Endogenous antioxidant capacity and oxidative stress and nitrosative stress after thoracic surgery

Association of Cardiothoracic Anaesthetists Grant 2009

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Study summary and objectives

This study was a prospective observational pilot study seeking to evaluate any association between preoperative total antioxidant capacity (TAC) and postoperative markers of lung injury in patients undergoing lung resection for primary lung cancer.

The role of oxidative stress has been well established in the pathophysiology of acute lung injury and acute respiratory distress syndrome (ALI/ARDS) after lung resection, with the magnitude of oxidative stress having been associated with major adverse events. We hypothesised that patients presenting for thoracic surgery may have dysfunctional antioxidant mechanisms and that such dysfunction may place them at increased risk of oxidative stress and lung injury following surgery.

The primary objective of this investigation therefore was to identify any association between total antioxidant capacity (TAC) and oxidative stress. TAC in patients presenting for thoracic surgery for lung resection was compared with a control group of healthy patients recruited from patients undergoing elective joint replacement. The group of patients going on to have lung resection were then followed up and a series of blood and urine samples taken for measurement of indices of oxidative and nitrosative stress. In addition we evaluated the novel lung injury biomarker Pentraxin 3 and its association with clinical outcomes in this patient group.

Principal findings

Thirty five patients undergoing lung resection were recruited to the study. One patient was excluded as their tumour was found not to be resectable at thoracotomy and so no lung resection was performed, and one patient was excluded because (contrary to the surgical plan at the time of recruitment), lung was resected via a video assisted thoracoscopic technique. Seventeen of the remaining 33 patients were male (51.5%) with a median age of 69.7 years (range 35.3-81.9). Patients underwent pneumonectomy (n=5, 15%), extended lobectomy (n=2, 6%), simple lobectomy (22, 67%) or sublobar resection (4, 12%).

Total antioxidant capacity versus peri-operative oxidative / nitrosative stress

Following recruitment and participation in the study, two patients were subsequently discovered to be taking excluded medications (antioxidant or anti-inflammatory drugs) and so were excluded from all TAC and oxidative / nitrosative stress analyses.

- Peri-operative changes in plasma malondialdehyde (MDA)

Plasma MDA was measured as a marker of oxidative stress. Levels fell post-operatively (Friedman's test, $p<0.01$, Figure 1), though there was no significant difference in plasma MDA immediately versus 24 hours post-operatively.

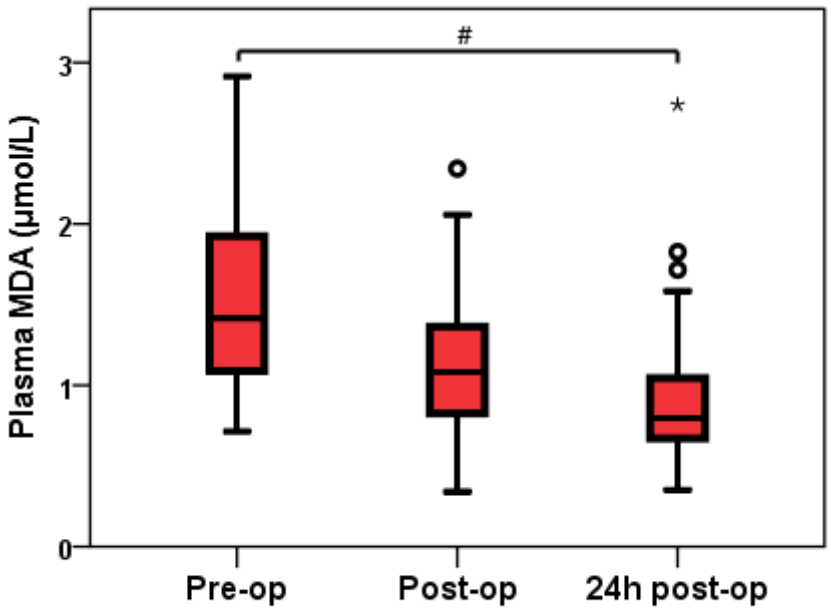


Figure 1. Peri-operative changes in plasma malondialdehyde (MDA) level.
#p<0.01, Friedman's test, n=30. Boxes show median and IQR, whiskers define range. Outliers defined as lying greater than 1.5 box-lengths from box edge (open circles), extreme outliers defined as lying greater than 3 box-lengths from box edge (asterisk).

