI, Thomas JJ, Bayman EO, Choi JY, Kimble KK, Todd MM. Are anesthesia and surgery during infancy associated with altered academic performance during childhood? Anesthesiology. 2012; **117**(3): 494-503.
29. Bong CL, Allen JC, Kim JT. The effects of exposure to general anesthesia in infancy on academic performance at age 12. Anesthesia and analgesia. 2013; **117**(6): 1419-28.
30. Wang X, Xu Z, Miao CH. Current clinical evidence on the effect of general anesthesia on neurodevelopment in children: an updated systematic review with meta-regression. PloS one. 2014; **9**(1): e85760.
31. Davidson AJ, Becke K, de Graaff J, Giribaldi G, Habre W, Hansen T, et al. Anesthesia and the developing brain: a way forward for clinical research. Paediatric anaesthesia. 2015; **25**(5): 447-52.
32. Davidson AJ, Morton NS, Arnup SJ, de Graaff JC, Disma N, Withington DE, et al. Apnea after Awake Regional and General Anesthesia in Infants: The General Anesthesia Compared to Spinal Anesthesia Study-Comparing Apnea and Neurodevelopmental Outcomes, A Randomized Controlled Trial. Anesthesiology. 2015; **123**(1): 38-54.
33. Frawley G, Bell G, Disma N, Withington DE, de Graaff JC, Morton NS, et al. Predictors of Failure of Awake Regional Anesthesia for Neonatal Hernia Repair: Data from the General Anesthesia Compared to Spinal Anesthesia Study-Comparing Apnea and Neurodevelopmental Outcomes. Anesthesiology. 2015; **123**(1): 55-65.
34. Bayley N. Bayley Scales of Infant and Toddler Development. 3rd ed. San Antonio: Harcourt Assessment Inc; 2006.
35. Fenson L, Marchman VA, Thal DJ, Dale PS, Reznic JS, Bates E. MacArthur-Bates Communicative Development Inventories: User's Guide and Technical Manual 2nd ed. Baltimore Brookes Publishing; 2007.
36. van Buuren S. Multiple imputation of discrete and continuous data by fully conditional specification. Statistical methods in medical research. 2007; **16**(3): 219-42.
37. Seaman SR, White IR. Review of inverse probability weighting for dealing with missing data. Statistical methods in medical research. 2013; **22**(3): 278-95.
38. Johnson SA, Young C, Olney JW. Isoflurane-induced neuroapoptosis in the developing brain of nonhypoglycemic mice. Journal of neurosurgical anesthesiology. 2008; **20**(1): 21-8.

39. Di Maggio C, Sun L, Kakavuoli A, Byrne M, Li G. A retrspective cohort study of the association of anesthesia and hernia repair surgery with behavioral and developmental disorders in young children. Journal of neurosurgical anesthesiology. 2009; **21**: 286-91.
40. Harris SR, Langkamp DL. Predictive value of the Bayley mental scale in the early detection of cognitive delays in high-risk infants. Journal of perinatology : official journal of the California Perinatal Association. 1994; **14**(4): 275-9.
41. Bode MM, D'Eugenio DB, Mettelman BB, Gross SJ. Predictive validity of the Bayley, Third Edition at 2 years for intelligence quotient at 4 years in preterm infants. Journal of developmental and behavioral pediatrics : JDBP. 2014; **35**(9): 570-5.
42. Lee BH, Chan JT, Kraeva E, Peterson K, Sall JW. Isoflurane exposure in newborn rats induces long-term cognitive dysfunction in males but not females. Neuropharmacology. 2014; **83**: 9-17.

Figure


4, 023 screened
3,301 excluded
1, 085 children met predefined exclusion criteria
1, 084 surgeon and/ or anaesthesiologist declined participation
728 parent or guardian did not consent
404 other reasons for not randomising (largely logistical reasons)
722 Randomised
363 RA
359 GA
2 misrandomised
1 withdrawal of consent
361 in intention to treat analysis
358 in intention to treat analysis
Protocol violation, n= 74
5= Surgery cancelled
69 = General anaesthetic required
Protocol violation, n=2
2 = Surgery cancelled
Lost to follow-up, no assessment, n = 14
Lost to follow-up, no assessment, n=1
Bayley completed, n = 54
Bayley completed, n = 1
Partial Assessment, n = 6
2 = child uncooperative
1 = child unwell
1 = visual defect
1 = hearing defect
1 = reason unknown
Lost to follow-up no assessment, n = 37
287 in per protocol analysis
356 in per protocol analysis
Lost to follow-up, no assessment, n = 47
Partial Assessment, n = 12
2 = administration error
2 = child uncooperative
1 = acquired brain injury
Partial Assessment, n = 15
4 = administration error
1 = child uncooperative
1 = child unwell
3 = visual defect
1 = developmental delay
Complete case, n = 238
Complete case, n = 294

Web Appendix

Click here to download Web Appendix: Supplemental appendix.docx

A multi-site RCT comparing regional and general anaesthesia for effects on neurodevelopmental outcome and apnoea in infants

-

The GAS study

Principal Investigators

Andrew Davidson
Department of Anaesthesia
Royal Children's Hospital
Flemington Road
Parkville 3052 Victoria,
AUSTRALIA
Tel: +61 3 9345 5233
Mobile +61 402 271 274
Fax +61 3 9345 6003
Email andrew.davidson@rch.org.au

Mary Ellen McCann
Department of Anesthesiology,
Perioperative and Pain Medicine,
Children's Hospital Boston,
300 Longwood Avenue,
Boston, MA 02115-5737
USA
Tel: 0011-1- 617-355-7737
Fax: 0011-1-617-730-0894
Email: Mary.McCann@childrens.harvard.edu

Neil Morton
Department of Anaesthesia
Royal Hospital for Sick Children
Yorkhill
Glasgow G3 8SJ
Tel: 0011- 44- 13 8983 0402
Fax: 0011- 44- 14 1201 0186
Email: neilmorton@mac.com

This trial is not sponsored by any pharmaceutical company or other commercial entity

Trial protocol version date: 18 October 2012

INVESTIGATORS ("The GAS Consortium")

(PI/chair first then alphabetical order)

Chief Investigators

A/Prof Andrew Davidson (PI),
A/Prof Mary Ellen McCann (PI),
Dr Neil Morton (PI),
Professor David Bellinger,
Professor Charles Berde,
Dr Jurgen de Graaff
Dr Nicola Disma,
Dr Geoff Frawley,
Ms Pollyanna Hardy,
Dr Rod Hunt,
Dr Robyn Stargatt,
Dr Davinia Withington,
Professor Andy Wolf

Associate investigators

Professor Vicki Anderson,
Professor John Carlin,
Mr Tom Clarnette,
Professor Lex Doyle,
A/Prof Kate Leslie,
A/Prof Sulpicio Soriano,
Dr Suellen Walker

Site investigators- Australia
A/Prof Andrew Davidson- Royal Children's Hospital- Melbourne
Dr David Costi- Women's and Children's Hospital- Adelaide
Dr Mark Fajgman- Department of Anaesthesia- Monash Medical Centre
Dr Bruce Hullett- Princess Margaret Hospital- Perth

Site investigators- US
A/Prof Mary Ellen McCann- Children's Hospital Boston- Boston
Dr Joss Thomas- The University of Iowa Hospital- Iowa
Dr Anne Lynn- Seattle Children's Hospital and Regional Medical Center- Seattle
Dr Santhanam Suresh- Ann and Robert H. Lurie Children's Hospital of Chicago - Chicago

Dr Lynn Maxwell- Children's Hospital of Philadelphia- Philadelphia
Dr Stephen Hays- Vanderbilt Children's Hospital- Nashville
Dr Andreas Taenzer- Dartmouth Hitchcock Medical Center- Lebanon
Dr David Polaner- Denver Children's Hospital- Colorado
Dr Peter Szmuk- Children's Medical Center at Dallas- Texas
Dr. Robert Williams - Vermont Children's Hospital at Fletcher Allen Health Care

Site investigators- Canada

Dr Davinia Withington, Montreal Children's Hospital, Montreal
Dr Koto Furue, Centre Hospitalier UniversitaireSainte-Justine, Montreal

Site Invesitgators- Italy
Dr Nicola Disma, Gaslini Children's Hospital, Genoa
Dr Bruno Guido Locatelli, Ospedali Riuniti, Bergamo
Dr Ida Salvo, Buzzi, Milan

Site Investigators- UK
Dr Neil Morton- Royal Hospital for Sick Children- Glasgow
Dr Peter Stoddart- Bristol Royal Hospital for Children- Bristol
Dr Anthony Chisakuta- Royal Belfast Hospital for Sick Children- Belfast
Dr Oliver Bagshaw- Birmingham Children's Hospital- Birmingham
Dr Annette Davis, Royal Liverpool Children's Hospital Alder Hey, Liverpool
Dr Ayman Eissa, Sheffield Children's Hospital, Sheffield

Site Investigators- NZ
Dr Niall Wilton- Starship Children's Health- Auckland

Site Investigators- The Netherlands
Dr Jurgen de Graaff- University Medical Centre Utrecht, Utrecht
Prof. Anthony Absalom- Universitair Medisch Centrum, Groningen

Project officers, Melbourne:
Ms Suzette Sheppard
Ms Gillian Ormond

National Study Coordinator for USA, Boston
Ms Jacki Marmor

Principal Nurse Researcher for USA, Boston
Ms Vanessa Young

Data management coordinator, Melbourne:
Ms Suzanna Vidmar

Trial statisticians
Pollyanna Hardy
Sarah Arnup

STUDY SITES

Australia
Department of Anaesthesia,
Flemington Road,
Royal Children's Hospital,
Parkville, VIC 3052
Ph: + 61 3 9345 5233

Department of Anaesthesia,
Monash Medical Centre,
246 Clayton Road,
Clayton VIC 3168
Ph: + 61 3 9594 3283

Department of Paediatric Anaesthesia
Adelaide Women's and Children's Hospital
72 King William Road,
North Adelaide, SA 5006
Ph: + 61 8 8161 7231

Department of Anaesthesia
Princess Margaret Hospital for Children
Roberts Road,
Subiaco, WA 6008
Ph: + 61 3 8 9340 8222

Cabrini Hospital
183 Wattletree Road
Malvern, VIC, 3144
Ph: +61 3 9508 1222

USA

Children's Hospital Boston,
Department of Anesthesiology,
Perioperative and Pain Medicine,

300 Longwood Avenue,
Boston, MA 02115
Ph: +1-857-218-4657

The University of Iowa Hospital
Department of Anesthesiology,
200 Hawkins Drive
Iowa City, Iowa 52242
Ph: +1-319-356-8878

Seattle Children's Hospital
University of Washington,
Department of Anesthesiology
4800 Sandpoint Way NE
Seattle, WA 98105
Ph: +1-206-987-3996

Children's Memorial Hospital,
Department of Pediatric Anesthesiology,

2300 Children's Plaza, Box 19,
Chicago, IL 60614
Ph: +1-773-975-8705

Children's Hospital of Philadelphia,
Department of Anesthesiology and
Critical Care Medicine,
9th Floor Main
34th Street and Civic Center Boulevard,
Philadelphia, PA 19104
Ph: +1-215-590-4925

Vanderbilt University Medical Cente,
Department of Anesthesiology
705A Medical Arts Building
1211 21st Avenue South,
Nashville, TN 37212
Ph: +1-615-343-6223

Dartmouth Hitchcock Medical Center,
Department of Anesthesiology,
One Medical Center Drive,
Lebanon, NH 03756
Ph: +1-603-650-6823

The Children's Hospital Denver
Anesthesiology
13123 East 16th Avenue
Aurora, Colorado 80045
Ph: +1-720-777-8485

Children's Medical Center at Dallas
Department of Anesthesiology,
1935 Medical district Drive
Dallas, Texas 75235
Ph: +1-214-456-8559

