Title

Randomisation to a Liberal versus Conservative Oxygenation Target: Redox Responses in Critically ill Children

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G.A.L.J, S.E, M.J.P, and S.R conceptualised the article. G.A.LJ., M.O., S.E. undertook laboratory analyses. G.A.L.J, S.E, M.J.P, S.R. analysed the data with input from D.W., P.R.M and D.A.H. G.A.L.J, S.E, M.J.P wrote the initial drafts with all authors providing critical feedback and edits to subsequent revisions. All Authors approved the final draft of the manuscript.

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Conflicts of interest

No conflicts of interest to declare.

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Abstract

Rationale

Optimal systemic oxygenation targets in pediatric critical illness are unknown. A U-shaped relationship exists between blood oxygen levels and Pediatric Intensive Care (PICU) mortality. Redox stress or iatrogenic injury from intensive treatments are potential mechanisms of harm from hyperoxia.

Objectives

To measure biomarkers of oxidative status in children admitted to PICU and randomized to Conservative (SpO_2 88-92%) versus Liberal (SpO_2>94%) peripheral oxygenation targets.

Design

Mechanistic sub-study nested within the Oxygen in Paediatric Intensive Care (Oxy-PICU) pilot randomized feasibility clinical trial (clinicaltrials.gov; NCT03040570).

Setting

Three United Kingdom mixed medical and surgical PICUs in University Hospitals.

Patients

Seventy-five eligible patients randomized to the Oxy-PICU randomized feasibility clinical trial.

Interventions

Randomization to a Conservative (SpO_2 88-92%) versus Liberal (SpO_2>94%) peripheral oxygenation target.

Measurement

Blood and urine samples were collected at two timepoints; <24 hours and up to 72 hours from randomisation in trial participants (March-July 2017). Plasma was analysed for markers of ischaemic/oxidative response, namely thiobarbituric acid-reactive substances (TBARS; lipid peroxidation marker) and ischaemia modified albumin (protein oxidation marker). Total urinary nitrate/nitrite was measured as a marker of reactive nitrogen species (RONS). Blood hypoxia-inducible factor (HIF)-1a mRNA expression (hypoxia response gene) was measured by Reverse transcription polymerase chain reaction.

Main Results

Total urinary nitrate/nitrite levels were greater in the liberal compared to conservative oxygenation group at 72 hours (median difference 32.6 μmol/mmol of creatinine [95% CI 13.7-93.6], p<0.002, Mann-Whitney test). HIF-1a mRNA expression was increased in the conservative group compared to liberal in <24-hour samples (6.0-fold [95% CI 1.3-24.0], p=0.032). There were no significant differences in TBARS or ischaemia modified albumin.

Conclusions

On comparing liberal with conservative oxygenation targets we show, first, significant redox response (increase in urinary markers of RONS), but no changes in markers of lipid or protein oxidation. We also show what appears to be an early hypoxic response (increase in HIF-1a gene expression) in subjects exposed to conservative rather than liberal oxygenation targets.

Abstract Word count: 305 words (excluding titles)

Information boxes

Research in Context

- There remains clinical uncertainty in the evidence from adult trials of conservative versus liberal oxygen targeting
- In children admitted to the PICU there is an association between exposure to extremes of SpO_2 or PaO_2 and mortality
- Oxidative damage may be a mechanism of harm in hyperoxia in experimental models, but the presence of such a mechanism has not been studied in the PICU

At the bedside

- In ventilated children requiring supplemental oxygenation, undergoing targeted support, we have identified differences in biological indicators of redox signalling and the hypoxia gene expression response
- We have observed increased redox signalling after randomized exposure to liberal rather than conservative oxygenation target. We have also observed hypoxic response within 24 hours of randomization to conservative rather than liberal oxygenation target.
- The contribution of iatrogenic injury associated with achieving liberal oxygenation targets is unknown. Future biomarker studies should focus on whether redox and hypoxic responses translate into clinically significant differences in patient outcomes.

We do not know whether we should be targeting higher or lower peripheral oxygen-hemoglobin saturation (SpO_2) or arterial oxygen tension (PaO_2) values in critically-ill children (1–4). There are no randomised control trials (RCTs) to inform this decision in the Pediatric Intensive Care unit (PICU). In critically-ill adults, although there are RCTs, the evidence of harm or benefit remains uncertain (5–9). The *Improving Oxygen Therapy in Acute-illness* systematic review and meta-analysis (with 16,037 adults) suggested harm with a liberal oxygen target (relative risk [RR] for death 1·21, 95% confidence interval [CI] 1·03–1·43) (10).

