ICS medal winners and research abstract presentations

Research Gold Medal Presentations winner

Simvastin reduces inflammation and improves clinical outcomes in ALI: results of the HARP study

T Craig, Royal Victoria Hospital, Belfast, Northern Ireland

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) account for 19% of ventilated patients in Irish ICUs[1] in keeping with international data. There is no effective pharmacological treatment. There is increasing evidence that statins may be useful in sepsis.[2] In addition, statins may also be a potential therapy in ALI as they modify many of the important underlying processes.[3] In an observational epidemiological study, ALI patients pre-treated with statins had a 73% lower relative risk of death (OR 0.27, 95% CI 0.06-1.21 p=0.09). We have shown that statins lower bronchoalveolar lavage (BAL) neutrophils in a healthy volunteer lipopolysaccharide model of ALI, in part by increasing neutrophil apoptosis. Statins also reduce tumour necrosis factor-α (TNF-α), matrixmetalloproteases -7,-8 and -9 in BAL fluid and lower plasma CRP.[4]

Extravascular lung water (EVLW) as a predictor of mortality

ALI/ARDS, characterised by non-cardiogenic pulmonary oedema can be assessed by measurement of EVLW. Traditionally EVLW has been indexed to actual body weight (EVLWa). As lung size is dependent on height rather than weight, we hypothesised indexing to predicted body weight (EVLWp) may be a better predictor of mortality in all aetiologies of ALI/ARDS.

We recorded demographics, severity of illness scores and EVLWa/EVLWp by thermodilution (Pulse Contour Continuous Cardiac Output system, PiCCO, Pulsion Medical, Münich, Germany). Using single regressor logistic regression, six variables were significantly related to mortality: APACHE II, PaO_2, PaO_2/FiO_2 ratio, oxygenation index (OI), EVLWa and EVLWp. In multiple logistic regression analysis, EVLWp but not EVLWa was a predictor of mortality with an OR of 4.3 (95% CI 1.5-12.9) per SD. A baseline EVLWp value of 16 mL/kg predicted ICU mortality with a sensitivity of 0.75 (CI 0.47-0.91) and specificity of 0.78 (CI 0.61-0.89). This study[5] showed that early measurement of EVLWp is a better predictor than EVLWa to identify patients at risk for death in ALI/ARDS and may be a useful surrogate endpoint in clinical trials in ALI.

Results of the HARP study – a randomised double blind phase II trial of simvastatin in acute lung injury

Patients admitted to the regional intensive care unit, Belfast were screened for ALI according to consensus guidelines. Demographic information, severity of illness scores including APACHE II, SAPS II, lung injury scores (LIS) and GOCA scores were recorded.

Patients were randomised to 80 mg simvastatin or placebo for up to 14 days. EVLW, PaO_2:FiO_2 ratio, OI, plateau pressure and sequential organ failure assessment (SOFA) score were recorded daily. Pulmonary inflammation was assessed by BAL cytokines and systemic inflammation by plasma CRP and cytokines. Safety data including CK, transaminases, need for renal replacement therapy (RRT), adverse events and outcome data was collected. Data were analysed by unpaired t tests or Mann Whitney U tests. A χ_2 or Fischer's exact test was used to compare proportions.

Sixty patients were recruited. At day 7 there was no difference in EVLW or other clinical outcomes. However by day 14, the simvastatin treated group had improvements in oxygenation, respiratory mechanics and overall non-pulmonary organ dysfunction (**Figure 1**) with improvement in the cardiovascular (p=0.0001), renal (0.003), and coagulation (p=0.04) SOFA components. At day 14, none of the simvastatin group required vasopressors or inotropes (0% *vs* 33%; p=0.05). There was a trend to a reduction in RRT in the statin group at day 14 (p=0.09). ICU mortality was 30% in both groups. Simvastatin was well tolerated with no increase in adverse events.

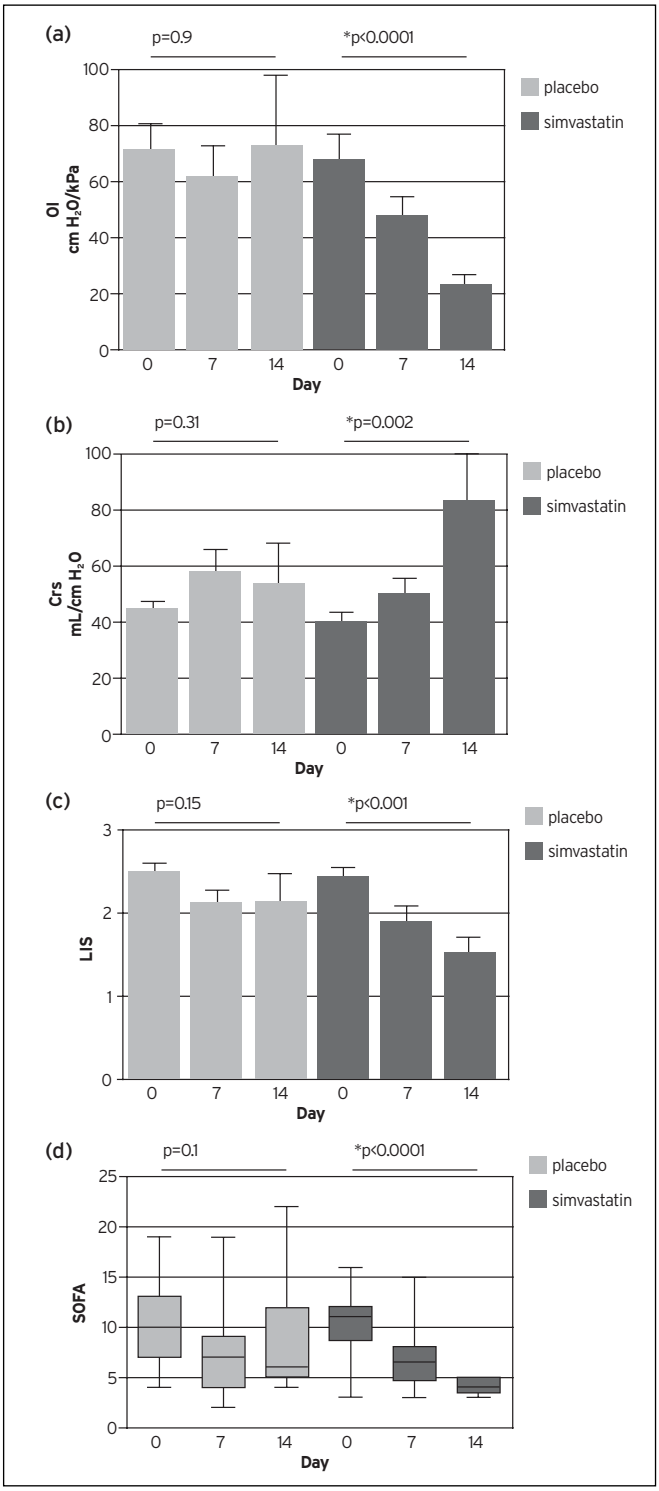

Figure 1. Treatment with simvastatin 80mg improved pulmonary dysfunction, as measured by oxygenation index (OI) (a), respiratory system compliance (Crs) (b) and lung injury score (LIS) (c) and non-pulmonary organ dysfunction, as measured by the Sequential Organ Failure Assessment (SOFA) score (d) at day 14 compared to day 0. Data are mean (SD) or median (IQR) as appropriate.

By day 3 simvastatin decreased BAL IL-8 by 2.5 fold (p=0.04), and IL-6 by 2.9 fold although this was not significant (p=0.07). Plasma CRP decreased in both groups at day 7 but was significantly reduced by day 14 in the statin group only (206.4 ±81.2 *vs* 77.6 ±72; p=0.0003). Simvastatin had no effect on plasma cytokines.

Translational and ongoing work

Investigations are currently ongoing to attempt to elucidate the potential mechanisms of statins in ALI. Based on data that MMP 9 may have a beneficial effect on epithelial repair,[6] we measured NGAL (neutrophil gelatinase associated lipocalin), a complex of lipocalin and MMP-9 in BAL fluid and plasma in ALI. Simvastatin had no effect on NGAL levels. Patients whose BAL NGAL increased over the first 72 hours had significantly lower EVLWp at day 3 than those whose BAL NGAL fell (12.7+/-1.4 *vs* 18.9+/-2.6 mL/kg, p<0.05, n=24). All patients who increased BAL NGAL concentration over the 72 hour period survived. Day 3 BAL NGAL was >3 fold higher in ICU survivors compared with non-survivors. In contrast, ICU survivors had a non-statistically significant decrease in plasma NGAL at day 3 compared with non-survivors (p=0.13). This suggests that an increase in NGAL in the pulmonary but not systemic compartment is associated with improved survival and clearance of EVLW.

We have found for the first time that simvastatin 80mg once daily is safe and is associated with improvement in pulmonary and non-pulmonary organ dysfunction in ALI. The reduction in inflammatory mediators indicates a potential mechanism for these effects. Phase III trials are warranted to further assess the impact of statins in ALI.

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Research Gold Medal Presentations runners-up

Endothelial dysfunction in critical illness

M Duffy, Ulster Hospital, Belfast, Northern Ireland

The endothelium has a key role in the maintenance of vascular homeostasis.[1] It actively regulates vascular tone, platelet aggregation, coagulation, fibrinolysis and leucocyte activation.[2] Endothelial function is impaired in critical illness and may be important in the pathophysiology of multiple organ failure.

In vivo assessment of endothelial function in critical illness is difficult to perform at the bedside. Estimates may be obtained indirectly by measuring plasma levels of endothelium-derived regulatory proteins and microalbuminuria. Microalbuminuria has been shown to be predictive of outcome in a wide variety of acute conditions including trauma, surgery, ischaemia-reperfusion injury, acute pancreatitis and meningitis. In a recent prospective observational study, microalbuminuria measured within 15 minutes of admission to intensive care was as good a predictor of outcome as other validated severity of illness scores such as APACHE II and SAPS II.[3]

Pulse wave analysis (PWA) is a relatively new technique which uses a validated transfer function to derive the central aortic waveform from the peripheral pulse pressure wave.[7] The central aortic pressure wave differs in morphology from the peripheral pressure wave in that there are two systolic peaks. The first systolic peak is due to ventricular systole and the second is due to wave reflection. The difference in amplitude between the first and second peaks, expressed as a percentage of the pulse pressure, is known as the augmentation index (AIx). The AIx falls in response to systemic vasodilatation. PWA combined with provocative pharmacological testing has been validated as a simple, repeatable, non-invasive means of assessing endothelial function in patients with cardiovascular disease. Its use within the critical care setting has never previously been described. Salbutamol is an endothelium-dependent vasodilator. Glyceryl trinitrate (GTN) causes vasodilatation independently of the endothelium. The change in AIx in response to salbutamol and GTN can be used to define endothelium-dependent vasodilatation (EDV) and endothelium-independent vasodilatation (EIDV) respectively. The endothelial function index (EFI) is the ratio of EDV to EIDV. EFI is therefore a surrogate marker of systemic endothelial function.[9]

To explore the role of endothelial function in critical illness two clinical studies were performed. The first, the Function of the Endothelium in Critical Care (FECC) Trial was a prospective observational study in an adult intensive care unit (ICU). Our hypothesis was that the EFI could be used as a predictor of ICU mortality. The other was a prospective double-blind randomised placebo-controlled clinical trial where PWA was utilised to assess the ability of ascorbic acid to protect endothelial function post ischaemia-reperfusion injury.

