Background: A biologically plausible link between gastro-oesophageal reflux (GOR), aspiration, and lung allograft dysfunction has been suggested, but there is no systematic evidence indicating the presence of gastric contents in the lung. We have tested the hypothesis that pepsin, as a marker of aspiration, is detectable in bronchoalveolar lavage (BAL) fluid of allograft recipients who had not reported symptoms of GOR.

Methods: Standardised 3 x 60 ml surveillance BAL fluid samples from 13 chronologically sequential stable lung allograft recipients without chronic rejection (10 patients treated with a prophylactic proton pump inhibitor) were studied. Lavage supernatants were assayed by an ELISA based on a monospecific goat antibody for pepsin/pepsinogen. Pepsin levels were compared with those from four normal volunteer controls.

Results: Pepsin levels were measurable in all allograft recipients, in keeping with gastric aspiration (median 109 ng/ml (range 35–1375)). In the control group the pepsin levels were below the limit of detection. Treatment with a proton pump inhibitor was not correlated with pepsin levels. There was no correlation between BAL fluid neutrophils and pepsin levels.

Conclusion: These data demonstrate lung epithelial lining fluid concentrations of pepsin in lung allograft recipients which are much higher than blood reference levels, with no detectable pepsin in controls. This provides direct evidence of gastric aspiration, which is potentially injurious to the allograft.
Bronchoscopy was carried out with patients in a semi-reclined position. Bronchoalveolar lavage was standardised to a 3×60 ml procedure with oxygen saturation routinely measured during the procedure. The BAL fluid sample was split and assessed for clinical microbiology and differential cell counts on Giemsa stained cytocentrifuge preparations. Cell free BAL supernatants were prepared by centrifugation (10 minutes, 1500 rpm, 10 minutes), aliquots snap frozen by immersion in liquid N₂, and stored at −80°C prior to ELISA.

Transbronchial biopsies were obtained from allograft patients only.

**Pepsin/pepsinogen ELISA**

A locally developed ELISA was performed using 100 µl of unconcentrated BAL supernatants. The assay, based on a monospecific antibody to porcine pepsin, measured both pepsin and total pepsinogens, referred to henceforth as “pepsin”, with a lower limit of detection of <1 ng/ml. All assays were performed by one individual and the coefficient of variation for the assay was 13%. Serum reference levels for pepsin are 49.8–86.6 µg/l. The ELISA on the samples from normal subjects were performed 9 months after the transplant patients.

**Processing of TBB samples**

Five to seven TBB samples were taken at each allograft bronchoscopy, fixed in 10% formalin, embedded in paraffin, and stained with haematoxylin and eosin to assess acute or chronic rejection according to standard criteria.

**Statistical analysis**

Non-parametric methods were used throughout using Minitab statistical software. The median pepsin levels in allograft recipients were compared with those in control transplant patients.

**RESULTS**

Patient demographic data, BAL and pathological rejection assessments are summarised in table 1. Five of the 13 subjects had clinically significant mild to moderate (a2) acute rejection, but all were free from long term irreversible loss of lung function.

**BAL fluid data**

The median BAL return was 90 ml (range 55–100) in allograft recipients and 80 ml (range 55–90) in controls, indicating technically satisfactory procedures. As in our previously published data on allograft patients, the percentage of neutrophils in the BAL fluid of allograft recipients was variable (median 2.0% (range 0.2–35.6)) and higher than our normal range (1.6% (range 0–2), p = 0.03).

**DISCUSSION**

Limited previous reports, largely retrospective but some with formal objective oesophageal pH monitoring, have suggested that GOR is a significant problem in lung allograft recipients, and treatment of GOR has been cited as a new therapeutic option to treat patients with the bronchiolitis obliterans syndrome.

In this study we have shown that high and variable levels of pepsin are detectable in BAL fluid of allograft recipients, with no pepsin detected in normal control BAL fluid samples. To our knowledge, this is the first systematic direct evidence of gastric aspiration into lung allografts. This may be a continuing and cumulative potential injury to allografts, and we provide mechanistic support for this contention.

Figure 1 BAL fluid pepsin levels in allograft recipients and controls.