In theory, harm with liberal clinical oxygen use may occur as a direct consequence of hyperoxia, or some indirect effect of an intervention also used to increase SpO_2/PaO_2 besides administering more oxygen, like mechanical ventilation and ventilator-induced lung injury (11). Regarding, the direct mechanism, the products of metabolic reduction-oxidation (redox) reactions may cause oxidative damage to proteins, nucleic acids, lipids, and carbohydrates and contribute to cell death or change of function. Some of these products – reactive oxygen and nitrogen species (RONS) – also have essential roles in redox-responsive signalling (11, 12). Thus linking fluctuations in tissue oxygen tension with activation of nuclear transcription factors, such as hypoxia inducible factor (HIF)-1a in response to hypoxia, and other nuclear factors in response to hyperoxia (13). Together, this physiology maintains redox balance and enhances cell survival during hypoxia or hyperoxia (14). Clinically, the production of RONS can be followed using urinary nitrate level as a surrogate (15, 16). Potential harmful effects of RONS during hypoxic or ischaemic conditions can be followed by measuring thiobarbituric acid-reactive substances (TBARS) or ischaemia modified albumin (IMA), respectively (14, 17).

In this exploratory mechanistic study we aimed to quantify the presence of hypoxic and hyperoxic responses using biomarkers of oxidative stress in a heterogenous population of critically-ill children randomised to two SpO_2 targets in the *Oxygen in Pediatric Intensive Care*

(Oxy-PICU trial) (18). The null hypothesis was no differences in HIF-1a expression, TBARS, IMA or total nitrate/nitrite production in response to the two different SpO_2 targets.

Methods

Oxy-PICU trial

The Oxy-PICU multicentre pilot RCT examined the feasibility of a trial comparing conservative (SpO_2 88-92%) versus liberal (SpO_2>94%) saturation targets in emergency PICU admissions receiving mechanical ventilation (Table 1). This pragmatic pilot feasibility trial did not specify strategies for maintaining SpO_2 targets. Health Research Authority (212228) and National Research Ethics Service (16/SC/0617) approvals were obtained and the protocol registered in advance (clinicaltrials.gov NCT03040570; February 2, 2017). The protocol and clinical results have been published previously (18, 19). A 'research without prior consent' approach was used, which was deemed appropriate in emergency situations where delay in commencing treatment allocation may be detrimental and when the treatments being compared are within the range of normal practice. Parents/legal representatives were approached as soon as practical after randomisation to obtain consent for continued inclusion in the study.

Randomisation and sampling

Participants were randomised (1:1) to either the conservative or liberal SpO_2 group by a secure web-based computer-generated dynamic procedure (minimisation) with a random component (80% chance of allocation to the group that minimises imbalance). Minimisation was performed on: age (<12 months vs. ≥12 months); study site; primary reason for admission (lower respiratory tract infection vs. 'other'); and severity of abnormality of gas exchange

(saturation to fraction of inspired oxygen ratio <221 with positive end expiratory pressure/continuous positive airway pressure >5 cm H_2O vs. other). Participants had two blood samples of 1 mL and one urine sample of up to 5 mL collected at two timepoints: within 24-hours and at 72-hours post-randomisation (or immediately before removal of invasive sampling lines in patients with a shorter length of stay). Samples were taken alongside clinical samples to avoid additional venepuncture or accessing of indwelling lines. Urine samples were taken from indwelling catheters, urine bags, cotton wool collection or directly into a urine pot. Blood was collected in lithium-heparin tubes and immediately separated into plasma and cellular components with a cooled ($4^{O}C$) centrifugation at 2000g. The cellular pellet was suspended in RNAlater^{TM} solution to preserve RNA stability. In exceptional circumstances where a delay in processing occurred, samples were stored at $4^{O}C$ and processed within 30 minutes of collection. Samples were stored at the local hospital at minus $80^{O}C$ before transportation on dry ice to a nearby University laboratory.

Plasma was analyzed for markers of oxidative/ischaemic stress. Spectrophotometric IMA measurement was based upon the cobalt binding assay of Bar-Or et al (20). IMA was expressed as a proportion of total serum albumin for each sample to standardize measurement. In the TBARS assay, sample (or standards) were added to TBA with oxidation products of unsaturated lipids reacting to form compounds detected by spectrophotometry (21). Urine was analyzed for nitrite and nitrate levels as markers of RONS as previously described by Miranda et al (22). Total urinary nitrate/nitrite values were expressed as a ratio to urinary creatinine to standardize measurement. Whole blood HIF-1a RNA expression was measured using reverse transcription-polymerase chain reaction (RT-PCR). RNA extraction was performed as per the Ribopure RNA purification kit (Blood) and subsequent cDNA synthesis

using the SensiFAST cDNA synthesis Kit (Bioline). cDNA yield was estimated prior to gene expression analysis of HIF-1a (gene of interest) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; housekeeper reference gene) using RT-PCR with Sensifast SYBR Hi-ROX kit (Bioline). The point in PCR cycling where gene amplification exceeded background signal (Cycle Threshold; C_T) was compared between GAPDH and HIF-1a (delta C_T). Lower C_T corresponded to higher gene expression.

