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ABSTRACTS

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(*The name of the person presenting the paper is shown in bold type. All authors have certified that, where appropriate, studies have been conducted with the approval of the relevant Human Ethics Committee or Animal Experimental Review Committee.*)

Communication within operating theatres: a multicentre service evaluation

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The drive for enhanced patient safety has led to the widespread implementation of surgical safety checklists.[1] We examined aspects of theatre communication surrounding surgical safety checklists throughout our region, using a newly established trainee-led audit and research collaboration (SHARC), which is partly inspired by a model from the Southwest Peninsula (SWARM).

We conducted a service evaluation across seven hospitals over a 6 week period. Electronic data capture and paper forms were used to prospectively collect data from a large variety of theatres, spanning all major surgical specialities. The project was approved by all trusts' Clinical Governance and Information Governance departments.

Data were collected from 392 theatre lists covering 19 different surgical specialities. Formal team briefs were held in 85% of theatre lists. Only 58% of these had all medical members of the team present, with surgical consultants and surgical trainees missing in 11% and 30% of cases, respectively, compared with a 5% non-attendance rate for each of their anaesthetic counterparts. Ninety-two per cent of the time, however, the anaesthetist felt the team brief had been adequate. Holding a team brief was not associated with a delay (>10 min) in the start of a theatre list (32% with team brief *vs* 37% without, $P = 0.47$, χ^2 test). A surgical safety checklist was completed in 96% of theatre lists and the anaesthetist thought it had been done properly in 90% of cases. Introduction by name and role occurred in 58%. Subsequently, consultant anaesthetists and consultant surgeons knew each other's names in 93% of theatre lists, whereas only 30% of trainee anaesthetists and trainee surgeons could name each other. The majority of anaesthetists (85%) found routine introductions useful and 74% would find it helpful to have a whiteboard with names and roles in theatre.

Completion of both a team brief and surgical safety checklist are widely established across our region. However, there is still a mismatch between our perception and the actual quality of these routine safety procedures, shown by inconsistent attendance at team briefs and poor rate of introductions, which form an essential part of the 'time out' step in the checklists. Furthermore, trainees' knowledge of their counterpart trainee colleagues appears to be poor. The aviation industry places strong emphasis on their 'first names only' rule, reducing potential barriers to effective communication.[2] In the theatre environment, it is highly desirable to ensure knowledge of each colleague's name and role, particularly across the large and often complex teams. We therefore advocate the consistent use of whiteboards with all names and roles for all theatre cases.

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Evaluation of the influence of ASA classification on duration of anaesthetic pre-assessment consultation time

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At Sheffield Teaching Hospitals, all patients undergoing anaesthetic preoperative assessment are allocated the same duration of time with a nurse practitioner, leading to potential inefficiencies in patient flow through clinic. We have validated a patient (self-completed) preoperative assessment questionnaire (ePAQ-PO)[1] that estimates ASA status. To evaluate the impact of introducing our online tool, we required information on current duration of preoperative assessment.

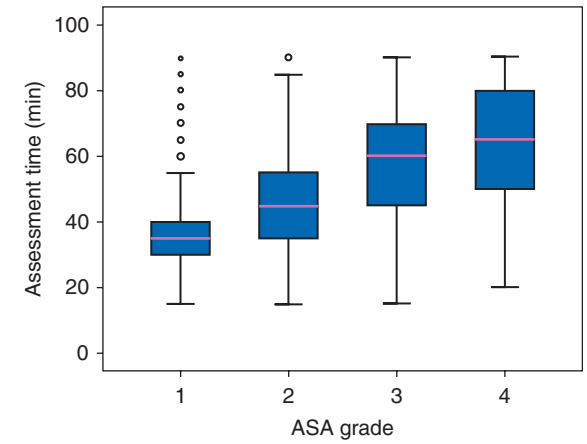

Fig 1 Time of consultation with nurse practitioner stratified by ASA grade. Box shows the median and IQR. Whiskers show the maximum and minimum values excluding outliers.

After registering the project with the trust service evaluation department, data were collected at the Sheffield Teaching Hospital preoperative assessment units, between May 2012 and January 2013. Twenty nurse practitioners recorded the following information on all patients they assessed: age, speciality, estimated ASA grade, and duration of consultation. Data were analysed using SPSS 21 and subjected to ANCOVA, the Pearson product-moment correlation, and regression analysis.

A total of 8519 patients were fully assessed. Two hundred and twenty-one were excluded from analysis due to incomplete time data. The mean (SD) age was 52 (18.1) yr; 55.8% were female, 20.9% were ASA I, 55.5% ASA II, 22.5% ASA III, and 1.1% ASA IV. The mean (SD) assessment duration for all patients was 47 (16) min and for each ASA group, the mean duration was: ASA I 36 (12), ASA II 46 (14), ASA III 58 (17), and ASA IV 63 (19).

Univariate analysis found that the assessment time was significantly influenced by age, ASA grade, surgical speciality, and nurse practitioner. Gender had no effect. Figure 1 illustrates that the duration of a preoperative assessment with a nurse practitioner increases with increasing ASA status ($P<0.001$). Regression analysis revealed an 11 min increase in assessment time for each progressive ASA grade.

Information about a patient's ASA status as estimated by ePAQ-PO would help with the efficient planning of clinics, potentially streamlining the preoperative assessment process and minimizing delays.

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Oxygen uptake efficiency slope and peak oxygen pulse predict outcome in abdominal aortic aneurysm repair

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Oxygen uptake efficiency slope (OUES) and oxygen pulse have not been extensively evaluated in the perioperative setting. Our aim was to determine the ability of OUES and oxygen pulse to predict length of stay and postoperative morbidity in abdominal aortic aneurysm (AAA) repair.

Consecutive patients undergoing cardiopulmonary exercise testing (CPET) before elective open or endovascular infrarenal AAA repair were recruited to a prospective multi-centre observational study. CPET was performed in the preoperative period and measurements were determined by clinicians experienced in CPET interpretation. Morbidity was measured using the postoperative morbidity survey (POMS). The length of stay (LOS) was logarithmically transformed before linear regression. One patient who died was removed from LOS analysis.

Forty-nine patients (46 male) were recruited. Twenty-seven underwent open AAA repair. The mean age was 72 yr. The median LOS was 6 days (EVAR = 4, open = 8). The median POMS count on the third postoperative day was 2 (EVAR = 0, open = 3). The mean (standard deviation) of each CPET variable was: AT 11.3 (2.1) ml kg^{-1} min^{-1}; peak VO_2 16.2 (3.6) ml kg^{-1} min^{-1}; VE/VCO_2 34.5 (5.3); peak O_2 pulse 11.6 (2.8) ml $beat^{-1}$; change in O_2 pulse from start of exercise to peak 6.9 (2.5) ml $beat^{-1}$; OUES 1708 (459). The predictive ability of each variable is shown in Table 1.

Table 1 Ability of CPET variables to predict outcome. Each variable was entered into a linear regression model with type of repair as a covariate. *$P<0.05$; **$P<0.01$

Variable	Change in outcome for a 1 SD increase in predictor	
	Mean LOS (%)	POMS count on day 3
AT (ml kg^{-1} min^{-1})	−11	−0.42
Peak $\dot{V}O_2$ (ml kg^{-1} min^{-1})	−23*	−0.33
VE/VCO_2	17	0.12
OUES	−30**	−0.49*
Peak O_2 pulse (ml $beat^{-1}$)	−28**	−0.36
Change in O_2 pulse (ml $beat^{-1}$)	−22*	−0.33

In our study population, both OUES and peak O_2 pulse were superior at predicting postoperative length of stay—and OUES early postoperative morbidity—compared with conventional CPET variables. An increase in OUES of 1 standard deviation reduced the mean length of stay by 30% [95% confidence interval (CI), 11–53%] and the number of POMS domains with morbidity by 0.49 (95% CI, 0.04–0.93). A 1 SD increase in peak oxygen pulse reduced the length of stay by 28% (95% CI, 10–48%). Our data support the use of OUES and oxygen pulse alongside conventional CPET variables. These findings need to be confirmed in larger samples and in other surgical populations.

Modelling neuronal synapses has the potential to guide theories on anaesthetic drug mechanisms

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A unifying theory of anaesthetic drug mechanism eludes us. Currently used experimental methods allow great spatial or temporal resolution but not simultaneously. We have built a model of a synapse based on standard synaptic transmission theory including glial glutamate/gaba glutamine metabolic recycling.[1]

The modelled synapse has an excitatory centre inhibitory surround receptive field made from eight inhibitory and a single excitatory 'presynaptic terminal'. The receptive field is presented with randomly generated input to induce quantal synaptic vesicle release into a theoretical synaptic cleft. The effect of released excitatory and inhibitory transmitters in the synaptic cleft on the post-synaptic membrane receptors is modelled using a simple mathematical function and includes temporal summative membrane voltage changes upon which action potentials are generated. The transmitter in the synaptic cleft is taken up by a theoretical glial cell and stored in an unprocessed intracellular pool. This unprocessed pool is converted by a modelled 'enzyme' function into substrate which is released into the synaptic cleft to be taken up by the presynaptic terminals to generate new transmitter vesicles. The 'enzyme' function does this at a rate determined by the ratios of the unprocessed:substrate pools; mimicking neuro-metabolic coupling.