Open abdominal aortic aneurysm (AAA) repair is associated with the systemic inflammatory response syndrome (SIRS), mediated in part by an increase in oxidative stress. Ischaemia-reperfusion injury induced by aortic clamping is associated with both an increase in oxidative stress and a decrease in antioxidant activity. Pro-inflammatory cytokines and markers of endothelial damage are increased by this insult.[10] Our hypothesis was that the intraoperative administration of intravenous ascorbic acid, an powerful water soluble antioxidant, may attenuate endothelial injury and this was investigated in the Ascorbic Acid in AAA (AAAAA) trial.

Methods

FECC

Adults admitted to the regional intensive care unit (RICU) in the Royal Victoria Hospital, Belfast were considered for inclusion. All consecutive patients were approached. PWA was performed within 24 hours of admission. Baseline demographic and physiological indices were recorded. Severity of illness scores (APACHE II, SAPS II, SOFA) were calculated. ACR was measured as were serum adhesion molecules. Follow-up was to hospital discharge.

AAAAA

Consecutive patients admitted to the regional vascular unit in the Royal Victoria Hospital, Belfast for elective open AAA repair were considered for recruitment. Ascorbic acid 2 g or placebo was given intravenously prior to application of the aortic clamp. Demographic and physiological indices were noted. Clamp time was recorded. Blood and urine samples were taken prior to and 4 hours after aortic clamping. Concomitantly, bedside analysis of endothelial function was performed using PWA. Serum adhesion molecules, plasma inflammatory cytokines and urinary ACR were measured. Exhaled breath condensate (EBC) was collected before and after aortic clamping and pH measured as a marker of pulmonary inflammation. The PaO_2:FiO_2 ratio (P:F Ratio) was calculated as a measure of pulmonary function. Data are presented as median (inter-quartile range).

Results

FECC

Ninety-nine participants were studied, there were 83 survivors and 16 non-survivors. No differences were found in demographics between survivors and non-survivors. The severity of illness scores, ACR and leucocyte count were significantly higher in non-survivors. A multiple logistic regression demonstrated that the EFI was the most accurate predictor of ICU mortality, and had the highest area under the ROC curve (**Figure 1**).

Predictive test	ROC AUC	95% CI	p-value
EFI	0.990	0-1	<0.0001
APACHE II	0.670	0.514-0.826	0.032
SAPS II	0.722	0.595-0.849	0.005
SOFA	0.707	0.560-0.853	0.009
Urinary ACR	0.694	0.579-0.809	0.017

Figure 1.

AAAAA

Thirty-one patients were studied. There were no differences in demographics or clamp times between groups. EBC pH fell after aortic clamping in both placebo and ascorbate groups, but with no difference between groups (**Figure 2a**). The P:F Ratio fell by 14.3 (2.0,38.6) kPa in the placebo group (p=0.008) and by 19.9 (3.6, 57.7) kPa in the ascorbate group (p=0.003), there was no significant difference between groups (p=0.48). There were no differences in levels of soluble adhesion molecules between groups. The ACR was significantly increased after aortic clamping with a rise of 9.6 (2.2,29.8) mg.mmol^{-1} in the placebo group (p=0.001) and 4.75 (0.9, 20.0) mg.mmol^{-1} in those who received ascorbic acid (p=0.001). Again there was no significant difference between groups (p=0.1).

EFI was improved after open AAA repair in the ascorbate group, whereas there was no significant change in the placebo group (**Figure 2b**). Regarding inflammatory cytokines, the post-operative level of IL-6 was lower in those who received ascorbic acid (**Figure 2c**).

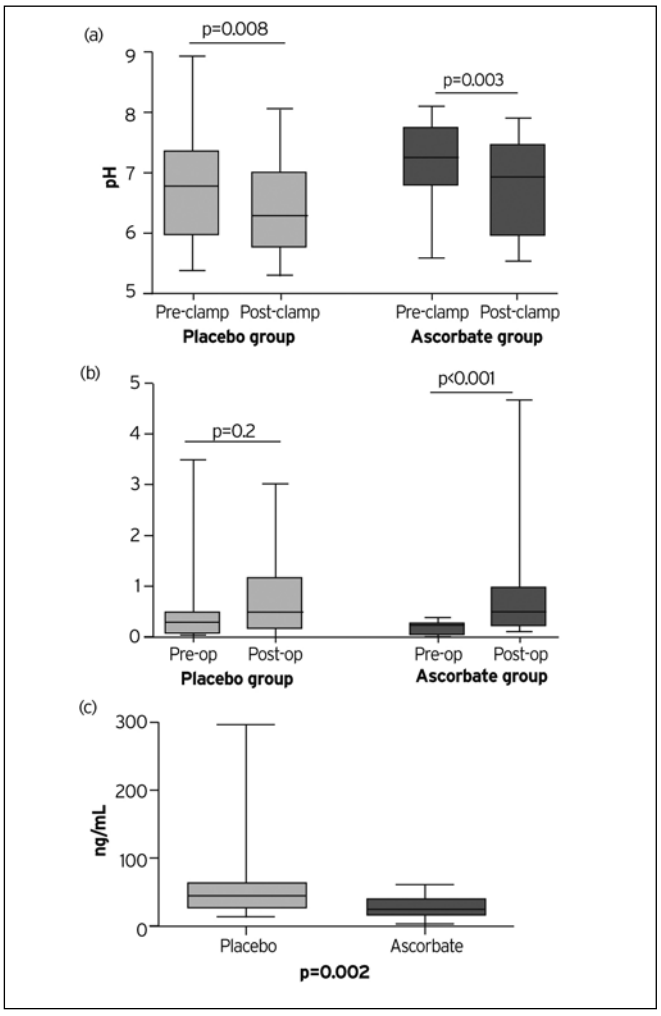

Figure 2. (a) EBC pH falls after aortic clamping. (b) EFI improves following open AAA repair after ascorbic acid administration. (c) IL-6 lower after open AAA repair in those who received ascorbic acid.

Conclusion

The EFI is a good independent predictor of mortality in intensive care. We have shown it to be superior to other validated severity of illness scores including APACHE II and SAPS II. *In vivo* bedside assessment of systemic endothelial function may enable the targeting of new endothelial therapies in critical illness.

Our AAAAA study has suggested that high-dose ascorbic acid may be beneficial to the endothelium during ischaemia-reperfusion injury. This important novel finding warrants further investigation to assess its clinical significance.

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Regional pathophysiology, tissue fate and outcome in traumatic brain injury; insights from diffusion tensor imaging

V Newcombe, University Division of Anaesthesia, Cambridge, UK

Introduction

Conventional imaging techniques are poor at demonstrating traumatic brain injury (TBI), particularly traumatic axonal injury (TAI), and there is limited information regarding its incidence, extent, temporal profile and possible resolution. MRI with diffusion tensor imaging (DTI) characterises the diffusion of water molecules in tissue environments, which is influenced by the microstructural organisation of tissues and their constituent cells, and can provide unique insights into pathophysiology, particularly in white matter (WM). The diffusion tensor can be used to represent the magnitude of water diffusion (quantified as the apparent diffusion coefficient, ADC), whether such diffusion is directionally non-uniform (fractional anisotropy, FA), and the orientation of that direction (eigenvectors/eigenvalues).

Previous DTI studies mainly assessed WM injury in TBI, and showed consistent reductions in fractional anisotropy (FA) in classical areas affected by TAI, even when conventional MRI showed no lesion.[1-7] Yet the evolution of these changes and their correlation with patient outcome is still poorly understood. The major aim of this project was to use diffusion tensor imaging (DTI) to characterise TBI in both the acute and chronic phases, and correlate findings with neurocognitive and functional outcome.

Lesion characterisation and evolution

Six patients (median Glasgow Coma Score (GCS) 6 (range 3 to 13)) with contusions were imaged twice while in intensive care at a median of 28 (range 20 to 44) and 182 (range 68 to 283) hours post injury (Figure 1).[8,9] Within the first 48 hours, all lesions showed three regions; a core, an area of raised ADC around the core, and a thin rim of hypointensity. While some lesions showed these regions more clearly than others, this pattern was universal. Over time, the ADC in the core increased, probably reflecting haematoma evolution. Early ADC reduction in the hypointense rim may represent cytotoxic oedema, perhaps due to critical ischaemia, resulting from microvascular changes.[10] This area evolves into a vasogenic picture over time, and is subsumed by the area of raised ADC. These imaging appearances may characterise the traumatic penumbra, where physiology is acutely deranged, but clinical interventions may enhance tissue survival.[11]

Understanding the microstructural basis of subtle white matter injury

DTI data were analysed in normal appearing whole brain WM (WBWM) in 33 patients with moderate to severe TBI (median GCS 6, range 3 to 11)

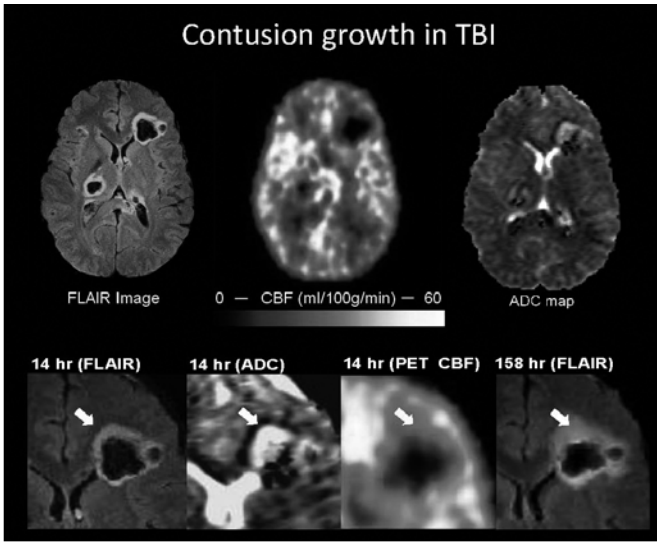

Figure 1. Heterogeneity in the cerebral pathophysiology following head injury.[12] The top panel shows FLAIR, positron emission tomography (PET) regional cerebral blood flow (rCBF), and ADC maps obtained at 14 hours post TBI. The lower panel shows a detail of these images, and a follow up image at 158 hours (6.5 days) post TBI. The shearing injury in the left basal ganglia shows regions of both increased and decreased rCBF. There is a reduction in rCBF around the right frontal contusion, which is peripheral to the pericontusional cuff of vasogenic oedema, and associated with a reduction in ADC, characteristic of cytotoxic oedema. The lower images (FLAIR, ADC map and PET CBF at 14 hours, with FLAIR at 6.5 days) illustrate how the rim of vasogenic oedema extends to include the rim of cytotoxic oedema seen at the early time point.

imaged at a median of 32 hours (range 7 to 132 hours) post injury. FA was decreased and ADC increased compared to controls ($p<0.001$ and $p=0.017$, respectively, Mann-Whitney U).[13,14] These changes resulted predominantly from selective increases in radial diffusivity (rather than decreases in axial diffusivity). In contrast, a decrease in ADC was seen in the grey matter.[15,16] The differences seen in the grey and white matter ROIs emphasise the need to separate these structures when investigating pathological changes. These data suggest that early pathology of TAI may be dominated by reversible cytotoxic oedema, rather than traumatic axotomy.

Longitudinal imaging

Eight patients were imaged sequentially at five time points: within the first week, ~10 days, 4 to 8 weeks, 6 to 12 months, and over 12 months post-injury (**Figure 2**). In the first week FA in WBWM was lower than controls, this decreased progressively with the second acute scan and the subacute scan. FA increased at the 6 month scan and increased further at the >12 month scan, but remained lower than the initial scan (Freidman's ANOVA, $p=0.004$ and <0.001 respectively). Although the initial reductions in FA may arise from WM oedema, most oedema should have resolved at the intermediate time point. Any ongoing FA reductions are likely to reflect inflammation, demyelination, atrophy, and plasticity. Such serial imaging may provide a valuable readout of ongoing tissue injury over time.