Statistical analysis

Graphpad Prism 8.3.0 and R programming language was used within R Studio Integrated development environment for Statistical analysis (23). We tested the null hypothesis at timepoints one and two. The results were presented as medians and interquartile ranges (IQR). Comparison of groups was carried out using an unpaired Mann-Whitney test. A secondary paired analysis was completed where available sample numbers allowed.

To explore the association between the oxygen dose and biomarker response, we used an SpO_2-time area under the curve prior to sampling as a measure of tissue exposure to oxygen. This was analysed using Spearman correlation coefficients as part of a univariable analysis, and using a mixed effects regression model with SpO_2-time area under curve, age, trial allocation and sampling timepoint (one or two) as fixed effect variables, and patient identifier as a random effect variable. The SpO_2-time area under the curve value is largely influenced by the timepoint as SpO_2 was kept within a narrow range of values; however, there is potential for selection bias in timepoint according to allocation (those in the conservative arm may liberate from support sooner and potentially less likely to have vascular sampling access in place for timepoint 2 samples. Hence, we used time-point and trial allocation as an interaction term. An a priori

power analysis was not possible due to unknown variability of distribution of markers of oxidative stress in this population.

Results

One hundred and seven critically-ill children were randomised in this feasibility study (Figure 1). Four participants declined consent for sampling. Seventy-five of 103 (73%) consented participants had one or more samples available (Conservative n=36; liberal n=39). Fewer samples were available at timepoint 2 for reasons including participants no longer being ventilated, staff not being available for sample processing and/or no indwelling urinary or vascular access (Supplementary table 1). Samples available for analysis at each timepoint varied due to available sample volumes and amount of cDNA extraction available for RT-PCR. Admission comorbidities at randomisation were similar between intervention groups.

Markers of oxidative stress

One-hundred and two samples were measured for TBARS, with a median level of 4.7 μM [IQR 3.5-6.4]). TBARS levels were not significantly different between conservative and liberal arms at timepoint 1 (conservative 4.4μM [IQR 3.2-5.5]; liberal 4.5 [IQR 3.2-7.2]; p=0.375; or time point 2 (conservative 5.1 [IQR 3.7-7.1]; liberal 4.9 [IQR 4.0-6.4]; p=0.815; Figure 2A). Ninety-six samples were measured for IMA, with a median level expressed as a corrected ratio to albumin was 0.0097 [IQR 0.0079-0.0113]. IMA was not significantly different between conservative and liberal arms at time point 1 (conservative 0.0089 [IQR 0.0068-0.0114]; liberal 0.0097 [IQR 0.0081-0.0110]; p=0.31; or time point 2 (conservative 0.0101 [IQR 0.0081-0.0113]; liberal 0.0104 [IQR 0.0082-0.0117]; p=0.732; Figure 2B).

Sixty-eight samples were measured for total urinary nitrate plus nitrite concentration, with a median of 78.4310 μmol/mmol [IQR 50.49-111.65]. Total nitrite/nitrate levels were significantly greater in the liberal oxygenation group at timepoint 2 compared to the conservative group (p<0.002, median difference 32.6, [95% CI 13.7-93.6], Mann-Whitney test Figure 2c). There was a significant increase in nitrate/nitrite levels in the liberal group between timepoint 1 to timepoint 2 (median difference; 26.1μmol/mmol creatinine, p=0.049, [95% CI -10.19 to 65.38]) in contrast to the conservative group (0.78 μmol/mmol creatinine, [95% CI -130.8 to 24.66], p=0.6, Wilcoxon test). When the analyses were repeated in the subset of cases (liberal n=14; conservative n=8) with both time points available, we observed a similar result of an increase in nitrate/nitrite levels in the liberal group (p= 0.031 median difference 26.11, [95% CI= -8.661 to 63.27]).

Response to hypoxia

HIF-1a gene expression was significantly increased in the conservative group compared to liberal group at timepoint 1 (Figure 3, p=0.032), corresponding to a 6.0-fold [95% CI 1.3-24.0] fold higher expression. HIF-1a expression was similar between groups at timepoint two. There were no differences in HIF-1a expression observed on paired analyses between timepoints (Conservative arm p= 0.21, liberal arm p = 0.56).