Vermont Children's Hospital at Fletcher Allen Health Care
Department of Anesthesiology
111 Colchester Avenue, 122WP2
Burlington, VT 05401
Ph: +1 -802- 847-2415

Canada

Department of Anesthesia
Montreal Children's Hospital
2300 Tupper Street,
D-1070, Montreal (Qc) H3H 1P3
Ph: +1-514-412-4400

Centre Hospitalier Universitaire Sainte-Justine,
3175 Côte-Sainte-Catherine
Montréal
Québec
H3T 1C5
Ph: +1-514-345-4733

Italy

Department of Anesthesia
IRCCS Gaslini Children's Hospital
L.go G. Gaslini, 5
16100 Genoa
Ph: +39 010 5636445

Department of Anesthesiology and Paediatric Intensive Care,
'Vittore Buzzi' Children's Hospital,
20100 Milano
Ph: +39 025 7995363

Department of Anesthesia
Ospedali Riuniti Di Bergamo
Via Tito Livio 2,
24100 Bergamo
Ph: +39 035 368611

UK

Bristol Royal Hospital for Children,
Upper Maudlin Street,
Bristol BS2 8BJ, UK
Ph: +44 117 342 8460

Department of Anaesthetics
Royal Belfast Hospital for Sick Children
Falls Road
Belfast BT12 6BE
Ph: 0289 024 0503

Department of Anaesthesia
Royal Hospital for Sick Children
Yorkhill
Glasgow G3 8SJ
Ph: +44 138 983 0402

Department of Anaesthesia
Sheffield Children's Hospital
Western Bank
Sheffield S10 2TS
Ph: 0114 271 7000

Department of Anaesthesia,
Birmingham Children's Hospital,
NHS Trust,
Steelhouse Lane B4 6NH
Ph: +44 121 333 9623

Department of Anaesthesia
Royal Liverpool Children's Hospital
Alder Hey
Eaton Road
Liverpool L12 2APS
Ph: 0151 228 4811

The Netherlands:

Wilhelmina Children's Hospital; University Medical Center Utrecht
Department of Anaesthesiology,
University Medical Centre Utrecht,
Mailstop Q 04.2.313,
PO Box 85500,
3508 GA Utrecht,
The Netherlands
Ph: +31 88 75 59677

Universitair Medisch Centrum Groningen
Hanzeplein 1, Postbus 30 001
9700 RB
Groningen
The Netherlands
Ph: 0011 31 (0) 50 361 1158

Trial Steering Committee

Chair: Prof Paul Myles, Anaesthetist, Alfred Hospital, Melbourne
Prof David Bellinger, Psychologist, Boston Children's Hospital, Boston USA
Prof Charles Berde, Paediatric Anaesthetist, Boston Children's Hospital, Boston USA
Prof John Carlin, Statistician, Murdoch Children's Research Institute, Melbourne
A/Prof Andrew Davidson (PI), Paediatric Anaesthetist, Royal Children's Hospital, Melbourne
Dr Rod Hunt, Neonatologist, Royal Children's Hospital, Melbourne
A/Prof Kate Leslie, Anaesthetist, Royal Melbourne Hospital, Melbourne
A/Prof Mary Ellen McCann (PI), Paediatric Anaesthetist, Boston Children's Hospital, Boston USA
Prof Neil McIntosh, Neonatologist, Edinburgh, UK
Dr Neil Morton (PI), Paediatric Anaesthetist, Royal Hospital for Sick Children, Glasgow, UK
Prof Andy Wolf, Paediatric Anaesthetist, Bristol, UK
A/Prof Davinia Withington, Paediatric Anaesthetist, Montreal, Canada
Dr Nicola Disma, Paediatric Anaesthetist, Genoa, Italy
Dr Jurgen de Graaff, Paediatric Anaesthetist, Utrecht, The Netherlands

Data Monitoring Committee

Chair: Dr Jonathan De Lima, Anaesthetist, Sydney, Australia
Prof David Field, Neonatologist, Leicester, UK
Prof Val Gebski, Statistician, Sydney, Australia
Prof Greg Hammer, Paediatric Anaesthesiologist, Stanford California, USA
Prof Dick Tibboel, Paediatrician, Sophia Children's Hospital, Rotterdam, Holland

TABLE OF CONTENTS

ABBREVIATIONS

GAS study – The acronym for the study

ABAS-II: Adaptive Behavioural Assessment System – Second Edition
AE: Non serious adverse events
BRIEF-P: Behavioural rating inventory of executive function- Preschool Version
BSID-III: Bayley scales of infant development, version III
CBCL: Child Behavior Checklist Caregiver
CMS: Children's Memory Scale
CPAP: Continuous positive airway pressure
CRF: Case report form
CSF: Cerebrospinal fluid
DMC: Data monitoring committee
ECG: Electrocardiograph
FLACC: Behavioural scale for scoring postoperative pain in young children measured in 5 areas: **f**ace, **l**egs, **a**ctivity, **c**ry, **c**onsolability
GA: General anaesthesia
GABA: Gamma amino butyric acid
HR: Heart rate
IV: Intra venous
IVH: Intra ventricular haemorrhage
NDNMB: Non-depolarizing neuromuscular blocking agent
NEPSY-II: Developmental neuropsychological assessment, Second Edition
NIBP: Non invasive blood pressure
NICU: Neonatal Intensive Care Unit or Neonatal unit
NMDA: n-methyl-d-aspartic acid
PACU: Post anaesthesia care unit – also known as the recovery ward
PI: Principal investigator
PMA: Postmenstrual age (see appendix one)
PVL: Peri ventricular leukomalacia
RA: Regional anaesthesia
RCH: Royal Children's Hospital, Melbourne
ROP: Retinopathy of prematurity
SAE: Serious adverse events
TSC: Trial steering committee
UTI: Urinary tract infection
WIAT-II Abbreviated: Wechsler Individual Achievement Test – Second Edition Abbreviated
WPPSI-III: Wechsler preschool and primary scale of intelligence- Third Edition

SYNOPSIS

Title: A multi-site RCT comparing regional and general anaesthesia for effects on neurodevelopmental outcome and apnoea in infants

Chief investigators: Andrew Davidson (PI), Mary Ellen McCann (PI), Neil Morton (PI), David Bellinger, Charles Berde, Jurgen de Graaff, Nicola Disma, Geoff Frawley, Pollyanna Hardy, Rod Hunt, Robyn Stargatt, Davinia Withington Andy Wolf

Objectives: The primary objective of this trial is to determine whether regional and general anaesthesia, given to infants undergoing inguinal hernia repair result in equivalent neurodevelopmental outcomes. Secondary objectives are to describe the frequency and characteristics of apnoea in the post-operative period, and determine the factors associated with apnoea.

Study population: 660 (plus 60) infants of postmenstrual age 60 weeks age or less, requiring inguinal hernia repair under anaesthesia. Exclusion criteria include pre-existing recognised risk factors for adverse neurodevelopmental outcome or previous exposure to general anaesthesia. Infants born at less than 26 weeks gestation will be excluded.

Design: Prospective, observer blind, multi-site, randomised, controlled, equivalence trial.

Treatment groups: The general anaesthesia group will receive sevoflurane for induction and maintenance. The airway can be maintained with a face mask, laryngeal mask or endotracheal tube, with or without neuromuscular blocking agents. Analgesia can be supplied with a caudal or ilioinguinal nerve block with bupivacaine up to a maximum dose of 2.5 mg/kg.

The regional group will receive no sedative agents. The regional blockade may be with spinal block alone, spinal block with caudal block, spinal with ilioinguinal block or caudal alone. A maximum dose of 2.5 mg/kg of bupivacaine can be used.

Outcome measure: Neurodevelopmental assessments will occur at 2 and 5 years with standard neuropsychological tools. Apnoea events and interventions for apnoea will also be recorded.

Primary outcome: The primary outcome will be the WPPSI-III Full Scale IQ score at 5 years chronological age.

1. INTRODUCTION and BACKGROUND

1.1 Title: A multi-site RCT comparing regional and general anaesthesia for effects on neurodevelopmental outcome and apnoea in infants

1.1.1 Study acronym: *The GAS study*

1.2 Agents to be investigated: This trial will compare outcome in infants receiving a): general anaesthesia with the volatile general anaesthetic sevoflurane plus regional nerve blockade with the local anaesthetic bupivacaine, or b): awake regional anaesthesia using regional nerve blockade with bupivacaine alone.

1.3 Population to be studied: 660 (plus 60) infants of postmenstrual age 60 weeks or less scheduled for hernia repair.

1.4 Rationale

Recent animal data has provided evidence to suggest that several commonly used anaesthetic agents (including volatile anaesthetic agents) may be neurotoxic to the developing brain [1, 2]

1.4.1 Anaesthesia in infancy

Anaesthesia in infants and neonates is becoming increasingly common. In 2002 in Western Australia 7.8% of male and 3.2% of female children aged less than 1 year were anaesthetised [3] . Of these infants 10% of the males and 20% of the females had the anaesthesia during their first 28 days of life. The use of anaesthesia in infants (and indeed in the foetus) is likely to further increase as more surgical options are available, as there is a greater appreciation of the ethical and developmental need for anaesthesia to reduce nociceptive stimuli, and as advances in anaesthesia practice reduce the short-term morbidity and mortality.

General anaesthesia in infants and neonates usually involves a volatile anaesthetic (e.g. sevoflurane, isoflurane or halothane) or an intravenous anaesthetic (e.g. barbiturate or propofol). The exact mode of action of anaesthesia is still unclear but there is usually some involvement of the GABA and/or NMDA receptors. Anaesthesia may also be provided with awake regional nerve blockade using a local anaesthetic agent (for example spinal anaesthesia).

1.4.2 Apoptosis and neurodevelopment

Studies have consistently demonstrated that neonatal rats, mice and guinea pigs exposed to GABA agonists, such as volatile anaesthetics, or to NMDA antagonists such as ketamine, have widespread neuronal apoptotic lesions and delays in achieving some developmental goals [4-13] . In rats the injury is most obvious when they are exposed to anaesthesia during the period of synaptogenesis at the age of 7 days. Recently neurotoxicity has also been demonstrated in prenatal rhesus monkeys exposed to ketamine and isoflurane [14-17] . The mechanism of neurotoxicity is also beginning to be unravelled [18]. Lastly changes in dendritic development have also been associated with exposure to anaesthesia [19-21].

Correlation of the stages of animal neural development with that of human brain development is imprecise. In the rat the period of synaptogenesis lasts from day 4 to day 10 and peaks at day 7. In the human this equates to the period from approximately the third trimester to one year of age. Thus, in theory humans may be susceptible to anaesthesia toxicity anywhere from ante-partum to late infancy.

Interspecies variation limits the generalisation of the animal data to humans. Similarly experimental conditions for rodents may be significantly different to conditions experienced by neonates undergoing surgery and anaesthesia. Differences include, concurrent oxygen, nutrition or blood glucose management, the dose of the agents used, the different organisation and plasticity of the human brain [2, 22-26]. Compared with the time of exposure to anaesthesia, the developmental period is considerably longer in a human compared to a rat, however from a molecular biological perspective, apoptosis could be triggered irrespective of the usual period of neurodevelopment. Due to greater plasticity it is possible that the human brain is capable of accommodating greater apoptotic injury than the rat brain without any clinically significant effect to neurological development, although the evidence for this premise is limited.

Several cohort studies have examined the association between anaesthesia and neurocognitive development [27-33]. These studies are difficult to interpret due to multiple confounding variables. It is unclear if the injury is due to anaesthetic drug toxicity, the inflammation and stress response associated with surgery or other factors associated with surgery and anaesthesia such as transport, ventilation and pain or confounding factors such as prematurity, sepsis or congenital abnormalities.

In another large study an association was found between exposure to multiple anaesthetics and poor school performance [34]. Similarly a large study from New York State found an association between hernia repair in infancy and behavioural and developmental disorders [35]. However, a twin study in the Netherlands found no evidence of a causal relationship between exposure to anaesthesia before the age of 3 and educational achievement. The authors came to this conclusion because even the unexposed co-twin from a discordant twin pair had the same increased risk of decreased cognitive performance as his/her twin exposed to anaesthesia [36].