Relationship to clinical outcomes

Clinical outcome, defined by the Glasgow Outcome Score, was related to the burden of white matter injury quantified by DTI late after TBI (**Figure 2**).[17,18] This study is the first DTI study to address the entire spectrum of survivors and provides a comprehensive context for DTI data interpretation. Mean FA and eigenvalues, were obtained for supratentorial white matter, the pons, midbrain and corpus callosum, in 64 patients at a median of 11.8 months post TBI, and in 36 healthy volunteers. Decreasing FA was related to poorer outcome (all p-values <0.001; corrected for multiple comparisons [Jonckheere-Terpstra Test for trend]). FA changes were accompanied by an increased radial, and to a lesser extent, axial diffusivity. This analysis showed that clinical outcome correlates with the burden of white matter injury. DTI abnormalities were also seen in the patients with the best outcomes, and patients in whom conventional MR was normal, suggesting that DTI can detect subtle injury missed by other approaches.

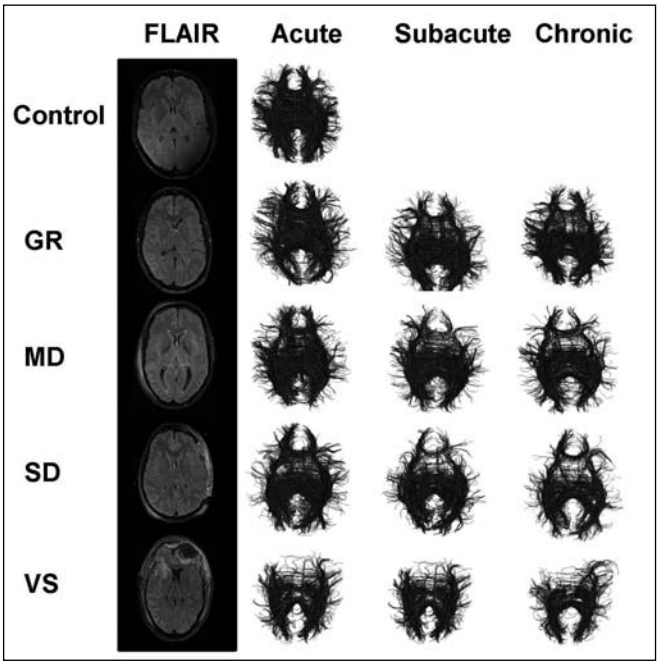

Figure 2. This image shows examples of FLAIR and "global" tractography maps created using DTIQuery[19] with representative examples from a control and GOS categories 2 to 5 to illustrate qualitative changes in white matter integrity over time. The FLAIR and acute scan was within the first week of injury, the subacute at approximately 4 to 8 weeks and the chronic at six months except for the VS patient whose third scan was at four months. It can be clearly seen that the "paths" able to be traced are far less in the patients than the controls despite similar appearing conventional structural imaging. The "paths" also tended to decrease over time. All patients shown did not have any contusions except for the VS patient who had bilateral frontal contusions. These appearances were underpinned by the gradations of change seen in the quantitative measures of DTI parameters. For ease of visualisation, tracts with lengths less than 7.5cm are not shown. GR = good recovery (GOS 5), MD = moderate disability (GOS 4), SD = severe disability (GOS 3) and VS = vegetative state (GOS 2).

Neuroanatomical substrates for late neurocognitive deficits

Neurocognitive dysfunction after traumatic brain injury (TBI) correlates imperfectly with lesion distribution, and may result from subtle insults to integrated neural systems, rather than overt lesions at focal injury sites. The hypothesis that the burden of microstructural injury, as defined by DTI, would correlate with neurocognitive performance was tested in two contexts: a visual memory task (Paired Associates Learning; PAL), involving cholinergic networks; and a decision making task (Cambridge Gamble Task; CGT), subserved by dopaminergic networks.[20] Structural MRI and DTI were undertaken, at least 6 months post-injury, in 42 patients (median admission GCS 6 (range 3 to 15), with traumatic axonal injury and no significant focal lesions. Testing with the Cambridge Neuropsychological Automated Test Battery (CANTAB) was performed on the same day. Multi-component ROIs were manually created for each task, based on prior knowledge of their neuroanatomical correlates. For both tasks, ADC in key areas identified from previous data, correlated significantly with task performance ($p<0.05$). Our data add to the evidence that loss of microstructural integrity, as detected by DTI, is an important determinant of function following TBI, and confirm the involvement of key neurochemical networks in these complex neurocognitive tasks.

Conclusions

The use of modern imaging tools such as DTI is challenging in the context of acute TBI, and clinical and logistic realities meant that we could not

recruit as many subjects that we wished to, or undertake imaging at tightly defined time points post-TBI. Nevertheless, this includes the largest collection of patient data of this detail available in the literature, and the analyses presented here show how DTI may be used to more fully characterise the disease processes post TBI *in vivo*. An improved *in vivo* understanding of the pathology of traumatic brain injury (TBI), including its distribution, extent and temporal profile, may enhance outcome evaluation and help to provide a mechanistic basis for deficits that remain unexplained by other approaches.

Statement of contributions and acknowledgements: The above analyses were performed as part of my PhD supervised by Professor David Menon and Dr Guy Williams and funded by a Gates Cambridge Scholarship and an Overseas Research Studentship. I was involved in the experimental design, data collection, data analysis, construction of new analytic tools, presentation of data and writing of the above manuscript. The data from patients in the vegetative state used in the analysis described in Section 5 were kindly provided by Dr Martin Coleman. Patient transport for scanning for these studies was undertaken by a cohort of Clinical Research Fellows in our group, who used the imaging procedure to collect data for distinct PhD/MD projects.

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Research Free Paper Presentations winner

Ventilator-associated pneumonia is characterised by elevated neutrophil proteases in the lung

A Conway Morris*, K Kefala*, C O'Kane†, DF McAuley†, TS Wilkinson*, K Dhaliwal*, H Reid‡ , TS Walsh§, AJ Simpson*. *MRC Centre for Inflammation Research, University of Edinburgh, Scotland, †Centre for Infection and Immunity, Queen's University, Belfast, Northern Ireland, §Department of Critical Care, Royal Infirmary of Edinburgh, Scotland, ‡Penicuik Medical Practice, Penicuik, Midlothian, Scotland, UK

Ventilator-associated pneumonia (VAP) is a significant problem in intensive care units, leading to increased morbidity, mortality and duration of stay.[1] Excessive release of proteases has been implicated in the pathogenesis in a variety of inflammatory lung diseases. We set out to quantify major proteases (matrix metalloproteinases (MMPs) and the serine protease neutrophil elastase (NE)) in VAP.

Fifty-five patients with clinically suspected VAP (chest X-ray infiltrates, plus two or more of elevated white cells, temperature above 38°C and muco-purulent sputum)[1] underwent standardised bronchoscopy and bronchoalveolar lavage (BAL). VAP was defined as growth of organisms above 10^4 colony forming units/mL. A group of 18 age- and sex-matched volunteers acted as a reference group and also underwent BAL. Samples were assayed for matrix metalloproteases (MMPs) 1,2,3,8 and 9 by Luminex assay. Tissue inhibitor of MMP (TIMP)1 and 2, neutrophil elastase (NE), and elastase inhibitors (α-1 antitrypsin, elafin and secretory leukocyte protease inhibitor (SLPI)) were assayed by sandwich ELISA.

Eleven (20%) patients had confirmed VAP, 44 (80%) fell into the 'non-VAP' group. Levels of the proteases assayed are shown in the **Table**, the neutrophil-derived proteases (MMP 8, 9 and NE) being significantly elevated in patients with VAP compared to those without VAP. Correcting for total neutrophil number did not alter this relationship.

Protease/inhibitor (median and inter-quartile range)	VAP	Non-VAP	P value (Mann-Whitney U test)	Matched volunteer reference group (median and inter-quartile range)
MMP1 (pg/mL)	142.0 (46.7-310.1)	116.0 (35.6-256.8)	0.69	All below limit of detection
MMP2 (ng/mL)	3.9 (3.3-8.6)	4.7 (1.4-14.7)	0.9	0.9 (0.4-19.2)
MMP3 (pg/mL)	408.8 (86.5-866.2)	115.8 (35.46-402.8)	0.07	77.5 (50.7- 80.0)
MMP8 (ng/mL)	184.4 (18.3-513.4)	5.2 (1.2-56.5)	0.002	2.4 (1.2-20.0)
MMP9 (ng/mL)	310.2 (21.8-746.4)	10.5 (0.2-90.0)	0.003	3 (0.94-59.8)
TIMP-1 (ng/mL)	58.0 (20.6-160.0)	29.9 (13.2-97.7)	0.3	4.3 (2.6-40.9)
TIMP-2 (pg/mL)	5.7 (2.4-10.5)	2.9 (1.1-6.0)	0.15	1.4 (0.9-4.9)
Neutrophil elastase (μg/mL)	2.7 (0.7-13.3)	0.3 (0.1-0.6)	0.0006	0 (0-0.4)
α-1 antitrypsin (mg/mL)	3.9 (1.1-20.6)	5.3 (1.7-16.3)	0.66	1.3 (0.7-7.4)
Elafin (ng/mL)	5.0 (3.4-19.0)	5.2 (1.1-11.9)	0.28	2.8 (1.1-11.5)
SLPI (ng/mL)	6.0 (4.0-70.8)	7.3 (1.0-38.0)	0.2	4.9 (0-38.0)
Neutrophils (10^5/mL)	2.8 (0.6-21.0)	3.2 (0.3-12.0)	0.6	0 (0-0.03)

This work suggests that microbiologically confirmed VAP is characterised by activation of neutrophils, resulting in the release of

proteolytic enzymes which may damage pulmonary tissue and contribute to respiratory failure. The difference in proteases is not mirrored by their inhibitors, suggesting that protease-inhibitor imbalances may play a role in the pathogenesis of VAP. Previous work by our group has shown alveolar neutrophils in VAP to have defective anti-bacterial function.[2] The emerging picture of VAP is of a 'worst case scenario', with neutrophils unable to efficiently clear bacteria but remaining toxic to host tissues.

Funding: This work was funded by the Sir Jules Thorn Charitable Trust.

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Clinical Practice Free Paper Presentations winner

Midazolam and brain stem testing

C Buss, J Brander, S Brown, D Gardiner, Nottingham University Hospitals Trust, Queens Medical Centre, Derby Road, Nottingham, UK

The new Academy of Medical Royal Colleges, *A Code of Practice for the Diagnosis and Confirmation of Death* states, "If midazolam levels are available brain-stem testing should not be undertaken if the level is >10µg/L."[1] Since the release of the code of practice our institution has had three patients where concern over potential midazolam levels have prohibited or deterred the clinician from brain stem testing.

The first case received 105 hours of sedation with midazolam (1291 mg), morphine (1285 mg) and propofol 1(6240 mg). Twenty-one hours after sedation was ceased the midazolam level was 5500 µg/L.

The second case received 27.5 hours of sedation with midazolam (496mg) and morphine (266 mg). Twenty and a half hours after sedation was ceased the midazolam level was 218 µg/L. A second level three and a half hours later was 140 µg/L giving a elimination $t_{1/2}$ of 4.4 hours, significantly higher than that suggested for the context sensitive half time for midazolam (75 minutes)[2] but within the range of other reports.[3]

The third case received 64 hours of sedation with midazolam (274 mg), morphine (247 mg) and propofol (400 mg). Twenty-two hours after sedation was ceased a non-urgent midazolam level was requested on the assumption the level would be high. The level was <10 µg/L. This patient had a six hour 'sedation hold' of midazolam during an operative procedure where remifentanil and volatile anaesthesia was used.

