S75  HIGH DOSE VITAMIN D SUPPLEMENTATION IMPROVES EXTRAVASCULAR LUNG WATER INDEX AND IN-VIVO TREG AND LL37 RESPONSES POST-OESOPHAGECTOMY

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Acute Lung Injury occurs in around 25% of patients post 2 stage oesophagectomy. We have previously shown that levels of 25-OH vitamin D are low in these patients and that those patients with the lowest levels of vitamin D are more likely to develop Acute Lung Injury post-operatively. In vitro, vitamin D has been shown to promote the differentiation of regulatory T cells (Treg) and the expression of LL37 (cathelicidin), an antimicrobial peptide.

We have given 13 patients a one-off high dose vitamin D supplement 3–14 days prior to oesophagectomy. Numbers of circulating regulatory T cells were analysed both pre- and post-supplementation. Plasma levels of LL37 were measured by ELISA. Extravascular Lung Water Index (EVLWi) was measured pre-operatively, post-operatively and on the day following surgery. Changes in LL37 and EVLWi were compared with results from a cohort of patients (n=50) who had not been supplemented.

Pre-operative vitamin D levels between the two groups were significantly different. The proportion of CD3+CD4+ T cells which were CD25+CD127loFoxP3+ increased post vitamin D supplementation. Plasma levels of LL37 were measured by ELISA. Extravascular Lung Water Index (EVLWi) was measured pre-operatively, post-operatively and on the day following surgery. Changes in LL37 and EVLWi were compared with results from a cohort of patients (n=50) who had not been supplemented.

Post-operative vitamin D levels were found to be significantly increased at the onset of ALI compared to healthy volunteers and fell significantly by day 7 compared to day 0. In contrast, levels of secretory leukocyte protease inhibitor (SLPI) did not decrease over time. This decrease in elafin was due to cleavage by the 20S proteasome which decreased within the pulmonary compartment over the course of ALI. The aim of this study was to characterise temporal changes in elafin concentration in patients with acute lung injury (ALI) and to evaluate whether a decrease in elafin levels is due to elevated protease activity. Previous work has shown that unregulated protease activity can cause proteolytic cleavage of elafin, impairing the innate immune function of the protein. Bronchoalveolar lavage fluid (BALF) was obtained from patients with ALI within 48 hours of onset of ALI (day 0), at day 3 and at day 7. Elafin levels were quantified by ELISA. Elafin susceptibility to proteolytic cleavage by ALI BALF was assessed by Western blot and by HPLC-Mass Spectrometry.

Conclusion The Aim of this study was to characterise temporal changes in elafin concentration in patients with acute lung injury (ALI) and to evaluate whether a decrease in elafin levels is due to elevated protease activity. Previous work has shown that unregulated protease activity can cause proteolytic cleavage of elafin, impairing the innate immune function of the protein. Bronchoalveolar lavage fluid (BALF) was obtained from patients with ALI within 48 hours of onset of ALI (day 0), at day 3 and at day 7. Elafin levels were quantified by ELISA. Elafin susceptibility to proteolytic cleavage by ALI BALF was assessed by Western blot and by HPLC-Mass Spectrometry. Elafin levels were found to be significantly increased at the onset of ALI compared to healthy volunteers and fell significantly by day 7 compared to day 0. In contrast, levels of secretory leukocyte protease inhibitor (SLPI) did not decrease over time. This decrease in elafin was due to cleavage by the 20S proteasome which was significantly increased in ALI BALF. Incubation of ALI BALF with the proteasome inhibitor epoxomicin confirmed that 20S proteasome activity was responsible for proteolytic cleavage of elafin resulting in diminished anti-elastase activity. In addition, free neutrophil elastase (NE) activity significantly increased in ALI BALF from day 0 to day 7. In conclusion, elafin concentrations decrease within the pulmonary compartment over the course of ALI as a result of proteolytic degradation. This loss of elafin may predispose, in part, to excessive inflammation in ALI.

Introduction Prone positioning and intravenous steroids represent a treatment option in patients with severe ARDS because most of the clinical manifestations in ARDS is secondary to excess fluid in the interstitium and alveoli and presence of inflammatory cells in the fluid.

Objective To assess the impact of prone positioning and steroids in severe ARDS and to assess and identify prognostic factors and potential predictors of mortality in ARDS patients.

Methods This is a retrospective study of 46 patients with severe ARDS from 2009–2011. The patient charts were reviewed and the impact of prone positioning and steroids was observed on severe ARDS patients. The primary outcome measured was ICU length of stay, number of ventilator days and 30 day mortality.

Abstract S75 Table 1: Effects of Vitamin D supplementation on median values of Vitamin D, EVLWi and LL37

<table>
<thead>
<tr>
<th>Vitamin D supplementation</th>
<th>No Vitamin D supplementation</th>
<th>p value (Mann-Whitney U test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-OH Vitamin D level</td>
<td>76 nmol/L</td>
<td>25 nmol/L</td>
</tr>
<tr>
<td>Change in EVLWi D0 post-op</td>
<td>–1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Change in EVLWi D1 post-op</td>
<td>–1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>% change in plasma LL37 as proportion of total protein post-op</td>
<td>10.9</td>
<td>–17.8</td>
</tr>
</tbody>
</table>

S76  PROTEOLYTIC CLEAVAGE OF ELAFIN BY 20S PROTEASOME MAY CONTRIBUTE TO INFLAMMATION IN ACUTE LUNG INJURY

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The Aim of this study was to characterise temporal changes in elafin concentration in patients with acute lung injury (ALI) and to evaluate whether a decrease in elafin levels is due to elevated protease activity. Previous work has shown that unregulated protease activity can cause proteolytic cleavage of elafin, impairing the innate immune function of the protein. Bronchoalveolar lavage fluid (BALF) was obtained from patients with ALI within 48 hours of onset of ALI (day 0), at day 3 and at day 7. Elafin levels were quantified by ELISA. Elafin susceptibility to proteolytic cleavage by ALI BALF was assessed by Western blot and by HPLC-Mass Spectrometry. Elafin levels were found to be significantly increased at the onset of ALI compared to healthy volunteers and fell significantly by day 7 compared to day 0. In contrast, levels of secretory leukocyte protease inhibitor (SLPI) did not decrease over time. This decrease in elafin was due to cleavage by the 20S proteasome which was significantly increased in ALI BALF. Incubation of ALI BALF with the proteasome inhibitor epoxomicin confirmed that 20S proteasome activity was responsible for proteolytic cleavage of elafin resulting in diminished anti-elastase activity. In addition, free neutrophil elastase (NE) activity significantly increased in ALI BALF from day 0 to day 7. In conclusion, elafin concentrations decrease within the pulmonary compartment over the course of ALI as a result of proteolytic degradation. This loss of elafin may predispose, in part, to excessive inflammation in ALI.

S77  PRONE POSITIONING AND INTRAVENOUS STEROIDS FOR THE MANAGEMENT OF SEVERE ARDS

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Introduction Prone positioning and intravenous steroids represents a treatment option in patients with severe ARDS because most of the clinical manifestations in ARDS is secondary to excess fluid in the interstitium and alveoli and presence of inflammatory cells in the fluid.

Objective To assess the impact of prone positioning and steroids in severe ARDS and to assess and identify prognostic factors and potential predictors of mortality in ARDS patients.

Methods This is a retrospective study of 46 patients with severe ARDS from 2009–2011. The patient charts were reviewed and the impact of prone positioning and steroids was observed on severe ARDS patients. The primary outcome measured was ICU length of stay, number of ventilator days and 30 day mortality.