Due to the difficulties with interpreting such cohort data, the best way to provide convincing evidence for the safety of volatile anaesthesia is with a prospective randomised trial[37]. Fortuitously, in many sites, anaesthesia is provided to infants undergoing inguinal hernia repair via one of two methods – general anaesthesia (GA) or regional anaesthesia (RA). RA can be with spinal or caudal anaesthesia or a combination of both. RA can be provided without exposure to any of the agents purported to be associated with neurotoxicity. Inguinal hernia repair is one of the most common procedures to be performed in infancy and provides a large population of infants having similar surgery. The reasons for choosing RA or GA may depend on the age of the child, the experience of the surgeon or anaesthetist, and the perceived risk of apnoea. As outlined below the risks of apnoea after modern anaesthesia are not clearly defined. The presence of equipoise in choice of anaesthesia for a single, relatively common procedure provides an ideal setting for a prospective randomised trial. The most important potential confounding variable is the influence of prematurity on developmental outcome and choice of anaesthesia. This potential confounding variable makes stratified randomisation and a subsequent stratified analysis essential.

1.4.3 Apnoea

Neonates, particularly those born pre-term, normally have periods of irregular (or periodic) breathing , but in some circumstances the pauses may be longer and may be described as apnoea. Apnoea is more common in the first 12 hours after anaesthesia [38] and the risk of post-anaesthesia apnoea is greater in neonates who were born pre-term [39, 40]. The exact significance of post-anaesthesia apnoea is unknown; the probable incidence of death or permanent injury due to post-anaesthesia apnoea being very low. It is assumed that there is a spectrum of severity and consequence.

Three small trials comparing general and spinal anaesthesia have reported a reduced risk of apnoea in high risk babies receiving spinal anaesthesia [38, 41, 42]. These studies are difficult to interpret due to small numbers, different ways of measuring apnoea and different general anaesthesia agents used [43]. A recent Cochrane review has called for a large well designed randomised trial to address this issue [44].

New general anaesthesia volatile agents such as sevoflurane and desflurane could, in theory, lower the risk of apnoea due to their faster elimination from the body [45]. However, one recent study has demonstrated that, like the older agents, the new agent sevoflurane is still associated with a higher incidence of post-operative apnoea than spinal anaesthesia [46]. Also, like earlier studies, the clinical relevance of this study is uncertain without a clear understanding of constitutes a “significant” episode of apnoea [47].

There is clearly a need for larger trials to evaluate the efficacy of apnoea prevention strategies in neonatal anaesthesia, including the use of RA. This study will provide useful data on the frequency and clinical significance of post-operative apnoea using RA and GA with modern anaesthesia techniques.

1.4.4 Success rate and complication rate of general and regional anaesthesia

Regional anaesthesia has varying popularity. Proponents for regional anaesthesia claim that it results in a fast recovery, early feeding and low rates of respiratory complication rate. Detractors of regional anaesthesia cite a substantial failure rate (with up to 25% requiring sedation or conversion to general anaesthesia) and potential for distress in the child as the block is performed. However, data supporting these comments are sparse and contradictory. This study will enable the collection of good quality data to help in answering these questions.

1.4.5 Impact

If volatile anaesthesia *is* associated with poor neurodevelopmental outcome, reducing exposure to volatile anaesthesia will have a substantial impact on the community. Although surgery in infants is often unavoidable, and withholding anaesthesia is not an option, there are alternatives to volatile anaesthesia such as purely opioid-based anaesthesia using newer agents such as remifentanil or increased use of regional local anaesthesia.

There is already considerable public and parental concern regarding the toxicity of drugs to the foetus and the newborn. Avoiding *potentially* neurotoxic drugs or environmental agents

during pregnancy has become a standard of care in our society. It is not surprising that a parent's concern continues after birth.

Previously the anaesthesia community has reassured the public that anaesthesia poses no specific toxic risk to neonates, however the recent animal data suggests such a reassurance cannot now be made without qualification. Resolving this issue, or at least being better able to inform the parents with better evidence-based data, should be a priority. This study will provide good evidence for any risk or not of clinically relevant neurotoxicity after exposure to brief sevoflurane anaesthesia in infants. It is important to note however that if no evidence of toxicity is found in this study of brief exposure, then this does not rule out possible significant toxicity with larger doses.

With respect to apnoea, current guidelines are based on little evidence [43]. Neonates are often routinely kept in hospital for observation after surgery. This is costly in terms of limited hospital resources and disruption to families. A better understanding of apnoea would have obvious and considerable cost and safety benefit. It is acknowledged that apnoea is a secondary outcome in this study and that the apnoea data are not without significant limitations. Due to limitations in resources the assessment of apnoea will not be blinded which introduces the risk of bias. It is also not possible to use more sophisticated measure of apnoea as it is logistically not possible to use the same monitors at all sites. This again introduces the possibility of error. In spite of these limitations this study should provide data on occurrence of significant apnoea after regional and general anaesthesia.

1.4.6 References:

1. Mellon, R.D., A.F. Simone, and B.A. Rappaport, *Use of anesthetic agents in neonates and young children.* Anesth Analg, 2007. **104**(3): p. 509-20.
2. McGowan, F.X., Jr. and P.J. Davis, *Anesthetic-related neurotoxicity in the developing infant: of mice, rats, monkeys and, possibly, humans.* Anesth Analg, 2008. **106**(6): p. 1599-602.
3. Sims, C., B. Stanley, and E. Milnes, *The frequency of and indications for general anaesthesia in children in Western Australia 2002-2003.* Anaesth Intensive Care, 2005. **33**(5): p. 623-8.
4. Jevtovic-Todorovic, V., et al., *Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits.* J Neurosci, 2003. **23**(3): p. 876-82.
5. Ikonomidou, C., et al., *Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain.* Science, 1999. **283**(5398): p. 70-4.
6. Hayashi, H., P. Dikkes, and S.G. Soriano, *Repeated administration of ketamine may lead to neuronal degeneration in the developing rat brain.* Paediatr Anaesth, 2002. **12**(9): p. 770-4.
7. Rizzi, S., L. Carter, and V. Jevtovic-Todorovic. *Clinically used general anesthetics induce neuroapoptosis in the developing piglet brain*. in *Society for Neuroscience Annual Meeting*. 2005. Washington Convention Center, Washington DC.
8. Johnson, S.A., C. Young, and J.W. Olney, *Isoflurane-induced neuroapoptosis in the developing brain of nonhypoglycemic mice.* J Neurosurg Anesthesiol, 2008. **20**(1): p. 21-8.
9. Fredriksson, A., et al., *Neonatal exposure to a combination of N-methyl-D-aspartate and gamma-aminobutyric acid type A receptor anesthetic agents potentiates apoptotic neurodegeneration and persistent behavioral deficits.* Anesthesiology, 2007. **107**(3): p. 427-36.

10. Cattano, D., et al., *Subanesthetic doses of propofol induce neuroapoptosis in the infant mouse brain.* Anesth Analg, 2008. **106**(6): p. 1712-4.
11. Wise-Faberowski, L., et al., *Isoflurane-induced neuronal degeneration: an evaluation in organotypic hippocampal slice cultures.* Anesth Analg, 2005. **101**(3): p. 651-7.
12. Yon, J.H., et al., *Anesthesia induces neuronal cell death in the developing rat brain via the intrinsic and extrinsic apoptotic pathways.* Neuroscience, 2005. **135**(3): p. 815-27.
13. Liang, G., et al., *Isoflurane causes greater neurodegeneration than an equivalent exposure of sevoflurane in the developing brain of neonatal mice.* Anesthesiology, 2010. **112**(6): p. 1325-34.
14. Zou, X., et al., *Prolonged exposure to ketamine increases neurodegeneration in the developing monkey brain.* Int J Dev Neurosci, 2009. **27**(7): p. 727-31.
15. Zou, X., et al. *Ketamine-induced neurotoxicity in developing monkeys: a histochemical study*. in *Society for Neuroscience Annual Meeting*. 2006. Georgia World Congress Centre.
16. Slikker, W., Jr., et al., *Ketamine-induced neuronal cell death in the perinatal rhesus monkey.* Toxicol Sci, 2007. **98**(1): p. 145-58.
17. Brambrink, A.M., et al., *Isoflurane-induced Neuroapoptosis in the Neonatal Rhesus Macaque Brain.* Anesthesiology, 2010. **112**: p. 834-841.
18. Head, B.P., et al., *Inhibition of p75 neurotrophin receptor attenuates isoflurane-mediated neuronal apoptosis in the neonatal central nervous system.* Anesthesiology, 2009. **110**(4): p. 813-25.
19. Vutskits, L., et al., *Clinically relevant concentrations of propofol but not midazolam alter in vitro dendritic development of isolated gamma-aminobutyric acid-positive interneurons.* Anesthesiology, 2005. **102**(5): p. 970-6.
20. Briner, A., et al., *Volatile anesthetics rapidly increase dendritic spine density in the rat medial prefrontal cortex during synaptogenesis.* Anesthesiology, 2010. **112**(3): p. 546-56.
21. De Roo, M., et al., *Anesthetics rapidly promote synaptogenesis during a critical period of brain development.* PLoS One, 2009. **4**(9): p. e7043.
22. Soriano, S.G., et al., *Of mice and men: should we extrapolate rodent experimental data to the care of human neonates?* Anesthesiology, 2005. **102**(4): p. 866-8; author reply 868-9.
23. Sanders, R.D. and A. Davidson, *Anesthetic-induced neurotoxicity of the neonate: time for clinical guidelines?* Paediatr Anaesth, 2009.
24. Davidson, A. and S. Soriano, *Does anaesthesia harm the developing brain--evidence or speculation?* Paediatr Anaesth, 2004. **14**(3): p. 199-200.
25. Anand, K.J. and S.G. Soriano, *Anesthetic agents and the immature brain: are these toxic or therapeutic?* Anesthesiology, 2004. **101**(2): p. 527-30.
26. Loepke, A.W., F.X. McGowan, Jr., and S.G. Soriano, *CON: The toxic effects of anesthetics in the developing brain: the clinical perspective.* Anesth Analg, 2008. **106**(6): p. 1664-9.
27. Loepke, A.W. and S.G. Soriano, *An assessment of the effects of general anesthetics on developing brain structure and neurocognitive function.* Anesth Analg, 2008. **106**(6): p. 1681-707.
28. Kabra, N.S., et al., *Neurosensory impairment after surgical closure of patent ductus arteriosus in extremely low birth weight infants: results from the Trial of Indomethacin Prophylaxis in Preterms.* J Pediatr, 2007. **150**(3): p. 229-34, 234 e1.
29. Ludman, L., L. Spitz, and A. Wade, *Educational attainments in early adolescence of infants who required major neonatal surgery.* J Pediatr Surg, 2001. **36**(6): p. 858-62.