To investigate the potential impact of the new code of practice on our institution's ability to carry out brain stem testing, a review was undertaken of brain stem tested patients during the two years prior to Case 1.

Twenty four patients were reviewed and of these 21 had received sedation on ICU. The median time for brain stem testing from cessation of sedation was 16 hours. Ten patients received propofol without midazolam or morphine.

Of the remaining 11 patients, seven received midazolam (median dose of 110 mg, median duration of sedation 26 hours and median time to brain stem testing after sedation ceased of 24.5 hours). Of potential conflict with the new Code of Practice one patient received a dose of midazolam similar to Case 2 and was brain stem tested at 32 hours and another patient received a midazolam dose of 3150mg and was tested at 33 hours.

The Code of Practice will impact on brain stem testing. It raises many issues regarding the pharmacokinetics of sedatives on ICU and neuro-critical care sedation in general.

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Research Poster Presentations winner

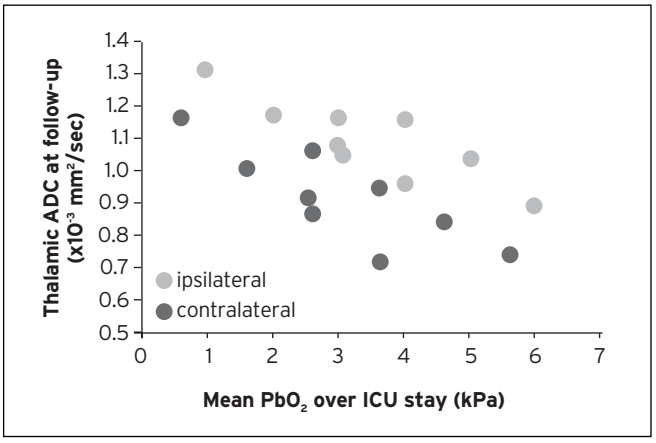
Early brain tissue oxygenation is related to selective neuronal loss following clinical head injury

V Newcombe*†, I Timofeev‡, C Williams*, D Chatfield*†, P Hutchinson†‡, J Coles*†, J Pickard†‡, G Williams†, A Gupta*, D Menon*†. *University Division of Anaesthesia, †Wolfson Brain Imaging Centre, Department of Clinical Neurosciences, ‡Academic Neurosurgery Unit, University of Cambridge, UK

We have previously used ^{11}C-flumazenil positron emission tomography to show that selective neuronal loss in the thalamus is pervasive after traumatic brain injury (TBI) and correlates with functional outcome.[1] The mechanisms responsible are unclear, but may involved global hypoxia/ischaemia as well as retrograde degeneration. We hypothesised that early brain tissue oxygenation would correlate with late magnetic resonance imaging (MRI) with diffusion tensor imaging (DTI) abnormalities in the thalamus, and therefore, help provide an explanation for late neuronal loss.

Nine patients underwent brain tissue oximetry (PbO_2) following acute TBI, using a Licox PbO_2 probe, sited in structurally normal frontal white matter. Mean PbO_2 was calculated for the duration of their ICU stay. At a median of 11.6 months (range 237 to 702 days) they underwent MRI with DTI. Apparent diffusion coefficient ADC (maps) were created, ADC calculated in regions of interest in the frontal lobes, splenium of the corpus callosum and thalami, and correlated with mean PbO_2 using Spearman's Rho. Research ethics approval and assent from next-of-kin were obtained.

Mean PbO_2 was inversely related to ADC in both frontal lobes (r=-0.73 & -0.72; p=0.025 & 0.031), and with the ADC in the thalamus bilaterally (r=-0.87 & -0.80; p=0.002 & p=0.010; **Figure**). In contrast, no correlation was seen between mean PbO_2 and ADC in the splenium of the corpus callosum, a common site of traumatic axonal injury (TAI; p=0.265).



The inverse correlation of mean PbO_2 with ADCs in the monitored brain region is unsurprising, but the correlations observed with contralateral regions and deep grey matter suggest that the burden of tissue hypoxia has a significant impact on secondary neuronal loss across the brain. In contrast, the lack of correlation with ADC changes in an area at risk of TAI suggests that mean PbO_2 levels may be less useful in monitoring the impact of secondary insults on the maturation of TAI. The correlations with measures of thalamic microstructural injury are particularly significant, since they establish a clear link between acute physiology, tissue fate in key brain regions, and clinical outcome.

Acknowledgments: MRC, NIHR, Royal College of Anaesthetists, Health Foundation, Academy of Medical Sciences, Gates Foundation, Cambridge.

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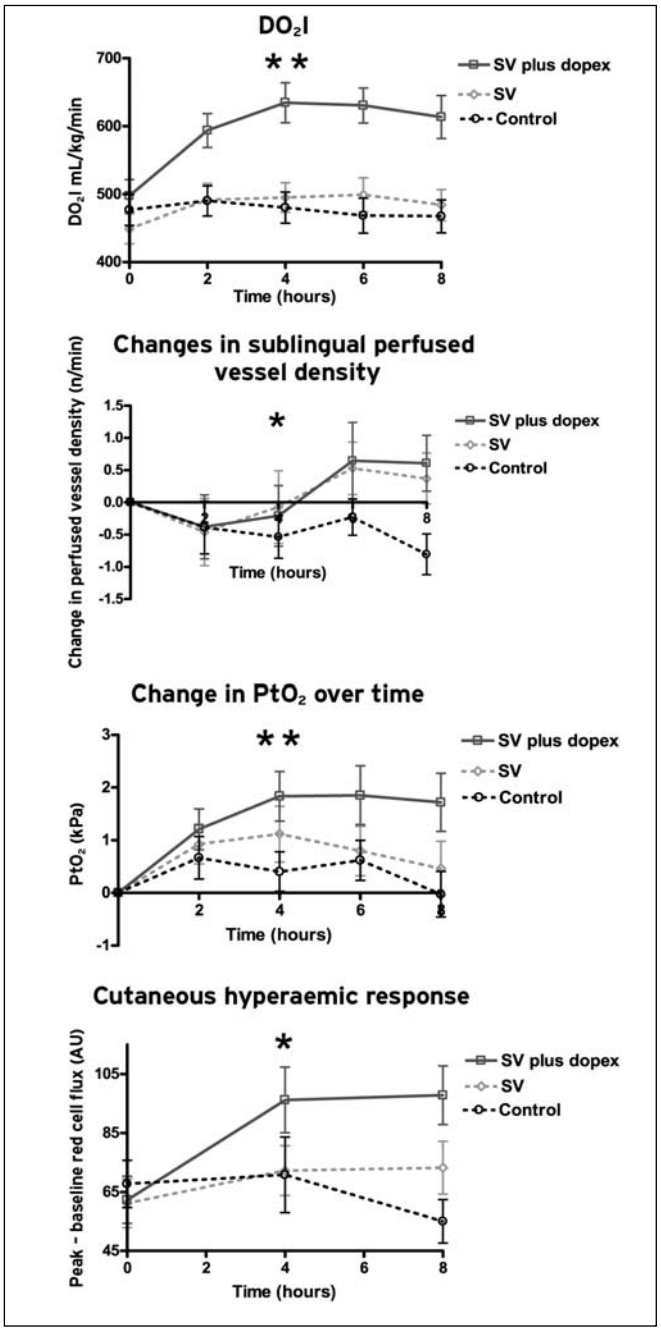
Research Free Papers Presentations runners-up

A randomised controlled trial of the effects of three haemodynamic therapies on microvascular flow, tissue oxygenation and inflammatory markers after major abdominal surgery

S Jhanji, A Vivian-Smith, S Lucena-Amaro, D Watson, CJ Hinds, RM Pearse, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, London, UK

Perioperative Goal-Directed Haemodynamic Therapy (GDHT) appears to improve outcome.[1] Importantly, the biological mechanisms underlying this therapy have not been investigated.[2] The objective of this study was to assess the effects of three haemodynamic regimens on tissue microvascular flow and oxygenation in patients after major abdominal surgery.



Approval from the local ethics committee and the competent authority were granted. Patients were stratified according to surgical specialty. Patients were then randomised to receive one of three haemodynamic protocols for eight hours immediately following major abdominal surgery. The central venous pressure (CVP) group received intravenous (IV) colloid boluses to achieve a sustained rise in CVP. The stroke volume (SV) group received IV colloid boluses to achieve a sustained rise in SV. In the SV plus dopexamine group, patients received IV colloid boluses to achieve a sustained rise in SV plus a fixed rate infusion of dopexamine (0.5 µg/kg/min). Data collected every two hours included mean arterial pressure (MAP), oxygen delivery index (DO_2I) (lithium dilution and pulse power analysis), central venous oxygen saturations ($ScvO_2$), sublingual microvascular flow videos (sidestream darkfield imaging), cutaneous microvascular flow (laser Doppler) and cutaneous tissue PtO_2 (Clark electrode). Serum Il-1ß, Il-6, Il-β, TNF-α and ICAM-1 were also measured. Data are presented as mean (SD) or absolute values (%).

One hundred and thirty-five patients were recruited in 15 months. Following surgery, SV-guided fluid therapy with dopexamine was associated with a significant increase in DO_2I, sublingual microvascular flow, the cutaneous hyperaemic response and tissue PtO_2 during the intervention period (**Figure 1**). There were no differences in serum inflammatory markers, post-operative complications, hospital stay or mortality.

GDHT with dopexamine results in an improvement in global haemodynamics, tissue microvascular flow and oxygenation but no change in systemic inflammatory markers.

Grant acknowledgement: ESICM/ECCRN Spacelabs Intelligent Monitoring Award 2006. RMP is an NIHR clinician scientist. SJ, CJH and RMP are named inventors on a patent application relating to dopexamine.

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Evolution of gene expression signatures in septic shock due to community-acquired pneumonia

J Radhakrishnan*, E Svoren†, P Ellis‡, C Langford‡, P Hutton§, C Garrard§, **CJ Hinds*** JC Knight# and the GAinS Investigators. *Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK, †Queen Mary University of London, Barts and The London School of Medicine and Dentistry, UK, ‡Wellcome Trust Sanger Institute, Cambridge, UK, §Intensive Care Unit, John Radcliffe Hospital, Oxford, UK

Severe sepsis and septic shock involve a dynamic process of gene reprogramming. Emerging evidence suggests that changes in gene expression influence clinical outcome, but our knowledge of the genome wide temporal evolution of these changes remains limited[1,2] and studies to date have typically involved non-homogeneous sepsis cohorts.

To investigate temporal changes in global gene expression profiles and identify predictors of mortality, we studied patterns of gene expression in patients admitted to intensive care units (ICU) with septic shock due to community acquired pneumonia (CAP). Following ethics committee approval and informed consent, serial samples of peripheral blood leukocytes were collected on days 1, 3 and 5 of ICU admission from 28 patients (15 survivors, 13 non-survivors). To provide a baseline for comparison, control samples were also obtained from 15 cardiac surgery patients prior to induction of anaesthesia. Leukocytes were collected using the LeukoLOCK system (Ambion). Total RNA was isolated and genome-wide gene expression profiles obtained using the Illumina Human WG-6 v3 Beadarray platform that interrogates more than 48,000 unique transcripts.

Gene expression data was analysed by fitting a linear model using a Bayesian algorithm. Samples from CAP survivors (S) and non-survivors (NS) were compared independently to the baseline control samples for each of the three time points. Between 600 and 1300 genes were at least 1.5 fold differentially expressed with an adjusted p value less than 0.01 for each time point comparison. Pathway analysis was performed with the Gene Set

Enrichment Algorithm using pathways derived from Gene Ontology database, KEGG and published literature. Fewer pathways were enriched in NS as compared to S. A mixture of immune related signalling, transport, and biosynthetic pathways were significantly enriched, with different patterns of enrichment observed at each of the time points.