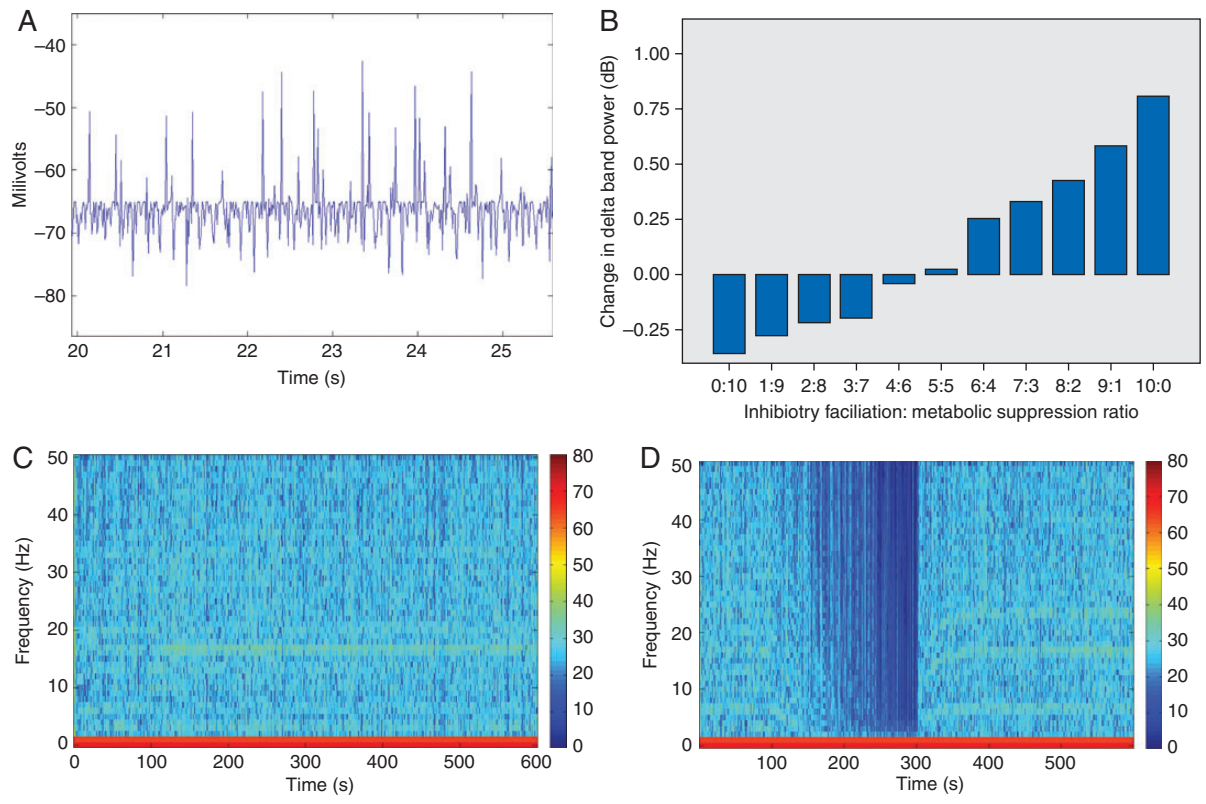

Fig 2 (A) Average pseudo-EEG output from four synapses (5 s sample). (B) Delta band absolute power change related to the ratio of inhibitory facilitation: metabolic suppression. (C) Spectogram of pseudo-EEG from four modelled synapses. (D) Spectogram of four modelled synapses with abolished metabolism for 200 s.

Drug action is simulated at: (i) the excitatory and (ii) inhibitory portions of the post-synaptic membrane function, (iii) the rate at which unprocessed transmitter is converted to substrate mimicking drug–metabolic interaction, and (iv) the presynaptic release of vesicles. These parts of the synaptic transmission cycle were chosen to provide the unique opportunity to separate different potential synapse drug interactions and estimate what proportions a real life anaesthetic might effect each synaptic transmission component by attempting to replicate real data.

Initial modelling based on four synapses produces a composite EEG output and spectrogram (Fig. 2A). To produce anaesthesia induced EEG delta band (1–3 Hz) changes,[2] considerably more inhibitory 'receptor' facilitation is required than metabolic suppression (Fig. 2B). Metabolic suppression produces wide spread reductions in all EEG bands (Fig. 2C). We hope that with further development of this model, it might be used to guide future experiments and help reveal more about the mechanisms of anaesthetic drug action.

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Influence of patient posture on respiratory signal size using RESpeck

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We have developed a device (RESpeck) that provides a reliable measure of respiratory rate at frequent intervals in patients receiving patient-controlled morphine analgesia after surgery.[1] However, almost all the patients we studied were recumbent and inactive. The chest wall moves with a different pattern when subjects are sitting because the abdominal muscles become tonically active.[2] To assess the capacity to measure respiration in patients who are not recumbent, we studied whether body orientation (e.g. sitting or recumbent) affected the size of the respiratory signal.

We approached 16 patients in a surgical ward to measure their movements. Eight had undergone surgery with general anaesthesia and were receiving opioids for analgesia. A RESpeck device containing an encapsulated tri-axial accelerometer (MMA8451Q, Freescale, East Kilbride, UK) was fixed below the costal margin in the midclavicular line. The device was oriented with the Y measure directed craniocaudally, so that the *Y*-axis reading would be zero if the patient and device were horizontal and −1 G if the patient were sitting vertically upright. Data were sampled at 12.5 Hz and transmitted using Bluetooth LE to an iPod Touch (Model A1421, Apple Inc., Cupertino, CA, USA) placed at the bedside. The axis data were combined to generate respiratory signals analogous to flow and volume, with a DC value of zero. The individual axis signals and combined breathing waveform were analysed using proprietary software (Spike 2, version 5.19, CED, Cambridge, UK). Movement was measured as the root mean square (RMS) of the signal amplitude.

Suitable data were obtained from 13 subjects aged 65 yr (range 48–82), mean weight 72 kg (SD = 20.2), mean height 166 cm (SD = 9.5). We recorded 159 h of breathing (mean 736 min per patient) and selected 100 time periods when the subjects were in a stable body position (12 h and 47 min of data).

One patient changed position very little (the 95% CI of the *Y*-axis readings was 0.04 G). A further three patients had 95% CI values <0.3 G. In the remainder, the *Y*-axis ranges (i.e. the range of posture) were 0.46 (0.34–0.54) G (median, quartiles) which we considered sufficient to influence the size of the respiratory signals. In each of these patients, we tested for a correlation between *Y*-axis value and signal size. We found no significant correlation between the mean *Y*-axis reading of each measurement and the RMS of the volume signal. Satisfactory signal amplitudes were present in all postures.

In this small sample of surgical patients, respiratory signal amplitude was not systematically affected by posture.

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Subclinical endotoxaemia enhances lung ischaemia–reperfusion injury

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Ischaemia and reperfusion (IR) of the pulmonary vasculature, associated with systemic inflammation due to infection or surgical stress, represent two principal mechanisms[1] in acute lung injury (ALI). In a mouse model of subclinical endotoxaemia, we have observed enhanced margination of inflammatory leucocytes ($Ly6C^{high}$ monocytes and neutrophils) to the lungs, where they are primed towards secondary insults and contribute to sepsis and high-stretch ventilation models of ALI.[2,3] We hypothesized that increased lung margination and responsiveness of inflammatory leucocytes during subclinical systemic inflammation would enhance the severity of IR-induced ALI.

Subclinical endotoxaemia was induced in mice by i.v. injection of low-dose LPS (20 ng) in animals terminally anaesthetized with ketamine/xylazine.[2] IR injury was modelled using an isolated perfused lung system. At 2 h after LPS injection, the pulmonary vasculature was flushed with sterile perfusate and no-flow ischaemia induced for 2 h at 37°C. The lungs were then perfused and ventilated for a further 2 h. Pulmonary oedema was assessed by lung wet–dry weight ratios; lung-marginated cell number and activation by flow

cytometry and perfusate samples were analysed by ELISA for TNF.

Low-dose endotoxaemia was found to exacerbate IR-induced lung oedema compared with IR alone ($P < 0.05$). Despite flushing of the pulmonary vasculature, in the non-LPS-treated IR group, leucocytes were largely retained in the lungs until the end of the IR procedure [neutrophils: 2.3 $(0.8) \times 10^5$, Ly-6C^{high} monocytes: 3.1 $(1.5) \times 10^5$] compared with unflushed controls [neutrophils: 3.3 $(1.8) \times 10^5$, Ly-6C^{high} monocytes: 4.2 $(1.4) \times 10^5$]. The number of leucocytes after IR in the LPS pretreated group was significantly increased compared with IR controls: approximately nine-fold neutrophils ($P < 0.0001$), and approximately four-fold Ly6C^{high} monocytes ($P < 0.01$). Leucocyte activation, evidenced by monocyte L-selectin shedding and increased neutrophil CD11b expression, was increased in both the IR and LPS-IR groups, compared with untreated controls. However, perfusate TNF levels increased only in the LPS-IR group, whereas it was undetectable in the IR group.

These results suggest that latent pulmonary inflammation associated with subclinical endotoxaemia could be an important determinant of the subsequent IR-induced ALI through the enhanced margination of inflammatory leucocytes. The observation of TNF release in endotoxin-treated mouse lungs provides evidence of priming towards IR, and is likely to represent a monocyte-derived response based on patterns of TNF expression in lung-marginated leucocytes.[4]

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Remote lung injury after renal graft ischaemia–reperfusion injury in rats

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Prolonged cold storage causes ischaemic injury of renal grafts and subsequent reperfusion injury after engraftment, which has been considered as one of the major deleterious factors associated with early dysfunction of transplanted grafts.[12] In addition, such renal graft injury could result in remote organ injury, including lung. Indeed, remote lung injury is a recognized clinical complication in patients suffering from acute renal injury and is associated with high morbidity.[34] However, the acute lung injury and underlying molecular mechanisms after renal transplantation remain elusive. The aim of this study was to investigate the acute lung injury at histological and cellular levels in a rat syngeneic transplantation model.

The Lewis rat renal graft was extracted and stored in 4°C Soltran preserving solution for 24 h and transplanted into the Lewis rat recipient, the lungs were harvested 24 h after grafting for immunofluorescence staining and histological analysis.

Cold ischaemia for 24 h in renal graft led to pulmonary injury 24 h after transplant surgery. The level of injury correlated strongly with the level of renal graft ischaemia–reperfusion injury (IRI). Upon grafting, expression of toll like receptor-4 (TLR-4) was significantly enhanced and nuclear factor kappa B (NF-κB) expression and nuclear translocation was significantly increased. The average injury score was significantly higher in the recipient receiving ischaemic renal grafts.

Renal graft IRI triggered distant lung injury, which is likely through activation of TLR-4/NF-κB pathway. This study could provide the molecular basis for strategies to be developed to prevent or treat such complications after renal transplantation.

Acknowledgement

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Microcirculatory and macrocirculatory responses in a porcine model of traumatic haemorrhagic shock and resuscitation

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Haemorrhagic shock is the leading cause of preventable death after traumatic injury. However, our understanding of the effects of tissue injury and haemorrhage on the microcirculation is incomplete. We aimed to examine the relationship between the microcirculation and global systemic circulation in a porcine model of injury and haemorrhage and to assess the impact of microcirculatory impairment on subsequent resuscitation.

Five terminally anaesthetized Large White pigs received a standardized limb injury followed by a controlled haemorrhage (35% blood volume). After a 30 min shock, phase resuscitation was commenced with 0.9% saline to maintain hypotensive arterial pressure targets (80 mm Hg SAP) for

60 min. Thereafter, cross-matched porcine blood products (1:1 ratio PRBC/FFP) were administered to all animals to maintain normotension. Sublingual SDF video microscopy was performed at the following time points: pre-injury baseline, shock, hypotensive resuscitation, normotensive resuscitation, and late normotensive resuscitation and analysed in accordance with agreed consensus criteria for assessing the microcirculation.[1] Global cardiovascular parameters were also recorded along with arterial and venous blood gas measurements.

