

Exhaled nitric oxide levels are decreased in children with cyanotic congenital heart disease and correlate with the inflammatory response to cardiopulmonary bypass

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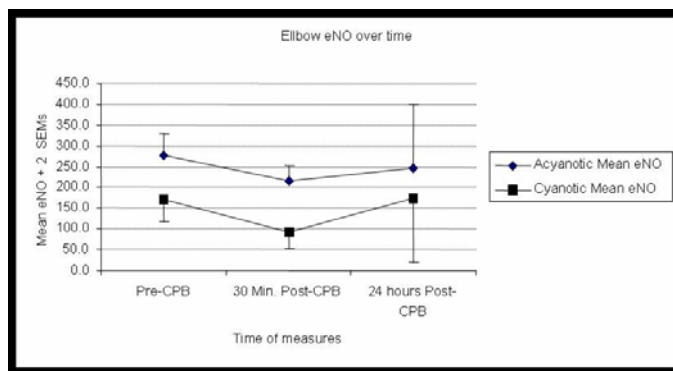
Introduction: The systemic inflammatory response syndrome (SIRS) to cardiopulmonary bypass (CPB) is well documented. It can cause pulmonary dysfunction by damaging the pulmonary vascular endothelium resulting in increased pulmonary vascular resistance and decreased airway compliance. Nitric oxide (NO) is produced continuously by the pulmonary vascular endothelial cells and airway epithelial cells and plays an essential role in the physiological control of the lung and in the pathophysiology of many disease states. Measuring exhaled NO (eNO) levels in children has recently become an important diagnostic and monitoring tool in asthma. There has been some interest in measuring eNO in patients undergoing CPB because changes in the levels may help monitor lung injury (1). The cytokine response to CPB is complex and it involves both pro-inflammatory mediators such as IL6 and anti-inflammatory mediators such as IL10. Other mediators such as VEGF are important in both pulmonary vascular leak and the regulation of NO production post-CPB. The cytokine inflammatory response may also be triggered by other stimuli such as hypoxia, so it is possible that children who have cyanotic congenital heart disease may have an ongoing chronic inflammatory state and differences in NO production. We therefore hypothesized that:

1. Children with cyanotic heart lesions would produce less eNO than children with acyanotic heart lesions.
2. IL6, IL10 and VEGF serum levels would correlate with eNO levels
3. Changes in airway pressures during mechanical ventilation would correlate with eNO levels

Methods: A prospective, observational, non-randomized study was designed. After IRB approval and informed consent, 50 children undergoing cardiac surgery with CPB were enrolled. Sampling of blood and eNO occurred at three time points: immediately before skin incision, and at 30 minutes and 24 hours after CPB. Exhaled NO was sampled under identical ventilator parameters for all subjects from the elbow of the endotracheal tube using a Sievers Nitric Oxide Analyzer NOA 280 (Ionics/GE, Boulder, CO). Blood was sampled from the arterial catheter placed for surgery and was immediately centrifuged and stored at -70C for later analysis. Biomarker analysis (IL6, IL10, VEGF) was performed using a Luminex platform assay. Clinical data was collected on each patient and stored in a secure database. Anesthetic and CPB management was standard for all cases.

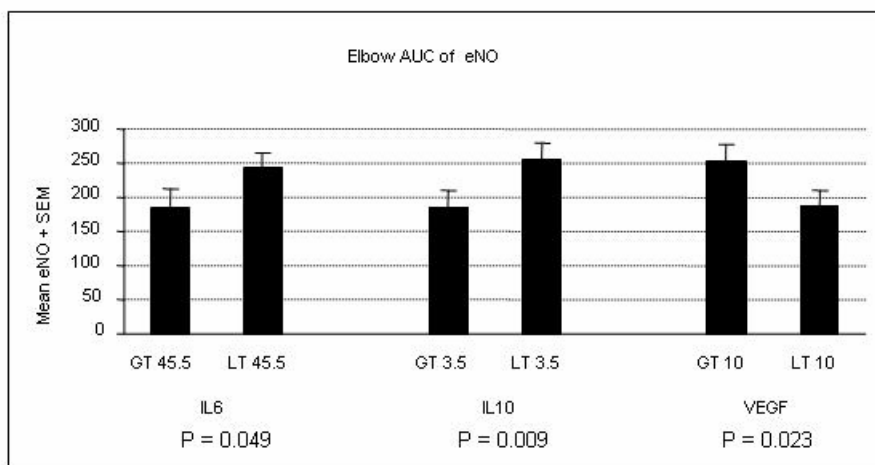
Results: Exhaled nitric oxide levels were analyzed as area under the curve and a mixed effect model was used which accounts for the correlation within repeated measures of a patient. In the analysis assessing the eNO response profiles between cyanotic and acyanotic patients, the main effect of cyanotic status was significant ($p=0.03$). Figure 1 demonstrates that both groups have a decreased eNO 30 minutes post-CPB but this level recovers to pre-CPB levels by 24 hours. However, at all time points the cyanotic group (16/50 patients) produce less eNO than the acyanotic group.