Models were also fitted to compare S and NS at different time points. Transcript levels of some of the classical protein markers of sepsis (TNF, IL6 and ILβ,) did not differ between S and NS. In contrast NS showed persistent up-regulation of key pattern recognition receptor genes (eg TLR2, TLR4, TLR6), suggesting on-going activation of the innate immune signalling pathways.

By studying sequential changes in genome wide gene expression profiles we have characterised evolving patterns of gene expression that differentiate S from NS of septic shock due to CAP. It is hoped that novel insights derived from such studies may lead to the development of genomic biomarkers for prognosis or individually targeted interventions, as well as suggesting new therapeutic approaches.

This study was supported by the Wellcome Trust and a Young Investigator Award from the Intensive Care Society.

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Research Poster Presentations

Plasma C5a, IL-6 and IL-10 are elevated prior to nosocomial infection in critically ill patients: a preliminary report

A Conway Morris*, J Antonelli†, C McCulloch† RO Jones‡, I Laurenson§, DG Swann‡, A Hay‡, TS Walsh‡, AJ Simpson*. *MRC Centre for Inflammation Research, University of Edinburgh, Scotland, UK, †Clinical Research Facility, Royal Infirmary of Edinburgh, Scotland, UK, ‡Department of Critical Care, Royal Infirmary of Edinburgh, Scotland, UK, §Department of Medical Microbiology, Royal Infirmary of Edinburgh, Scotland, UK

Nosocomial infections are a significant problem within the intensive care unit. The frequency of these is thought to reflect dysfunction of the immune system, however debate continues as to the mediating factors and the temporal pattern of immune dysfunction. We have previously demonstrated that C5a, a pro-inflammatory complement fragment, can drive neutrophil dysfunction.[1] This work is a preliminary report from a project examining the relationship between immune function and the acquisition of nosocomial infection.

Critically ill patients (patients requiring support of ≥1 organ systems for ≥48 hours) admitted to a single intensive care unit were recruited within 48 hours of admission. Serial samples of blood were taken and analysed for plasma immuno-tropic molecules. Infection was determined by pre-defined criteria, in cases of dubiety an expert consensus panel was asked to adjudicate. Patient data was censored from 48 hours before nosocomial infection occurred to reduce the risk of infection itself causing the effect seen.

Twenty-six patients have been recruited to date, 8 (31%) have developed confirmed or probable infection. Patients with IL-6 and C5a above median at admission, and those with levels of IL-6 or IL-10 elevated above median during their ICU stay were at significantly increased risk of infection (see **Table**). Other measured cytokines (IL-8, IL-1, TNF-α and IL-12) showed no relationship with nosocomial infection. There was no difference in severity of illness (APACHE II score) or organ failures (SOFA score) at admission between those who developed infection and those who did not. There was no relationship between these scores and the inflammatory mediators measured.

	Elevated at admission (n)	Relative risk of nosocomial infection	Elevated during ICU episode (n)	Relative risk of nosocomial infection
C5a	11	9 (1.3-66)	13	3 (0.7-12)
IL-6	13	7 (1.0-49)	9	5.7 (1.4-22)
IL-10	11	2.3 (0.68-7.8)	7	6.8 (1.7-26)

This work suggests that hyper-inflammation is associated with, and precedes, the acquisition of nosocomial infection. This may be due to pro-inflammatory molecules driving dysfunction, or indirectly by driving an anti-inflammatory response. However further work and patient recruitment is required to determin whether these molecules are independent predictors or simply reflecting other processes.

Funding: This work is funded by the Chief Scientist Office.

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1. **Conway Morris A, Kefala K, Wilkinson TS *et al.*** C5a mediates peripheral blood neutrophil dysfunction in critically ill patients. *Am J Respir Crit Care Med* 2009;180:19-28.

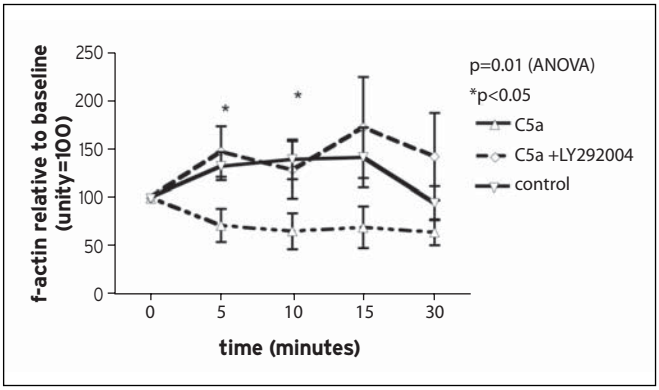
C5a drives phagocytic dysfunction via a PI3K delta-dependent inhibition of actin polymerisation

A Conway Morris*, AG Rossi*, TS Walsh†, AJ Simpson*. *MRC Centre for Inflammation Research, University of Edinburgh, Scotland, UK, †Department of Critical Care, Royal Infirmary of Edinburgh, Scotland, UK

The complement split-product and anaphylotoxin, C5a, causes defects in neutrophil phagocytosis in animals[1] and humans[2] with sepsis. Although dependency on the phosphoinositol 3-kinase (PI3K) pathway has been identified,[2] the mechanism of dysfunction is poorly understood.

An *in-vitro* model of C5a-mediated neutrophil dysfunction was created by treating healthy human donor neutrophils with C5a at concentrations found in sepsis (100nM). Phagocytosis was assessed using serum-opsonised zymosan particles. The involvement of the PI3K pathway was investigated using pan-PI3K inhibitors wortmannin and LY292004. Iso-form specific inhibitors IC87114 and AS605240, which inhibit the δ and γ iso-form respectively, were used to further define the pathways involved. Actin polymerisation was assessed using a fluorescent probe for f-actin (phalloidin). The ability of granulocyte-macrophage colony stimulating factor (GM-CSF) to 'rescue' actin polymerisation was investigated. Phagocytosis by neutrophils from critically ill patients was investigated, actin polymerisation determined and the effects of *ex-vivo* GM-CSF evaluated.

C5a inhibited phagocytosis of zymosan; an effect prevented by pre-treatment with pan-PI3K inhibitors. Pre-treatment with the PI3Kγ inhibitor failed to prevent the effect of C5a. In contrast, pre-treatment with the PI3Kδ inhibitor was effective. C5a treated neutrophils failed to polymerise actin in response to zymosan and this effect could be prevented by treatment with a PI3K inhibitor (see figure). This defect was reversed by treatment with GM-CSF. PI3K inhibitors applied after treatment with C5a were unable to rescue phagocytosis. GM-CSF, applied *ex-vivo*, improved phagocytosis in neutrophils from patients with critical



illness. In these patients phagocytosis correlated with the change in polymerised actin in neutrophils following exposure to a phagocytic target (r=0.62 p=0.003).

These data suggests that C5a activates PI3Kδ which in turn inhibits actin polymerisation, so preventing phagocytosis. GM-CSF is able to reverse the inhibition of actin polymerisation. This work increases understanding of the pathways underpinning phagocytic defects in critically ill patients and provides further insight into how GM-CSF is able to rescue neutrophil function.

Funding: This work was funded by grants from the Chief Scientist Office and Sir Jules Thorn charitable trust.

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Ischaemic ECG changes in septic shock: a comparison of vasopressin and noradrenaline

AC Gordon*, S Mehta†, S Lapinsky†, G Newton†, D Ayers‡, J Singer‡, KR Walley‡, JA Russell‡ for the VASST Investigators.
*Critical Care, Charing Cross Hospital, Imperial College London, UK.
†University of Toronto, Canada, ‡University of British Columbia, Canada

There is concern that vasopressin may increase myocardial ischaemia when used in the treatment of septic shock. We therefore compared rates of ischaemic ECG changes in vasopressin and noradrenaline treated patients who had septic shock enrolled in the VASST study.[1]

Patients who had septic shock were randomised to receive a blinded infusion of low-dose vasopressin or noradrenaline in addition to open-label vasopressors. The ECG substudy was carried out in eight centres who participated in the VASST study. Patients who had a 12-lead ECG at baseline and at least one further ECG within four days were included. ECGs were analysed independently and in duplicate by a cardiologist and intensivist, blinded to treatment allocation. Definitions of abnormalities and a checklist were drawn up before analysis. Ischaemia was defined as present if identified in two analyses. Categorical variables were analysed using the chi-square test and continuous variables using the T-test. Multivariate regression analysis was run to adjust for age, APACHE II score, sex, ischaemic heart disease and dose of noradrenaline at baseline.

	Vasopressin (n=53)	Noradrenaline (n=50)
Patients with any ischaemic changes	**10 18.9%**	**8 16.0%**
AF/Flutter/PVST	3	1
Bundle Branch Block	0	0
Q waves	1	1
ST changes	10	7
T wave inversion	5	2

ECG abnormalities reported in all ischaemic ECGs

One hundred and three patients and 366 ECGs were included. The mean age was 62.8 ± 16.8 years, mean APACHE II score 28.5 ± 7.5, 76 (73.8%) were male, and 53 patients were treated with vasopressin and 50 patients with noradrenaline. Eighteen patients (17.5%) developed new ischaemic changes. Patients who developed ischaemia were older 70.7 *v* 61.2 years (p=0.03), had higher APACHE II scores 33.8 *v* 27.3 (p=0.001) and had a trend to a higher 28-day mortality rate 58.8 *v* 36.5% (p=0.1). There was no difference in rates of ischaemic changes in the vasopressin group (18.9%) compared to the noradrenaline group (16.0%, p=0.8). On multivariate analysis the vasopressin group had an odds ratio for ischaemic changes of 1.49 (95%CI 0.48 - 4.60).

Ischaemic ECG changes were common (17.5%) but there was no difference in occurrence between vasopressin and noradrenaline treated patients in this substudy from VASST.

Funding: Canadian Institutes of Health Research and UK NIHR BRC funding scheme.

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Is non-invasive cerebral saturation a good indicator of invasive oxygen extraction?

MS Thorniley, Z Bashir, C Slinger, S Haynes, A Mortimer, CN McCollum.
Department of Academic Surgery, University Hospital of South Manchester, Manchester, UK

Major vascular surgery is frequently associated with blood loss and the current clinical decision to transfuse red cells is mutifactorial. The indicators to transfuse include haemoglobin concentration (Hb), blood loss, cardiovascular parameters and mixed venous oxygen saturation. The decision to transfuse should be to maintain adequate tissue oxygenation. The physiological response to blood loss is to increase cardiac output to maintain delivery. If the cardiac output cannot be improved the oxygen extraction ratio will increase to maintain tissue oxygenation. Systemic OERs can be a sensitive indication of changes in oxygen consumption/ delivery and it has been reported by Orlov et al[1] that they can be used as a more appropriate transfusion trigger. However, OER is invasive. Near infrared spectroscopy measures cerebral haemoglobin oxygen saturation (CsO_2) and has been shown in previous work by our group to fall significantly during aortic aneurysm surgery. The aim of this study was to determine if the noninvasive measurements of CsO_2 correlated with OER measurements in patients undergoing elective abdominal aortic aneurysm surgery (AAA). The target Hb for blood transfusion is 70-90 g/L[2] and Hb in this region is frequently associated with higher than normal Lac levels. Furthermore, regression analysis was determined for all patients with a Hb value <90g/L and a Lac level greater than the mean 2mM to determine the relationship of CsO_2 with OER in this critical region.