30. Walker, K., et al., *Neurodevelopmental outcomes and surgery in neonates.* J Paediatr Child Health, 2006. **42**(12): p. 749-51.
31. TVICS, G., *Surgery and the tiny baby: sensorineural outcome at 5 years of age. The Victorian Infant Collaborative Study Group.* J Paediatr Child Health, 1996. **32**(2): p. 167-72.
32. Bouman, N.H., H.M. Koot, and F.W. Hazebroek, *Long-term physical, psychological, and social functioning of children with esophageal atresia.* J Pediatr Surg, 1999. **34**(3): p. 399-404.
33. Chacko, J., W.D. Ford, and R. Haslam, *Growth and neurodevelopmental outcome in extremely-low-birth-weight infants after laparotomy.* Pediatr Surg Int, 1999. **15**(7): p. 496-9.
34. Wilder, R.T., et al., *Early exposure to anesthesia and learning disabilities in a population-based birth cohort.* Anesthesiology, 2009. **110**(4): p. 796-804.
35. Di Maggio, C., et al., *A retrspective cohort study of the association of anesthesia and hernia repair surgery with behavioral and developmental disorders in young children.* J Neurosurg Anesthesiol, 2009. **21**: p. 286-291.
36. Bartels, M., R.R. Althoff, and D.I. Boomsma, *Anesthesia and cognitive performance in children: no evidence for a causal relationship.* Twin Res Hum Genet, 2009. **12**(3): p. 246-53.
37. Davidson, A.J., et al., *Anesthesia and outcome after neonatal surgery: the role for randomized trials.* Anesthesiology, 2008. **109**(6): p. 941-4.
38. Krane, E.J., C.M. Haberkern, and L.E. Jacobson, *Postoperative apnea, bradycardia, and oxygen desaturation in formerly premature infants: prospective comparison of spinal and general anesthesia.* Anesth Analg, 1995. **80**(1): p. 7-13.
39. Malviya, S., J. Swartz, and J. Lerman, *Are all preterm infants younger than 60 weeks postconceptual age at risk for postanesthetic apnea?* Anesthesiology, 1993. **78**(6): p. 1076-81.
40. Cote, C.J., et al., *Postoperative apnea in former preterm infants after inguinal herniorrhaphy. A combined analysis.* Anesthesiology, 1995. **82**(4): p. 809-22.
41. Welborn, L.G., et al., *Postoperative apnea in former preterm infants: prospective comparison of spinal and general anesthesia.* Anesthesiology, 1990. **72**(5): p. 838-42.
42. Somri, M., et al., *Postoperative outcome in high-risk infants undergoing herniorrhaphy: comparison between spinal and general anaesthesia.* Anaesthesia, 1998. **53**(8): p. 762-6.
43. Fisher, D.M., *When is the ex-premature infant no longer at risk for apnea?* Anesthesiology, 1995. **82**(4): p. 807-8.
44. Craven, P.D., et al., *Regional (spinal, epidural, caudal) versus general anaesthesia in preterm infants undergoing inguinal herniorrhaphy in early infancy.* Cochrane Database Syst Rev, 2003(3): p. CD003669.
45. O'Brien, K., D.N. Robinson, and N.S. Morton, *Induction and emergence in infants less than 60 weeks post-conceptual age: comparison of thiopental, halothane, sevoflurane and desflurane.* Br J Anaesth, 1998. **80**(4): p. 456-9.
46. William, J.M., et al., *Post-operative recovery after inguinal herniotomy in ex-premature infants: comparison between sevoflurane and spinal anaesthesia.* Br J Anaesth, 2001. **86**(3): p. 366-71.
47. Rochette, A., et al., *Clonidine added to bupivacaine in neonatal spinal anesthesia: a prospective comparison in 124 preterm and term infants.* Paediatr Anaesth, 2005. **15**(12): p. 1072-1077.

1.5 Compliance

The trial will be conducted in compliance with the protocol, Good Clinical Practice (Therapeutic Goods Administration, Australia) and all applicable regulatory requirements. The trial is registered with the NHMRC Australian and New Zealand Clinical Trials Registry (ACTRN012606000441516) and on Clinicaltrials.gov (NCT00756600).

2. OBJECTIVES

2.1 Primary Aim

The primary aim of this prospective, observer blind, randomised, multi-site, controlled, clinical, equivalence trial is to determine whether different types of anaesthesia [regional vs general] given to infants undergoing inguinal hernia repair result in equivalent neurodevelopmental outcomes at 5 years of age (chronological).

2.2 Secondary Aims

2.2.1 Neurodevelopmental outcome: Compare a range of secondary neurodevelopmental measures between groups at 2 years (corrected) and 5 years of age (chronological).

2.2.2 Apnoea: Describe the frequency and characteristics of apnoea in the post-operative period after both regional and general anaesthesia for inguinal hernia repair in infants, and determine other factors associated with increased risk of apnoea.

2.2.3 Recovery: Collect data on the ease of performance, complication and success rate and the early recovery profile of infants in the post-operative period for both regional and general anaesthesia for inguinal hernia repair.

2.3 Hypotheses

2.3.1 Primary Hypothesis: The WPPSI-III Full Scale IQ score at 5 years chronological age in infants who are anaesthetised for hernia repair is equivalent when using general anaesthesia compared with regional anaesthesia.

2.3.2 Secondary Hypothesis: Cognitive component of the Bayley III scale of infant development measured at 2 years of age in infants who are anaesthetised for hernia repair is equivalent when using general anaesthesia compared with regional anaesthesia.

3. STUDY DESIGN

3.1 Experimental Design: Prospective, observer blind, multi-site, randomised, controlled, equivalence trial.

3.2 Randomisation: Randomisation to either of the two treatment groups (GA or RA) will be by block and stratified by site and gestational age at birth. Gestational age at birth strata will be:

- 26 weeks to 29 weeks and 6 days
- 30 weeks to 36 weeks and 6 days

- 37 weeks and more

Access to randomisation numbers will be via a 24 hour web-based randomisation service set up and maintained by A/Prof Ryan at The Data Management & Analysis Centre, Department of Public Health, University of Adelaide, South Australia:
http://www.dmac.adelaide.edu.au/gasRandomisation
Entry to the website is password protected.

3.3 Blinding: The anaesthetist will be aware of the group allocation. The psychologist and paediatrician performing the 2 and 5 year neurodevelopmental assessments will be blinded to study group. Parents will not be told which group their child was in but they will be allowed to discover the group and will be told if asked. Parents will be clearly instructed that if they do discover the group then they should not inform the assessing psychologist. A record will be made of whether or not the parent or the psychologist became aware of the allocation. The apnoea assessment and recovery assessment will not be blinded.

3.4 Study duration: The duration of subject participation is five years.

- Children will be enrolled before surgery.
- Data will be collected before and during anaesthesia and in the early post-operative period.
- Formal neurodevelopmental assessment will occur at 2 years corrected age and 5 years chronological age (see appendix 1 for definition of corrected and chronological age).

3.5 Source data

The following data will be collected onto the Case Report Forms (CRFs) at the corresponding times.

3.5.1 Data for eligibility

Data for potential eligibility will be collected as per the inclusion and exclusion criteria

3.5.2 Data for randomisation

For randomisation the following extra data is collected:

- Mother's expected date of delivery
- Birth date

3.5.3 Identifying information

The following identifiable data will be collected. Identifying data will be handled separately.

- Child's name
- Family address and phone numbers
- Child's paediatrician or family doctor
- An alternate contact to find the family (For example, the mother's mother)

3.5.4 Medical and social history

The following medical and social history data will be collected:

- Language spoken at home
- Family structure with details of parents including who is primary income earner, who is primary carer and details of employment status and education
- Maternal age at birth of child
- Gender
- Method of calculating expected date of delivery
- Birth weight, in kg
- Apgar score at 1 and 5 minutes, mode of delivery, if the child was part of a multiple pregnancy, presence of labour, antenatal steroids, history of prolonged rupture of membranes, pre-eclampsia or chorioamnionitis
- History and details of previous apnoea or respiratory support
- Details of any previous neurological injury including intraventricular haemorrhage
- Previous ROP or hearing defects
- Previous sepsis
- Days the child spent in hospital after birth and date child last discharged from hospital

3.5.5 Pre anaesthesia details. Both regional and general groups

The following data will be collected prior to anaesthesia:

- Date of surgery
- Current respiratory support
- Current medication
- Weight
- Timing and details of last feed
- Baseline HR, NIBP, SpO_2, temperature in a calm comforted child
- Details of premedication and fluids

3.5.6 Anaesthesia details

The following data during anaesthesia will be collected

3.5.6.1 Anaesthesia details. Regional group only

- Seniority of anaesthetist
- Times for anaesthesia blocks and surgery
- Blocks used and details of block technique including, dose and concentration of bupivacaine, presence of blood in needle and flow of CSF through needle
- Lowest SpO_2 and HR during the block
- Volume and type of fluid given in theatre
- Blood glucose and haemoglobin
- Details of any rescue therapy for low glucose, hypotension or hypoxia
- Efficacy of block and activity of child during the procedure
- Details of any respiratory support or apnoea during procedure
- HR, NIBP, SpO_2 and activity of child every 5 minutes during surgery

3.5.6.2 Anaesthesia details. General group only

- Seniority of anaesthetist
- Times for anaesthesia induction and times for blocks and surgery
- Blocks used and details of block technique including dose and concentration of bupivacaine, presence of blood in needle and flow of CSF through needle
- Details of anaesthesia induction
- Details of airway management and use of any NDNMB
- Volume and type of fluid given in theatre
- Blood glucose and haemoglobin
- Details of any rescue therapy for low glucose, hypotension or hypoxia
- Efficacy of blocks
- Details of awakening, airway removal and reversal of any NDNMB
- Details of any respiratory complications or apnoea during awakening or after airway removed.
- HR, NIBP, inspiratory oxygen, SpO_2 and end tidal CO_2 and sevoflurane concentrations every 5 minutes during surgery

The total dose of sevoflurane will be calculated as concentration*hours dose equivalent

3.5.6.3 Details of protocol violations

- Details of any protocol violation including need for opioids or nitrous oxide
- Details of management for failed block

3.5.7 Surgical details

The following surgical data will be collected:

- Presence of unilateral or bilateral hernia on pre-op exam
- Unilateral or bilateral exploration and presence of unilateral or bilateral hernia on exploration
- Details of any other procedure performed (such as circumcision)
- Seniority of surgeon

3.5.8 Post anaesthesia care unit data

In PACU (or during the first 30 minutes on the ward if PACU is bypassed) the following data will be collected:

- Details of monitoring for apnoea
- Drugs given
- Number of significant apnoeas
- Details of any intervention for apnoea or respiratory support needed
- HR, SpO_2 every 5 minutes during PACU stay and lowest SpO_2
- FLACC scores and details of any analgesia
- Presence of stridor

- Time to first feed

3.5.9 Post PACU details

For the 12 hours after PACU or up until routine discharge the following data will be collected.

- Details of monitoring for apnoea
- Drugs given
- Details of any intervention for apnoea or respiratory support needed
- Number of significant apnoeas
- Presence of stridor
- Time child discharged home

3.5.10 Follow-up day 1-5

In the period from 1-5 days post anaesthesia the following data will be collected where relevant.

- Day discharged home
- Details of any medical interventions initiated on day 1-5
- Was the child readmitted to hospital?
- Details of any evidence for infection associated with nerve blockade or presence of stridor

If child stays longer than 5 days:

- Length of stay in hospital
- Details of any medical intervention in the period between surgery and discharge from hospital

3.6 Details of apnoea data collection

At all sites HR, respiration and SpO_2will be continuously recorded in PACU. This data may be downloaded electronically where possible. While in PACU the child shall be watched continuously by a research assistant who shall watch for any pauses in breathing. Details of any intervention for apnoea in PACU will be recorded. An activity sheet will be completed to identify feeding and other activity that may contribute to artefact when data is electronically collected.

After PACU all sites will monitor for apnoea using their established practices. For all patients any post-PACU intervention or change in management due to apnoea will be recorded. After PACU some children will have continuous monitoring with HR SpO_2 and respiratory rate for up to 24 hours. Where possible this data may be recorded and downloaded electronically. As for PACU electronic data will be accompanied by an activity sheet.

3.7 Two year assessment data

The following data will be collected at 2 years corrected age.

3.7.1 Data for family/environmental factors at two years

- Support for special needs such as audiologist, optometrist, physiotherapy, speech/language therapy, occupational therapy, orthoptist, psychologist.
- Child Care arrangements, if child attends playgroup and age started at group.
- Social history as per 3.5.4

3.7.2 Medical history at two years

- Details of all hospital attendances and admissions to hospital after discharge following hernia repair.
- Exposure to any anaesthetic agents or benzodiazepines since discharge from hospital after hernia repair.
- Details of any recurrence of hernia on either side since repair

Questions on hearing and visual acuity

3.7.3 Neurodevelopmental assessment at two years:

- Bayley Scales of Infant Development III (BSID-III): Cognitive, language and motor scales, social-emotional scale and adaptive behaviour scale
- Paediatric assessment including neurological examination to determine presence of cerebral palsy
- Macarthur-Bates Communicative Development Inventory

It will be noted whether or not the parent, psychologist or paediatrician are aware of allocation.