Biomarker oxygen dose-response relationship

Univariable analysis of all biomarkers according to dose of exposure to oxygen (SpO_2 -time AUC) found no significant relationship between oxygen exposure prior to sampling and biomarker levels (Spearman correlation coefficient; IMA 0.18 [95% CI -0.01, 0.36]; TBARS 0.13

[95% CI -0.07, 0.31]; Nitrate/nitrite -0.14 [95% CI -0.37, 0. 10]; HIF-1a -0.12 95% [CI -0.35, 0.13]). IMA was positively associated with SpO_2-time AUC in the multivariable model, although the effect size was small, with a 7% increase in IMA for a 1% increase in the SpO_2-time AUC (Beta coefficient 0.07 [95% CI 0.01, 0.12]). There were no significant associations between TBARS, Nitrate/nitrite or HIF-1a and SpO_2-time AUC (Beta coefficient; TBARS 0.01 [95% CI -0.09, 0.10]; Nitrate/nitrite -3 x 10^{-6} [95% CI -0.19, 0.19]; HIF-1a 0.01 [95% CI -0.41, 0.42]; Supplementary table 2).

Discussion

In this exploratory study of redox-response nested within a pilot RCT of oxygenation targets, we demonstrate an association between a liberal oxygenation target and significantly lower HIF-1a gene expression, with subsequent increase in biomarkers of RONS production. These changes occurred in the absence of significant changes in markers of lipid peroxidation or protein oxidation. Numbers of clinical trial participants with available samples (71%) were similar in the liberal (n=39) and conservative (n=36) groups. This compares favourably with the adult *Conservative Oxygen Therapy during Mechanical Ventilation in the ICU* (ICUrox) nested study of markers of oxidative stress in participants randomised to a conservative SpO_2 threshold (normal practice of >90% or conservative SpO_2 targets of <97%.) (24). The ICUrox sub-study also found no significant effect of conservative oxygenation therapy on markers protein or lipid oxidation despite a significant difference in PaO_2 between intervention groups.

The increase in the urinary marker of RONS that we have demonstrated provides a potential mechanism of hyperoxic respiratory tissue damage in mechanically ventilated critically-ill children via direct oxidative injury. The contribution of these oxidative responses from iatrogenic injury of intensive care treatments to achieve saturation targets cannot be

completely appreciated from our results. In animal and in vitro human cell studies of hyperoxia, an increase in RONS occurs due to excess oxygen utilisation leading to redox imbalance (25, 26). RONS are key effectors of damage in animal models of hyperoxia associated lung injury via protein, lipid and carbohydrate oxidation and activation of the innate immune response (27, 28). Animal models of hyperoxia associated lung injury demonstrate similar histopathology to acute respiratory distress syndrome with subsequent fibrosis and reduction in pulmonary function (29). Response to oxygen levels is tissue specific within respective microenvironments (30). It is not possible to conclude the relative contributions of specific tissues to the oxidative signals we have measured.

The primary outcome of the pilot Oxy-PICU study was feasibility. In this sub-study IMA and TBARS serve as surrogate markers of oxidative stress in the absence of clinical outcomes of trial participants. Much of the TBARS signal comes from malondialdehyde (MDA); the stability of MDA under biological conditions is variable. Raised MDA levels in critically-ill adults with sepsis can be used in prognostication of 30-day mortality but the specificity of the assay used can influence measurement (31). The absence of significant changes in IMA and TBARS may represent a true insignificant effect of conservative versus liberal SpO_2 targets or that measured RONS were contributing to redox signalling rather than redox stress (32). Conversely, given that both HIF-1a mRNA and total urinary nitrate/nitrite showed significant changes, our results may reflect insufficient power to detect a significant difference in crude markers of protein and lipid oxidation. Based on our results, we calculated post-hoc sample size estimates for each biomarker to detect a difference between the conservative and liberal groups at timepoint 2. To detect any difference with 80% power and an alpha of 0.05, for IMA 621 samples per group would be needed; for TBARS 640 samples per group; for HIF-1a 46 samples per group. For RONS, 17 samples per group would be needed to detect any difference

(Supplementary table 4); 14 samples per group would be needed to detect higher levels of RONS in the liberal group compared to the conservative group (i.e. a one-sided difference).

In our study, the early increase in HIF-1a mRNA expression without increased RONS markers in the conservative group demonstrates a response in the absence of potentially damaging tissue ischaemia or redox stress. Our trial represents utilisation of a physiologically appropriate conservative SpO_2 target as seen in the Liberal or Conservative Oxygen Therapy for Acute Respiratory Distress Syndrome ($LOCO_2$) trial (SpO_2 targets of 88-92 or >96%) that causes relative hypoxic stress without redox imbalance in a heterogenous patient population as seen in the ICUrox trial. We demonstrate an increase in HIF-1a mRNA expression but not a resultant increase in the translation of HIF-1a nuclear factor. HIF-1a and Nrf2 nuclear factors each have over 200 target genes including those regulating cellular survival (33). Further work, for instance RNAseq-based transcriptomics, is necessary to explore the precise pathways that are downstream HIF-1a and Nrf2 targets.