Both macrocirculatory and microcirculatory parameters were reduced during shock and hypotensive resuscitation and recovered during normotensive and late resuscitation, although not to baseline levels. Overall microcirculatory flow index (MFI) showed a good correlation with both cardiac output ($r = 0.86$; $P < 0.01$) and mean arterial pressure ($r = 0.78$; $P < 0.01$). However, while the macrocirculation was uniformly impaired, significant heterogeneity was observed in microcirculatory flow, particularly during the shock phase ($P = 0.03$).

We have demonstrated that the microcirculation broadly follows the macrocirculation during injury, haemorrhagic shock, and subsequent resuscitation. However, the microcirculation exhibits marked heterogeneity during low flow states.

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Observational study investigating bispectral index trends in patients undergoing elective surgical resection of non-small cell lung cancer

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Bispectral index (BIS) monitoring is a non-invasive method of quantifying depth of general anaesthesia (GA). Secondary analysis of randomized studies has shown that prolonged deep GA, or *cumulative deep hypnotic time*, is associated with excess mortality.[1] However, this relationship is less pronounced when cancer patients are excluded,[2] indicating that cancer patients are more sensitive to deep anaesthesia or that cumulative low BIS (BIS < 45) is a surrogate for intrinsic vulnerability of this patient population. This study was performed to investigate depth of anaesthesia of patients undergoing thoracic surgery for tumour resection, and if this varies with anaesthetic technique and surgical procedure.

This prospective observational study was performed between May and July 2012. Forty patients undergoing elective resection of non-small cell lung cancer (NSCLC) at Birmingham Heartlands Hospital (BHH), either by video-assisted thoracoscopic surgery (VATS) or by open thoracotomy, were recruited. Before induction of anaesthesia, a BIS monitor was attached to each patient and removed at the end of surgery. Intraoperative BIS data were collected blindly, and also total anaesthetic and surgical times. A mean BIS value of between 45 and 60 was selected as the target for optimal depth of anaesthesia. Normality of data was tested with the Shapiro–Wilk test, and relative proportions of patients under, within, and over the target range were tested via χ^2 analysis ($P < 0.05$). Statistical differences in means were tested via Fisher's least significance difference test ($P < 0.05$).

Out of the 40 patients, 15 (37.5%) had a VATS procedure, while 20 patients (50%) underwent true thoracotomies (excluding VATS conversions). More patients undergoing open thoracotomies (90%, $n = 18$) had a mean BIS of <45 ($P < 0.05$) than VATS procedures (60%, $n = 9$). Cumulative deep hypnotic time was twice as great in patients having thoracotomy compared with VATS [126.6 (53.4) *vs* 63.6 (46.9) min, $P < 0.05$; mean (SD)]. In addition, the 10 patients (50% of total thoracotomies) who had open thoracotomy under GA and thoracic epidural had a mean BIS of 39.3 (SD 5.29), compared with 40.2 (SD 4.04) for the five patients (25% of total thoracotomies) who underwent thoracotomy under GA and intrathecal morphine.

Our data show that BIS values were lower for longer in patients undergoing surgical resection of NSCLC via open thoracotomy compared with those having VATS. Furthermore, this seems to be more pronounced in patients who have their thoracotomy under GA and thoracic epidural. Therefore, thoracotomy patients, anaesthetized under GA and thoracic epidural, may provide a suitable target population for future interventional studies investigating depth of anaesthesia and its effects on postoperative outcomes of surgical lung cancer patients.

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Noxious stimulation under sevoflurane monoanaesthesia in children results in an increase in δ band activity in the EEG

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In the UK, more than 235 000[1] children admitted to hospital each year receive an operation or investigation under general anaesthesia. It is unknown whether noxious stimuli evoke a change in the brain activity of children receiving a general anaesthetic. The insertion of an i.v. cannula provides an opportunity to measure electrophysiological responses after a clinically noxious stimulus using EEG, EMG, and ECG. In this study, we present a novel approach to investigating clinically noxious procedures, and demonstrate that noxious stimuli evoke changes in the brain activity of the anaesthetized child.

Twenty children aged between 2 and 8 yr were studied under sevoflurane monoanaesthesia. Induction of anaesthesia was performed using volatile agents as per routine anaesthetic practice. Once stable, patients were maintained under sevoflurane monoanaesthesia. Premedication or analgesia was not administered to any participants. Sevoflurane was kept constant at an end-tidal volume of 2.5%, relating to 1 MAC of sevoflurane across this age range.[2] Experimental noxious (pin-prick), non-noxious (tactile), and cannulation were performed on the dorsum of the hand while electrophysiological activity was recorded. In a subset of children, topical local anaesthetic cream (Ametop) was applied to the stimulus site.

Changing patterns of neuronal activity evoked by noxious and non-noxious stimuli were 'time-locked' to electrophysiological recordings by means of a high-speed camera and an event-detection interface. Video recordings were captured at 220 fps, a precision of 9 ms and accuracy of 4.5 ms, and touch events marked with a precision and accuracy of 624 and 256 μs, respectively.

Baseline EEG activity was dominated by δ (<3Hz) and α (8–12 Hz) band frequencies which is consistent with previously reported anaesthetic literature.[3] The baseline remained stable throughout the experimental protocol ($P = 0.23$, three-way ANOVA). There was a significant increase in δ band activity after the noxious stimuli, which was not observed after non-noxious tactile stimuli ($P < 0.05$, ANOVA). In the children receiving local anaesthetic, the response to noxious stimuli was diminished. No changes in EMG and ECG activity were observed after both noxious and non-noxious stimuli.

Studies describing the effect of noxious stimulation on the EEG during general anaesthesia describe two response patterns a 'classical' (shift towards high frequencies) and 'paradoxical' (shift towards low frequencies) arousal. In animals, both responses can be elicited by electrical stimulation of the reticular formation. In humans, the evoked activity is dependent on stimulus intensity, anaesthetic depth, and age. These studies demonstrate that noxious stimulation in anaesthetized children evokes a change in brain activity, even though no concomitant behavioural or autonomic changes were observed.

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Comparison of measured *vs* predicted blood propofol concentration in children undergoing spinal surgery, preliminary analysis

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Target-controlled infusion (TCI) of propofol is used during spinal surgery in children because spinal-evoked potentials are suppressed by vapour anaesthetics. Because the data used to develop TCI algorithms are limited, the relationship between predicted and actual blood propofol concentrations is uncertain in individual patients. Some children having spinal surgery have had delayed return to consciousness, and cardiovascular depression has occurred, possibly caused by excessive blood levels. Also, during major blood loss, blood levels may be reduced. This pilot study aimed to identify the difference between the predicted and measured blood propofol concentration during spinal surgery.

The study had ethical committee approval and was registered with MHRA. Children aged 5–18 yr undergoing spinal surgery predicted to last over 3 h were studied. Children or parents gave written consent. Patients were excluded if they had major hepatic or renal disease.

During anaesthesia, propofol concentrations were measured from arterial blood samples approximately every 30 min: up to 10 samples per patient. Routine anaesthesia was unaltered. The Paedfusor TCI model was used. The anaesthetist was blinded from the propofol measurements. Propofol concentrations were determined using a Pelorus 1500 analyser (analysis takes ~3 min). Predicted propofol levels were extracted from the TCI syringe driver and downloaded onto a PC for off-line analysis.

The performance error (PE) was calculated [PE = (Cm-Cp/Cp) × 100]. The main outcome of interest was the median performance error (MDPE) and the median absolute performance error (MDAPE = median |PE|) in each patient. MDAPE >30% was considered clinically significant. Wobble (PE–MDPE) and divergence (regression coefficient of PE *vs* Time) were also calculated. The study aimed to recruit 20 patients. The following data are preliminary and are of the first 10 participants.

Participants had a median age of 13 (range 9–17) yr and a median weight of 48 (range 24.5–95) kg. All 10 had posterior spinal fusion surgery for scoliosis. A total of 85 samples were taken, ranging from 6 to 10 per patient. Nine patients had MDAPE >30%. The median MDAPE for all 10 patients was 64.2% (range −16.3% to 103.3%), and the median wobble for all patients was 13.2% (range 7.3–42.4%). Divergence showed a decrease in this difference over time, with the median value recorded as $\times 0.3\%\ h^{-1}$ (range -1.2 to $0.1\%\ h^{-1}$).

There was a major difference between the predicted and measured propofol concentration: the Paedfusor model underestimated propofol concentration. This difference was greatest

within the first hour of anaesthesia. Blood levels were not excessively high towards the end of surgery and there was no evidence of propofol accumulation.

Systemic concentrations of levobupivacaine administered by rectus sheath catheter

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Rectus sheath catheter (RSC) local anaesthetic infusions are a popular option for analgesia after midline laparotomy due to the higher dependency and risks of perioperative epidurals.[1] Concentrations of local anaesthetic during RSC infusion are unknown. Single-shot ropivacaine demonstrated a long absorption profile with peak concentrations at 45 min and significant concentrations at 180 min.[2] Bupivacaine concentrations >2.5 μg ml^{-1} risk facial paraesthesia, slurred speech,[3] hypotension,[4] seizures, and cardiac arrest.

Patients undergoing cystectomy had RSCs placed under ultrasound guidance after induction of anaesthesia. Twenty millilitres of 0.375% levobupivacaine were administered to each side on the completion of surgery (dose did not exceed 2 mg kg^{-1}). Top-up doses of 20 ml 0.25% levobupivacaine bilaterally were administered every 4 h while the patient was on the intensive care unit (ICU) and every 6 h when on the ward. Arterial blood samples were collected around the first dose with pre- and post-dose samples for subsequent doses on ICU. Similarly, two venous samples were obtained on the ward. Total bupivacaine concentration was measured by high performance liquid chromatography and ultraviolet detection.

Bupivacaine concentration increased with each top-up, never reaching a plateau and passing the toxic level of 2.5 μg ml^{-1} (Fig. 3).

There is a need to review current dosing regimens and to perform appropriate dose finding studies supported by pharmacokinetic modelling.