3.8 Five years assessment data

The following data will be collected at 5 years chronological age.

3.8.1 Data for family/environmental factors at five years

Data as per 3.7.1

3.8.2 Medical history at five years

- Details of all hospital attendances and admissions to hospital since 2 year assessment
- Details of any recurrence of hernia on either side since repair
- Details of any intervention initiated as a result of the findings at the 2 year neurodevelopmental assessment

3.8.3 Neurodevelopmental assessment at five years

- A paediatric assessment as per 2 years including hearing and visual acuity tests
- General intellectual ability, Verbal, Visuo-spatial and Processing Speed Skills from the Wechsler Preschool and Primary Scale of Intelligence - Third Edition (WPPSI-III)

- Attention and executive function, verbal skills, memory and learning, social perception, sensorimotor skills and visuomotor integration will be measured using the Neuro psychological – Second Edition (NEPSY II)
- Memory and learning and attention and executive function will be measured using the Children's Memory Scale (CMS)
- Academic skills will be measured using the Wechsler Individual Achievement Test – Second Edition Abbreviated (WIAT-II Abbreviated)

- The parent/caregiver of the child will be asked to fill out:
    - Behavioural Regulation Index and Meta Cognition Index of the Behavioural Rating of Executive Function- Preschool Version Parent Form (BRIEF-P) to assess neurobehaviour
    - Adaptive Behavioural Assessment System – Second Edition (ABAS-II) to measure adaptive behaviour
    - Child Behavior Checklist Caregiver (CBCL) to measure the child's general behaviour.

It will be noted whether or not the parent, psychologist or paediatrician are aware of allocation.

4. SELECTION OF SUBJECTS

4.1 Number of subjects: 660 (plus 60)

4.2 Inclusion criteria: Any child scheduled for unilateral or bilateral inguinal hernia repair (with or without circumcision).

4.3 Exclusion criteria:

- Any child older than 60 weeks postmenstrual age
- Any child born at less than 26 weeks gestation
- Any contraindication to general *or* spinal/caudal anaesthesia (for example: neuromuscular disorder or coagulopathy)
- Pre-operative ventilation immediately prior to surgery
- Congenital heart disease that has required surgery or will require surgery or which requires ongoing pharmacotherapy (PDA alone is not an exclusion criterion)
- Known chromosomal abnormality or any other known congenital or acquired abnormalities (apart from prematurity) which are likely to affect development
- Children where follow-up would be difficult for geographic or social reasons
- Families where the primary language spoken at home is *not* the language in which the WPPSI-III will be given [i.e. English for Australian sites, English or French for Canada sites, English or Italian for Italian sites,English or Spanish for US sites and English or Dutch for the Netherlands site]
- Known neurological injury such as cystic periventricular leukomalacia (PVL), or grade 3 or 4 intra ventricular haemorrhage (IVH) (+/- post haemorrhage ventricular dilatation)
- Previous exposure to volatile anaesthesia or benzodiazepines as a neonate or in the third trimester *in utero*

4.4 Enrolment

Potential participants will be identified from theatre bookings or direct contact from surgeons or anaesthetists. After identification investigators will:

- Check medical history to confirm all inclusion criteria are met and no exclusion criteria are met.
- Confirm from both surgeon and anaesthetist that participant may be suitable and seek their approval to approach the family.
- Explain the project to the child's family and obtain written consent.
- In the US, the treating physician/neonatologist must also be consulted before the child is entered into the study.

4.5 Subject withdrawal criteria

4.5.1 Failed regional block with general anaesthesia

If the child is having a general anaesthetic (with sevoflurane) and the local anaesthesia block is inadequate (persistent tachycardia or hypertension), then opioids or nitrous oxide may be given at the discretion of the anaesthetist. Post-operative recovery and apnoea data, and neurodevelopmental data will still be collected.. These children will also be followed up in hospital and with a phone call 5 days post-anaesthesia as per study protocol..

4.5.2 Voluntary withdrawal

Families may withdraw from the study at any stage. If they withdraw, data collected up to that point will still be used for analysis unless the family requests that it is not used.

4.5.3 Cancellation of surgery or delay between randomisation and surgery

Every effort should be made to minimise the period between randomisation and anaesthesia.

If the surgery is cancelled after randomisation and not rescheduled, then the reason for cancellation must be noted and the case will be treated as a protocol violation. If the surgery is delayed and the child has a hernia repair at a later date then every effort should be made to follow the allocated treatment group as per the protocol. If the child has become ineligible for any reason after randomisation then this will be noted in the protocol violation section.

Note that as per any other violations, if a child is ever randomised then the child must receive two and five year developmental assessments as per protocol whether they had the surgery or not.

In the event of delay or cancellation, a child will never be randomised twice.

The treating staff always have the opportunity to not follow the protocol if they consider this to be in the child's best interests, but only the parents can withdraw the child completely from the trial.

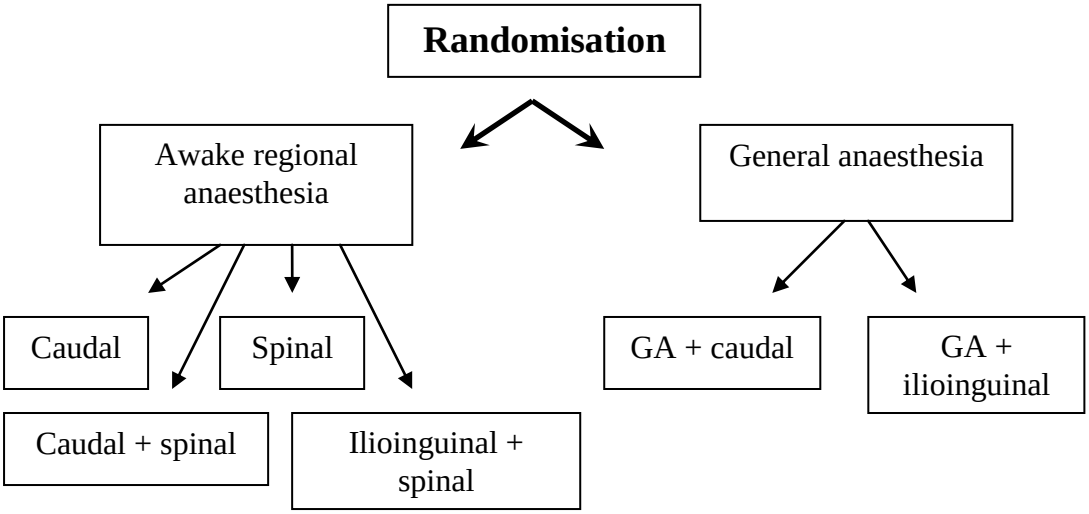
5. TREATMENT

5.1 Treatment groups: There are two groups

5.1.1 ***General anaesthesia group***: Sevoflurane for induction and maintenance of anaesthesia plus nerve blockade with caudal or ilioinguinal bupivacaine

5.1.2 ***Awake regional group***: No general anaesthetic agent and one of the following blocks:
a) Spinal block alone
b) Spinal and caudal block
c) Spinal and ilioinguinal block
d) Caudal block alone

Note: If two blocks are used, the anaesthetist may choose to give one on completion of surgery.



5.2 Performing nerve block

5.2.1 Agent: All blocks will be performed with the local anaesthetic agent bupivacaine.

5.2.2 Choice of nerve block: Within each treatment group the choice of nerve block is at the discretion of the anaesthetist.

5.2.3 Experience of doctor performing nerve block

An essential component of the trial is a high success rate for regional anaesthesia. At each site awake spinal and caudal may be performed only by anaesthetists with an established high rate of success for the block (>90%). This anaesthetist may be called just to provide the block for the treating anaesthetist.

5.2.4 Asepsis: An aseptic technique is required for all blocks. This includes:

- Hands washed and sterile gloves worn
- Surgical masks worn
- Skin preparation with an antiseptic
- Area covered with sterile drapes

5.3 Details of General Anaesthesia group treatment protocol

5.3.1 Pre-operative management:

- Pre-operative fasting in accordance with institutional guidelines
- Premedication with oral paracetamol (acetaminophen) 20mg/kg [optional]

5.3.2 Induction:

- Sevoflurane up to 8% induction in air/oxygen mix
- No nitrous oxide
- Circuit and fresh gas flow at discretion of anaesthetist
- Oxygen concentration at discretion of anaesthetist
- IV insertion before or after induction

5.3.3 Maintenance:

- Airway maintenance: Endo-tracheal intubation, laryngeal mask or face mask only with or without an oral Guedel airway.
- A non-depolarizing neuromuscular blocking agent (NDNMB) may be used to facilitate endo-tracheal intubation.
- Analgesia with caudal local anaesthesia blockade with bupivacaine or ilioinguinal nerve local anaesthesia up to a maximum dose of 2.5mg/kg bupivacaine
- Maintenance with sevoflurane in an air/oxygen mix. Sevoflurane concentration at discretion of anaesthetist. No nitrous oxide.
- Mechanical or spontaneous ventilation to maintain end tidal CO_2 between 35 and 45 mmHg. Fresh gas flows and circuit at discretion of anaesthetist.
- Oxygen concentration at discretion of anaesthetist to maintain arterial oxygen saturation > 95%
- Reversal with neostigmine 0.05 mg/kg and atropine 0.02 mg/kg if NDNMB used.
- During anaesthesia administer Ringer's lactate (Hartman's) solution at 4ml/kg/hr
- Warming in accordance with institutional practice

5.4 Details of Awake Regional group treatment protocol

5.4.1 Pre-operative management:

- Pre-operative fasting in accordance with institutional guidelines
- Premedication with oral paracetamol (acetaminophen) 20mg/kg [optional]

5.4.2 During anaesthesia:

- IV insertion before or after spinal/caudal, IV fluids – Ringer's lactate (Hartman's) solution at 4ml/kg/hr
- Oral sucrose for sedation/analgesia. <u>No other sedation</u>
- <u>No volatile anaesthetic agents may be given at any stage</u>
- Warming in accordance with institutional practice
- Oxygen by face mask or blow by at discretion of anaesthetist to maintain arterial oxygen saturation > 95%

5.4.3 Nerve blockade

5.4.3.1 Spinal anaesthesia nerve blockade:

- 25 gauge needle lumbar puncture at L2-3 or L3-4,
- Dose of bupivacaine 0.5% up to 0.2 ml/kg 0.5% bupivacaine (minimum volume of 0.5ml) (note: US may use 0.75% bupivacaine- see appendix III)

- Child may be in lateral or sitting position

5.4.3.2 Caudal blockade

- Dose of bupivacaine up to 2.5 mg/kg bupivacaine, concentration at discretion of anaesthetist
- Via needle or cannula

5.5 Post-operative management for both groups:

- Paracetamol (acetaminophen) 20mg/kg oral or intravenous at discretion of anaesthetist if not given pre-operatively
- IV fluids Ringer's lactate(Hartman's) solution @ 4ml/kg/hr until feeding,
- Temperature maintenance as per institutional practice
- Oxygen by face mask or blow by at discretion of anaesthetist or nurse in PACU to maintain arterial oxygen saturation > 95%

5.6 Treatments not permitted during anaesthesia

- Opioids may not be given.
- Benzodiazepines, nitrous oxide, ketamine and clonidine may not be given.
- No drugs apart from bupivacaine may be added to any nerve block

5.7 Triggers to abandon awake regional and administer general anaesthesia

If there is a failed awake regional anaesthetic on clinical assessment then the infant is given a general anaesthetic with sevoflurane alone and continues in the study. In this situation the airway management is at the discretion of the anaesthetist. Regional failure may be due to movement impeding surgery or a vigorous crying child that cannot be comforted.

