

## **SSA Research Grant 2014 Report (£1000)**

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The work was undertaken by CS as part of his Medical Research Scotland (MRS) funded Vacation Scholarship under the supervision of PM, JK and BS.

### **Project: Does Procollagen Peptide III (PCP-III) predict susceptibility to lung injury after cardiac surgery?**

#### **Lay Summary**

Some patients become unwell with breathing problems following heart-surgery. This can be as a result of the surgery or the supportive techniques used during the operation. Breathing problems range from mild to severe with some patients requiring extra support from a ventilator.

It is difficult to predict which patients will develop difficulties. Measurements performed in blood samples may help predict which patients are at risk. This study will analyse samples already collected from heart-surgery patients to see if we can predict which patients will develop breathing problems. In the future this may allow care to be tailored for individual patients.

#### **Original Project Aims and Objectives**

*To determine if pre-operative Procollagen Peptide-III levels are predictive of post-operative oxygenation and lung injury in patients undergoing cardiac surgery.*

We previously demonstrated that pre-operative PCP-III levels were associated with post-operative oxygenation following lung resection. Cardiac surgery is associated with high rates of complications, with poor oxygenation and lung injury being common. It was hypothesised that PCP-III may be able to predict susceptibility to lung injury and / or poor oxygenation after cardiac surgery.

#### **Methods**

With research ethics approval and informed patient consent, serum samples were collected from 90 patients undergoing cardiac surgery. Preoperative PCP-III analysis was performed using commercially available enzyme-linked immunosorbent assay kits by a trained laboratory researcher.

Oxygenation was calculated as a ratio of arterial oxygen to the fraction of inspired oxygen (P:F Ratio) on postoperative (POD) days 1 and 2. A P:F ratio of <26.6kPa (200mmHg) was

deemed 'poor oxygenation' whilst a P:F ratio above this was deemed 'normal' in keeping with the definition of ARDs and other research<sup>1,2</sup>.

A lung injury score (LIS), was calculated on POD 1 and 2<sup>3</sup>. This score had the following parameters scored out of 4: number of lung quadrants affected by alveolar consolidation, level of hypoxaemia, amount of PEEP required, respiratory compliance. A chest x-ray and a hypoxaemia value were the minimum parameters required for a score to be calculated. Lung injury is not present if the score is 0, mild-to-moderate if 0.1-2.5 and severe if >2.5. All required data was collected and synthesised from an ICU clinical information system. Worst values for P:F ratios and LIS were used for each day. All X-rays were reported by CS and a sample were reported by BS and PM for the assessment of inter-observer variability.

Statistical analysis was performed using SPSS. Data was assessed for normality. Categorical data is presented as frequency (%) and continuous data is presented as mean (SD) or median (IQR) as appropriate to distribution. Correlations were assessed using Spearman's coefficient. Between groups comparisons were performed using the Mann-Whitney U-test or independent T-test. Kruskal-Wallis test or ANOVA was used to compare multiple groups. Area Under the Receiver Operator Curve (AUROC) was calculated to evaluate the discriminative capability of PCP-III to predict poor oxygenation or lung injury. Statistical significance was  $p < 0.05$ .

## **Results**

Ninety patients were recruited and 88 (19 female, 69 male) were included for analysis. Of the original cohort, two patients had their surgeries cancelled for clinical reasons. Eighty-one participants had samples available and were included in the PreOP PCP-III analysis.

Median PCP-III levels were 0.078ng/mL (0.078-0.136) for all patients.

### *P:F Ratio*

On POD1, the median P:F ratio was 18.1kPa (11.9-32.6). Twenty-eight patients had normal oxygenation and sixty had poor oxygenation. PreOp PCP-III levels were 0.078ng/mL (0.078-0.111) for normal and 0.078ng/mL (0.078-0.194) for poor oxygenation and were similar when compared using a Mann-Whitney U test ( $p=0.34$ ). On POD2, the median P:F ratio based on 44 patients was 13.18kPa (10.8-23.0). Four patients had normal oxygenation and forty had poor oxygenation. PreOp PCP-III levels were 0.235ng/mL (0.110-0.437) for normal and 0.078ng/mL (0.078-0.213) for poor oxygenation respectively and were similar when compared using a Mann-Whitney U test ( $p=0.18$ ) (Figure 1). There was no correlation between PreOp PCP-III and P:F ratios on POD1 ( $r=-0.06$ ;  $p=0.60$ ) and POD2 ( $r=0.15$ ;  $p=0.34$ , Spearman's correlation coefficient).

