

## Airway Surfactant Protein D Deficiency in Adults With Severe Asthma

*Rose-Marie A. Mackay, PhD; Christopher L. Grainge, MD, PhD; Laurie C. Lau, PhD; Clair Barber, BSc; Howard W. Clark, MD, DPhil; and Peter H. Howarth, MD, DM*

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**e-Appendix 1.****SP-D antibodies.**

The same 'in house' antibody was used as the capture antibody and for the detection of SP-D by ELISA. The antibodies were raised in rabbits against a recombinant fragment of SP-D, which consists of the head neck and a small part of the collagen-like region. This is considered to be the active part of the protein although cleavage effects functionality, the fragment is not as efficient as agglutination as the native full-length protein, it remains active. Therefore, because there is evidence of degradation only in the severe asthmatics it suggests that complete cleavage is the possible mechanism for lower levels of SP-D seen in this group.

**Enzyme linked immunosorbent assay (ELISA)**

Maxisorp 96-well plates (Nunc) were coated overnight at 4 °C with rabbit anti- SP-D antibody (d: 1/1000) in sodium carbonate coating buffer (15mM Na<sub>2</sub>CO<sub>3</sub>, 35mMNaHCO<sub>3</sub>, pH 9.6). Plates were washed 3 times with 300µl per well PBS-T and blocked for 1 hour at room temperature with 300 µl per well of blocking buffer (PBS-T with 3% BSA). Plates were washed as before and incubated for 1 hour at room temperature with 100 µl per well of biotinylated rabbit anti-SP-D antibody in blocking buffer (d: 1/1000). Plates were washed again and incubated with 100 µl per well of Horseradish peroxidase-linked anti-rabbit IgG in blocking buffer (d: 1/1000) for 1 h at room temperature. Plates were washed once more and incubated for 15 min with 100 µl per well of TMB peroxidase reagent (BioRad). The reaction was terminated with 50 µl per well of 1N H<sub>2</sub>SO<sub>4</sub> and plates were read at 450 nm.

**e-Table 1. Patient characteristics.**

| <b>Asthma</b>  | <b>Age<br/>Mean (range)<br/>SD</b> | <b>N</b> | <b>Male</b> | <b>Ex-<br/>Smokers</b> | <b>Atopy</b> | <b>Percentage<br/>predicted FEV<sub>1</sub><br/>Mean (range)<br/>SD</b> | <b>Percentage<br/>predicted FVC<br/>Mean (range)<br/>SD</b> | <b>Inhaled steroid<br/>(µg)<br/>Median (IQR)</b> | <b>Oral<br/>steroid<br/>(n)</b> |
|----------------|------------------------------------|----------|-------------|------------------------|--------------|---|---|--|---------------------------------|
| <b>Healthy</b> | 30.2 (19-55) 14.1                  | 10       | 2           | 1                      | 2            | 108 (101-115)<br>7.1  | 112 (96-114) 8.8  |  |                                 |
| <b>Mild</b>    | 24.4 (18-56) 7.9                   | 22       | 7           | 0                      | 22           | 90 (82-103) 15  | 110 (102-114)<br>7.6  |  |                                 |
| <b>Severe</b>  | 41.5(20-69) 13.2                   | 28       | 8           | 14                     | 19           | 70 (43-86) 26   | 82 (63-102) 27  | 1600-2400 (2000)                                 | 15                              |

**e-Table 2. Inflammatory markers**

|                    | <b>Healthy<br/>(n=10)</b> | <b>Mild<br/>(n=22)</b> | <b>Severe<br/>(n=28)</b> | <b>P value</b>      |
|--------------------|---------------------------|------------------------|--------------------------|---------------------|
| <b>Median MPO</b>  | 2.3                       | 4.1                    | 37                       | ❖ <b>&lt;0.001</b>  |
| <b>(IQR)</b>       | (0.89 to 20)              | (2.4-14)               | (8.4 to 57)              | # <b>&lt;0.01</b>   |
| <b>Median IL-8</b> | 4.5                       | 20.48                  | 72.4                     | * <b>NS</b>         |
| <b>(IQR)</b>       | (0 to 34)                 | (11 to 24)             | (31 to 170)              | ❖ <b>&lt;0.0001</b> |
| <b>Median ECP</b>  | 0.81                      | 3.1                    | 14                       | # <b>&lt;0.01</b>   |
| <b>(IQR)</b>       | (0 to 1.7)                | (0.47 to 5.9)          | (2.9 to 45)              | * <b>NS</b>         |
| <b>Median NE</b>   | 9.7                       | 3.0                    | 30                       | ❖ <b>&lt;0.0001</b> |
| <b>(IQR)</b>       | (2.8 to 16)               | (1.9 to 5.4)           | (8.1 to 72)              | # <b>&lt;0.01</b>   |
| <b>Median LPS</b>  | ND                        | 2.8                    | 14                       | * <b>NS</b>         |
| <b>(IQR)</b>       |                           | (2.6 to 3.6)           | (3.4 to 72)              | ❖ <b>&lt;0.001</b>  |

**e-Table 3. Linear regression of age as a confounding factor**

|                   | <b>Beta</b> | <b>CI 95%</b>   | <b>N</b> | <b>P Value</b> |
|-------------------|-------------|-----------------|----------|----------------|
| <b>BAL SP-D</b>   | -126.0      | -185.4 to -66.7 | 60       | 0.001          |
| <b>Serum SP-D</b> | 48.3        | 21.0 to 75.5    | 60       | 0.001          |

**e-Figure 1. Western blots showing SP-D in BAL from healthy control subjects (HC) and mild asthmatics (MA)**