Note: If there is total failure of a spinal then the spinal may be attempted again with another 1mg/kg of bupivacaine. Total failure is defined as follows: after 5 minutes the infant continuing to vigorously spontaneously move both their legs with no evidence of motor block and withdrawing both their legs after gentle pinch to either thigh. If only partial failure then the child should not receive another spinal attempt. The total dose of bupivacaine for entire case may not exceed 2.5 mg/kg.

5.7.1 Field Block

Note that if the ilioinguinal, caudal or spinal block in either regional or general group is thought to be inadequate then the anaesthetist or surgeon may perform a field block with bupivacaine. A field block may also be performed at any stage in any child pre-emptively without evidence of inadequate ilioinguinal, caudal or spinal block. In all cases the total dose of bupivacaine should not exceed 2.5 mg/kg. Performing a field block is not a protocol violation.

5.8 Rescue treatments

5.8.1 Blood pressure

Intra-operative and PACU; if blood pressure falls more than 20% below baseline give IV bolus of 20 ml/kg Ringers lactate (Hartman's) solution. Vasoactive drugs if deemed

necessary by anaesthetist. Note baseline blood pressure defined as blood pressure in a comforted non-distressed child.

5.8.2 Blood glucose

Give a bolus of intravenous 10% dextrose 5ml/kg for hypoglycaemia (blood sugar level <3.0 mmol/L)

5.8.3 Hypoxia

Hypoxia to be managed with oxygen via mask and/or intubation in regional group and increasing FiO_2 in GA group as deemed necessary by anaesthetist.

5.9 Follow-up

For success, this trial requires a high rate of retention for the 2 and 5 year assessments. Therefore a family will be excluded if geographic or extreme social circumstances make follow up difficult. Families will receive a phone call one week after the hernia repair and then cards on the child's birthdays and regular newsletters.
Families will also receive a phone call one year after hernia repair to check that their contact details are current.

6. OUTCOME

6.1 Primary outcome

The primary outcome will be the WPPSI-III Full Scale IQ score at 5 years chronological age.

6. 2 Secondary outcomes

Secondary outcomes fall into four areas:
1) Other measures of neurodevelopmental outcome
2) Incidence of apnoea
3) Other outcomes relating to anaesthesia group
4) Incidental outcomes not related to choice of anaesthesia

6.2.1 Other measures of neurodevelopmental outcome

6.2.1.1 Neurodevelopmental secondary outcomes at 2 years:

- Cognitive, motor and language scales of the Bayley Scales of Infant Development III [Bayley 2005].
- Social-emotional scale and adaptive behaviour scale from the Bayley III
- The MacArthur-Bates Communicative Development Inventory
- A paediatric assessment including a neurological examination to determine the presence of cerebral palsy will be conducted by a paediatrician blinded to the type of anaesthetic used.

6.2.1.2 School age developmental outcome at 5 years:

- A paediatric assessment as per 2 years including hearing and visual acuity tests.

- General intellectual ability, verbal, visuo-spatial and processing speed skills are incorporated into the Wechsler Preschool and Primary Scale of Intelligence - Third Edition (WPPSI-III) The primary outcome is the full scale, while subscales will be computed as secondary outcomes.
- Selected NEPSY II subtests to assess attention and executive function
    - Attention/Executive Function – Span – Sentence Repetition
    - Attention/Executive Function – Sustained – Auditory Attention
    - Attention/Executive Function – Inhibition/Switch – Inhibition
    - Verbal/Language Skills – Word Generation
    - Verbal/Language Skills – Speeded Naming
    - Memory & Learning - Memory for Names/Delay
    - Social Perception - Affect Recognition
    - Social Perception - Theory of Mind
    - Sensorimotor Skills - Fingertip Tapping
    - Visuomotor integration - Design Copy
- The Wechsler Individual Achievement Test – Second Edition Abbreviated (WIAT- II Abbreviated) will be used to assess the academic skills of the child using the screening test
- The Children's Memory Scale (CMS) will be used to assess
    - Attention/Executive Function – Span - CMS Numbers Forwards
    - Working Memory - CMS Numbers Backwards
    - Memory and Learning – CMS Word List/ Delay
- The behaviour of the children will be measured by their parent/caregiver using three parent/caregiver rated scales. These are:
    - The Behavioural Regulation Index and Meta Cognition Index of the Behavioural Rating of Executive Function – Preschool Version Parent Form (BRIEF-P) to measure neurobehaviour
    - The Adaptive Behavioural Assessment System(ABAS) to measure the child's adaptive behaviour
    - The Child Behaviour Checklist Caregiver Questionnaire (CBCL) will be used to measure the child's general behaviour

6.2.2 Apnoea

Apnoea will be defined in terms of changes in recorded vital signs and need for intervention. The timing of the apnoea will also be early or late.

6.2.2.1 Significant apnoea by vital signs

A significant apnoea will be defined as a pause in breathing of greater than 15 seconds or a pause greater than 10 seconds if associated with oxygen saturation <80% or bradycardia (20% decrease in heart rate).

6.2.2.2 Significant apnoea by intervention

Any intervention for apnoea will be recorded as frequency, time and nature of intervention. Types of intervention recorded will include need for stimulation, assisted ventilation, CPAP, endo-tracheal intubation, administration of a methylxanthine stimulant, and delay in discharge home due to apnoea or discharge from hospital with a home apnoea monitor.

6.2.2.3 Early apnoea

Any significant apnoea in the PACU or the first 30 minutes of the NICU if PACU is bypassed or in the operating room after completion of surgery, discontinuation of general anaesthesia and removal of laryngeal mask or endo-tracheal tube.

6.2.2.4 Late apnoea

Any documented significant apnoea occurring in NICU or other hospital ward within 24 hours of completion of surgery, excluding early apnoeas.

6.2.3 Other outcomes relating to anaesthesia group:

- Success rate of nerve block
- Time taken for nerve block
- Behaviour of child during nerve block, during surgery and in PACU
- Time to first feeding

6.2.4. Incidental outcomes not related to choice of anaesthesia:

- Incidence of hernia recurrence at 2 years will be recorded. If the repair was unilateral the incidence of contralateral occurrence will be noted.

7. SAFETY

7.1 Monitoring during anaesthesia and in PACU

During all nerve blockade there will be ECG, NIBP and SpO_2 monitoring. During anaesthesia for both groups there will be ECG, NIBP, temperature and SpO_2 monitoring. In the general anaesthesia group there will also be agent analysis and end-tidalCO_2 monitoring. Blood glucose and haemoglobin will be measured during anaesthesia. In PACU there will be monitoring of SpO_2, temperature, and HR.

7.2 Data monitoring committee

An independent DMC has been established. The DMC operates as per the charter listed in the appendix. The DMC will receive interim reports every 6 months. Serious adverse events will be reported to the DMC every 6 months. Non-serious adverse events will be recorded and reported to the DMC be reported every 6 months. The DMC will report to the Trial Steering Committee.

The data monitoring committee will receive interim reports every 6 months.
The DMC will consider the rate of failed awake regional anaesthesia requiring conversion to general anaesthesia and the rate of apnoea in each group. The DMC will also consider all relevant publications and findings from other studies that are relevant to the conduct of this trial.

Codes will only be broken for reports to the DMC or if requested by the TSC or DMC.

The DMC has stopping rules on which to recommend to the TSC that the study should be halted. (See charter in appendix)

7.3 Adverse events

Investigators at each site will review the child daily for 5 days during admission after hernia repair or until discharge. They will perform a follow-up phone call one week after hernia repair if the child is discharged.

For each SAE the site investigator will complete an SAE form.SAE forms will be emailed without delay to the project coordinator responsible for the region. The project coordinator will then forward the SAE form to all three PIs without delay and will also notify all regulatory bodies as required including ethics committees, institutional review boards, sponsors and government regulatory authorities.

SAEs will also be reported by the site investigator to the child's paediatrician, anaesthetist and surgeon and they will initiate appropriate management and inform the family if the family is not already aware of the event.

7.3.1 Serious adverse events: Serious adverse events are the following

- Death due entirely or in part to anaesthesia
- Any death within 48 hours of anaesthesia
- Readmission to hospital due to anaesthesia complications apart from apnoea.
- Delay in discharge from hospital due to any complications apart from apnoea.
- Inability to satisfactorily complete surgery, or surgery abandoned due to inadequate anaesthesia (not including surgery successfully completed after conversion of regional to general anaesthesia)
- Evidence of local anaesthesia toxicity such as seizures or arrhythmia
- High neuraxial blockade requiring intubation
- Any persisting neurological deficit from neuraxial blockade
- Meningitis or epidural abscess within 5 days of anaesthesia
- Evidence for bowel puncture with ilio-inguinal or caudal nerve blockade defined as aspiration of bowel contents
- Barotrauma during general anesthesia resulting in pneumothorax
- Cardiac arrest or bradyarythmia requiring chest compressions
- Any other event not mentioned above that is a life threatening event or jeopardises the patient or requires medical or surgical intervention to prevent a serious adverse event. The interpretation of whether or not an event fulfils this particular criterion is the responsibility of the site investigator.

SAEs will also be reported to the DMC and all ethics committees involved with the trial.

7.3.2 Adverse events: Adverse events are as follows

- Apnoea requiring readmission to hospital within 48 hours of anaesthesia
- Delay in discharge due to apnoea.
- Failure of regional anaesthesia requiring sedation or analgesia (excluding oral glucose)
- Failure of regional anaesthesia requiring conversion to general anaesthesia (from either technical failure of insufficient blockade for length or scale of surgery)
- Post-anaesthesia apnoea requiring endo-tracheal intubation

- Delay in extubation for greater than 1 hour after surgery
- Hypoxia during anaesthesia with SpO_2 < 80% for 5 minutes
- The administration of vasoactive drugs to support circulation
- Increase in post operative oxygen requirement or increased respiratory support for greater than 1 hour post anaesthesia
- Any other event not mentioned above that results in the child needing medical attention whether or not it is related to the allocated anaesthetic technique or measurement interventions.

7.4 Report to family

The family will receive a report of the neurodevelopmental assessment at 2 and 5 years. If any significant problems are identified these will be highlighted for the family, and with the permission of the family, the child's paediatrician or family doctor may also be notified. The study team or child's paediatrician or family doctor may offer relevant referral to appropriate medical or allied health professionals when indicated.

8. STATISTICAL CONSIDERATIONS

8.1 Analysis of primary outcome

Since this an equivalence study, the primary outcome will be analysed on a per-protocol analysis basis to ensure a conservative estimate in the direction of non-equivalence. The means will be adjusted for gestational age at birth. The adjusted mean difference in Full Scale IQ Score between arms will be presented with a 2-sided 95% confidence interval. Equivalence will be accepted if the confidence interval of the difference in means lies within -5 and +5 points.

8.2 Analyses of Secondary outcomes

8.2.1 Other measures of neurodevelopmental outcome

A per-protocol analysis of the mean difference of the Cognitive component of the Bayley III scale between arms adjusted for gestational age will be presented with a 2-sided 95% confidence interval. Equivalence will be accepted if the confidence interval of the difference in means lies within -5 and +5 points.

For other continuous data, groups will be compared as for the primary outcome with the gestation-adjusted mean difference in scores presented with a 2-sided 95% confidence interval. For frequency data difference in frequency between groups will be presented with a 2-sided 95% confidence interval.

8.2.2 Apnoea:

There will be four Apnoea outcomes.

- Early significant apnoea on vital signs
- Late significant apnoea on vital signs
- Intervention for early apnoea
- Intervention for late apnoea

For each apnoea outcome an adjusted regression analysis will be performed to assess associations between risk factors and apnoea.

8.2.3 Other outcomes related to anaesthesia group

All other outcomes related to anaesthesia group will be reported for each group with 2-sided 95% confidence interval.