- Peri-operative changes in plasma F2-isoprostane

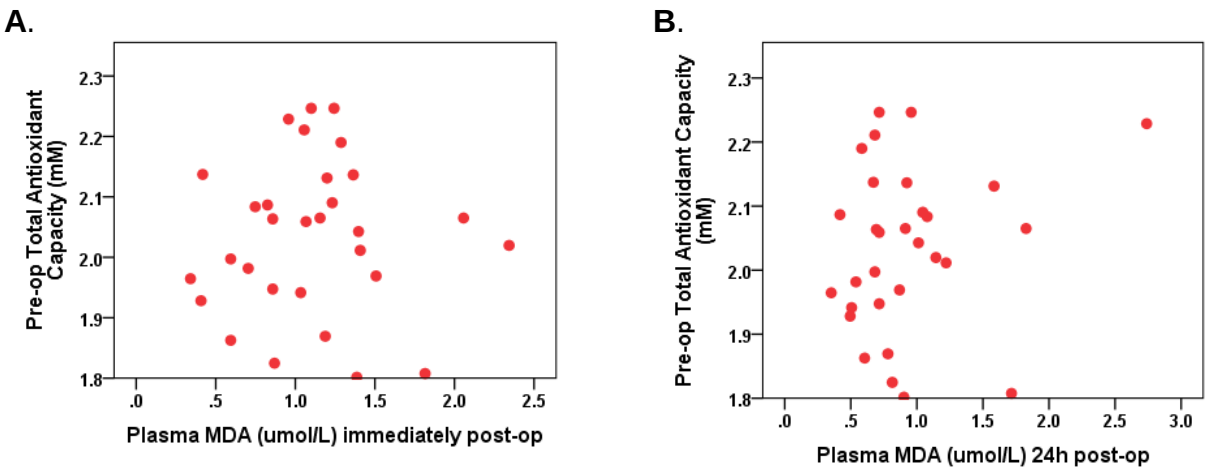
Plasma F2-isoprostane is another measure of oxidative stress; there were no significant changes in plasma F2-Isoprostane in the post-operative period (Friedman's test, $p=0.64$, data not shown).

- Peri-operative changes in urinary nitrate to creatinine ratio

Nitrate to creatinine ratio was used as a measure of nitrosative stress. Urine samples were available in 25 patients. There were no significant peri-operative changes in urinary nitrate to creatinine ratio (Friedman's test, $p=0.16$, not shown). These results were unchanged following exclusion of one patient whose results were extreme outliers at all time points ($p=0.16$).

- Association between TAC and post-operative oxidative and nitrosative stress

There was no association between pre-operative TAC and plasma MDA immediately nor 24-hours post-operatively ($r=0.07$, $p=0.70$ and $r=0.18$, $p=0.35$ respectively; Figures 2A & B).



Figures 2A and B. Total antioxidant capacity (TAC) versus post-operative oxidative stress as plasma malondialdehyde (MDA).
A: TAC v $MDA_{post\text{-}op}$, r=0.07, p=0.70. B: TAC v $MDA_{24h\ post\text{-}op}$, r=0.18, p=0.35. Spearman correlation for both, n=30.

Similarly, there was no association between pre-operative TAC and post-operative F2-Isoprostane or urinary nitrate/creatinine at either time point (Table 1).

Table 1. Association between pre-operative TAC and post-operative F2-isoprostane and urinary nitrate/creatinine

		Immediately post-op		**24 hours post-op**	
		F2-Isoprostane	*Urine nitrate/creatinine*	*F2-Isoprostane*	*Urine nitrate/creatinine*
Total Antioxidant Capacity	*r*	*0.00*	*-0.01*	*-0.03*	*-0.22*
	p	*0.98*	*0.98*	*0.88*	*0.30*
	n	*30*	*24*	*31*	*24*

Spearman correlation for all. *n,* no of patients included in analysis.