Acknowledgement

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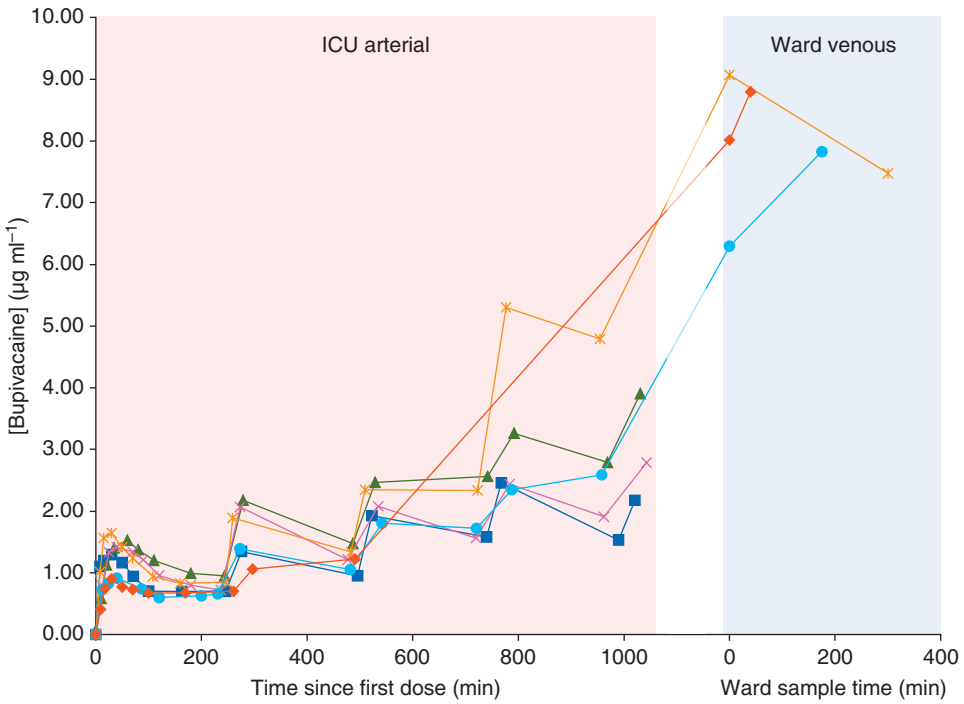

Fig 3 Plasma bupivacaine concentrations in six patients receiving levobupivacaine infusions.

Pain during epidural catheter insertion: a prospective audit

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Pain during epidural insertion despite local anaesthetic infiltration of the skin and subcutaneous tissues is a complication which is poorly understood and may be associated with adverse effects such as nausea, sweating, and the vasovagal reflex.[12] Furthermore, the fear of pain during epidural insertion may be associated with an increased risk of movement, thus increasing the risk of injury to spinal nerves during epidural placement.[3] This audit aimed to determine the incidence and severity of procedural pain during epidural catheter insertion in non-anaesthetized patients.

Patients were identified using the epidural analgesia database (APIS) at St James's University Hospital, Leeds, between September 2012 and February 2013. Thirty-seven patients were included in the audit. Patients were interviewed at the bedside using a structured questionnaire within a maximum of 48 h after operation. Pain scores were measured using a visual analogue score (VAS) 0–10. Retrospective analysis of the medical notes at the end of the data collection period ensured consistency of the data.

The incidence of procedural pain during epidural catheter insertion was 43.2% ($n = 16$), with a mean pain score of 4.5 (2.22), range 1–10. The predominant pattern of pain was 'brief' (95%) and either dull (37.5%) or sharp (37.5%). Patients who were nervous before having an epidural in the sitting position had statistically significantly higher mean pain scores when compared with patients who were not nervous ($P = 0.02$).

Mild-to-moderate pain during epidural catheter insertion was common despite skin and subcutaneous tissue infiltration with local anaesthetic before epidural catheter insertion. The impact of nervousness on procedural pain could be minimized by adopting measures to increase patient reassurance, knowledge of the procedure, and familiarity with the team and preoperative room surroundings (Fig. 4).

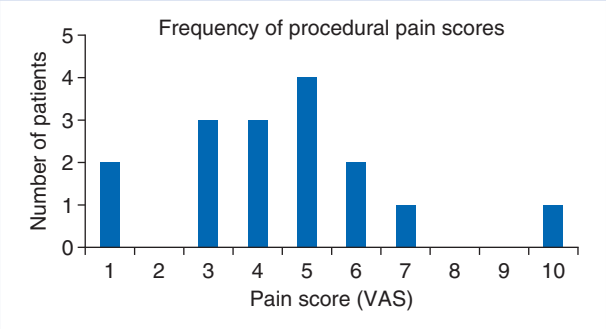

Fig 4 Frequency of procedural pain scores reported during epidural catheter insertion.

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Laboratory evaluation of a novel anaesthesia delivery device

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Anaesthetic vaporizers generally consist of a means to separate gas flows into two pathways: one going through the vaporization chamber and one as a bypass. This is required because the saturated vapour pressure of volatile anaesthetics is much greater than needed clinically. Vaporizers also tend to be heavy, bulky items of equipment to provide thermal stability. Incorrect placement on the backbar of the anaesthetic machine can lead to leaks and awareness in patients.[1] We have developed a novel method of delivering volatile anaesthetics where the liquid anaesthetic is formed into an emulsion. The emulsion is contained in a compact, lightweight device through which all the gas flows. The extent of vaporization of the anaesthetic, and hence the vapour pressure, is limited because of the emulsion, providing controlled release of the anaesthetic. The concentration of delivered anaesthetic is further precisely controlled by a magnetic stirrer placed in the emulsion.

Initial tests were carried out to measure the concentration of anaesthetic delivered at temperatures ranging from 10 to 30°C at a flow of 1 litre min^{-1} with the volatile anaesthetics sevoflurane and isoflurane formulated separately into emulsions. The sevoflurane formulation was prepared by mixing 50 ml sevoflurane with 90 ml of aqueous Zonyl FSN-100 (a commercial non-ionic surfactant, ABCR, Karlsruhe, Germany) solution (20 w/w %). The isoflurane formulation is a mixture of 30 ml isoflurane and 110 ml of aqueous Zonyl FSN-100 solution (30 w/w %). Concentrations of anaesthetic were measured using a calibrated Capnomac Ultima monitor (Datex Instrumentarium Inc., Heslinki, Finland).

The output of the device was repeatable and reproducible and could be adjusted readily by altering the stirring rate, for example, to maintain output at various temperatures (Fig. 5). The output could be maintained within 0.1% (v/v) of the intended setting and the device can deliver a controlled level of anaesthetic for at least 70 min.

Forming liquid anaesthetics into an emulsion offers a simple, inexpensive method of delivering safe concentrations of volatile agents. The water in the emulsion provides good

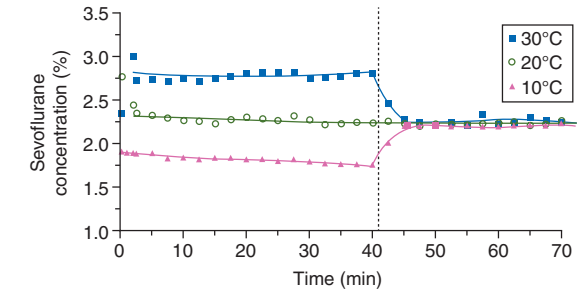

Fig 5 Output of the device at three different temperatures. Note change of stirring rate at about 40 min to control output.

thermal stability, so that a heavy metallic container is not required.

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Effect of a perioperative haemodynamic therapy algorithm on outcomes after major gastrointestinal surgery: a multi-centre randomized controlled trial

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More than 230 million patients undergo surgery worldwide[1] each year with reported hospital mortality between 1% and 4%.[2] Complications appear most frequent among high-risk patients who are older patients or have co-morbid disease and undergo major gastrointestinal or vascular surgery. Patients who develop complications but survive to leave hospital often suffer reductions in functional independence and long-term survival.[3] Small trials suggest surgical outcomes may be improved by cardiac output-guided haemodynamic therapy, but this remains uncertain.[4 5] We evaluated this treatment in specified high-risk surgical patient group.

This was a multi-centre, randomized, observer-blinded trial in 17 UK hospitals. The high-risk group was defined as patients over 65 undergoing major gastrointestinal surgery or patients over 50 with any of the following risk factors: diabetes mellitus, renal insufficiency, a predefined cardiorespiratory risk factor, or emergency surgery. Patients were randomized to a cardiac output-guided haemodynamic therapy algorithm for i.v. fluid and low-dose dopexamine during and 6 h after surgery or usual care. Randomization was performed through a secure Internet-based data entry system. Assuming a type I error rate of 5%, 345 patients per group (690 total) were required to detect with 90% power a reduction in 30 day complications from 50% in the control group to 37.5% in the intervention group (absolute risk reduction 12.5%; relative risk reduction 25%). Allowing for a 3% one-way cross-over rate due to the use of cardiac output monitoring in the usual care group, this was increased to 367 per group (734 total). An interim analysis was performed after the recruitment of 376 patients. Predefined stopping guidelines permitted early termination of the trial for harm but not effectiveness. The primary outcome was moderate or major complications or death within 30 days. Secondary outcomes included 30 and 180 day mortality.

We enrolled 734 patients between June 2010 and November 2012; 368 patients were allocated to the haemodynamic intervention and 366 to usual care. Baseline characteristics were similar between both groups. Patient care outside the trial intervention was also similar, including admissions to critical care. Protocol compliance was good with fewer than 10% of patients in each group experiencing a deviation from the allocated intervention. This was achieved through the presence of trained investigators where necessary, to observe, advise, or deliver the intervention. Investigator self-assessment of blinding also suggested a high rate of compliance with trial procedures. We found that the intervention in this patient group was both feasible and was easily implementable.

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Blood transfusion and outcome after hip fracture: systematic review and meta-analysis

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Anaemia is common in patients with hip fracture.[1] Our previous meta-analysis showed a significant association between admission haemoglobin and mortality in hip fracture patients.[2] However, it is not clear if this increase in mortality is due to blood transfusion, as patients with anaemia are more likely to be transfused than those without.[3] We performed a systematic review and meta-analysis on the association of blood transfusion and outcome in hip fracture.

A systematic search was conducted of MEDLINE, EMBASE, Cinahl, and AMED databases. Titles and abstracts were screened to identify papers ($n = 17\,747$) containing data on anaemia in hip fracture patients and rejecting: duplicates; comparisons of surgical procedures; letters; review articles; and editorials. Full-text papers were assessed using The Newcastle–Ottawa quality assessment tool by two separate reviewers and agreement reached by consensus. Fourteen studies were included. Blood transfusion was associated with an increased odds ratio

of unadjusted mortality of 1.58 (1.39–1.79). When mortality was adjusted for confounding variables such as haemoglobin level, clinical status, age, sex, or mobility, the odds ratio for mortality was non-significant [0.99 (0.76–1.29)] as were adjusted hazard ratios [1.1 (0.98–1.23)].

