**EFFECT OF CIGARETTE SMOKE EXTRACT ON NEUTROPHILS ISOLATED FROM HUMAN PERIPHERAL BLOOD**

Heng Zhong¹, Melinda Tooze¹, Lisa G Wood¹,², Jodie L Simpson¹,², Peter AB Wark¹,²
¹Centre for Asthma and Respiratory Disease, The University of Newcastle, NSW, 2305. ²Department of Respiratory and Sleep Medicine, John Hunter Hospital, NSW, 2305.

**Background**
Increased numbers of neutrophils are found in the lungs of COPD patients, which contribute to airway inflammation. While cigarette smoke exposure is a major risk factor for COPD it is unclear how cigarette smoke modifies neutrophil function and activity.

**Aims**
This study aimed to assess the effect of cigarette smoke extract (CSE) on neutrophils in an in vitro model.

**Methods**
Neutrophils were isolated from peripheral blood donated by four (4) healthy subjects using Percoll density gradient centrifugation. Neutrophil purity was measured by May-Grunwald Giemsa (MGG) and Chromotrope 2R (C2R) staining. Neutrophils were only used if purity was ≥95%.

Cigarette smoke extract (CSE) was made according to the following method. Briefly, a research-grade cigarette (Tobacco Health Research - University of Kentucky, USA), with the filter removed, was connected, via tubing, to a 60mL syringe. The cigarette was then lit and the smoke was drawn in to the syringe. Once the syringe was full, the collected smoke was expelled, or bubbled, in to 10mL RPMI media containing 1% foetal bovine serum (FBS) (1% RPMI). This process was repeated until the entire cigarette had been ‘smoked’. The smoke extract from one cigarette bubbled through 10mL media was regarded as 100%. This was diluted, using 1% RPMI, to prepare the 1% and 10% CSE solutions.

Neutrophils were seeded into 24-well cell culture plates at a final concentration of 1x10⁶ cells/well. Wells were then exposed to either CSE (1% or 10%) or a media control (1% RPMI). Samples were collected at 2, 4 and 18hrs and analysed for viability of neutrophils, by trypan blue exclusion, and CXCL8 release, by ELISA (R&D Systems).

**Participant Information**
All four (4) subjects were no-smoking controls with no evidence of airflow obstruction or chronic respiratory symptoms.

<table>
<thead>
<tr>
<th>Subject Group</th>
<th>FEV1%, Predicted Mean (SD)</th>
<th>Age (Years), Mean (SD)</th>
<th>Sex (M:F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Controls</td>
<td>99.2 (17.14)</td>
<td>27.75 (2.06)</td>
<td>2:2</td>
</tr>
</tbody>
</table>

**Results**
At all concentrations of CSE, neutrophil viability was significantly reduced at 18hrs when compared with the 2 and 4hr timepoints. The 10% CSE also resulted in a significant reduction in the viability of neutrophils at 18hrs when compared to 1% CSE and the media control.

**Conclusion**
Cigarette smoke extract altered neutrophil viability and release of CXCL8. A lower dose (1%) of CSE led to more CXCL8 release from neutrophils compared to 10% CSE and media. This may be due to oxidative stress and activation of the NF-κb pathway. A higher dose (10%) of CSE led to less CXCL8 release, which may be due to increased neutrophil death.

**Future Work**
We will examine the effect of cigarette smoke extract on neutrophils from healthy controls, smoking controls and subjects with COPD, in terms of apoptosis/necrosis, TLRs and NF-κb signalling pathways, oxidative stress and chemotaxis ability.

**Acknowledgement**
The authors would like to thank Amber Smith and Kevin Oreo for their clinical and technical assistance and all the volunteers’ for their participation.

In partnership with our community

**References**

1. Centre for Asthma and Respiratory Disease, The University of Newcastle, NSW, 2305.
2. Department of Respiratory and Sleep Medicine, John Hunter Hospital, NSW, 2305.