We are not able to conclude whether increases in urinary nitrate/nitrite at timepoint two in the liberal study group are due to increases in redox signalling or due to decreased production (or increased consumption) in the conservative group. Our analysis did not consider factors that lower levels of nitrate/nitrite (via decreased production, increased consumption or reduction) or varying nitrogen load which may contribute to RONS production from other sources, (i.e. diet, parenteral nutrition, immune or epithelial cells induced nitric oxide synthase) (34). The relative contributions of the globin superfamily of proteins that convert nitrite to other nitrogen species were not available in our analysis (35). Although not measured in our study, the presence of bacteria (or absence due to antibiotic administration) in urine would have enabled us to comment on potential bacterial denitrification reducing measured nitrates/nitrites levels (36). The TBARS assay we used primarily measures MDA but

also other minor products of lipid peroxidation; whilst MDA may itself react to form other unmeasured products (37). Further studies should use a more specific test of lipid peroxidation.

The number of patients recruited for this mechanistic sub study was limited and we note that the distribution of some participant diagnoses between the liberal and conservative groups is unequally distributed. We recognise that sample timings in our study, in particular timepoint 2, were variable. Study samples were only taken when routine clinical samples were taken to avoid oversampling. In the case of the timepoint 2 samples, this led to both variability of when samples were taken and the availability of samples (Supplementary Table 1). Interestingly, in our multivariable analysis there was a positive association between SpO_2-time area under the curve and IMA, but no significant associations between the TBARS, Nitrate/nitrite and HIF-1a, and SpO_2-time area under the curve.

In conclusion, we have demonstrated significant biological responses of randomisation to liberal versus conservative peripheral oxygenation targets in critically-ill ventilated children that appear early in critical illness. These responses range from changes in total urinary nitrate and nitrite, through to a genetic response in HIF-1a mRNA production. As Oxy-PICU was a pilot feasibility trial with a primary outcome of feasibility, we are not able to conclude whether these responses represent physiologically appropriate redox signalling or redox stress that may lead to harm. A larger definitive Oxy-PICU clinical study is currently aiming to recruit 2040 participants and will achieve 90% power in detection of a combined end point of mortality and length of organ support (38). Measurement of markers of redox stress and their downstream targets in this larger study could reveal more about the biological response to a conservative SpO_2 target.

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Figure legends

Figure 1. Flow chart of samples analysed from the Oxy-PICU pilot randomised clinical trial. *See supplementary table 3 for reasons samples not available.

Figure 2: (A) TBARS, (B) IMA, (C) Total urinary nitrate + nitrite in ventilated children randomised to a conservative or liberal oxygenation target. Time Points: TP1= sample taken within 24 hour of randomisation, TP2= Samples taken at 72 hours post-randomisation (or immediately prior to removal of invasive sampling lines in patients with an anticipated shorter length of stay). *$p<0.002$ compared with conservative TP1, $p<0.05$ vs. liberal TP2. Unpaired analyses are shown. For paired samples, a paired Mann-Whitney analysis revealed similar results.

Figure 3: HIF-1a expression in ventilated children randomised to a conservative or liberal oxygenation target. Time Points: TP1= sample taken within 24 hour of randomisation, TP2= Samples taken at 72 hours post-randomisation (or immediately prior to removal of invasive sampling lines in patients with an anticipated shorter length of stay). Unpaired analyses are shown but paired analyses where samples were available revealed similar results.

Research in Context

Observational studies in children admitted to intensive care show a U-shaped relationship between mortality and exposure to extremes of SpO_2 or PaO_2. We know of no randomised trials of SpO_2 targets in a Paediatric Intensive Care population.

The non-PICU Bronchiolitis of Infancy Discharge trial of conservative vs liberal SpO_2 targets showed equivalent safety profile and primary outcome of cough resolution. Adult trials of conservative oxygen targets show harm, benefit, or no significant difference in mortality or morbidity.

Association between oxidative damage and harm have been demonstrated in adult ischemic heart disease and animal hyperoxia models. No causal relationship has been established between hyperoxia, increased redox signals and poor outcomes (or mild-hypoxia and benefit) in Paediatric Intensive Care.

At the bedside

We provide evidence of a significant redox response in children randomised to a liberal oxygenation target versus a significant hypoxic response in children randomised to a conservative oxygenation target without associated redox response.

The time course of the hypoxic response in the conservative group was within 24 hours of randomisation to the study which illustrates the need for appropriate oxygenation targets early in a patients' PICU journey.