Despite no difference in mortality, blood transfusion was associated with an increased odds ratio of infection of 2.36 (1.60–3.48). This increased risk persisted even with adjusted outcomes. Although there was significant heterogeneity between studies, all studies reported increased risk with transfusion.

Adjusted mortality figures show no evidence of increased mortality in patients receiving blood transfusion in hip fracture. There is evidence that blood transfusion increases the risk of postoperative infection.

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Evaluation of current practice with regard to the maintenance of intraoperative arterial pressure

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Intraoperative hypotension (IOH) is common. There is evidence that patients who experience a reduction in intraoperative arterial pressure of $\geq$20% are at increased risk of complications.[1–3] The aim was to evaluate the incidence of IOH in patients undergoing surgery for a fractured neck of femur.

Data were collected from 15 patients (12 males), with a median age of 81.5 (59–95) yr. The mean arterial pressures (MAPs) were collected from the anaesthetic monitor at 5 min intervals. MAP 20% below preoperative pressure was defined as hypotension. MAP data are presented as the median (range). The burden of hypotension was calculated as the area under the curve (AUC). *Post hoc* comparisons for the type of anaesthesia and type of operation were made.

All 15 patients were >20% below their preoperative MAP for more than 5 min, and 93% ($n = 14$) were below this threshold for >10 min (Fig. 6). The median duration of IOH was 65 (5–95) min. The median AUC was 94 (3–2388) mm Hg min. Patients receiving a general anaesthetic compared with neuraxial block tended to have lower MAPs; the difference was not statistically significant ($P = 0.13$). Patients undergoing hemiarthroplasty had lower MAPs compared with all other fracture neck of femur operations; the difference was not statistically significant in this small sample ($P = 0.16$). The median AUC values for general anaesthetic (1199 mm Hg min) were higher than neuraxial block (466 mm Hg min). AUC values for hemiarthroplasty were higher than those for all other fracture neck of femur operations (1492 and 635 mm Hg min, respectively).

IOH is common in patients undergoing fracture neck of femur surgery. Such hypotension may be associated with

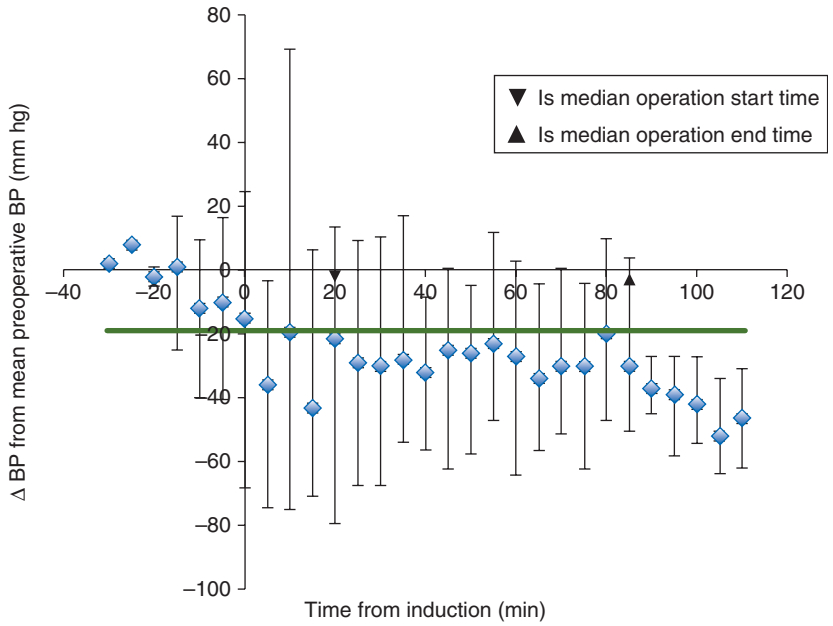

Fig 6 Median (range) perioperative MAP. Zero on the *x*-axis marks the point of induction and zero on the *y*-axis is the preoperative BP. The line marks the IOH threshold.

adverse outcomes and an interventional study to intraoperative arterial pressure control may be justified.

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Impact of sarcopenia on postoperative morbidity in colorectal cancer patients undergoing curative resection

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Preoperative identification of patients at risk of complications and increased length of stay after colorectal cancer resection has many potential benefits such as targeted post-surgical care, discharge planning, and risk stratification. It has been suggested that sarcopenia (degenerative loss of muscle mass) may impact on postoperative morbidity.

We identified 122 patients undergoing elective colorectal cancer resection; of which, 100 had preoperative computerized tomography (CT) staging scans. Lean muscle mass (LMM) was estimated using total psoas area (TPA) normalized for patient height. Psoas cross-sectional area was measured at the L3 vertebral level by two independent assessors. Patient characteristic data, clinico-pathological details, and length of stay were collected from patient notes. Complications were graded according to the Clavien Dindo classification.[1]

Inter-rater correlation for TPA was high with an $r^2 = 0.97$. Similarly, observer assessed TPA correlated with image analysis software analysis ($r^2 = 0.94$, $P < 0.001$). Sarcopenia was defined using sex-specific cut-off points of $<385\ mm^2\ m^{-2}$ for females and $<545\ mm^2\ m^{-2}$ for males[2] with 15% of patients in our study identified as being sarcopenic. Sarcopenia was associated with a significantly increased risk of developing major complications (grade ≥ 3, OR 5.41, $P = 0.01$). Sarcopenia had no effect on length of stay or time to mobilization.

Sarcopenia was associated with a significant increased risk for major complication after colorectal cancer resection. Preoperative CT and measurement of TPA may augment other strategies to identify patients at high risk of postoperative complications.

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Mechanistic profile of argon neuroprotection *vs* injury *in vitro*

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Argon has been shown to be neuroprotective in *in vivo* and *in vitro* models[1–4] like other noble gases, namely xenon.[5] However, it is cheaper and more readily available, but little is known about its mechanism of action. Glycogen synthase kinase 3β (GSK3β) is involved in numerous signalling cascades, yet a link between this kinase and argon has yet to be established. Perinatal hypoxic–ischaemic encephalopathy (HIE), one of the biggest contributors to neonatal brain injury, has a high mortality rate. Twenty-five per cent of surviving individuals suffer from neuropsychological impairment such as epilepsy.[6] Current treatments for HIE are not fool proof with the need to discover new therapies. The objective of this study was to investigate the mechanisms through which argon is neuroprotective *in vitro* on cortical cultures looking at the cell apoptotic and cell signalling cascades to see its potential application for HIE treatment.

For this study, cortical neuronal cell cultures (n=10) derived from rat fetuses were exposed to several gases (nitrogen and argon). The neuroprotective effects of argon were analysed with immunofluorescence staining at 4 and 24 h after treatment with argon alone or superposed with culture medium deprived of oxygen and glucose.

Argon treatment of cortical cell cultures was shown to have a significantly higher increase in extracellular receptor kinase 1/2 (ERK) compared with nitrogen [1.8 (0.9) *vs* 1.2 (0.8), $P < 0.01$ and 1.3 (0.5) *vs* 2.5 (1.2), $P < 0.01$]. There was an increase in phosphatidylinositide 3-kinase (P13K) 4 h after exposure [1.1 (0.5) *vs* 1.9 (1.1), $P < 0.05$]. Protein levels of cleaved caspase 3 [1.0 (0.1) *vs* 0.5 (0.1), $P < 0.001$ and 1.0 (0.1) *vs* 0.9 (0.2), $P < 0.05$], cytochrome *c* [1.2 (0.2) *vs* 0.9 (0.2), $P < 0.01$], and GSK3β, activated tyrosine 216 isoform [1.0 (0.1) *vs* 0.9 (0.1), $P < 0.05$ and 0.9 (0.1) *vs* 0.7 (0.1), $P < 0.01$] were significantly lower in the argon-treated group compared with the nitrogen-treated group after oxygen glucose deprivation. Argon also increased expression of Bcl-2 compared with nitrogen [2.6 (0.8) *vs* 1.7 (0.6), $P < 0.01$].

In conclusion, argon was shown to have neuroprotective properties via the inhibition of the intrinsic apoptotic pathway and the up-regulation of cellular survival pathways. These data may provide increasing evidence for its use as a neuroprotectant in HIE.

Acknowledgement

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Neurotoxic potential of N_2O *in vitro*

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Nitrous oxide (N_2O) is commonly used as labour pain relief,[1] but its potential neurotoxicity to the neonatal brain[2] has been largely ignored. N_2O causes accumulation of homocysteine by irreversibly inactivating vitamin B_{12}, an important co-factor in the remethylation pathway.[3 4] Homocysteine has been found to be neurotoxic due to its injurious effect on mitochondria.[5 6] Argon, a noble gas, is neuroprotective and has the potential to be used during labour as protection against hypoxia/ischaemia-induced brain injury.[7] The aim of this study was to determine the effects of these gases, in *in vitro* neuronal cultures.

Primary rat cortical neurones (n=10) from E18 rats were cultured. For 'pretreated' groups only, vitamin B_{12} (10^{-5} M) was added to the medium on plating. Cultures were exposed to N_2O or argon (each at 70%, 20% O_2, and 5% CO_2) for 2, 4, and 6 h. Additionally, homocysteine (250 μM) was added for 4 h as positive control. Cleaved caspase-3 (apoptotic marker), cytochrome *c* (mitochondria injurious marker), and Bcl-2 expression (cell survival marker) were measured with *in situ* immunofluorescence staining.

All markers produced their highest levels of expression after 4 h gas exposure. N_2O exposure for 4 h increased caspase-3 ($P < 0.001$), cytochrome *c* ($P < 0.001$), and Bcl-2 expression ($P < 0.001$). Argon exposure for 4 h increased Bcl-2 expression ($P < 0.001$). Vitamin B_{12} pretreatment reduced expression of caspase-3 ($P < 0.001$), and cytochrome *c* ($P < 0.001$) when exposed to N_2O for 4 h.

N_2O has neurotoxic effects via activation of the intrinsic apoptotic pathway, which can be abolished by vitamin B_{12} pretreatment. Unlike N_2O, argon has no such effect but promotes neuronal survival signal. The implications of our work could be that argon, when combined with N_2O, may prevent hypoxic–ischaemic brain injury in the newborn at delivery and may also eliminate the side-effects of N_2O.