Total antioxidant capacity in patients with lung cancer versus healthy patient

A group of 13 'healthy' controls of similar age and sex distribution were recruited from a population of patients presenting for elective orthopaedic surgery. TAC was significantly higher in surgical patients with lung cancer than in 'healthy' controls (p<0.01, independent samples t-test; Figure 3).

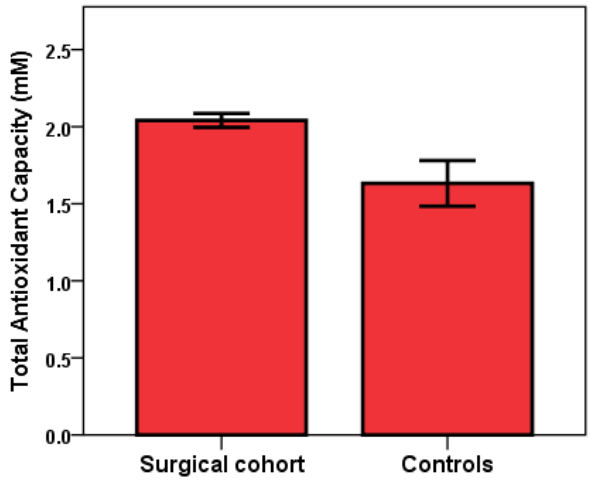


Figure 3. Total antioxidant capacity in surgical patients with lung cancer and in healthy control patients.
p<0.01, independent samples t-test. Bars are mean, error bars represent 95% CI. n=33 and 13 for surgical cohort and control group respectively.

Pentraxin 3

- Peri-operative changes in Pentraxin 3 (PTX3) level

PTX3 levels were not available immediately post-operatively for one patient; this patient was excluded from the longitudinal analysis of biomarker levels. Pre-operative PTX3 levels were <20 pg/ml in all patients whilst 24 hours post-operatively PTX3 values varied from <2 pg/ml to 2630 pg/ml reflecting in excess of a thousand fold variation in PTX3 response ($p<0.001$). Pre-operative and immediate post op levels were similar. In three patients, 24 hours post-operative PTX3 level remained below the level of detection of the assay (<2 pg/ml), a sensitivity analysis excluding these three patients was performed throughout.

Following the observation of strong association between PTX3 level and post-operative oxygenation, a number of post-hoc comparisons were undertaken in order to evaluate the utility of PTX3 as a plasma biomarker of lung injury. The results of these analyses are shown below.

Discussion

The principal findings of this study are that thoracic surgical patients with lung cancer undergoing lung resection have elevated TAC compared to healthy controls but there was no evidence of any increased peri-operative oxidative or nitrosative stress detectable by measurement of plasma malondialdehyde, F2-isoprostane or urinary nitrate / creatinine ratio. It is notable however, that oxidative stress at baseline (i.e. pre-operatively) in this surgical cohort was apparent, with plasma MDA and F2-isoprostane levels significantly elevated compared to reference values.

In this single centre study, we recruited 35 patients undergoing lung resection or lung cancer. In addition, in the absence of a well validated reference range, we recruited and measured baseline TAC in a group of ‘healthy’ patients free of the attributes of the thoracic population known to be

deleterious to antioxidant function (e.g. smoking, malignancy and severe lung disease). In view of the volatility of redox relationships being studied, samples were collected, stored and analysed according to a strict protocol and stored at -70^{o}C prior to analysis.

