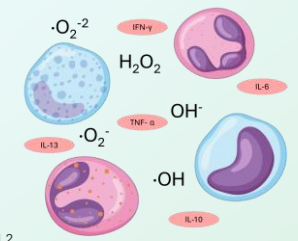


## Redox, inflammatory and immunological results from the EPIVA trial

### (The EPigenetic consequences in children of Intravenous vs Volatile Anaesthesia for surgery – A randomised, feasibility trial)

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#### Summary

General anaesthesia (GA) may have long-term effects on child health. EPIVA randomised 16 children having hypospadias surgery to maintenance of GA with Total Intravenous Anaesthesia (TIVA) or Volatile Anaesthesia (1:1); analysing blood samples post-induction and pre-emergence. The two types of Anaesthetic were associated with distinct patterns of change in immunological, inflammatory and redox status. The temporal relation of these to potential epigenetic change is unclear, as is subsequent progression to phenotypic change. EPIVA has demonstrated the feasibility of a paediatric perioperative trial of this nature and highlighted areas for further investigation.

#### Background

General anaesthetic agents alter cellular function and there remain concerns regarding their potential long-term impacts on children, including on the developing brain (1). Epigenetic processes represent a potential causative mechanism for harm (2), and these may be triggered by and/or lead to alterations in redox, inflammatory and immunological status (3).

#### Aim

The EPIVA trial (Ethics:23/NW/0191, ClinicalTrials.gov: NCT05936853) aimed to compare two strategies for maintaining general anaesthesia (Volatile vs. Total intravenous anaesthesia (TIVA)), investigating potential epigenetic changes, and the biological markers/pathways that may be associated with them. The primary objective of the study was to demonstrate whether a paediatric perioperative trial of this nature was feasible.

#### Methods

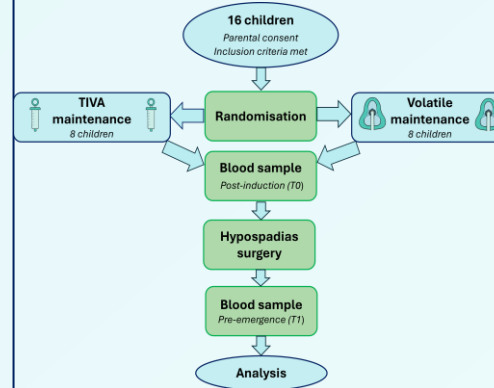


Figure 1. Overview of the stages from recruitment to analysis

- Participant and outcome-assessor blinded, single centre (tertiary), randomised trial.
- 16 children aged between 0.5 and 3 years, undergoing hypospadias surgery, with an anaesthetic time estimated to be >1 hour.
- Inclusion criteria included no previous anaesthetic exposure and no neurodevelopmental/neurodisability diagnosis.
- Randomised to GA maintenance with Volatile anaesthesia or TIVA (1:1 ratio).
- Peripheral blood samples were taken post-induction (T0) and pre-emergence (T1).
- Sample processing included centrifugation and snap-freezing of plasma.
- Analysis included redox, inflammatory, and immunological status.
- Statistical analysis between T0 and T1 for each group was performed using paired t-test, post normality testing.

#### Results

##### Leukocytes

- The TIVA group showed a significant increase in Neutrophils between time-points (MD 81.9%, (95% CI:13.6% to 150.2%) p=0.025).
- The TIVA group showed significant decreases in Lymphocytes (-57.1% (-37.6% to -76%) p<0.001), Monocytes (-48.9% (-14.6% to -83.2%) p=0.012) and Eosinophils (-44.4% (-18.0% to -70.9%) p=0.005)
- The Volatile anaesthesia group showed a significant decrease in Lymphocytes (-50.7% (-31.4% to -70.1%) p=0.001).

##### Inflammatory cytokines

- The Volatile anaesthesia group demonstrated significant decreases in TNF- $\alpha$  (-15.9% (-5.2% to -26.6%) p=0.010) & IFN- $\gamma$  (-21.7% (-11.2% to -32.2%) p=0.002)

##### Redox markers

- The Volatile anaesthesia group demonstrated significant increases in FRAP (Ferric Reducing Antioxidant Power) (31.4% (10.9% to 51.9%) p=0.009) and TFTs (Total free thiols) (7.7% (0.8% to 14.7%) p=0.034).