Cerebral and peripheral continuous NIRS (Somanetics INVOS instrument) measurements were made in 66 patients undergoing AAA surgery. Measurements were made from incision to closure. Hb measurements were made from a central venous line. Statistical analysis was determined using SPSS 15.0.

A significant moderate negative relationship was found between CsO_2 and OER (r=-0.44 p<0.001). Linear regression analysis showed that in patients with Hb<90g/L the relationship was closer r^2= 0.45, p<0.001 and in patients with Hb<90g/L and Lac >2mM the relationship was r^2=0.77, p<0.001 (**Figure 1**).

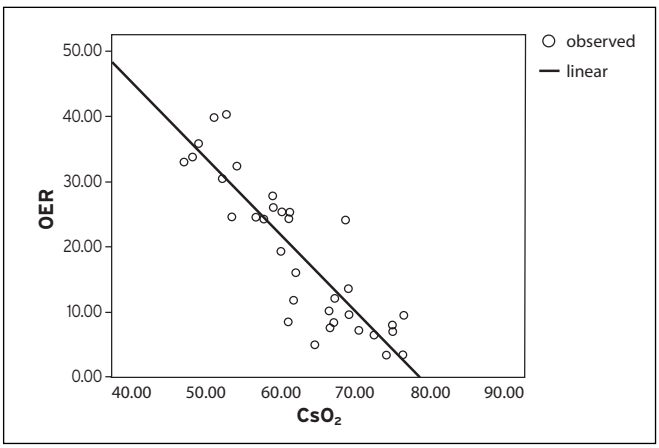

Figure 1. Relationship between CsO_2 and OER at Hb <90 h/L and Lac >2.37 mM (r^2=0.77, p<0.001).

The noninvasive NIRS measured CsO_2 can be used as a surrogate marker of oxygen extraction. Non invasive oximetry is a reproducible method and demonstrates that as the critical region of anaerobic metabolism begins with Hb falling and lactate rising the relationship between CsO_2 and OER is closer. This suggests a use for CsO_2 in providing critical information in the development of a transfusion trigger.

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Thomas the Tank Engine significantly improves the understanding of oxygen delivery and hypoxaemia

JF Cosgrove*, ID Nesbitt*, DJ Kennedy†, M Sawdon‡, K Fordy§, P Laws*, J Green*. *Perioperative and Critical Care, Newcastle upon Tyne Hospitals NHS Trust, Newcastle upon Tyne, UK, †Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK, ‡School of Medicine and Health, University of Durham, Stockton-on-Tees, UK, §Perioperative and Critical Care, Sunderland Royal Hospitals, Sunderland, UK

Despite adequate background knowledge many physiological concepts are poorly understood; students "know" but do not "understand".[1] Analogous imagery can enhance understanding and Thomas the Tank Engine has been used previously to demonstrate hypoxaemia.[2-5] Such imagery has not been evaluated in medical student education.

The aim of the study was to assess the effectiveness of Thomas the Tank Engine in improving the understanding of hypoxaemia in Year One Medical Students at the Universities of Newcastle and Durham. Two 30-minute lectures on the subject were delivered to students. The control lecture was a conventional presentation; the study lecture contained additional images of Thomas the Tank Engine.[4,5] Local Research Ethics approval was advised as being unnecessary; HiT Entertainment-UK granted permission for the use of the imagery of Thomas the Tank Engine.

Students were randomised into four groups (A-D.) A and B received the control lecture, C and D the study lecture. All students undertook a 20-question post-lecture multiple choice questionnaire (MCQ) on oxygen delivery and hypoxaemia; A and C sat a similar pre-lecture MCQ as an "evaluative preparatory drill" assessing background knowledge and monitoring for priming.[6,7] Pre and post-lecture MCQ scores for groups A and C were compared to assess for lecture effectiveness (A *vs* A and C *vs* C) and for differences between the control and study lecture (pre-MCQ A vs. pre-MCQ C and post-MCQ A *vs* post-MCQ C.) The effect of priming was assessed by comparing post-lecture MCQ scores (A *vs* B and C *vs* D.)

Students also completed a post-lecture qualitative evaluation of eight aspects of lecture quality: organisation, relevance, lecture "pitched" at an appropriate level, difficulty, comprehension of oxygen delivery, assistance of visual aids, interest, worthwhile. They were scored 1 to 5: strongly agree/ agree/ undecided/ disagree/ strongly disagree. All scores were collected using an ARS-KEEpad system and compared using the Mann-Whitney U-test for non-parametric data. A p value <0.05 was regarded as significant.

Group numbers were A n=73, B n=56, C n=59, D n=53. Both lectures significantly improved post-lecture MCQ scores (p<0.001.) Group A had a significantly higher pre-lecture MCQ score compared to group C (median 16 *vs* 12, p<0.001); there was no difference post-lecture between A and C (median 18 *vs* 17, p=0.4). Post-lecture MCQ scores were no different between A and B (median 18 *vs* 18, p=0.14) or C and D (median 17 *vs* 17, p=0.6.) With respect to qualitative evaluation the imagery also made the lecture significantly more organised (p=0.006), interesting and stimulating (p<0.001) and improved qualitative understanding (p<0.001.)

Images of Thomas the Tank Engine can significantly improve the understanding of oxygen delivery and hypoxaemia in Year One Medical Students. A pre-lecture MCQ did not create a priming effect in either group.

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Acute respiratory distress syndrome (ARDS): outcomes in a tertiary referral intensive care unit (ICU)

RH Johns, KAE Elliott, A Karim, S Boluda, C Matejowsky, GJ Bellingan, DCJ Howell. Dept of Critical Care, University College Hospital, London, UK

ARDS is a severe life-threatening respiratory condition. A recent large meta-analysis, which included patients with acute lung injury (ALI) as well as ARDS, reported an ICU mortality of rate 44.3%.[1] Reported ICU and hospital mortality rates for ARDS alone are 53% and 61% in Scotland;[2] and 49% and 58% in Europe.[3] Our ICU manages a unique and complex cohort of ARDS patients who often have high APACHE scores at admission. In this study we examined outcomes in our ARDS patients and compared results to published literature.

All patients admitted to the ICU between May 2008 and Jan 2009 were included (n=1402). Patients meeting ARDS defining criteria were identified prospectively and further data from our ICU CIMS (clinical information management system) was assessed retrospectively by two independent observers. ARDS was defined by ERS/ATS Consensus criteria and absence of echocardiographic or clinical evidence of significant left ventricular impairment or pulmonary oedema. In ARDS patients, the level of compliance with low tidal volume (v_t) ventilation, achievement of peak airway pressures (PAP) of <30 cm H_2O and neutral/negative fluid balance (FB) strategies, during admission, was assessed.

Of 1402 admissions (665 non-elective (ne), 737 elective (e)) ARDS occurred in 67 patients (63 ne; 4 e). Overall incidence was 4.8%, and 9.5% amongst non-elective admissions (63/665). Mean (± standard error of the mean) values were: Age 57 ± 2 years, APACHE II 27 ± 1, ICU length of stay 19 ± 3 days, duration of mechanical ventilation 14 ± 2 days, ICU mortality 48%, and hospital mortality 55%. Overall compliance with low v_t ventilation was 84%, PAP <30 cm H_2O: 71% and neutral/negative FB: 62%. In survivors, compliance was 83%, 86% and 83% respectively.

Pneumonia and sepsis were the commonest precipitants of ARDS. Our incidence is lower than the 8.1% reported in Scotland,[2] which most likely reflects the high number of elective surgical patients admitted to our unit. Our admission APACHE II scores are higher than reported in this study consistent with greater severity of illness in our ARDS patients. Despite this, our mortality rates are lower than other published studies[2-4] which may be explained by our good adherence to current evidence-based ventilatory and supportive strategies, as recommended by published ARDSNet studies.

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Cognitive dysfunction following abdominal aortic surgery: a pilot study

T Kelley, MS Thorniley, V Sekar, CN McCollum. Department of Academic Surgery, University Hospital of South Manchester, Manchester, UK

Cognitive dysfunction is a frequent problem following major surgery. However, not much is known about the role of cerebral haemodynamics on postoperative cognitive function.

We recruited eight consecutive patients (mean age 70.2 years [63-90 years]) with an initial mini mental state exam (MMSE) †23 undergoing elective abdominal aortic surgery were recruited. Cognitive testing using CANTAB Eclipse (computerised test battery) was performed preoperatively and at five and 14 days postoperatively. The middle cerebral arteries (MCA) were insonated using a 2 MHz pulsed transcranial Doppler (TCD) probe. The flow velocities, pulsatility and resistance indicies were monitored continuously at all times.

Four out of eight patients (50%) had cognitive impairment at five days (20% drop in 20% of the tests), with a return to pre-op levels at 14 days in seven out of eight patients (88%). Total duration of the procedure and increased clamp times strongly predicted impaired reaction time at five days ($p=0.01$ and $p<0.01$ respectively). The mean difference in spatial working memory within errors test from pre-op to five days was 3.0, ($p=0.5$). However, it strongly correlated with the greatest change in MCA flow velocities following aortic clamping ($p=0.037$). The other test scores assessing spatial working memory (total errors, between errors and mean time to last response) just failed to achieve significance ($p=0.08$, $p=0.09$ and $p=0.09$ respectively).

The duration of operation and clamping are associated with postoperative cognitive dysfunction (POCD) in patients undergoing abdominal aortic surgery. Intraoperative changes in blood flow velocities may contribute to the development of POCD. The relevance of these findings to everyday life after abdominal aortic surgery needs to be studied in greater detail.

Do noise-cancelling headphones improve sleep quality on the intensive care unit?

Hassall I, Latif I, **McGrattan K**, Pugh M. Critical Care Unit, Royal Preston Hospital, Preston, Lancashire, UK

Patients do not sleep well on the intensive care unit (ICU), at least in part due to the noisy environment.[1] Poor sleep quality is associated with immune system dysfunction, cardiovascular instability, depression and delirium.[2] Delirium alone is associated with an increase in ICU and hospital stay and an increase in mortality.[3]

Noise-cancelling headphones are designed to minimise background noise. We hypothesised that the wearing of these headphones may improve the sleep quality of ICU patients.

Extubated patients following elective gynaecological or general surgery admitted to the ICU post operatively were randomised to wearing noise-cancelling headphones (Audio-Technica, Quiet point, ATH-ANC7) or not for the first two postoperative nights. After each night patients completed the Richards-Campbell Sleep Questionnaire (RCSQ).[4] This consists of five visual analogue scales covering the sleep domains of depth, latency, awakenings, percentage time awake, and quality of sleep. The five scores are averaged to produce a figure for overall sleep quality from 0-100mm where 0 is good quality and 100 poor. We also collected information on analgesics and sedatives prescribed to patients.

There were 11 patients in the treatment group and seven in the control group. Five of the patients in the treatment group found the headphones uncomfortable and were unable to wear them during the night. They did not complete RCSQs. Of the remaining six in the treatment group three patients stayed for two nights on the ICU. Of the seven in the control group one stayed for two nights.

Visual analogue scores in the treatment group were consistently lower (better sleep quality) than in the control group. The average sleep quality score derived from the RCSQ was 24.7mm in the treatment group compared with 89.3mm in the control group.

It is difficult to derive conclusions from this study since the numbers are so small. Moreover, nearly half of the patients randomised to wearing headphones found them too uncomfortable. In this small study patients who were able to tolerate wearing headphones had a better quality of sleep. We intend to repeat this study to include larger numbers of patients.