Future studies should focus on whether redox and hypoxic responses translate into clinically significant differences in patient outcomes and downstream targets of our measured biomarkers.

Tables and figures

INCLUSION CRITERIA	EXCLUSION CRITERIA
• Children more than 38 weeks corrected gestational age and less than 16 years of age • Within the first 6 hours of face-to-face contact with PICU staff or transport team • An emergency admission accepted to a participating PICU • Requiring invasive or non-invasive respiratory support (including: invasive mechanical ventilation, non-invasive ventilation or high-flow humidified oxygen therapy) • Receiving supplemental oxygen for abnormal gas exchange	• Death perceived as imminent • Brain pathology/injury as primary reason for admission (e.g. traumatic brain injury, post-cardiac arrest, stroke, convulsive status epilepticus) • Known pulmonary hypertension • Known or suspected sickle cell disease • Known or suspected uncorrected congenital cardiac disease • End-of-life care plan in place with limitation of resuscitation • Receiving long-term mechanical ventilation prior to this admission • Recruited to Oxy-PICU in a previous admission

Table 1. Oxy-PICU pilot randomised clinical trial inclusion and exclusion criteria

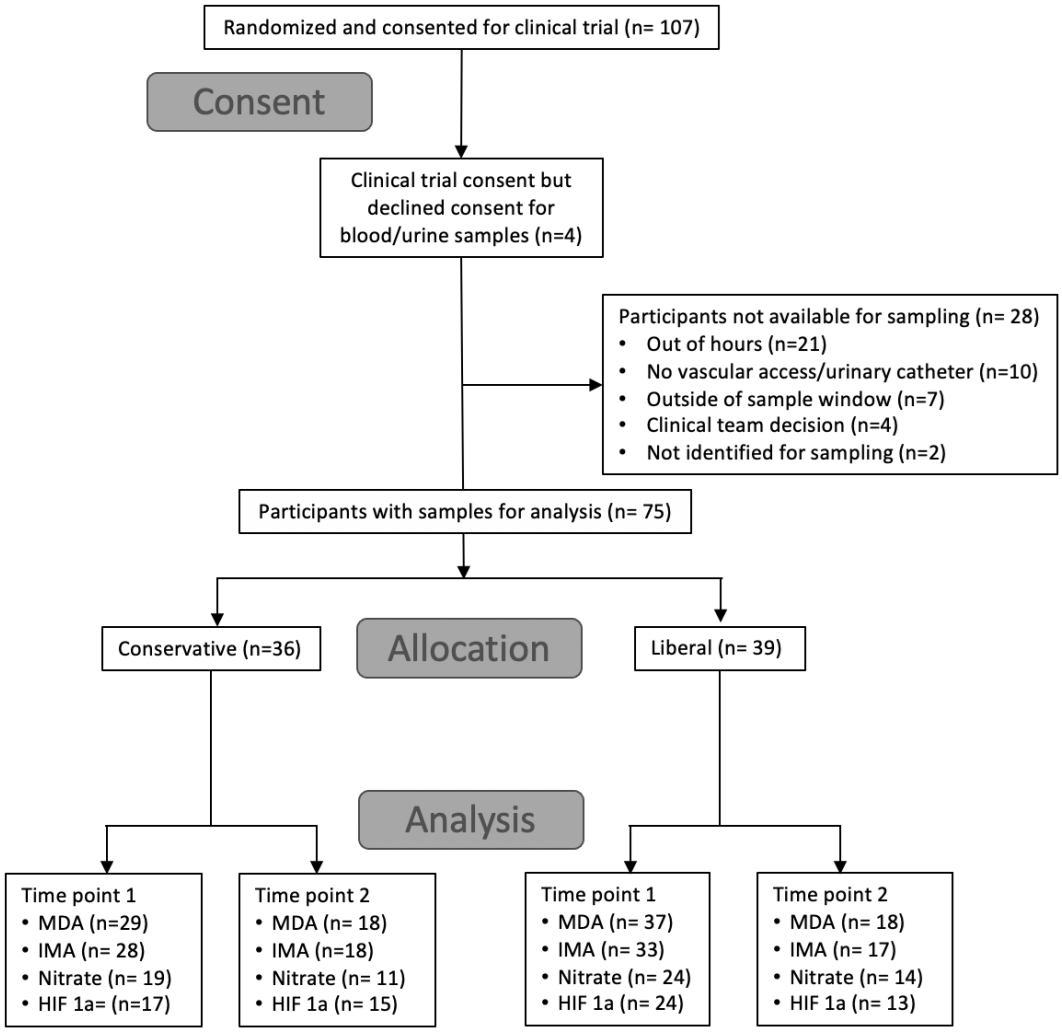

Figure 1. Flow chart of samples analysed from the Oxy-PICU pilot randomised clinical trial