Acknowledgement

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Non-invasive measurement of cerebrovascular response to hypercapnia in the brainstem using arterial spin labelling

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The cerebrovascular response (CVR) to hypercapnia is defined as the percentage change in cerebral blood flow (CBF) per unit change in carbon dioxide. It is a potentially clinically useful tool but relies on being able to accurately measure regional CBF. Until recently, accurate measurement of brainstem CBF using fMRI has proven difficult due to breathing and cardiac-related noise, MRI signal loss, and a high arterial blood volume that confounds measurements. Recent advances in arterial spin labelling (ASL) techniques have provided us with accurate, non-invasive measurements of regional CBF in areas of the brain other than the brainstem. A pilot study published by our group showed that ASL was a suitable technique for studying brainstem response to hypercapnic challenges. Here we develop our previous work and present a study of human brainstem CVR to hypercapnia using pulsed arterial spin labelling.

After approval from the local research ethics committee, 10 participants were recruited (seven males, three females). Each participant underwent a scan with periods of normocapnia and hypercapnia. Hypercapnia was defined as an increase in end-tidal carbon dioxide of 8 mm Hg above baseline and was achieved by mixing of medical air and 5% carbon dioxide using a semi-closed anaesthetic breathing circuit. Multi-inversion time-pulsed ASL was carried out during both tasks. The resulting perfusion maps enabled calculation of CBF and therefore CVR to hypercapnia in cortical grey matter and brainstem.

Results are summarized in Table 2.

Multi-inversion time-pulsed ASL has resulted in values for grey matter CVR to hypercapnia that are similar to those quoted in perfusion literature. The values calculated for

Table 2 Mean values (standard deviation) for brainstem and grey matter CBF at normocapnia and hypercapnia and the CVR to hypercapnia

Region of interest	Normocapnia	Hypercapnia	CVR to hypercapnia (% increase mm Hg increase CO_2^{-1})
Brainstem (ml 100 g^{-1} min^{-1})	43.8 (11.5)	55.7 (15.9)	3.8 (5.7)
Grey matter (ml 100 g^{-1} min^{-1})	52.6 (10.4)	67.3 (12.8)	3.5 (1.9)

brainstem CVR to hypercapnia are comparable with those obtained by positron emission tomography. This is the first time brainstem CVR to hypercapnia has been measured using ASL. Quantification of a person's CVR to hypercapnia could provide optimization of CBF on an individual basis, which has potential clinical uses in patients who have suffered cerebrovascular accidents, acute brain injury, and the perioperative management of neurosurgical tumours.

Correlation between end-tidal and arterial carbon dioxide measurements in neurosurgical patients

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End-tidal P_{CO_2} ($P_{E'CO_2}$) is commonly used as a surrogate for arterial P_{CO_2} (Pa_{CO_2}). This clinical usefulness is dependent upon the correlation between Pa_{CO_2} and $P_{E'CO_2}$, which has been shown to be poor in the pre-hospital setting[1] and in critically ill polytrauma patients.[2] The correlation between Pa_{CO_2} and $P_{E'CO_2}$ in neurosurgical patients has not previously been examined.

Over a 6 month period (February to August 2013), a retrospective analysis of two cohorts of neurosurgical patients [level 3 patients on intensive care unit (ICU) with traumatic brain injury (TBI) and elective neurosurgical patients] was undertaken. Contemporaneous Pa_{CO_2} and $P_{E'CO_2}$ measurements were collected from electronic records (Metavision, iMDSoft, Needham, MA, USA) and anaesthetic records. Data were analysed using regression coefficients and the Bland–Altman plots.

Twenty-two patients were identified; 10 had undergone elective neurosurgery [40% male, mean age (SD) 52 (16) yr] and 12 had suffered TBI and had been ventilated on ICU [92% male, mean age (SD) 43 (16) yr]. In total, 600 paired Pa_{CO_2} and $P_{E'CO_2}$ measurements were analysed (39 in the surgical group and 561 in the ICU group).

The correlation between Pa_{CO_2} and $P_{E'CO_2}$ for the whole study population was highly variable (Fig. 7).

The mean (SD) $Pa_{CO_2} - P_{E'CO_2}$ difference was 0.75 (0.41) kPa [95% confidence interval (CI) −0.05 to 1.55] for the surgical patients and 0.65 (0.63) kPa (95% CI −0.59 to 1.89) for the ICU patients. Correlation between Pa_{CO_2} and $P_{E'CO_2}$ was moderate for the surgical patients ($R^2 = 0.50$) and poor for the ICU patients ($R^2 = 0.27$).

In neurosurgical patients, correlation between Pa_{CO_2} and $P_{E'CO_2}$ is generally poor and varies significantly. $P_{E'CO_2}$ levels should not be relied upon to maintain Pa_{CO_2} levels within a narrow therapeutic range, and regular checking of Pa_{CO_2} values is recommended for neurosurgical patients.

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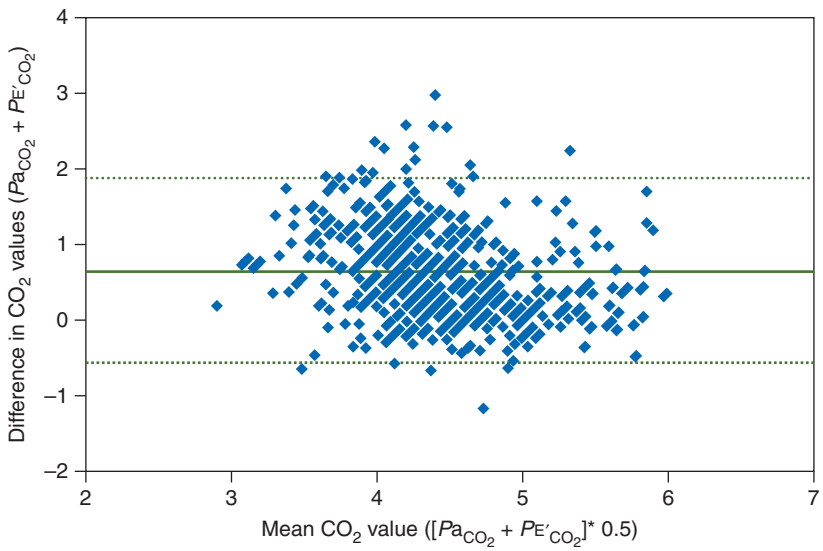

Fig 7 Bland-Altman plot showing end-tidal P_{CO_2} ($P_{E'CO_2}$) and arterial P_{CO_2} (Pa_{CO_2}). Bias is represented by the solid lines and the limits of agreement by dashed lines.

Variability of cerebral oximetry 'baseline' in patients undergoing cardiac surgery

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Cerebral near-infrared spectroscopy (NIRS) is increasingly being used as a perioperative monitor to guide anaesthesia and clinical perfusion and is most often based on NIRS thresholds that predict cerebral hypoxia. While some choose an absolute threshold of regional cerebral oxygen saturation ($rScO_2$), more choose a threshold based on a percentage below what is usually termed 'baseline'. This term is subject to two main sources of error: first, the timing of the 'baseline' varies. It may be the period before induction of anaesthesia or immediately before the insult (carotid clamping, cardiopulmonary bypass). Secondly, 'baseline' readings may be affected by several interventions. The F_{IO_2} (0.21–1.0) varies between publications. This increases the $rScO_2$ by up to 8%[12] due to an increase in dissolved oxygen in the blood.[3] $rScO_2$ is also influenced by the cardiac variables left ventricular (LV) function and dilatation. The purpose of this preliminary investigation was to define the variability in $rScO_2$ at different time points around a standard anaesthetic induction in a group of patients with different cardiac function.

Data from 29 patients [22 men, 70 (9.6) yr] were included. $rScO_2$ was monitored in both frontal lobes using a NIRO 200NX (Hamamatsu Photonics, UK). Readings were made at 'baseline' on room air, during 3 min of pre-oxygenation (F_{IO_2} 0.8), for 6 min after induction of anaesthesia, and for 12 min of 'steady state' anaesthesia (F_{IO_2} 0.3–0.5 with 1% isoflurane). Sample rate was 1 Hz (average from left and right hemispheres) of tissue oxygenation index (TOI), and the tissue haemoglobin index (THI), an indexed measure of cerebral blood volume. Preoperative LV function was classed as good (ejection fraction $>$50%) or moderate/poor ($<$ 50%). Data were downloaded into an excel spreadsheet and analysed using independent samples *t*-test.

Seventeen patients with a good LV had baseline TOI 68.6% (5.4), 12 with a moderate/poor LV had baseline TOI 62.4 (6.5) ($P < 0.01$). These increased by similar amounts during preoxygenation and induction and intubation. At steady state, TOI was 1.0 and 4.4 (NS) higher than baseline. The increase in TOI was most closely associated with a significant increase in THI (cerebral blood flow and dilatation) in the patients with moderate/poor LV compared with those with a good LV ($P < 0.001$).

These preliminary data reinforce the view that the concept of 'baseline' $rScO_2$ must be more clearly defined and that variables such as LV function must form part of the analysis when defining thresholds for intervention.

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Cerebral near-infrared spectroscopy during trans-catheter aortic valve implantation: an observational study

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Near-infrared spectroscopy (NIRS) is a non-invasive tool for assessing cerebral oxygenation. Studies in cardiac surgery demonstrate that intraoperative cerebral desaturation leads to a greater incidence of early postoperative cognitive decline and prolonged hospital stay.[1] During trans-catheter aortic valve implantation (TAVI), the hypoperfusion during rapid ventricular pacing (RVP) may contribute to postoperative outcomes. This observational study examines cerebral oxygenation during TAVI to assess changes in cerebral oxygenation associated with RVP.

Thirty-six patients [80.4 (5.6) yr, 18 male] undergoing TAVI with complete perioperative NIRS data were enrolled. Bi-frontal recordings of tissue oxygenation index (TOI) were made using a NIRO 200NX (Hamamatsu Photonics, UK) with a sampling rate of 0.5–1 Hz until 20 min after aortic valve (AV) deployment. Results were analysed in Microsoft Excel and R. Baseline TOI was calculated as the median TOI for 3 and 20 min of steady-state anaesthesia before RVP. The median TOI was calculated for each minute after commencing RVP. Significant deviations from baseline (10% decrease) were calculated and reported as both seconds spent 10% below baseline and as the area under the curve (AUC) of the saturation-time plot (% s). Comparisons between variables were made using the Wilcoxon signed-rank test.