No adverse events were recorded.

#### Conclusions

EPIVA shows that a paediatric perioperative trial of this nature is feasible, and highlights the impact that different agents used for the maintenance of anaesthesia can have on biological processes:

- TIVA was associated with significant changes in leukocyte populations.
- Volatile anaesthesia was associated with significant changes in redox and inflammatory markers

Analysis of potential epigenetic modifications in these two groups is ongoing, and any identified could feasibly be upstream, or downstream, of the changes in redox, inflammatory, and immunological status, shown here. Whether these translate to phenotypic change warrants further investigation.

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**References:** 1) Liu X et al. General anesthesia affecting on developing brain: evidence from animal to clinical research. *J Anesth.* 2020 Oct;34(5):765-772. PMID: 32601887; 2) Wu Z et al. Epigenetic Alterations in Anesthesia-Induced Neurotoxicity in the Developing Brain. *Front Physiol.* 2018 Jul 31;9:1024. 3) Riti SA et al. The Impact of General Anesthesia on Redox Stability and Epigenetic Inflammation Pathways: Crosstalk on Perioperative Antioxidant Therapy. *Cells.* 2022 Jun 9;11(12):1880PMID: 35741011

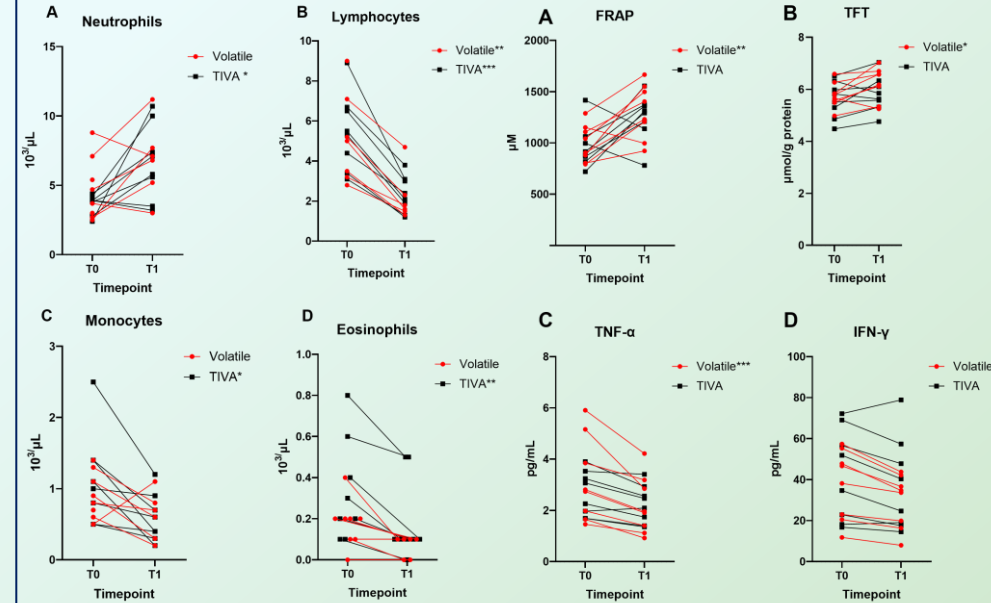


Figure 2. Changes in leukocyte populations between T0 (post-induction) and T1 (pre-emergence), separated by anaesthetic type. A) Neutrophils. B) Lymphocytes. C) Monocytes. D) Eosinophils (graph staggered to facilitate individual data-point identification). Statistical significance was determined by performing two-tailed paired samples t-tests following the Shapiro-Wilk test for normality. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

Figure 3. Changes in inflammatory markers between T0 (post-induction) and T1 (pre-emergence), separated by anaesthetic type. A) Ferric reducing ability of plasma (FRAP). B) Total free thiols (TFTs). C) Tumour necrosis factor - alpha (TNF- $\alpha$ ). D) Interferon - gamma (IFN- $\gamma$ ). Statistical significance was determined by performing two-tailed paired samples t-tests following the Shapiro-Wilk test for normality. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001