	CONSERVATIVE		**LIBERAL**	
	N	**(%)**	**N**	**(%)**
ADMISSION DIAGNOSIS	**36**		**39**	
SEVERE SEPSIS/SEPTIC SHOCK	6	17	3	8
RESPIRATORY	25	70	30	79
OTHER INFECTION	4	11	0	0
CARDIAC ARRYTHMIA	0	0	1	3
DKA	1	3	0	0
TRAUMA	0	0	1	3
OTHER METABOLIC	0	0	2	5
SOLID TUMOUR	0	0	1	3

SURGICAL - ACUTE ABDOMEN	0	0	1	3
COMORBIDITIES	**N**	**(%)**	**N**	**(%)**
CARDIAC ARREST PRIOR TO PICU ADMISSION	0	0	1	3
CARDIOMYOPATHY OR MYOCARDITIS	1	3	0	0
SCID	1	3	0	0
LEUKAEMIA	0	0	1	3
NEURODEGENERATIVE DISORDER	1	3	0	0
BONE MARROW TRANSPLANT RECIPIENT	1	3	1	3
ORGAN SUPPORT	**N**	**(%)**	**N**	**(%)**
CONTINUOUS INFUSION OF INOTROPE	16	44	8	21
VASODILATOR	0	0	1	3
EXTRACORPOREAL MEMBRANE OXYGENATION	0	0	1	3

Table 2. Participants' baseline characteristics at randomisation.

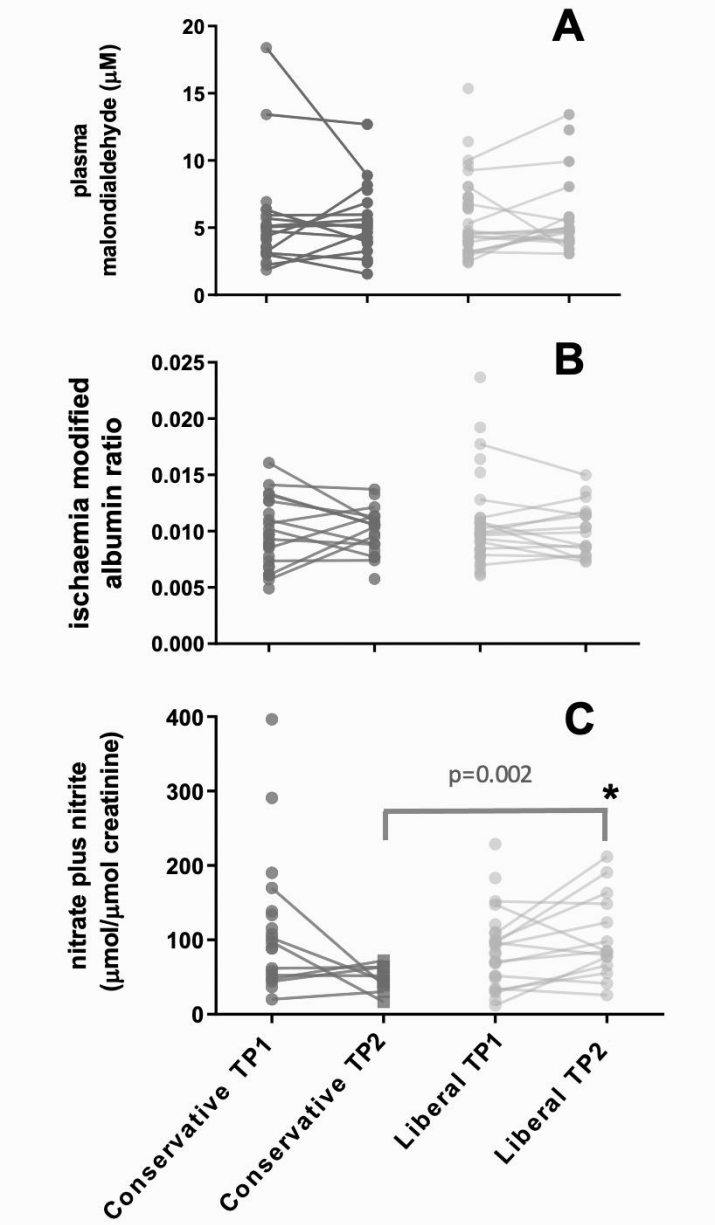

Figure 2: (A) MDA, (B) IMA, (C) Total urinary nitrate + nitrite in ventilated children randomised to a conservative or liberal oxygenation target. Time Points: TP1= sample taken within 24 hour of randomisation, TP2= Samples taken at 72 hours post-randomisation (or immediately prior to removal of invasive sampling lines in patients with an anticipated shorter length of stay). *p<0.002 compared with conservative TP1, p<0.05 vs. liberal TP2. Unpaired analyses are shown. For paired samples, a paired Mann-Whitney analysis revealed similar results.