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Dose-dependent effects of dopexamine on indices of shock and organ dysfunction in a rodent model of laparotomy and endotoxaemia

MN Bangash*, NS Patel*, CJ Hinds*, R Lever†, C Thiemermann*, RM Pearse*. *Barts and The London School of Medicine and Dentistry, Queen Mary's University of London, UK, †School of Pharmacy, University of London, UK

Sepsis related organ failure is associated with abnormal haemodynamics, altered tissue microcirculation and oxygenation and an abnormal immune response. Dopexamine is a synthetic catecholamine which may have important clinical benefits but the effects of this agent on organ failure is not fully understood. The objective of this study was to investigate the effect of dopexamine on indices of organ failure in a rodent laparotomy and endotoxaemia model.

Male Wistar rats (220-400 g) were allocated to one of five groups. All procedures were performed in accordance with Home Office regulations. Animals were anaesthetised with intra-peritoneal thiopental sodium 30 mg/kg following which tracheal, carotid artery and internal jugular catheters were sited. A midline laparotomy was performed with small bowel exteriorisation in to a Saran wrap pouch. Sham group animals underwent surgery and four hours of 4.3 mL/kg/h normal saline fluid resuscitation with prior administration of endotoxin vehicle. Control group animals received the same management and in addition received 6 mg/kg *E.Coli* endotoxin over 10 minutes prior to fluid resuscitation. Dopexamine groups received intra venous fluid, endotoxin and dopexamine infusion at 0.5 µg/kg/min (Dopex 0.5 group), 1.0 µg/kg/min (Dopex 1.0 group) or 2.0 µg/kg/min (Dopex 2.0 group). Blood samples were taken for determination of lactate, base excess, leucocyte expression of CD11b, urea, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). At the end of the four hour protocol, organ tissue was harvested to measure myeloperoxidase levels (marker of leucocyte infiltration).

Forty animals were studied. Groups were similar in all respects though Dopex groups received 1.5 to 1.7 mL/kg more normal saline over the course of the experiment compared to Controls. Endotoxin administration was associated with a significant rise in plasma lactate, urea, creatinine, AST and ALT. These changes appeared to be attenuated by dopexamine infusion (**Table**). These effects were more marked in the Dopex 1.0 and Dopex 2.0 groups. Leucocyte expression of CD11a integrin was decreased in association with endotoxin administration while expression of CD11b integrin was increased. Increasing doses of dopexamine were associated with a non-significant decreasing trend in CD11b and CD11a. Tissue myeloperoxidase levels are awaited but will be available for presentation at the meeting.

In a rodent model of laparotomy and endotoxaemia, abnormalities of tissue perfusion and organ failure were less severe in animals receiving

dopexamine infusion. This effect was more obvious at doses of 1.0 µg/kg/min and above. Further research is required to explore the effects of dopexamine on leucocyte-endothelial adhesion and tissue micro-vascular flow.

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Conflict of Interest: RP has received a grant from Circassia Holdings Ltd. RP & CH are named inventors on a patent application relating to dopexamine.

	Sham (n=8)	Control (n=7)	Dopex 0.5 (n=8)	Dopex 1.0 (n=8)	Dopex 2.0 (n=9)
IV fluid (mL/kg)	21.7 (0.6)	21.1 (0.6)	22.6 (0.9)*	22.6 (0.4)*	22.8 (1.4)**
Heart rate (bpm)	415 (18)	453 (42)	506 (41)**	487 (16)	483 (13)
Δ Base excess (mmol/L)	0.7 (3.8)**	-7.3 (4.6)	-5.9 (3.2)	-3.5 (2.4)	-1.8 (3.5)*
Δ Lactate (mmol/L)	0.6 (1.0)*	3.1 (2.2)	2.0 (1.4)	0.4 (1.1)**	0.5 (1.6)**
Urea (mmol/L)	8.4 (2.9)**	17.1 (4.6)	15.1 (1.7)	14.7 (1.8)	12.7 (2.9)*
Creatinine (mol/L)	36 (6)**	86 (26)	54 (14)*	40 (18)**	52 (24)**
ALT (IU/L)	56 (18)**	162 (90)	88 (15)**	79 (19)**	77 (22)**
AST (IU/L)	187 (94)**	472 (235)	349 (69)	275 (88)*	289 (90)*

Effects of dopexamine on heart rate and parameters of shock and organ dysfunction (*p<0.05 vs control group, **p<0.01 *vs* control group, Δ refers to change from baseline to end).

Ascorbate improves endothelial function and reduces systemic inflammation after open aneurysm repair

MJ Duffy*‡, BA Mullan*, TR Craig*‡, DW Harkin†, DF McAuley*‡.
*Regional Intensive Care Unit, Royal Victoria Hospital, Belfast, Northern Ireland, UK. †Regional Vascular Unit, Royal Victoria Hospital, Belfast, Northern Ireland, UK. ‡Centre for Infection and Immunity, Queens University of Belfast, Belfast, Northern Ireland, UK

Open abdominal aortic aneurysm (AAA) repair is associated with the systemic inflammatory response syndrome (SIRS) which may lead to organ failure and death. This is mediated by ischaemia-reperfusion injury which is associated with an increase in oxidative stress resulting in endothelial dysfunction. In ischaemia-reperfusion injury there is a decrease in plasma ascorbate, an aqueous phase antioxidant.

Our hypothesis was that intraoperative ascorbate administration could reduce endothelial dysfunction associated with AAA repair.

A prospective double-blind randomised placebo-controlled clinical trial was performed. Consecutive patients admitted for elective open AAA repair were considered for recruitment. Ascorbate 2 g or placebo was given intravenously prior to application of the aortic clamp.

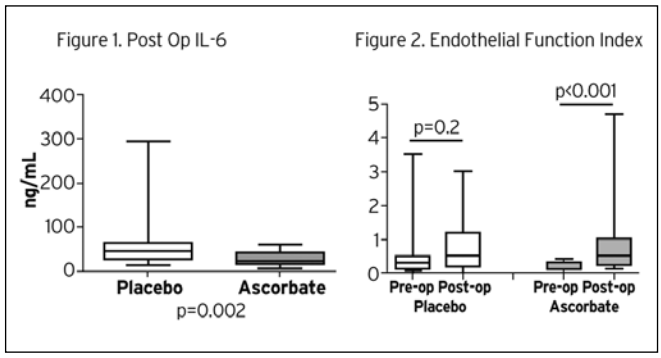
Blood and urine samples were taken prior to and 4 hours after aortic clamping. Microalbuminuria assessed by albumin:creatinine ratio(ACR) was measured as a surrogate marker of systemic endothelial dysfunction. Plasma levels of TNFα, IL-6 and IL-8 were measured by luminex bead array.

Concomitantly, bedside analysis of endothelial function was performed using pulse wave analysis and calculation of the endothelial function index (EFI) by measuring the response to endothelial dependent (salbutamol) and independent (GTN) vasodilators.

Exhaled breath condensate(EBC) was collected prior to and after aortic clamping and pH measured immediately as a marker of pulmonary inflammation. Pulmonary function was measured by PF ratio. Patient follow-up was until hospital discharge.

Thirty-one patients were studied, of which 13 received ascorbate and 18 placebo. There were no differences in demographics or clamp times between groups. There was a significant rise in IL-6 in both treatment (p=0.002) and placebo (p<0.001) groups. However, the post-operative level was lower in those who received ascorbate (p=0.03, **Figure 1**).

EFI improved in the ascorbate group (p<0.001), whereas there was no significant change in the placebo group (p=0.2, **Figure 2**). The ACR was significantly increased with a similar rise encountered in both placebo and treatment groups.



EBC pH fell after aortic clamping in both placebo (p=0.008) and ascorbate (p=0.003) groups, but with no difference between groups. The PF ratio also fell significantly in placebo (p=0.002) and ascorbate (p<0.001) groups with no difference between groups. There were no differences in duration of critical care or hospital stay. There was a single post-operative fatality in the placebo group.

The intraoperative administration of parenteral ascorbate prevented the increases in IL-6 encountered after aortic clamping. A bedside marker of endothelial function was significantly improved by ascorbate administration. Ascorbate may therefore play a role in modulating systemic inflammation in open AAA repair. This study also highlights the ability of aortic clamping to have an indirect effect on pulmonary inflammation as evidenced by the fall in EBC pH. This important finding warrants further investigation.

Cerebral oxygen saturation during abdominal aortic surgery predicts postoperative cognitive dysfunction

T Kelley, V Sekar, Z Bashir, CN McCollum, MS Thorniley.
Department of Academic Surgery, University Hospital of South Manchester, Manchester, UK

Confusion and cognitive dysfunction are common following major vascular surgery, but little is known about the causes. We studied the role of cerebral oxygen saturation (CsO_2) during surgery.

We recruited eight consecutive patients (mean age 70.2 years [63-90 years]) with an initial mini mental state exam (MMSE) †23 undergoing elective abdominal aortic surgery were recruited. Cerebral oximetry was studied preoperatively, intraoperatively (from surgical incision to the end of surgery), and at five and 14 days postoperatively. Cognitive testing using CANTAB Eclipse (computerised test battery) was performed preoperatively and at Five and 14 days postoperatively.

Four out of eight patients (50%) had cognitive impairment at five days (>20% fall in at least 20% of the tests), with a return to preop levels at 14 days in seven of the patients (88%). The maximum percentage fall in CsO_2 during surgery correlated significantly with a worsening reaction time at five days (p=0.031). The oxygen extraction ratio at declamp also correlated significantly with the reaction times at five days (p=0.035).

Abdominal aortic surgery is associated with cognitive impairment five days later which recovered to pre-op levels at 14 days. Changes in cerebral oxygen saturations during surgery may explain confusion and cognitive dysfunction following major surgery,

Defective efferocytosis due to aberrant intra-pulmonary steroid metabolism contributes to persistent inflammation in ARDS

CR Bassford*, S McKeown†, C OKane‡, F Gao*§, DF McAuley‡, GD Perkins*§, D Thickett†. *Academic Department of Critical Care, Anaesthesia and Pain, Heart of England NHS Foundation Trust, Birmingham, UK, †Lung Inflammation Investigation Unit, University of Birmingham, Birmingham, UK, ‡Respiratory Medicine Research Programme, Queen's University, Belfast, UK, §Warwick Medical School Clinical Trials Unit, University of Warwick, Coventry, UK

Efferocytosis is the removal of spent and apoptotic cells, and is necessary for the resolution of inflammation. Failure of efferocytosis results in pro-inflammatory mediator release and further tissue damage.[1] In the acute respiratory distress syndrome (ARDS) a defect in the removal of spent neutrophils by alveolar macrophages (AM) may contribute to persistent inflammation. Corticosteroids increase efferocytosis in many types of phagosome; and we have previously demonstrated a decrease in cortisol levels in the broncho alveolar lavage fluid (BALF) of patients with persistently severe ARDS.[2] We therefore hypothesised that efferocytosis in ARDS was influenced by the availability of active glucocorticoids within the lung, and the intra-pulmonary metabolism of corticosteroids by hydroxysteroid dehydrogenase (HSD).

An efferocytosis assay using fluorescently labelled apoptotic neutrophils was used in conjunction with established HSD activity assays and flow cytometric techniques.

Efferocytosis was decreased by BALF taken from patients at onset of ARDS, compared to controls (p=0.01). Efferocytosis induced by BALF taken at day 4 of ARDS was higher than that of day 0 BALF (p=0.008), but no different from that of control (p=0.067). BALF had no effect on cell viability. Efferocytosis is up-regulated by cortisol and cortisone compared to controls (mean efferocytosis index: control=0.05, cortisone=0.09, cortisol=0.11; n=9, p=0.001). Efferocytosis up-regulation by cortisone is suppressed by HSD inhibitors (n=7, p=0.037). Salbutamol also up-regulates efferocytosis in a dose dependent manner (ANOVA: p=0.001), and has an additional effect when combined with cortisol. Alveolar macrophages from ARDS patients have decreased HSD conversion of cortisone to cortisol at diagnosis of ARDS compared to day 4 (0.10 *vs* 0.57 pmol/million cells/hr, p=0.039, n=12). Annexin V/PI flow cytometry of ARDS BALF neutrophils (n=14) demonstrates an increase in late apoptotic and necrotic cells compared to human LPS challenge (n=20).