In this study there was no post-operative increase in any of the measured indices of oxidative / nitrosative stress. This is especially surprising in the case of plasma malondialdehyde; MDA had been selected as the studies primary outcome on the basis of several previous authors demonstrating consistent post-operative increases in plasma MDA[1-4]. Oxidative stress is the result of complex interactions resulting in accumulation of stable end products such as lipid hydroperoxides and aldehydes, which can be measured in the blood and urine. Isoprostanes are produced from free radical mediated peroxdation of arachidonic acid, in a manner which is independent from cyclooxygenase enzyme activity. F2-isoprostane levels are said to be a more reliable index of oxidative stress compared to other biomarkers[5]. Malondialdehyde is a product of lipid peroxidation and was commonly measured previously as thiobarbituric acid reacting substances, but this assay is non-specific and so more recently methods which are specific for MDA have been developed (such as high performance liquid chromatography as used in this study). Since these measures involved changes to lipids it should be realised that dietary changes may impact on circulating levels and it is recommended that several measures are undertaken[6]. With all measures of oxidative stress, the poor stability of the substances in plasma is an important consideration. We have shown previously that plasma MDA was markedly elevated in other inflammatory conditions such as sepsis[7].

There are several reasons which may account for the lack of further increases in biomarkers of oxidative stress. Firstly, previous studies have used volatile anaesthetic agents, compared to our local standard practice of total intravenous anaesthesia with propofol (as used in the current study). Propofol has a structure not unlike tocopherol (vitamin E) with potent antioxidant properties[8] and it is conceivable therefore that propofol 'protected' the patient from peri-operative oxidative stress.

Secondly, there may have been a dilutional effect of peri-operative intravenous fluids. Inselman et al adjusted plasma MDA level for plasma albumin concentration to mitigate the effects of haemodilution in patients undergoing cardiopulmonary bypass[9], though such an adjustment was not performed in the previously described thoracic studies[1, 2, 4]. In our institution however, it is standard practice to 'fluid restrict' thoracic surgical patients; as such any effect of haemodilution might be expected to be minimal.

Thirdly, the studies by Misthos et al demonstrated that post-operative increases in MDA was associated with the duration of one-lung ventilation (OLV)[1, 2]. In these studies two thirds of the study cohort underwent OLV for greater than 90 minutes. In contrast, in the current study mean (SD) duration of OLV was 75.1 (24.2) minutes. It is possible therefore that there was less oxidative insult than expected.

As the function of endogenous antioxidant mechanisms relies on a complicated interplay between enzymatic and non-enzymatic antioxidants both within the cell and in the extracellular fluid, TAC in plasma was chosen as a composite measure of 'total antioxidant' function to provide more biologically relevant information than that obtained by measuring levels of individual selected antioxidants[10]. Fundamental to this, we hypothesised that the secondary oxidative burden of lung cancer would result in lower TAC, in patients undergoing lung resection compared to healthy patients without cancer. Previous studies reported an 11% increase in TAC in individual patients

three months after lung resection of tumour[11] and a 10% reduction in TAC in patients with lung cancer compared to controls (smokers with respiratory disease)[12]. In contrast to these findings, we found that patients scheduled for lung resection had significantly elevated TAC when compared to healthy patients of a similar age and sex distribution. This could be due to genuinely higher measured antioxidant capacity, as a result of method artefact, or due to interference from other substances not taken into consideration such as drugs or metabolic/breakdown products.

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Post-hoc analyses: Pentraxin 3 as a novel biomarker of lung injury - Evidence from a human one-lung ventilation model

Measurement of biomarkers informative of the pathogenesis or clinical progress of lung injury could offer the potential to allow early identification of patients at risk, aid with diagnosis, guide clinical management, assess severity of disease, stratify risk and predict outcome.

Putative properties of the 'ideal' lung injury biomarker include:

- High sensitivity and specificity
- Variation in proportion to the severity of injury
- Association with clinically important outcomes

Pentraxin 3 was thus compared against these properties. Chest X-ray scores were determined by two observers, blinded to the patients clinical and biochemical progress. A modified (for use after lung resection) lung injury score (LIS) was then calculated, where the chest x-score was 'corrected' for the number of 'scoreable' quadrants (i.e. excluding those from which lung had been resected or where there was residual pneumothorax). A (modified) LIS of > 1.5 was defined as a 'positive' outcome (Indicating presence of 'lung injury').