There was no statistical difference in baseline TOI averaged over 3 *vs* 20 min of steady-state anaesthesia ($P = 0.800$). The median TOI for the left and right hemispheres were 65.4% (range 37.7–81.3%) and 61.7% (49.2–82.8%), respectively. The median TOI decreased by an absolute 0.86% for the minute after RVP commenced in the left [95% confidence interval (CI) 0.35–1.40%, $P = 0.002$] and 0.55% in the right hemisphere (CI 0.10–1.00%, $P = 0.035$). At 2 min post-RVP, the median TOI were not statistically different to baseline. After RVP, the average time per minute spent below 10% of baseline increased by 7.4 s (CI = 4.9–13.1 s, $P < 0.001$) in the left and 9.3 s (CI = 6.1–13.9 s, $P < 0.001$) in the right hemisphere. The AUC increased by 24.5% (CI = 11.4–39.8%, $P < 0.001$) and 22.8% (CI = 14.6–33.1%, $P < 0.001$), respectively. The median TOI showed an absolute increase from baseline of 2.35% in the left hemisphere (CI = 1.2–3.3%, $P = 0.001$) and 2.05% in the right (CI = 1.0–3.2%, $P = 0.001$) at 5 min post AV deployment.

Three minutes of steady-state anaesthesia were adequate to determine baseline TOI. The small decrease in the average TOI that occurs after RVP is of dubious clinical significance. However, shorter periods of marked desaturation may be important. Periods spent below 10% of baseline saturations were shown to increase in the period after RVP until AV deployment. Larger studies are needed with patient follow-up to determine whether these periods are of clinical significance in the TAVI cohort.

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In vitro hyporesponsiveness of CD4+ and CD8+ T cells in septic patients with faecal peritonitis

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Sepsis is associated with immune hyporesponsiveness. This study quantified the population differences of peripheral blood mononuclear cells (PBMCs) between septic patients with faecal peritonitis, age- and gender-matched surgical patients (without sepsis), and age- and gender-matched healthy participants. PBMCs were stimulated with anti-CD3 and anti-CD3+anti-CD28, in an *in vitro* model. After 4 days incubation, T and B lymphocyte proliferation was measured by CFSE (Carboxy-Fluorescein Succinimidyl Ester) dilution assay. Cell activation was determined by expression of surface markers CD25 and CD69 on T lymphocytes and CD86 on B lymphocytes. Cell viability was determined using UV live–dead staining and an apoptosis assay.

Initial analysis of PBMC populations, on the day of blood sampling, showed reduced percentages of $CD4^+$ T lymphocytes ($P = 0.0061$) and increased percentages of $CD19^+$ B lymphocytes ($P = 0.0063$) in septic patients compared with both surgical patients and healthy participants. *In vitro*, after 4 days incubation, with and without stimuli, the percentage of $CD19^+$ B cells was higher in septic patients compared with both surgical and healthy controls. Reduced proliferation of $CD4^+$ and $CD8^+$ T lymphocytes from septic patients was observed when incubated with anti-CD3+anti-CD28 ($P = 0.0314$), but not with anti-CD3 alone (Fig. 8). Both $CD4^+$ and $CD8^+$ T lymphocytes from septic patients were less activated when exposed to anti-CD3+anti-CD28 stimulation ($P = 0.0328$). No difference was found in levels of CD69. No differences in PBMC viability were found on the day of blood sampling. However, in our *in vitro* model, across all conditions, septic patients had lower proportions of live PBMCs compared with healthy participants at day 4.

In summary, we demonstrated a reduction in B and T lymphocyte populations in blood from septic patients, but not surgical patients. We also demonstrated an *in vitro* functional impairment in response to T lymphocyte stimuli together with reduced cell viability. Surgical patients demonstrated a trend towards reduced T and B lymphocyte numbers. To our knowledge, this is the first attempt to perform an immune functional assay across these three groups simultaneously.

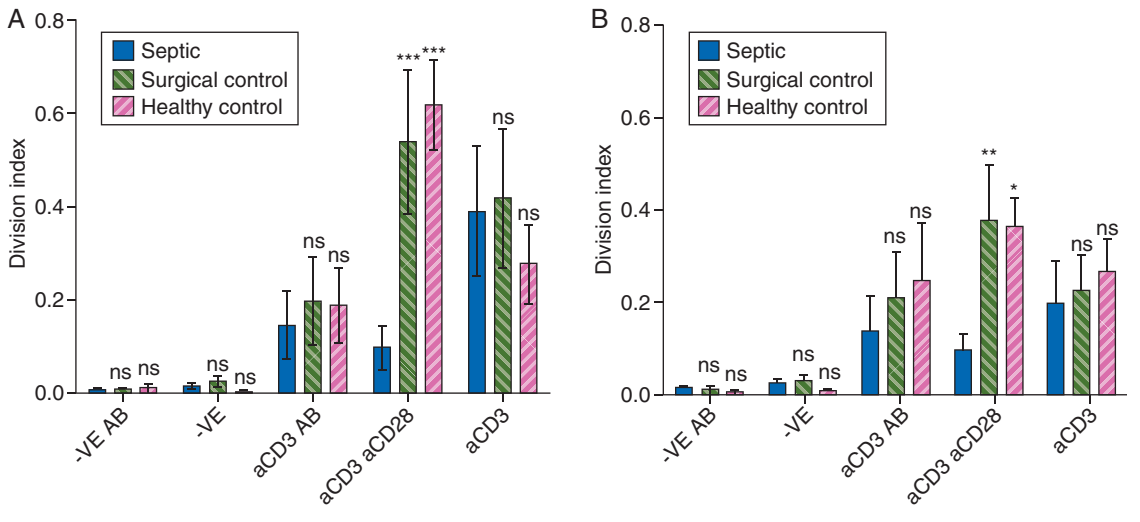

Fig 8 Demonstrating reduced CD4+ (A) and CD8+ (B) T Q5 lymphocyte proliferation in response to the stimuli anti-CD3+- anti-CD28.

Role of the PGC1α pathway in mitochondrial function under conditions of sepsis

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Mitochondria are the major physiological producer of reactive oxygen species (ROS). During sepsis, mitochondrial ROS production exceeds antioxidant defences, leading to a state of oxidative stress which fuels inflammation and causes direct mitochondrial damage. The resulting mitochondrial dysfunction and subsequent bioenergetic failure is suggested to play a central role in sepsis-induced organ dysfunction. Dampening of inflammatory responses, restoration of endogenous antioxidant levels, and maintaining or restoring mitochondrial energetic function is likely to be beneficial in sepsis. The peroxisome proliferator-activated receptor gamma (PPARγ) coactivator 1-alpha (PGC1α) pathway is a potential target due to its effects on antioxidant expression, inflammation, and mitochondrial biogenesis (generation of new mitochondria).

Human endothelial cells were treated with bis-(2-hydroxybenzylidene) acetone (2HBA), which activates downstream transcription factor targets of PGC1α, or hexadecyl-azelaoyl phosphatidylcholine (Azelaoyl PAF), a PPARγ activator, at several concentrations, with and without lipopolysaccharide (LPS) plus peptidoglycan G (PepG) to mimic sepsis. Mitochondrial function was measured in intact cells as membrane potential, metabolic activity and mitochondrial volume, and total glutathione as an index of oxidative stress. Interleukin-6 (IL-6) was measured in culture medium to determine effects on inflammation. Measures of mitochondrial function were lower in cells treated with LPS/PepG than control cells, while IL-6 concentrations were higher (Fig. 9, $P < 0.001$). Under conditions of sepsis, 2HBA protected against mitochondrial dysfunction, seen as maintenance of membrane potential and metabolic activity, lower IL-6 and increased mitochondrial volume and glutathione levels (all $P < 0.05$). Likewise, Azelaoyl PAF maintained membrane potential, maintained metabolic activity, and increased glutathione levels (all $P < 0.05$).

These data show that promoting activation of the PGC1α pathway protects against mitochondrial dysfunction and inflammation in cells under conditions mimicking those seen in sepsis and may be a novel future therapeutic target.

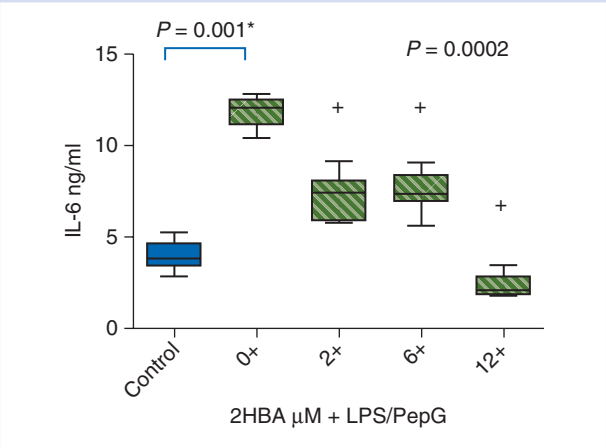

Fig 9 IL-6 concentrations in culture media from cells exposed to 2 μg ml^{-1} LPS and 20 μg ml^{-1} PepG for 24 h and treated with 2HBA. Data are shown as median, interquartile and full range (n=6). P value shown is Kruskal Wallis. + = significantly lower than cells without 2HBA ($P<0.05$, Mann–Whitney U-test).

Mitochondrial sirtuin activation in endothelial cells under conditions of sepsis

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Sepsis remains a significant cause of mortality in the population due to lack of effective treatment options. Current evidence suggests that mitochondrial dysfunction is central to the development of multiorgan dysfunction syndrome, the primary cause of death in sepsis. The protein peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) regulates mitochondrial biogenesis, metabolism, and antioxidant production, three key processes which become dysregulated in sepsis. Regulation of PGC-1α is achieved through a number of post-translational modifications which include deacetylation. Deacetylation occurs through the action of the enzyme sirtuin 1 (SIRT1) which increases the activity of PGC-1α. Chemical activators such as SIRT1 activator 3 can also activate SIRT1. This project aimed to investigate whether SIRT1 activation had a protective effect on mitochondria under conditions mimicking sepsis.

Human endothelial cells were cultured and treated with 0–8 μM SIRT1 activator 3 with and without 2 μg ml^{-1} lipopolysaccharide (LPS) and 20 μg ml^{-1} peptidoglycan (PepG) to mimic sepsis. Mitochondrial metabolic activity, membrane potential, volume, antioxidant expression, and interleukin-6 (IL-6) production was measured after 24 h or 7 days.