### *Lung Injury Score*

On POD1, the median LIS based on 76 patients was 1.13 (0.5-2.0). Thirteen patients had no lung injury, fifty-five patients had mild-to-moderate lung injury and eight patients had severe lung injury with median PreOp PCP-III levels of 0.078ng/mL (0.078-0.078), 0.078ng/mL (0.078-0.126) and 0.078ng/mL (0.082-0.310) respectively. These were similar when compared using a Kruskal-Wallis test ( $p=0.16$ ). On POD2, the median LIS based on 31

patients was 1.0 (1.0-1.0). Three patients had no lung injury, twenty-three patients had mild-to-moderate lung injury and five patients had severe lung injury with median PreOp PCP-III levels of 0.116ng/mL, 0.083ng/mL(0.078-0.159) and 0.078ng/mL(0.078-0.149) respectively. These were similar when compared using a Kruskal-Wallis test ( $p=0.42$ )(Figure 2). There is no correlation between PreOp PCP-III and LIS on POD1 ( $r=0.16$ ;  $p=0.19$ ) and POD2 ( $r=-0.17$ ;  $p=0.38$ , Spearman's correlation coefficient).

In view of similar PCP-III levels between groups, ROC curves were not produced.

### **Discussion**

The main findings of this study were that PCP-III levels did not correlate to LIS or to oxygenation levels. Further, PCP-III median levels did not differ between those patients that had poor oxygenation levels and those that had normal oxygenation levels, nor did levels differ between patients that had non-existent, mild-to-moderate or severe LIS. As a result of this, there was no discriminative capability of PCP-III in identifying patients with lung injury or poor oxygenation.

PCP-III has been shown to be a marker of fibroproliferation in ARDS, as well as having prognostic capabilities in patients with acute lung injury<sup>4,5</sup>. Rising levels of PCP-III (and PCP-III by-products) are thought to reflect increased collagen synthesis and fibro-proliferation<sup>4</sup>. When this increase in collagen occurs in the lungs, oxygenation decreases as a result of impaired gas exchange<sup>6</sup>.

In a recent pilot study, PCP-III levels demonstrated a negative association to oxygenation immediately after and 24 hours after surgery<sup>7</sup>. This negative association also existed between *preoperative* PCP-III levels and 6 hour post-operative oxygenation. This study looked at 22 patients undergoing lung resection for primary lung cancer, and was a one lung ventilation population. Our study looked to expand on these findings by looking at lung injury and oxygenation in a large cohort of cardiac surgery patients.

Cardiac surgery has been shown to be associated with lung injury<sup>8</sup>. It was therefore hypothesised that preoperative PCP-III could be used to predict the development of this complication in a cardiac surgery population. Considering the previous findings, the non-significant results were surprising. This may be for a number of reasons. Firstly, it should be considered that our study examined a novel population - cardiac surgery patients - and that these patients may respond physiologically differently to lung injury than those in other cohorts. Although, ARDS and lung injury have been shown to have a homogenous pathophysiology<sup>6</sup>. Also, the patients from the previous study all had lung cancer and, as a result, were more likely to have a greater extent of lung fibrosis due to the nature and causes of their disease. PCP-III levels have been shown to be elevated in patients with lung fibrosis and lung cancer<sup>9,10</sup>. It may be that these patients have a higher basal level of PCP-III, thereby explaining the difference between our study and the previous pilot study. It's possible that thoracic surgery patients are drastically different from cardiac patients in regards to susceptibility to lung injury and that PCP-III may be predictive in one population but not the other. A final explanation could be that the results from the pilot study were due to a type 1 alpha error caused by small number of patients, meaning that there is no effect in the wider population.

In summary, this study showed that there was no association between preoperative PCP-III levels and lung injury and oxygenation.