A PTX3 level of 767.2pg/ml 24h post-op had 100% sensitivity and 67% specificity in predicting a modified LIS of >1.5 24h post-operatively with an area under the receiver operating characteristic curve (AUROCC) of 0.81 (95% CI 0.63-0.99).

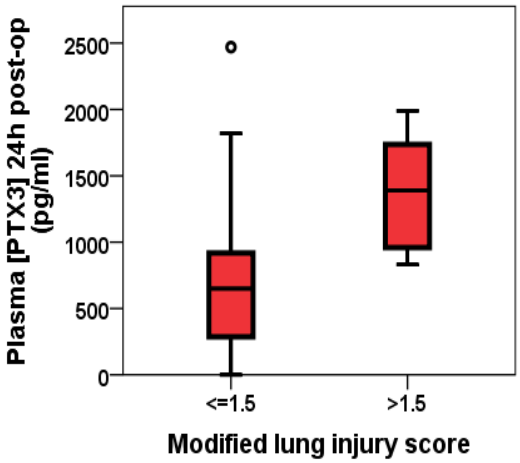

Figure 1. Modified lung injury score on post-operative day one versus PTX3 concentration. p=0.03; Mann-Whitney U test. n=21 (ABG results were not available on POD 1 in 12 patients).

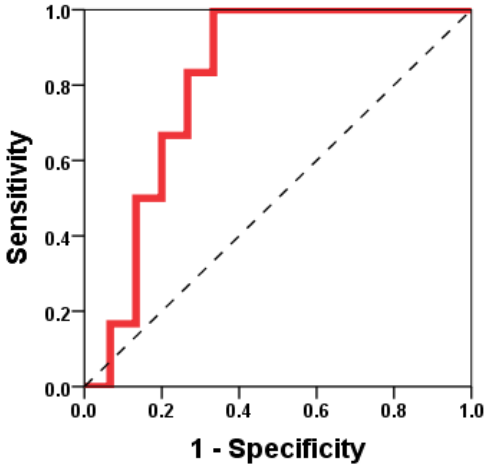

Figure 2. Predictive value of PTX3 24h post-operatively for modified lung injury score greater than 1.5 on post-operative day one. AUC=0.81 (95% CI=0.62-0.99).

PTX3 level 24h post-op was positively associated with CXR score (r=0.38; p=0.04) and there was a trend towards negative association with PaO_2:FiO_2 ratio (r=-0.40; p=0.08). PTX3 level 24h post op tended to be higher in patients with prolonged critical care unit stay (defined as greater than 48h,

p=0.08) [Figure 3], and was associated with significantly longer hospital stay (r=0.44; p=0.01) [Figure 4].

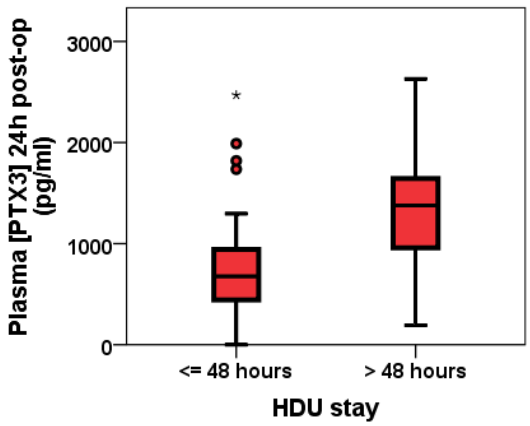

Figure 3. PTX3 level 24 hours post-operatively value versus duration of HDU stay. p=0.08, Mann-Whitney U test, n=33.