Metabolic activity after 7 days was significantly lower in cells treated with LPS/PepG compared with untreated controls ($P = 0.004$, Fig. 10A). Treatment of LPS/PepG-exposed cells with SIRT1 activator 3 protected against decreased metabolic activity (Fig. 10A). IL-6 concentrations in culture medium were higher in cells treated with LPS/PepG for 24 h compared with untreated cells ($P = 0.002$) and SIRT1 activator 3 dampened this increase (Fig. 10B). SIRT1 activator 3 also protected against changes in mitochondrial membrane potential and volume, and total glutathione in cells treated with LPS plus PepG.

These results suggest that SIRT1 activation prevents against mitochondrial dysfunction in cells under conditions of sepsis. Further research will fully elucidate the exact mechanisms involved and may suggest novel therapeutic intervention strategies in the future.

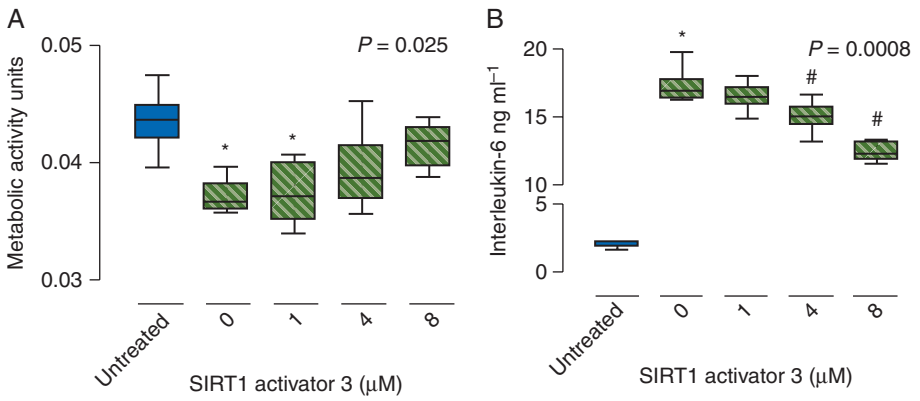

Fig 10 (A) Mitochondrial metabolic activity after 7 days and (B) IL-6 levels after 24 h in culture medium in cells treated with LPS plus PepG with and without SIRT1-activator 3. Box and whisker plots show median, IQR, and full range (n=6). P-values are Kruskal–Wallis. *Significantly different from untreated cells ($P<0.05$). #Significantly different from cells treated with LPS/PepG without SIRT1-activator 3 ($P<0.05$). (Mann–Whitney U-test).

Investigation of indole-derivative melatonin-like compounds as novel treatments for sepsis

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Sepsis is a significant cause of morbidity and mortality worldwide. The multiple organ dysfunction syndrome (MODS) is a common complication of sepsis. Mitochondrial dysfunction associated with oxidative stress has been implicated as the key process in the pathogenesis of MODS. Melatonin is an endogenous indole hormone with major effects on sleeping patterns, but it is also a potent antioxidant and anti-inflammatory agent and may be of benefit in the treatment of MODS. Indole precursors to melatonin may possess similar properties and may have the added benefit of not affecting sleeping patterns. This project investigated the potential antioxidant effects of the indoles 5-hydroxytryptophan (5-HTP) and *N*-acetylserotonin (NAS) in endothelial cells under conditions mimicking sepsis.

Human umbilical vein endothelial cells were cultured and treated with 0–100 μM 5-HTP or NAS in the presence and absence of 2 μg ml^{-1} lipopolysaccharide (LPS) plus 20 μg ml^{-1} peptidoglycan (PepG) to mimic a mixed polymicrobial sepsis. Cell viability, mitochondrial volume, inner mitochondrial membrane potential and metabolic activity were measured after 24 h and 7 d culture in six replicate independent experiments.

Neither of the compounds studied had any detrimental effect on cell viability at the concentrations investigated. Treatment of cells with LPS/PepG for 7 d resulted in a decrease in mitochondrial inner membrane potential ($P < 0.05$) which was also decreased in the presence of 5-HTP ($P < 0.05$), such that at 100 μM, 5-HTP membrane potential was significantly lower than in cells treated with LPS/PepG alone ($P < 0.05$). Mitochondrial volume and metabolic activity also decreased in cells treated with LPS/PepG for 7 d ($P < 0.05$). Concurrent treatment of cells with 5-HTP blunted the decrease in mitochondrial volume ($P = 0.036$, Fig. 11) and at 100 μM, 5-HTP, mitochondrial volume was higher than in cells exposed to LPS/PepG alone ($P < 0.05$, Fig. 11). Neither 5-HTP nor NAS had any effect on metabolic activity and NAS also did not alter mitochondrial volume or membrane potential in cells exposed to LPS/PepG.

This study showed that there were varying effects of NAS and 5-HTP on measures of mitochondrial function, but not all these effects were beneficial.

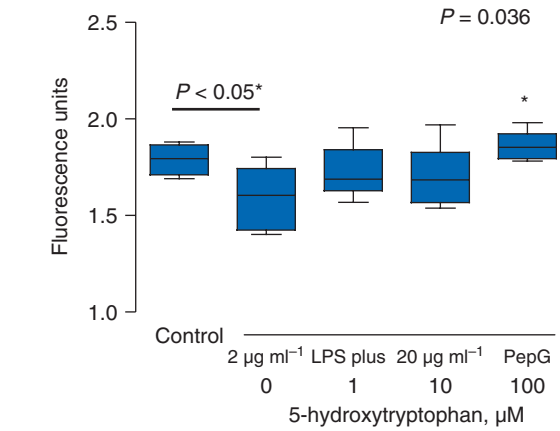

Fig 11 Mitochondrial volume in endothelial cells after 7 days treatment with LPS and PepG plus with 5-hydroxytryptophan (5-HTP). Control is cells without LPS/PepG or 5-HTP. Box and whisker plots show median, interquartile and full range (n=6). P-value shown is Kruskal–Wallis across LPS/PepG groups. *=$P<0.05$ in comparison to cells without 5-HTP.

Towards an efficient biosensor for the detection of lipopolysaccharide in sepsis using molecularly imprinted polymers

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The true potential of circulating biomarker detection and surveillance in patients with sepsis is yet to be elucidated. The focus of this project is lipopolysaccharide (LPS), a major constituent of gram-negative bacteria outer cell walls, and its use as a sepsis marker. The overarching hypothesis is the detection of circulating LPS will facilitate the early diagnosis of sepsis.

Molecular imprinting describes the generation of synthetic, polymeric receptors through the polymerization of monomers around a template molecule (Fig. 12). The resultant polymers possess recognition properties akin to an antibody with the ability to recognize the original target molecule and related species. Efficient molecularly imprinted polymers rely on strong interactions between the target, in this case, LPS, and the functional monomers that form the polymer matrix. Polymyxin B (PMB), a peptide antibiotic, has high affinity for LPS and is utilized in this study as a functional monomer. LPS is not a suitable template for conventional imprinting techniques; therefore, techniques that circumvent problems associated with the imprinting of biological macromolecules are needed and modified approaches that localize LPS at a surface via chemical immobilization at a solid–solvent interface (surface imprinting on a solid support) or via self-assembly at a solvent–solvent interface (suspension polymerization/microfluidic techniques) have been investigated.

Microfluidics (MF) describes the science and technology that manipulates very small (10–9 to 10–18 litre) volumes of fluid. A system that generates segmented flow within polytetrafluoroethylene (PTFE) tubing has been constructed, providing small pockets of an aqueous monomer solution travelling in an organic solvent continuous phase. Using a bespoke cavity resonator, the *in situ* polymerization of the 'beads' of aqueous monomer solution in this MF system has been achieved using microwaves. Furthermore, the process of polymerization can be monitored by sensing the changes in resonant frequency imposed by the changing monomer solution as it polymerizes. Continued troubleshooting of suspension polymerization and microfluidic processes forms the foundation of ongoing work as the successful imprinting of LPS in polymer beads with integrated PMB becomes a possibility.

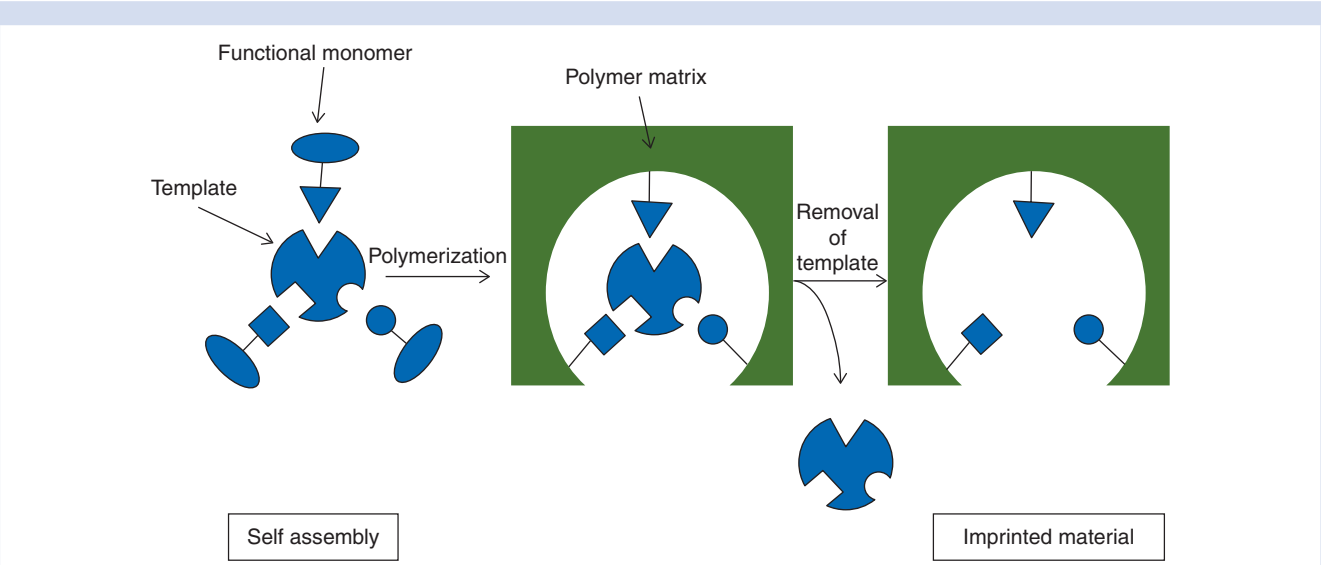

Fig 12 Schematic representation of traditional molecular imprinting method. A solution phase complex of the template molecule (LPS) and functional monomers (polymerizable PMB and acrylamide) forms, which is 'locked' in place during polymerization. Following removal of the template species, an imprint of the template within the polymer matrix remains; this imprinted material demonstrates a natural affinity for the template molecule.