28th September 2015

## References

1. Ranucci M, Ballotta A, Rovere MT, Castelvechio S. Postoperative Hypoxia and Length of Intensive Care Unit Stay after Cardiac Surgery: The Underweight Paradox? *PLoS One*. 2014;9(4): e93992
2. Suzuki T, Kurazumi T, Toyonaga S et al. Evaluation of noninvasive positive pressure ventilation after extubation from moderate positive end-expiratory pressure level in patients undergoing cardiovascular surgery: a prospective observational study. *J Intensive Care*. 2014 Jan 23;2(1):5
3. Murray JF, Matthay MA, Luce JM, Flick MR. An expanded definition of the adult respiratory distress syndrome. *Am Rev Respir Dis*. 1988;4(3):720–723.
4. Forel JM, Guervilly C, Hraiech S, Voillet F et al. Type III procollagen is a reliable marker of ARDS-associated lung fibroproliferation. *Intensive Care Med* 2015;41(1):1–11.
5. Chesnutt AN, Matthay MA, Tibayan FA, Clark JG. Early detection of type III procollagen peptide in acute lung injury—pathogenetic and prognostic significance. *Am J Respir Crit Care Med* 1997;156(3):840–845.
6. Ragaller M and Richter T. Acute Lung Injury and Acute Respiratory Distress Syndrome. *J Emerg Trauma Shock*. 2010;3(1): 43–5
7. Arthur A, McCall PJ, Macfie A, Jolly L, Kinsella J, Kirk A, Shelley B. Type III procollagen peptide as a biomarker of susceptibility to ARDs? *Intensive Care Med, Correspondence*. 2015;41:568–569
8. Stephens RS, Shah AS, Whitman GJ. Lung injury and acute respiratory distress syndrome after cardiac surgery. *Ann Thorac Surg*. 2013 Mar;95(3):1122-9
9. Maasilta P, Salonen EM, Vaheri A, Kivisaari L, Holsti LR, Mattson K. Procollagen-III in serum, plasminogen activation and fibronectin in bronchoalveolar lavage fluid during and following irradiation of human lung. *Int J Radiat Oncol Biol Phys*.1991;20(5):973-80.
10. Hatahara T, Igarashi S, Funaki N. High concentrations of N-terminal peptide of type III procollagen in the sera of patients with various cancers, with special reference to liver cancer. *Gan*. 1984 Feb;75(2):130-5.

**Table 1. Baseline Characteristics; Values are number (%), mean (SD) or median (IQR)**

<b>Characteristics</b>	<b>All Patients (n=88)</b>
<b>Age (Years)</b>	66 (59-72)
<b>Female Gender n(%)</b>	19/88 (21.6%)
<b>Weight (Kg)</b>	81 (15.8)
<b>Actual Mortality (%)</b>	1/88 (1.1%)
<b>Co-Morbidities n (%)</b>	68/88 (77.3%)
Previous MI*	28/88 (31.8%)
Arterial Hypertension	59/88 (67.0%)
Left Main Stenosis	50/82 (61.0%)
Triple Vessel Disease	42/82 (51.2%)
<b>Intervention type n(%)</b>	
CABG	56/88 (63.6%)
AVR	15/88 (17.0%)
MVR	8/88 (9.1%)
CABG + AVR	4/88 (4.5%)
Other	5/88 (5.7%)
<b>CPB Time (Min)</b>	84 (66-112.5)
<b>Aorta Clamp Time (Min)</b>	58 (40.5-74.5)
<b>Surgical time (Min)</b>	202.5 (180-255)
<b>Ventilation Duration (Hr)</b>	7 (4.1-12.4)
<b>Intensive Care Unit Stay (Hr)</b>	23 (21.5-46)
<b>Hospital stay (Days)</b>	7 (6-12)