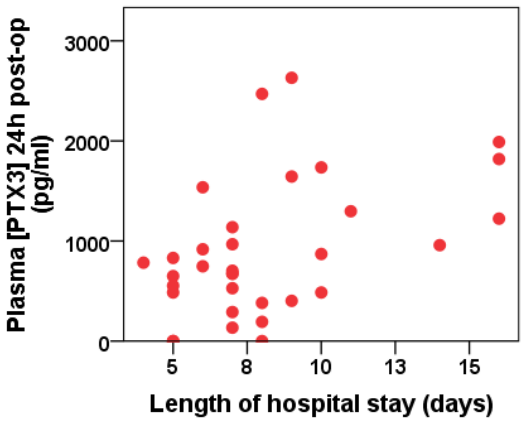

Figure 4. Association between hospital stay and PTX3 level 24 hours post-operatively. r=0.44, p=0.01, Spearman's rho; n=33.

Discussion

Pentraxin 3 (PTX3) compared favourably with the properties of the ideal lung injury biomarker and appeared to identify a population of patients with elevated post-operative modified Lung Injury Score with high sensitivity. *Johnson* has suggested that in the field of clinical risk prediction, an AUROCC of *"0.75 is good and greater than 0.8 is exciting"*[1]. In this context the values obtained in the current study are encouraging, though it must be acknowledged the confidence intervals for these estimates are wide, reflecting the modesty of the sample size.

Future work

We a currently working to further validate Pentraxin 3 as a biomarker of lung injury in a larger, combined cardiac and thoracic surgical population.

References

1. Johnson RG. The ghost of christmas future: predicting pneumonia after cardiac operations. Crit Care Med. 2014;42(5):1302-3.

Dissemination

Oral presentations

Endogenous antioxidant function and oxidative stress following lung resection 2015
Anaesthetic Research Society (London)

Plasma dimethylarginines in a post-surgical model of the acute inflammatory response 2012
Anaesthetic Research Society (Aberdeen)

The novel biomarker pentraxin 3 may aid risk stratification in the early post-operative period following lung resection 2012
European Association of Cardiothoracic Anaesthetisists (Amsterdam)

Early Experience with a Panel of Acute Lung Injury Biomarkers after Lung Resection 2011
Glasgow and West of Scotland Society of Anaesthetists (Glasgow)

Early Experience with a Panel of Acute Lung Injury Biomarkers after Lung Resection 2011
Association of Cardiothoracic Anaesthetisists (Leicester)

Poster Presentations

Changes in plasma 25-hydroxy-vitamin D in a surgical model of the acute inflammatory response 2013
Scottish Intensive Care Society (St Andrews)

Plasma dimethylarginines in a post-surgical model of the acute inflammatory response 2012
Medical Research Council (London)

Early Experience with a Panel of Acute Lung Injury Biomarkers after Lung Resection 2012
Scottish Intensive Care Society (St Andrews)

Publications

1. Arthur A, McCall PJ, Macfie A, Jolly L, Kinsella J, Kirk A, Shelley B. Type III procollagen as a biomarker of susceptibility to ARDS (Letter)? *Intensive Care Medicine* 2015; *in press.*

2. Shelley B, Macfie A, Tanner O, Galley H, Kinsella J. Pentraxin 3 as a novel biomarker of lung injury; evidence from a human one-lung ventilation model (Abstract). *Crit Care Med* 2014; 42: A1525.

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5. Shelley B, McSharry C, Macfie A, Kinsella J. Biomarkers of acute lung injury after lung resection (Abstract). *Anaesthesia* 2012; 67: 554.

Further funding

- resulting from / building upon pilot data obtained during the conduct of this study.

The novel inflammatory biomarker 'soluble urokinase-type plasminogen activator receptor' (suPAR); does it have a prognostic role following cardiac surgery? 2014
National Institute of Academic Anaesthesia / Royal College of Anaesthetists – Ernest Leach Fund (Dr Philip McCall - £2851)

Does Pro-collagen Peptide-III predict susceptibility to ALI after cardiac surgery? 2014
Scottish Society of Anaesthetists (£1000)

The pulmonary vascular / right ventricular response to lung resection. 2012
National Institute of Academic Anaesthesia - Association of Cardiothoracic Anaesthetists Project Grant (£26932)

Utility of a panel of acute lung injury biomarkers following lung resection – a pilot study. 2011
National Institute of Academic Anaesthesia / Royal College of Anaesthetists – Nuffield Award (£2500)