**Table 1 Summary of patient demographic data, BAL and pathological rejection assessments**

<table>
<thead>
<tr>
<th>Subject no</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Months after transplant</th>
<th>BAL return (ml)</th>
<th>Cell count (×10⁶/ml)</th>
<th>PMN (%)</th>
<th>AM (%)</th>
<th>Lymph (%)</th>
<th>Microbiology</th>
<th>Biopsy</th>
<th>PPI/H₂</th>
<th>Pepsin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>CF</td>
<td>3</td>
<td>70</td>
<td>0.2</td>
<td>99.6</td>
<td>0.2</td>
<td></td>
<td>Negative</td>
<td>a2/b1</td>
<td>Yes</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>Bronchiectasis</td>
<td>6</td>
<td>70</td>
<td>0.2</td>
<td>96.4</td>
<td>3.4</td>
<td></td>
<td>Negative</td>
<td>a0/b1</td>
<td>Yes</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>PPH</td>
<td>2.5</td>
<td>100</td>
<td>1.0</td>
<td>98.8</td>
<td>0.2</td>
<td></td>
<td>Negative</td>
<td>a2/b1</td>
<td>Yes</td>
<td>172</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>OB</td>
<td>3</td>
<td>100</td>
<td>3.4</td>
<td>66.2</td>
<td>3.4</td>
<td></td>
<td>Negative</td>
<td>a1/bx</td>
<td>Yes</td>
<td>68</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>CF</td>
<td>0.25</td>
<td>55</td>
<td>35.6</td>
<td>61.0</td>
<td>3.0</td>
<td></td>
<td>Negative</td>
<td>a2/bx</td>
<td>No</td>
<td>129</td>
</tr>
<tr>
<td>6</td>
<td>41</td>
<td>A1AT</td>
<td>2.5</td>
<td>85</td>
<td>7.4</td>
<td>86.4</td>
<td>5.0</td>
<td></td>
<td>Negative</td>
<td>a2/b0</td>
<td>No</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>44</td>
<td>LAM</td>
<td>6</td>
<td>100</td>
<td>1.0</td>
<td>94.6</td>
<td>4.4</td>
<td></td>
<td>Negative</td>
<td>a1/b1</td>
<td>Yes</td>
<td>34</td>
</tr>
<tr>
<td>8</td>
<td>44</td>
<td>VSD-EISEN</td>
<td>87</td>
<td>95</td>
<td>1.4</td>
<td>95.0</td>
<td>5.0</td>
<td>75.8</td>
<td>Aspergillus</td>
<td>a0/b0</td>
<td>Yes</td>
<td>67</td>
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<tr>
<td>9</td>
<td>38</td>
<td>CF</td>
<td>6</td>
<td>80</td>
<td>4.7</td>
<td>75.8</td>
<td>18.4</td>
<td></td>
<td>Klebsiella</td>
<td>a1/b1</td>
<td>Yes</td>
<td>107</td>
</tr>
<tr>
<td>10</td>
<td>62</td>
<td>LAM</td>
<td>12</td>
<td>90</td>
<td>2.0</td>
<td>94.4</td>
<td>3.6</td>
<td>39.9</td>
<td>Negative</td>
<td>a0/bx</td>
<td>No</td>
<td>1375</td>
</tr>
<tr>
<td>11</td>
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<td>Bronchiectasis</td>
<td>3</td>
<td>75</td>
<td>8.0</td>
<td>76.0</td>
<td>15.8</td>
<td></td>
<td>Negative</td>
<td>a2/b0</td>
<td>Yes</td>
<td>225</td>
</tr>
<tr>
<td>12</td>
<td>47</td>
<td>A1AT</td>
<td>2</td>
<td>75</td>
<td>2.6</td>
<td>56.4</td>
<td>0.2</td>
<td></td>
<td>Negative</td>
<td>a2/b0</td>
<td>Yes</td>
<td>1200</td>
</tr>
<tr>
<td>13</td>
<td>21</td>
<td>CF</td>
<td>12</td>
<td>95</td>
<td>8.5</td>
<td>98.2</td>
<td>0.6</td>
<td></td>
<td>Penicillium</td>
<td>a5/b0</td>
<td>Yes</td>
<td>237</td>
</tr>
</tbody>
</table>

CF, cystic fibrosis; PPH, primary pulmonary hypertension; OB, obliterative bronchiolitis (patient had a second lung transplant due to failure of the first); A1AT, α1-antitrypsin deficiency; LAM, lymphangiomatosis; VSD-EISEN, Eisenmenger’s syndrome; PMN, neutrophils; AM, alveolar macrophages; Lymph, lymphocytes; Biopsy, pathological assessment for rejection according to International Society for Heart and Lung Transplantation criteria: a = acute rejection < a1 non-significant, b = airway assessment, a+b = no material for assessment; PPI/H₂, prophylactic treatment with proton pump inhibitor or H₂ receptor antagonist.
approximately 1 in 200 of the ELF sampled, with our present data consistent with ELF concentrations of pepsin 10–10^5 times higher than serum reference levels. In contrast, our published data on BAL fluid levels of albumin in allografts are consistent with ELF levels substantially lower than those found in serum. Overall, our data indicate a gastric source of the pepsin detected.

Pepsin is a proteolytic enzyme, active at acidic pH. There are no data of which we are aware regarding the pH of allograft ELF, but acidic breath condensate is increasingly reported as a marker of inflammation in asthma, chronic obstructive pulmonary disease, bronchiectasis, cystic fibrosis, and following cardiothoracic surgery. These pH levels are consistent with potential proteolytic activity for pepsin. Aspiration of gastric contents into the lung would be anticipated to cause epithelial damage in allografts, stimulation of cytokine production, and an airway inflammatory/remodelling response, potentially contributing to irreversible loss of allograft function and eventual failure. It was noteworthy that most of the patients we studied were being treated with a prophylactic proton pump inhibitor at a low maintenance dose. This reflects widespread empirical use in allograft recipients in view of concurrent oral corticosteroid use and their role in patients with cystic fibrosis to prevent pancreatic enzyme supplement degradation. Such medication would be expected to suppress symptoms associated with GOR caused by acid, but a potential concern highlighted by our study is that “clinically occult” aspiration of other gastric contents would still be possible.

Our study, though novel, is preliminary and our control information is limited. However, we specifically adopted a rigorous approach to this by recruiting normal volunteers, and no pepsin was detected in these controls. Our results therefore indicate the presence of unexpectedly high, potentially deleterious levels of pepsin in lung allografts. This may be significant, irrespective of aetiology, with lung allografts singularly vulnerable to injury. Longitudinal studies are now required to assess whether the presence of BAL pepsin and other markers of GOR are related to long term allograft failure and chronic rejection, and such techniques may be broadly useful in studying other patients with GOR who develop lung disease.

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**REFERENCES**


Pepsin like activity in bronchoalveolar lavage fluid is suggestive of gastric aspiration in lung allografts

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