Local steroid metabolism by HSD is dysregulated early in ARDS. Low cortisol levels and defective HSD activity is associated in-vitro with defective efferocytosis. ARDS BALF suppresses efferocytosis of apoptotic neutrophils suggesting that the alveolar phagosome may be defective in ARDS. This hypothesis is supported by flow cytometry data suggesting that these changes may contribute to persistence of inflammation in ARDS.

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Intensive care outcomes and quality of life in lung transplant patients requiring intensive care admission

R Shetty*, P Nair†, AR Glanville†. *Intensive Care Unit, UCL Hospital, London, UK, †Intensive Care Unit and Lung Transplantation, St Vincent's Hospital, Sydney, Australia

ICU admission is common in lung transplant recipients.[1] The reported mortality is around 37%.[2] Clinicians face a difficult challenge determining which patients would benefit from being admitted to ICU. Unfortunately, little is known about the quality of life of patients who survive after admission to ICU. In this study, our aim was to gain a better understanding of this aspect.

All patients who underwent Single/Bilateral or Heart-Lung transplant from 2000 to 2006 were included in the study. Demographic data and details of ICU admissions were collected. Patients were requested to complete the SF36v2 questionnaire to assess quality of life. Sixty-six of 183 patients required one or more admissions to ICU from the time of their lung transplant until Dec 2007 when the data was collected (Study Group). ICU admissions in the immediate post-op period were excluded. Sixty-six patients were randomly selected from the remaining 117 patients to form the Control Group. Eleven patients (16.7%) in each group died prior to study commencement. Overall response rate to the questionnaire was 73%.

Commonest indications for lung transplant included cystic fibrosis and emphysema. The number of transplants performed each year over the study period was found to increase. Fewer patients whose transplant operation occurred in the recent years were admitted to ICU. Despite this the annual admissions to ICU have increased over the years indicating increased transplant load. The major indications for ICU admission (76%) were graft related problems – 55% of patients were admitted within one year of their transplant surgery. The mean length of ICU stay was 4.5 days (range 1-32 days). Twenty-four patients (38%) required mechanical ventilation (MV). Mean duration of MV was 3.8 days (range 1-19 days). There were no deaths in ICU. There was no statistically significant difference in quality of life scores, either in the physical (42.67 *vs* 45.32, p= 0.322) or the mental health domains between the study group and the control group (45.09 *vs* 47.79, p= 0.381). Furthermore, encouragingly, these scores were not significantly different from the Australian Norms for quality of life in a general population.

We can conclude from our study that both in terms of ICU outcomes as well as long-term quality of life in lung transplant patients who required admission to ICU, the risk-benefit ratio appears favourable. Despite its limitations, this is the only study, which looks in to quality of life in this patient group, and has the highest number of patients compared to other studies. Prospective, matched multi-centre studies would help to confirm our findings.

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A retrospective study estimating the prevalence of human immunodeficiency virus amongst critically ill patients in a London teaching hospital

D Bruce-Hickman*, **SR Strachan†.** *University College London Medical School, King's College Hospital, London, UK, †Intensive Care Units, King's College Hospital, London, UK

The estimated prevalence of human immunodeficiency virus (HIV) in the UK is 0.127% with a regional variation between 0.025% and 1.192%; King's College Hospital is situated within a high prevalence area. The prevalence of HIV in critical care is unknown and with over a quarter of HIV-positive people unaware of their serostatus the Health Protection Agency (HPA) has called for increased testing.[1] This retrospective study was undertaken in order to measure the prevalence of HIV in our ICU from clinically-directed testing and compare it with the estimated prevalence from the population we serve, calculated from HPA data.

HIV testing in critical care is problematic due to the current requirement for consent;[2] therefore we also noted the proportion of patients tested who had capacity to consent. Furthermore, we looked for demographic or clinical indicators to enable critical care clinicians to more appropriately direct HIV testing in the absence of ability to consent.

We calculated the HIV prevalence for all ICU admissions from 01/04/2008 to 31/03/2009 by Primary Care Trust (PCT) HPA data to be

0.59%. HIV tests were performed on 20 of 1589 ICU patients during the year studied, by including a further 14 known HIV-positive patients our overall testing rate was 2.14%; we measured the HIV prevalence from this clinically-directed testing to be 1.13%. These results are statistically different (p=0.008) suggesting patients with HIV are more likely to require critical care compared with the general population.

If there is no hidden cohort, 78% (14/18) of HIV-positive patients had their status known on ICU admission. Forty-five percent (9/20) of the patients tested for HIV following ICU admission had capacity to consent at time of testing, 30% (6/20) lacked capacity and for 25% (5/20) capacity was unknown. There were no real clinical indicators of serostatus and no patient tested had more than one risk factor for HIV known when tested. The only indicators of increased HIV risk were demographic, with seropositive patients originating from either a high prevalence country or PCT.

The HIV prevalence in our ICU measured from clinically-directed testing was found to be nearly twice that estimated from HPA population data. The UK National Guidelines for HIV testing[3] recommend testing where the diagnosed HIV prevalence in the local population exceeds 0.2% so it could be argued that in our hospital testing should be routine. The true prevalence of HIV in critical care will only be known by testing all ICU admissions and this study provides the information required to calculate the sample size needed for a prospective study to ascertain a more robust prevalence of HIV in our ICU by anonymous testing of all admissions. By comparing the true prevalence with the clinically-directed prevalence measured from this study we shall then be able to determine whether there are additional seropositive critically ill patients and be able to allocate appropriate resources for this cohort of patients. The prospective study will also examine the acceptability of routine HIV testing within the critical care community.

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Sequential gene expression profiles following cardiac surgery involving cardiopulmonary bypass

E Svoren*, J Radhakhrishnan†, P Ellis‡, C Langford‡, JC Knight†, **CJ Hinds***. *Queen Mary University of London, Barts and The London School of Medicine and Dentistry, UK, †Wellcome Trust Centre for Human Genetics, Oxford, UK, ‡Wellcome Trust Sanger Institute, Cambridge, UK

Differential expression of specific genes has been associated with postoperative brain injury and individual variation in the severity of the host response following cardiac surgery.[1,2] The aim of this study was to document sequential changes in global gene expression and provide a genome wide description of the evolution of gene expression during the host response to low risk, elective cardiac surgery.

Having obtained ethics committee approval and informed consent, 15 Caucasian patients scheduled for elective cardiac surgery involving cardiopulmonary bypass (CPB) were recruited (10 coronary artery bypass graft, three valve replacements and three combined procedures, age 65 ±11 years, 12 male). Postoperative recovery was uneventful with only minor complications. All patients were alive 28 days after surgery. Peripheral blood leukocytes were isolated before induction of anaesthesia, after admission to intensive care and 24 hours after surgery, using the LeukoLOCK system (Ambion). Total RNA was isolated and genome-wide gene expression profiles obtained using the Illumina Human WG-6 v3 array that interrogates over 48,000 unique transcripts.

Gene expression profiles were homogeneous across all patients. Principal components analysis confirmed that the timing of the sample collection with respect to surgery was the primary determinant of changes in gene expression. Multiple genes involved in the innate immune response were found to be differentially regulated following cardiac surgery by fitting a linear model using the R package "limma". In the immediate postoperative period genes encoding Toll-like receptors TLR2, TLR4 and TLR6 were significantly up-regulated. NF_KB1 was unchanged in the immediate post operative period but was up-regulated on the first post-operative day. In the acute phase response pathway IL1β was up-regulated in both postoperative samples. The transcription factors STAT3 and CEBPB were also up regulated, while STAT4 and IFNγ were down-regulated. Pathways analysis was performed for each comparison using the gene-set enrichment algorithm. The significance of the enriched pathways was evaluated by a permutation algorithm involving phenotype switching. Pathways enriched in the postoperative period ($p<0.05$) included those related to the acute inflammatory response (IL-18, IL-6, IL-1β, IL8, IL10), signalling (M_YD88, MYH9), apoptosis (AKT-1), myeloid differentiation (BCL6, TIMP1), coagulation (CD-36, GNA-13), complement (C1QA, CR-1), carbohydrate metabolism (LDHA, G6PD), lipid catabolic processes (PLCB2) and fluid transport (AQP9). In general similar pathways were enriched at both time-points but the pattern of gene enrichment changed over time.

These data provide novel insights into the evolution of the host response to surgical and inflammatory insults. This pilot data will inform further studies in which we will investigate the influence of DNA sequence variation on changes in gene expression during the systemic inflammatory response and will provide a model of sterile systemic inflammation for comparison with sepsis survivors and non-survivors.

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Hand position for external chest compressions – centre of the chest or the inter-nipple line?

T Butler‡, **J Yeung***†, JW Digby‡, J Hughes‡, D Higgie‡, M Minshall‡, B Miller§, F Gao*†, GD Perkins*†. *University of Warwick, Warwick Medical School, UK, †Academic Department of Critical Care, Anaesthesia and Pain, Heart of England NHS Foundation Trust, Birmingham, UK, ‡College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK, §Department of Radiology, Heart of England NHS Foundation Trust, Birmingham, UK

Effective cardio-pulmonary resuscitation is a key link in the chain of survival.[1] There remains uncertainty over the optimal method for identifying the position on the chest where hands should be placed in order to perform external chest compression. There is little scientific evidence regarding the optimum hand position for chest compressions and currently the International Liaison Committee on Resuscitation (ILCOR) has concluded that there is 'insufficient evidence for and against a specific hand position for chest compressions during CPR in adults'.[2] The paucity of evidence supporting one method over another has led disparity between international guidelines with the American Heart Association (AHA) advocating that the rescuer should compress 'the lower half of the victim's sternum in the middle of the chest between the nipples', referred to as the inter-nipple line, whereas the European Resuscitation Council (ERC) guidelines taught Basic Life Support (BLS) providers to 'place the heel of one hand in the centre of the victim's chest' for chest compressions.[2,3]

The aim of this study is to identify and compare BLS providers' assessment of the 'inter-nipple line' (INL) and 'centre of the chest' (CoC), and the relationship between these positions and underlying anatomical structures.

Following approval from research ethics committee, 30 patients (22 males, mean age 60.5±15.0 and eight females average age 50.0 ± 14.8) having elective CT scans of the thorax at Birmingham Heartlands Hospital

between February and May 2009 were recruited. Two photos were taken, one photo with anatomical markers for the sternum and one without markers. Copies of unmarked photographs were distributed to 30 healthcare students trained in BLS who marked the CoC and INL for each patient. Mean values of CoC and INL were calculated and underlying structures identified from CT scans. Additional questions explored BLS providers' preference of hand positions.

CoC was identified at 0.69 (CI 0.63-0.75) of total length of sternum measured from the sternal notch compared to INL at 0.78 (CI 0.75-0.82) ($p<0.001$). Sixty percent INL and 46% CoC fell on ascending aorta, 3.3% of hand positions using the INL approach were inferior to the ventricles. The majority (80%) of BLS providers would not expose the chest before starting chest compressions whilst 60% preferred the CoC approach.

BLS providers' perception of the INL was significantly lower than the CoC with increased risk of causing harm to patients. The CoC technique for hand placement for chest compressions was preferred.

Conflict of Interest: None

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