8.2.4 Outcomes not related to anaesthesia group

These outcomes will be reported using descriptive statistics only.

8.3 Justification of numbers

The WPPSI-III full scale IQ score is a standardised score with a mean of 100 and standard deviation of 15. Cohort studies of ex-preterm babies at 5 years of age commonly show a mean of 95 and standard deviation of 15 (term is 105). A difference of 5 points (1/3 of a standard deviation) will be taken as the largest difference that would be acceptable to demonstrate equivalence.

The sample size is chosen so that if the two methods of anaesthesia really are close to equivalent and we assume an expected difference of only 1 standardised score point, there is a 90% chance that a 95% confidence interval will exclude a difference of more than 5. On these assumptions, the trial would need 598 infants in total. Enrolling 660 (plus 60) would allow for an average of 10% loss to follow-up.

8.4 Replacement

Subjects will be replaced when excluded from an as per protocol analysis due to major protocol violation. As this is an equivalence study it is necessary to recruit extra children to ensure randomisation occurs equally in each arm of the study.

9. DATA MANAGEMENT

The Clinical Epidemiology and Biostatistics Unit (CEBU) at the Royal Children's Hospital in Melbourne will be the Data Coordinating Centre for the study, and will be receiving original CRFs from central locations in the USA and the UK and all other study sites and entering these CRFS into a database. The CRFs will be double-entered to check for accuracy in data entry. All sites will retain a photocopy of the CRFs from participants enrolled at that site. All original CRFs will be sent to Melbourne

Melbourne, Boston and Glasgow will be responsible for identifying and chasing missing data in their region. Melbourne, Boston and Glasgow will keep all identifying data and be responsible for arranging follow-up within their region.

10. QUALITY CONTROL

The WPPSI-III is an individually administered assessment. It takes 60-90 minutes to administer. Examiners will be trained and have prior experience administering and

interpreting standardised assessments with infants. Typically examiners will have psychological training at the masters or doctoral level. At regular intervals the assessor will be video recorded during an assessment and the recording sent to Dr Stargatt in Melbourne to compare assessment quality across sites.

11. ETHICS

There is a strong argument to believe that equipoise exists in this trial. Awake regional and general anaesthesia are both accepted standards of care for inguinal hernia repair in children. Regional anaesthesia may be more technically demanding and preferences for one or the other may vary depending on the experience of the paediatric anaesthetist. In younger patients regional anaesthesia may be associated with less apnoea than general anaesthesia but this may be less apparent with the newer general anaesthesia agents. Similarly although there is animal data for toxicity, the evidence for risk of general anaesthesia is sufficiently weak to accept general anaesthesia as an arm in the study.

When recruiting patients the researcher approaching the patient must first ensure that both treating surgeon and anaesthetist are comfortable with randomisation.

All sites will obtain approval from their local Human Research Ethics Committees. In all cases written informed consent will be obtained from parents or guardians.

An independent Data Monitoring Committee and a Trial Steering Committee have been established to oversee the trial. Both committees have independent chairs and consist of statisticians, neonatologists, a psychologist and anaesthetists experienced in trial governance drawn from Australia, USA and Europe.

12. FINANCE AND INSURANCE

See attached budget in appendix II. Insurance will be provided by institutions where the study is being performed. In the UK Glasgow will be the sponsoring institution.

13. PUBLICATION POLICY

It is expected that the neurodevelopmental outcome at 2 and 5 years will be published in a high impact journal such as Lancet. A number of other publications will result from secondary outcome analysis. All publication submissions will need TSC approval. Apnoea data and other data pertinent to short term outcome will be published after all children have been enrolled and all apnoea data and recovery data have been collected and analysed. This will be before the 2 and 5 year data are available.

Appendix I

DEFINITIONS OF AGE

Calculating age is an imprecise science and there are various definitions in use. The dates cannot be exact but to avoid confusion the following definitions will be used:

Expected Date of Delivery (EDD): Date mother expected the child to be born – calculated *either* by 40 weeks after first day of last menstrual period, *or* from an early ultrasound scan, *or* 2 weeks plus date of conception if assisted reproduction

Gestational Age (GA):
Calculated as: GA (in days) = 280 days - (Expected Date of Delivery – date of birth)

Chronological Age: Time elapsed since birth.
Chronological age (in days) = date of randomisation – date of birth

Postmenstrual Age (PMA): PMA = Gestational Age + Chronological Age,
Calculated as: PMA (in days) = date of randomisation – EDD + 280 days

Corrected Age: Chronological Age from Expected Date of Delivery

Inclusion criteria are:

- Gestational Age, 26 weeks or more (GA $\geq$ 182 days)
- Postmenstrual Age, up to 60 weeks (PMA $\leq$ 426 days)

By entering EDD and date of birth, the randomisation program will automatically calculate Gestational Age and assign the child to the appropriate strata. If the calculated Gestational Age is less than 182 days or the Postmenstrual Age is greater than 426 days then an error message will appear and the child cannot be randomised.

Do not use the terms “Conceptual Age” or “Post Conceptual Age” and try to avoid using the term “Prematurity”. When recording EDD, note on the CRF which method is used to calculate EDD (menstrual period, assisted reproduction or scan).

Note: the date of randomisation used in the program will be the current date Australian Eastern Standard Time.

Reference:
American Academy of Pediatrics Policy Statement. Age terminology during the Perinatal Period. *Pediatrics.* 2004;114:1362-1364.

Appendix II

Funding

Seed funding

- The Murdoch Childrens Research Institute, Project Grant: **AUS$40,000**
- The Australian and New Zealand Collage of Anaesthetists, Project Grant: **AUS$48,000** & **$60,000**
- "The Smith Award" from the Department of Anesthesiology, Perioperative and Pain Medicine, Children's Hospital Boston: **US$50,000**
- Canadian Anesthesiologists' Society, "Dr. R A Gordon Patient Safety Research Award": **Can$20,000**
- Pfizer Canada Inc: **Can$4,000**

Major funding

- Australia: NHMRC project grant (2011-2015): **AUS$721,338**
- Australia: NHMRC project grant (2008-2010): **AUS$490,750**
- Canada: Canadian Institutes of Health Research (2009-2015): **Can$86,747**
- USA: FDA contract: Safety of Key Inhaled and Intravenous Drugs in Pediatrics (SAFEKIDS) Initiative, FDA-SOL-08-SAFEKIDS Clin 002-Project 2: (2008) **US$308,000** & (2009) **US$100,000**
- USA: 2010-2015 NIH 1-R01 HD06 1136-01A1 Grant Award **$2,421,699**
- UK: HTA: **£195,000**
- Italy: Italian Ministry of Health; Italian NHS Grant for Young Researchers (2009-2012) E178,000.

Appendix III

US sites protocol amendment

In the US, spinal bupivacaine 0.5% is not commercially available so 0.75% is diluted. This added step is a safety issue so all US sites will use bupivacaine at 0.75% strength. The US local ethics committees have approved this amendment.

CHU Sainte-Justine, Montreal, Canada Protocol Amendment (I)

In CHU Sainte-Justine, 0.75% bupivacaine is used as standard practise in spinals. Therefore CHU Sainte-Justine will be allowed to use 0.75% bupivicaine instead of 0.5% bupivacaine for spinals.

Appendix IV

UK sites protocol amendment

In the UK, bupivacaine is not commercially available. Therefore all UK sites will use levobupivacaine for their spinal, caudal, ilioingunal and field blocks. The UK local ethics committees have approved this amendment.

Universitair Medisch Centrun Groningen, The Netherlands Protocol Amendment (I)

In Universitair Medisch Centrum Groningen, The Netherlands levobupivacaine is used as standard of care for their caudals. Therefore this site will be allowed use levobupivacaine in caudals.

Appendix V

ANALYSIS PLAN

1. ANALYSES

1.1 Definitions

The primary comparison for this equivalence trial will be carried out on a per-protocol (PP) basis defined as follows:

> The analysis will exclude all infants classified as having a major protocol violation the majority of whom will be infants who were randomised to receive regional anaesthesia but were given general anaesthesia (see Section 2 'Protocol violations' below).

A secondary analysis will be carried out on an intention-to-treat (ITT) basis as follows:-

> The analysis will include all children as randomised irrespective of the treatment received and for whom final outcome data are available.

Both the PP and the ITT analyses will be performed on the primary outcome and all secondary outcomes as defined in section 6 of the Protocol, and adjusted for gestational age at birth according to the pre-specified groupings used for the randomisation. Results from both the PP and the ITT analysis will be reported.

1.2 Rationale

In general it is best practice to analyse outcomes on an ITT basis where all participants are included according to their randomised allocation and issues of selection bias are avoided.

In this study there are unavoidable protocol violations the majority of which are babies allocated to regional anaesthesia who have some exposure to general anaesthesia particularly if the regional anaesthesia fails (e.g. due to movement impeding surgery or a vigorous crying child that cannot be comforted). If all infants were analysed according to their randomised allocation in an ITT analysis, this switching from one randomised treatment to the other could dilute the potential effect of general anaesthesia and thus bias the trial towards equivalence as these children will receive both study interventions.

Selection bias in a PP analysis for this study may occur if the underlying reason for a protocol violation is related to failure of a regional anaesthetic and the neurodevelopmental outcome of the child. For example, infants who are switched may have an underlying neurodevelopmental problem at baseline, and thus be more likely to have a worse neurodevelopmental outcome at 2 and 5 years; or larger babies, who are more likely to require some supplementary general anaesthesia, may have a better neurodevelopmental outcome at 2 and 5 years. Selection bias can be partially assessed by comparing infants with failed and successful regional anaesthesia on baseline characteristics including the weight of the baby (see Section 5 'Assessing Bias' below).

It is considered that the main source of bias for this trial will be failures in the regional group with switching to the general anaesthetic group. A PP analysis is therefore considered the best approach in this trial where the prime focus is on the causal effect of the two types of

anaesthesia. An ITT analysis will provide interpretations as to the benefit of the treatment policy.

2. PROTOCOL VIOLATIONS

During the study information will be collected on all protocol violations. After data collection, these data will be classified by an independent review panel as either major or minor according to their likely impact on the child's primary outcome, with major violations defined as those that could potentially affect the comparison of the interventions. Major violations are expected to primarily involve children in the regional arm who received any sevoflurane irrespective of the reason.

3. MAINTAINING STUDY POWER

Since the exclusion of protocol violations for the PP analysis will reduce the sample size and hence the power of the study, the sample size will be adjusted to allow for these exclusions so that the target sample size of 660 (plus 60) patients is maintained for this analysis.

4. MISSING OUTCOME DATA

Infants with a non-missing outcome will be included in the PP and ITT analysis (complete-case analysis). In addition, a sensitivity analysis will be carried out on a PP and an ITT basis, using multiple-imputation methods to impute missing 5 year outcome data. These imputations will use baseline data and known outcomes measured at 2 and 5 years.

Numbers and reasons why data are missing will be captured and reported where possible. Potential imbalances in missing and observed outcomes between intervention groups will be assessed according to Section 5 'Assessing bias' below.

5. ASSESSING BIAS

Any patterns of departure from randomised treatment will be assessed by comparing infants having no major protocol violations with those having major protocol violations by treatment arm, on infant characteristics.

Patterns of missing outcome data will be assessed by comparing infants with and without outcome data by treatment arm on infant characteristics.

6. RANDOMISATION ERRORS

Occasionally babies may be randomised under the incorrect stratum e.g. if there is a data entry error in the babies date of birth during the randomisation process. In this situation the child will be analysed under their correct stratum with no additional adjustment although this will be noted in the trial report.

BIBLIOGRAPHY

Gilda Piaggio, PhD; Diana R. Elbourne, PhD; Douglas G. Altman, DSc; Stuart J. Pocock, PhD; Stephen J. W. Evans, MSc; for the CONSORT Group. Reporting of Noninferiority and Equivalence Randomized Trials: An Extension of the CONSORT Statement. *JAMA* 2006; **295**:1152-1160.