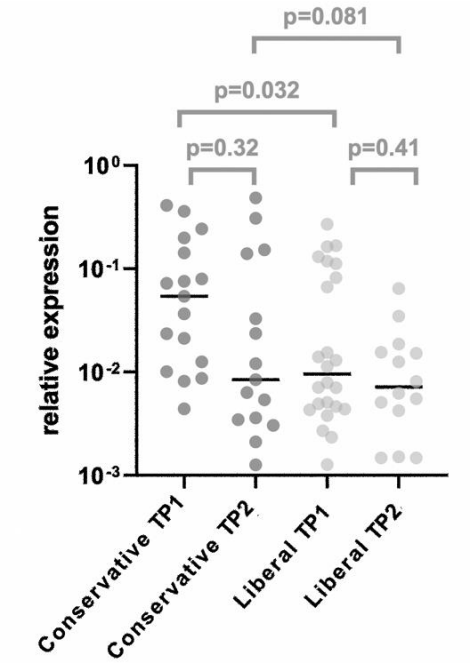

Figure 3: HIF 1a expression in ventilated children randomised to a conservative or liberal oxygenation target. Time Points: TP1= sample taken within 24 hour of randomisation, TP2= Samples taken at 72 hours post-randomisation (or immediately prior to removal of invasive sampling lines in patients with an anticipated shorter length of stay). Unpaired analyses are shown but paired analyses where samples were available revealed similar results.

Biomarker	Odds ratio	95% Confidence Interval
IMA		
SpO2-time AUC	0.07	0.01, 0.12
Time point (2 cf 1)	-0.10	-0.26, 0.06
Allocation (Liberal cf Conservative)	0.04	-0.9, 0.18
Time point 2 * Liberal arm	0	-0.17, 0.17
Age in years	-0.03	-0.06, -0.01
TBARS		
SpO2-time AUC	0.01	-0.09, 0.10
Time point (2 cf 1)	0.04	-0.23, 0.31
Allocation (Liberal cf Conservative)	0.07	-0.16, 0.31
Time point 2 * Liberal arm	0.06	-0.23, 0.35
Age in years	-0.02	-0.06, 0.01
Nitrate/nitrite		
SpO2-time AUC	-3 x 10-6	-0.19, 0.19
Time point (2 cf 1)	-0.60	-1.17, -0.08
Allocation (Liberal cf Conservative)	-0.36	-0.78, 0.06
Time point 2 * Liberal arm	0.86	0.32, 1.43
Age in years	-0.04	-0.11, 0.02
HIF1α		
SpO2-time AUC	0.01	-0.41, 0.42
Time point (2 cf 1)	-0.91	-2.03, 0.22
Allocation (Liberal cf Conservative)	-1.17	-2.22, -0.12
Time point 2 * Liberal arm	0.76	-0.42, 1.88
Age in years	0.04	-0.08, 0.15

Supplementary Table: Odds ratios following multi-variable mixed effect regression analysis, with biomarker as the response variables, SpO2-time area under the curve, age, and a time point and allocation interaction term as the fixed effects variables and patient identifiers as the random effects variables. All continuous values and SpO2-time area under the curve values were log transformed for the purposes of the model given skewed distributions. The model used time point and allocation as a categorical variables, with the odds-ratio of time-point 2 in relation to time-point 1, and liberal in relation to conservative arms as the output. There is a positive association between SpO2-time area under the curve and IMA (both log transformed), but no significant associations between the TBARS, Nitrate/nitrite and HIF1α, and SpO2-time area under the curve.

Biomarker	Liberal group mean	Conservative group mean	Standard deviation	Effect size	Sample size (per group)
IMA					
- Timepoint 1	0.0104	0.0093	0.0035	0.3239	174
- Timepoint 2	0.0103	0.0099	0.0022	0.1707	621
MDA					
- Timepoint 1	5.56	5.08	3.09	0.16	729
- Timepoint 2	5.93	5.46	2.83	0.17	640
RONS					
- Timepoint 1	87.0	117.9	74.1	-0.42	105
- Timepoint 2	103.6	49.3	50.7	1.07	17
HIF-1a					
- Timepoint 1	0.02	0.10	0.10	0.52	67
- Timepoint 2	0.02	0.09	0.11	0.64	46

Supplementary table: Sample size estimates needed to detect a difference with 80% and a two-sided alpha of 0.05 for each biomarker measured. The sample size was scaled up by 15% to account for non-parametric tests.