**MI (Myocardial Infarction)**

**CPB (Cardiopulmonary Bypass)**

**CABG (Coronary Artery Bypass Graft)**

**AVR (Aortic Valve Replacement)**

**MVR (Mitral Valve Replacement)**

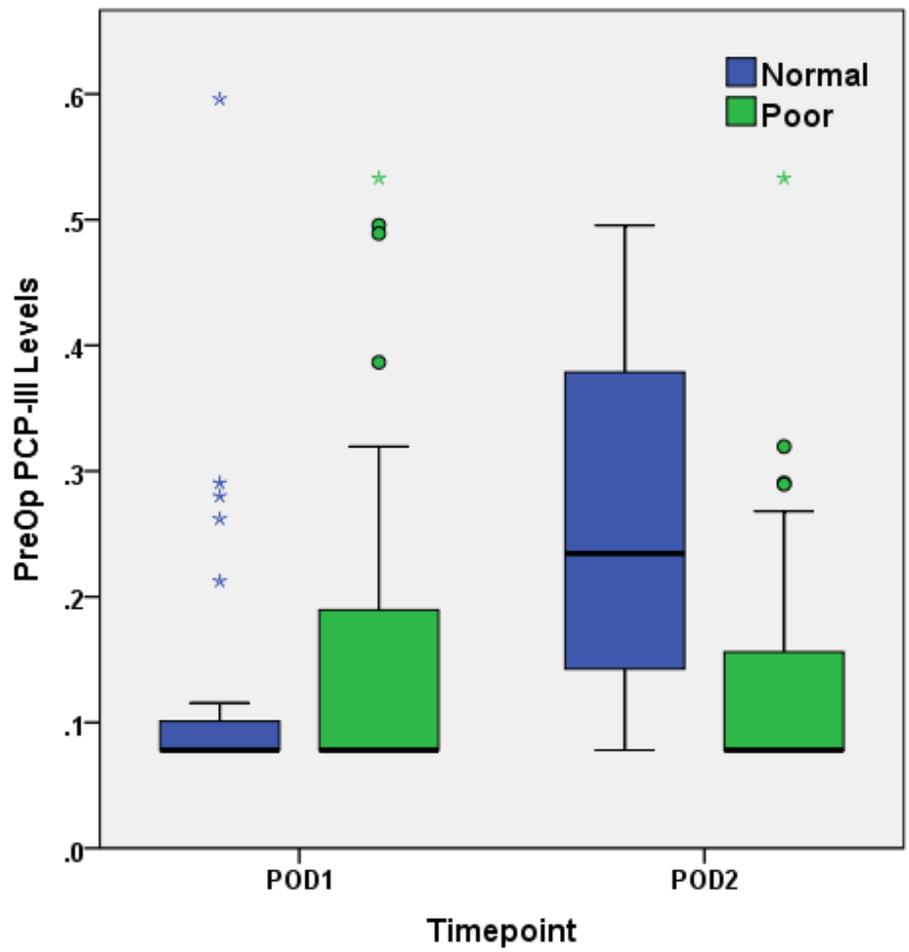


Figure 1: Oxygenation and Procollagen Peptide III (PCP-III) levels on postoperative days (POD) 1 and 2

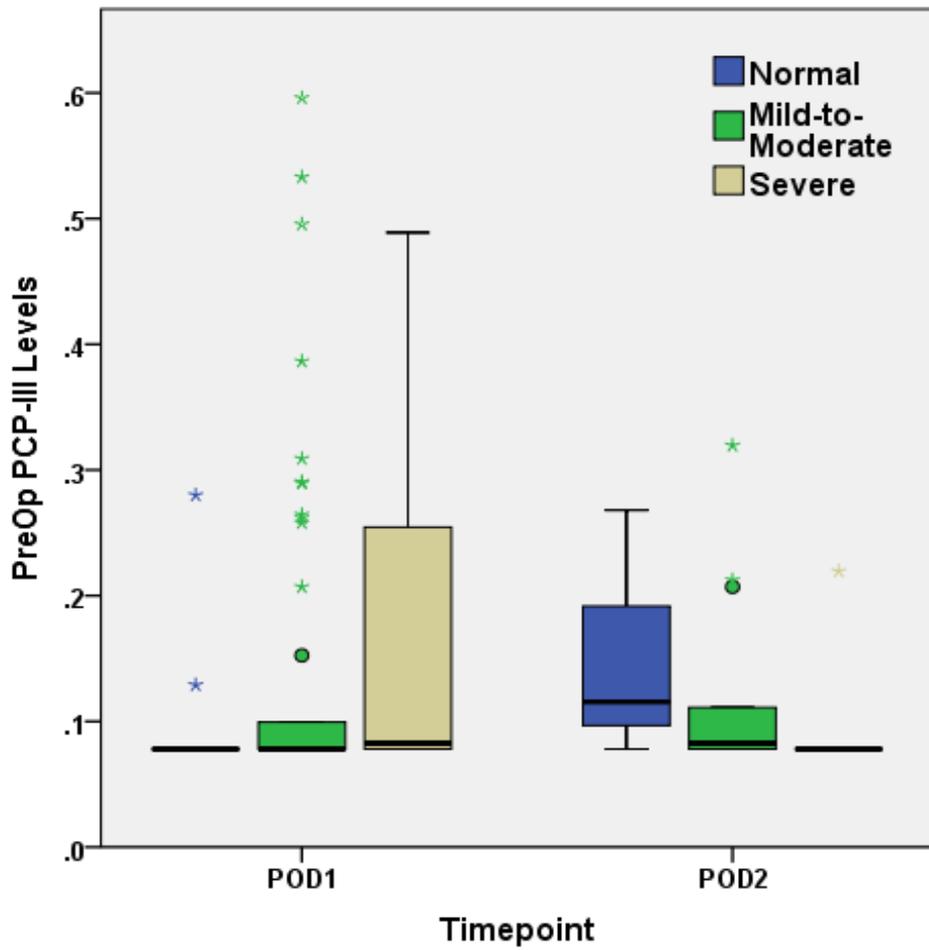


Figure 2: Lung Injury Score and Procollagen Peptide III (PCP-III) levels on postoperative days (POD) 1 and 2