Figure 1



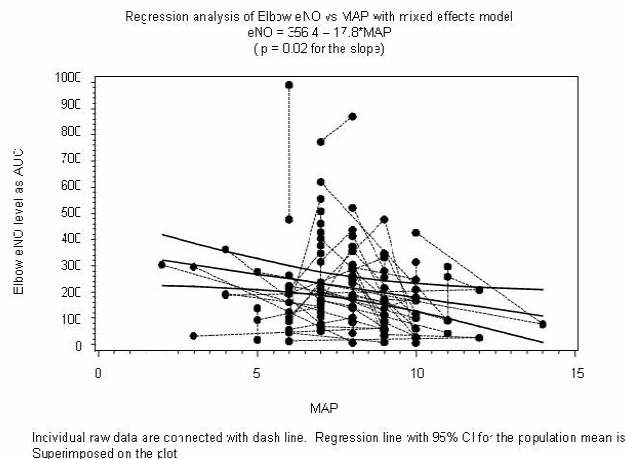
In the analysis of the biomarkers, both IL6 and IL10 increased at 30 minutes post-CPB and then decreased again at the 24 hour time point (up-down pattern). For VEGF, there was an opposite effect with a decrease 30 minutes post-CPB followed by an increase at 24 hours (down-up pattern). Some of the biomarker results were outside the lower or upper range of the assays. Therefore in the statistical analysis, assay results were dichotomized as either greater than (GT) or less than (LT) the median observed value and compared using the mixed effects model. Figure 2 demonstrates the negative correlation between IL6 and IL10 with eNO, and the positive correlation between VEGF with eNO.

Figure 2



In the analysis of ventilator airway pressures and eNO levels as area under the curve (AUC), mean airway pressures (MAP) and peak inspiratory pressures (PIP) were entered in the mixed effect model as continuous variables. The estimates of slope were significant with p-values of 0.045 for PIP and 0.02 for MAP. Figure 3 shows the data for MAP.

Figure 3



Discussion: These results show that children with cyanotic congenital heart disease produce less eNO than acyanotic children both at baseline and post-CPB. Humpl et al. have already demonstrated that changes in eNO levels are not due to changes in pulmonary blood flow (2) so one explanation may be that chronic hypoxia causes an inflammatory response which causes pulmonary cellular injury and decreased production of NO. The results show that eNO is a useful marker of the inflammatory changes taking place during CPB. The expected increase and then decrease in both IL6 and IL10 (up-down pattern) show a significant negative correlation with changes in eNO levels (down-up pattern). One mechanism which may explain this is the SIRS causes an acute decrease in constitutive nitric oxide synthase (NOS) production followed by a later recovery in NO levels from inducible NOS production. The results also show a positive correlation of VEGF and NO levels which suggest that as VEGF accumulates it causes increased NO production at the 24 hour time point by upregulation of NOS. The increase in MAP as eNO levels decrease also suggests pulmonary cellular dysfunction following CPB.

Conclusion: These results suggest that eNO may be a useful monitor of the inflammatory response to CPB and could be used to monitor various anti-inflammatory strategies. This is very attractive as eNO levels can be measured in real-time and are non-invasive, safe and inexpensive.

Refs:

1. Marczin, N., D. Royston, and M. Yacoub, *Exhaled nitric oxide in patients undergoing cardiothoracic surgery: a new diagnostic tool? in Acute Respiratory Distress Syndrome: cellular and molecular mechanisms and clinical management*, Matalon and Sznajder, Editors. 1998, Plenum Press: New York. p. 365-374
2. Humpl, T., et al., *Levels of exhaled nitric oxide before and after surgical and transcatheter device closure of atrial septal defects in children*. *J Thorac Cardiovasc Surg*, 2002. **124**(4): p. 806-10