Ian R. White. Uses and limitations of randomisation-based efficacy estimators. *Statistical Methods in Medical Research* 2005; **14**:327-347

ICH Harmonised Tripartite Guideline: Statistical Principles for Clinical Trials E9

Erica Brittain and Daphne Lin. A comparison of intent-to-treat and per-protocol results in antibiotic non-inferiority trials. *Statistics in Medicine* 2005; **24**:1-10**Appendix VI**

DMC CHARTER

1. Appointment and Membership

The members of the Independent Safety and Data Monitoring Committee (ISDMC) are appointed by the Chairman of the GAS Study Trial Steering Committee. The ISDMC comprises the following members:
Jonathan De Lima (Paediatric anaesthesia, Sydney – Chair)
Professor Val Gebski (Statistician Clinical Trials Centre in Sydney)
Professor Greg Hammer (Paediatric anaesthesia, Stanford, US)
Professor Dick Tibboel (Surgeon, Holland)
Professor David Field (Neonatologist, Leicester, UK)

Further appointments to the ISDMC may be made if there are resignations from the membership, or if the ISDMC members believe that additional expertise is required. Any such appointments would be made by the Chairman of the GAS Study Trial Steering Committee after consultation with the members of the ISDMC.

2. Role of the Independent Safety and Data Monitoring Committee (ISDMC)

The primary role of the ISDMC will be to:
- monitor and review patient safety in the trial;
- monitor efficacy based on interim analyses of results.

The ISDMC will function independently of all other individuals and bodies associated with the conduct of the GAS study, including the investigators, the Study Principal Investigators and other members of the Steering Committee, and study sponsors.

In the event that there is concern about the efficacy of either anaesthetic technique (GA or spinal), or the balance of benefits and risks conferred by the allocation to either of the anaesthetic techniques or if there is safety concern about trial continuation, this should be communicated immediately to the Study Principal Investigators and subsequently to the Steering Committee.

Outcome and adverse event data will be prepared by the study statistician by blinded treatment group. The ISDMC will be the sole body responsible for monitoring the frequency of serious adverse events *by treatment group*, and will be the only body, with access to summary data *by treatment group*. The ISDMC may request that the data be unblinded at any stage. The reasons for unblinding will be documented in the minutes. Nobody outside the ISDMC will have access to unblinded protocol violations, adverse event and outcome data.

3. Conflict of Interest

Members of the ISDMC should not otherwise be involved in the conduct of the GAS Study. They should not be employees of any of the study sponsors, other than that relating to their duties on the ISDMC. Any other potential conflicts of interest should be declared to the GAS Study Principal Investigators and to the ISDMC Chairman.

4. Terms of Reference

Every 6 months during the recruitment and follow-up phases of the study or when the ISDMC request, the ISDMC will be sent an interim report that includes:

- updated external evidence from other studies or systematic reviews,
- names and number of centres actively recruiting study participants,
- number of study participants recruited, against target recruitment rates,
- number of participants recruited who are classified as 'major protocol violations' (including babies who were 'switched' i.e. allocated to spinal but received GA),
- details of serious, unexpected or minor adverse events,
- outcome data for apnoea and the 2 year neurodevelopmental assessment.

4.1 Monitoring of Serious Adverse Events

Every 6 months, or when the ISDMC request, the ISDMC chairman will be sent a report of serious and unexpected adverse events that are possibly, probably or definitely related to the anaesthetic technique employed. This will be in the form of a cumulative summary of all such serious events, and will be by blinded treatment group (as stated above the ISDMC may request this data to be unblinded).

The report will include the frequency of serious adverse events and non serious events and details of post-op apnoeas. To avoid revealing treatment group, the incidence of 'failed spinals' will be presented separately. While these reports will focus primarily on serious adverse events, other adverse experiences (together with relevant clinical details) will also be summarised. The format of these reports will be determined by the ISDMC in consultation with the study statistician/s.

After consideration of each report, the ISDMC will issue a statement. In the event that there is no cause for concern about the safety of patients in the study, the ISDMC will issue a statement to that effect. In the event that the ISDMC has concerns about some aspect of safety, such that this may have implications for the trial design or continuation, this advice would be communicated immediately to the GAS Study Principal Investigators and through them to the Steering Committee.

4.2 Interim Analysis of Outcomes and stopping guidelines

Interim analyses for this study occur every 6 months or as requested by the ISDMC where outcome data are presented by treatment group.

The stopping guidelines for this study will be based on outcomes at 2 years (since there will be no 5 year outcome data available while the trial is still recruiting). Since recruitment is expected to continue until the end of 2011

there will be at least another two further ISDMC meetings while the trial is still recruiting and where the stopping guidelines will apply (from when this version of this document was compiled). If recruitment is much slower than expected, a third ISDMC meeting where the stopping guidelines apply may take place.

The stopping guidelines will be considered for the following outcomes:

1. the proportion of children with any of cerebral palsy, hearing defect or visual defect at 2 year follow up assessment
2. the standardised cognitive scale of the Bailey at the 2 year follow up assessment

For the first criterion the proportion of children with each outcome will be presented separately for the two groups along with a risk ratio (and its 95% confidence interval and associated p-value) comparing the proportions between the two groups. For the cognitive scale the mean outcome and its standard deviation will be presented for the two groups along with an estimate of the difference in means and its 95% confidence interval and associated p-value. For each outcome, the stopping guidelines will be based on the Peto stopping rule which suggests that a trial be stopped if $p<0.001$. To allow for the fact that the stopping guidelines are to be assessed for three outcomes, a stopping guideline of $p<0.0003$ ($p=0.001/3$) will be used for each outcome.

It is noted that this stopping rule be used as a guideline rather than an absolute rule, considering both safety data from the study and external evidence from other studies in deciding whether a recommendation is made to stop the trial or not. In particular, in assessing the stopping guidelines based on the transformed cognitive scale of the Bailey at 2 years, the decision to stop the study should also be based on the clinical relevance of the difference and its confidence interval, with a difference of 5 on the transformed scale thought to be a clinically important difference. Reasons should be recorded if the stopping guideline is disregarded. In the event that clinically relevant findings occur before the completion of the trial, these may be published, but the follow-up of the study participants will continue.

Once all participants have been recruited the ISDMC may be presented with a comparison of these outcomes *if the ISDMC requests it* although the study will not be stopped after this time. It is possible, however, that interim data may be published if there are findings which are thought to be essential for the public domain.

In addition to the analyses of these relevant endpoints, the ISDMC may request analyses on any other outcome.

4.3 Responsibility for Decisions About Early Termination of the Trial

While the ISDMC may make recommendations to the Steering Committee through the Study Principal Investigators about early termination of the trial, the final decision to stop the trial early or modify the protocol will be made by

the GAS Study Steering Committee. (In the event of this situation arising at any time, the decision of the Steering Committee will be discussed with the ISDMC immediately).

4.4 Study Statisticians

The ISDMC will receive all data and statistical reports from statisticians appointed by the GAS Study Principal Investigators.

4.5 Meetings of the Safety and Data Monitoring Committee

The ISDMC will meet in person, by conference call or via email at least yearly during the recruitment and follow-up phases of the trial.

The ISDMC may meet on other occasions, if this is deemed necessary. Minutes of each of the meetings will be recorded from secretarial assistance provided by the GAS study and held in confidence until completion of the trial.

4.6 Communications to the Safety and Data Monitoring Committee

At any time during the study, drug regulatory authorities, institutional review boards ethics committees, the study Steering Committee, the study sponsors or any other body or individual involved with the conduct of the trial may seek the advice of the ISDMC about any concern that they may have about the conduct, outcome or continuation of the study. Any such requests should be forwarded in writing to the ISDMC Chairman at the following address:

Dr Jonathan De Lima
Department of Anaesthesia
Children's Hospital at Westmead
Hawkesbury Road
Westmead NSW 2145
Tel: (02) 9845 0000
Fax: (02) 9845 3959

4.7 Support for the Activities of the Safety and Data Monitoring Committee

The GAS Study will organise conference call expenses. The GAS Study will also provide logistic support for the organisation of meetings of the ISDMC as well as administrative assistance.

5. Definitions

5.1 Serious Adverse Events (SAE)

Serious adverse events comprise those events that result in:

- Death due entirely or in part to anaesthesia
- Any death within 48 hours of anaesthesia

- Readmission to hospital due to anaesthesia complications apart from apnoea.
- Delay in discharge from hospital due to any complications apart from apnoea.
- Inability to satisfactorily complete surgery, or surgery abandoned due to inadequate anaesthesia (not including surgery successfully completed after conversion of regional to general anaesthesia)
- Evidence of local anaesthesia toxicity such as seizures or arrhythmia
- High neuraxial blockade requiring intubation
- Any persisting neurological deficit from neuraxial blockade
- Meningitis or epidural abscess within 5 days of anaesthesia
- Evidence for bowel puncture with ilio-inguinal or caudal nerve blockade defined as aspiration of bowel contents
- Barotrauma during general anesthesia resulting in pneumothorax
- Cardiac arrest or bradyarythmia requiring chest compression
- Any other event not mentioned above that is a life threatening event or jeopardises the patient or requires medical or surgical intervention to prevent a serious adverse event. The interpretation of whether or not an event fulfils this particular criterion is the responsibility of the site investigator.

5.2 Non Serious Adverse Events (AE)

A non serious adverse event is any undesirable experience occurring to a patient during the trial for which the patient seeks medical attention whether or not it is considered to be related to the allocated anaesthetic technique or measurement interventions.

6. Confidentiality of DMC discussions/ reports

The ISDMC meetings are structured with an open and a closed session. The open session is attended by the study statistician. The closed session is attended by the ISDMC members only.

Minutes of both the open and closed sessions are taken by an independent administrator not connected with the study.

All ISDMC discussions and draft reports of the ISDMC remain confidential until formally delivered to the Trial steering committee.

7. Liability status of the ISDMC members

As the ISDMC is an advisory body alone, ISDMC members are not liable for damage, harm, morbidity or mortality to recruited patients or investigators.

Appendix VI

US sites protocol amendment (II)

For the Regional arm of the study:
In cases where the anaesthesiologist feels that the surgery may last longer than one hour and/or where the anaesthesiologist feels that the spinal anesthetic may not be adequate for the procedure, at his/her discretion he/she may intermittently dose caudal space via cannula with chloroprocaine in order to maintain adequate analgesia for the procedure. Specifically, patients may be given a loading dose of 3% chloroprocaine of 1ml/kg in divided doses of no more than 0.25ml/kg per 15 seconds or in an infusion over 10 minutes and then an infusion of 1-2 ml/kg/hr. There are no additional risks to using chloroprocaine other than the standard risks of using any regional anesthetic.

Appendix VII

US Sites Protocol Amendment (III)

The Parents' Evaluation of Developmental Status: Developmental Milestones (PEDS:DM) is a parent questionnaire which will also need to be completed during either the initial visit if time allows or during the follow-up phone call around post-operative day 5. Parents will be given the option to complete the questionnaire on their own or with assistance from the research staff. The questionnaire will take several minutes to complete and it will assess the following domains of development in children traditionally, between birth and 8 years of age: gross motor, fine motor, expressive language, self-help, and social-emotional. It provides cut-off scores and age-equivalent scores. It was standardized on a sample of 1,600 children in North America and items are written at the first-grade reading level.

This additional assessment was added at the request of the National Institute of Health (NIH).

Appendix VIII

US Sites Protocol Amendment (IV)

and

Wilhelmina Children's Hospital, University Medical Centre Utrecht, the Netherlands Protocol Amendment (I)

Epinephrine/Adrenaline may be used for the caudal block at sites where it is standard practice to do so, but should be avoided if possible.

Up until this amendment, the use of Epinephrine/Adrenaline has been documented as a protocol violation. For this reason, its use should continue to be avoided where possible.

Supplementary Material

Click here to download Supplementary Material: CONSORT 2010 Checklist.doc