Serum sialyl Lewis X-i antigen in lung adenocarcinoma and idiopathic pulmonary fibrosis

H Satoh, H Ishikawa, Y T Yamashita, M Ohtsuka, K Sekizawa

Background: Sialyl Lewis X-i antigen has been used as a diagnostic tool for lung adenocarcinoma. However, serum levels of the antigen are also raised in some patients with idiopathic pulmonary fibrosis (IPF) without coexistent malignancy. Expression of the antigen in serum samples of patients with lung adenocarcinoma was evaluated and compared with that of patients with IPF by Western blotting in order to establish a specific laboratory test to differentiate lung adenocarcinoma from IPF.

Methods: The pattern of antigen expression in serum samples from 23 patients with either lung adenocarcinoma or non-malignant lung disease in whom serum levels of sialyl Lewis X-i antigen (>50 U/ml) were significantly increased was studied by Western blotting.

Results: Thirteen of the 14 serum samples from patients with lung adenocarcinoma had a molecular weight band at 120 or 130 kD, while five of the six patients with IPF had two or three bands at <97.4 kD. The pattern of antigen expression was apparently different between the two diseases. The sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio of this test in 20 patients with lung adenocarcinoma and IPF were 92.9%, 83.3%, 5.57, and 0.09, respectively.

Conclusions: Western blotting analysis of serum samples from patients with raised levels of sialyl Lewis X-i antigen provides some clinical information for a differential diagnosis between lung adenocarcinoma and IPF.
Western blotting analysis

Electrophoresis and immunostaining procedures were performed as reported previously. The serum samples from each patient were electrophoresed in a 6% linear gradient SDS polyacrylamide gel and the glycoproteins were transferred to a nitrocellulose filter. The filter was blocked with a 3% BSA solution and incubated with FH-6. The filter was washed extensively with PBS containing 0.05% Tween-20 and exposed to radiography using ECL (Amersham Life Science, Buckinghamshire, UK). Western blotting was repeated at least three times.

RESULTS

The study population consisted of 23 patients with lung adenocarcinoma and non-malignant lung diseases whose serum levels of sialyl Lewis X-i antigen were >50 U/ml. Malignancy was ruled out clinically in all six patients with IPF, the two patients with diffuse panbronchiolitis, and the one patient with bronchiectasis. Of the nine patients with non-malignant lung diseases, five died of respiratory failure and four are still alive after a 3-year follow-up period to December 2000.

Table 1 shows the age, sex, smoking status (expressed as the Brinkman index), and serum level of sialyl Lewis X-i antigen of each patient.

Protein profiles on Coomassie blue staining showed similarities in serum samples from patients with lung adenocarcinoma and those with non-malignant lung disease. In both lung adenocarcinoma and non-malignant lung disease the antigen carries glycoproteins in broad bands with a polydispersed pattern which is usually observed in Western blotting analysis of glycoproteins.

Figures 1 and 2 show the results of Western blotting. In each group of patients with the same diagnosis the sialyl Lewis X-i antigen at a level >50 U/ml essentially had the same pattern. Eleven of 14 serum samples from patients with lung adenocarcinoma had a band at molecular weight 120 kD. Two patients with lung adenocarcinoma (nos 12 and 16 in fig 2) with antigen levels of 135.0 and 129.6 U/ml, respectively, had a clear band with dense staining at 120 and 130 kD as well as three bands at <97.4 kD. The pattern of sialyl Lewis X-i antigen from other patients with lung adenocarcinoma was essentially the same, although it was not as clear as in these two patients. Only one of the 14 patients with lung adenocarcinoma had two bands at <97.4 kD (no 4 in fig 1).

In six patients with IPF the overall pattern of sialyl Lewis X-i antigen on Western blotting was different from the pattern seen for patients with lung adenocarcinoma. The most notable difference was observed at molecular weight bands 120 and 130 kD which were either faint or absent. However, two or three bands at <97.4 kD that were seen in cancer patients 4, 12, and 16 were also seen in five patients with IPF (nos 8, 9, 10,

Table 1 Detection of sialyl Lewis X-i antigen in serum

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Sex</th>
<th>Brinkman index</th>
<th>Serum sialyl Lewis X-i antigen (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lung adenocarcinoma</td>
<td>47</td>
<td>M</td>
<td>1500</td>
<td>100.0</td>
</tr>
<tr>
<td>2</td>
<td>Lung adenocarcinoma</td>
<td>77</td>
<td>F</td>
<td>0</td>
<td>140.0</td>
</tr>
<tr>
<td>3</td>
<td>Lung adenocarcinoma</td>
<td>50</td>
<td>M</td>
<td>1200</td>
<td>150.0</td>
</tr>
<tr>
<td>4</td>
<td>Lung adenocarcinoma</td>
<td>45</td>
<td>M</td>
<td>300</td>
<td>51.4</td>
</tr>
<tr>
<td>5</td>
<td>Lung adenocarcinoma</td>
<td>56</td>
<td>F</td>
<td>800</td>
<td>157.0</td>
</tr>
<tr>
<td>6</td>
<td>Lung adenocarcinoma</td>
<td>65</td>
<td>F</td>
<td>300</td>
<td>102.0</td>
</tr>
<tr>
<td>7</td>
<td>Lung adenocarcinoma</td>
<td>36</td>
<td>M</td>
<td>100</td>
<td>550.0</td>
</tr>
<tr>
<td>8</td>
<td>IPF</td>
<td>66</td>
<td>M</td>
<td>600</td>
<td>317.0</td>
</tr>
<tr>
<td>9</td>
<td>IPF</td>
<td>58</td>
<td>M</td>
<td>1000</td>
<td>145.0</td>
</tr>
<tr>
<td>10</td>
<td>IPF</td>
<td>74</td>
<td>M</td>
<td>1800</td>
<td>100.0</td>
</tr>
<tr>
<td>11</td>
<td>Lung adenocarcinoma</td>
<td>65</td>
<td>F</td>
<td></td>
<td>109.0</td>
</tr>
<tr>
<td>12</td>
<td>Lung adenocarcinoma</td>
<td>49</td>
<td>M</td>
<td>600</td>
<td>155.0</td>
</tr>
<tr>
<td>13</td>
<td>Lung adenocarcinoma</td>
<td>60</td>
<td>F</td>
<td>0</td>
<td>230.0</td>
</tr>
<tr>
<td>14</td>
<td>Lung adenocarcinoma</td>
<td>52</td>
<td>M</td>
<td>640</td>
<td>145.0</td>
</tr>
<tr>
<td>15</td>
<td>Lung adenocarcioma</td>
<td>44</td>
<td>M</td>
<td>600</td>
<td>116.0</td>
</tr>
<tr>
<td>16</td>
<td>Lung adenocarcioma</td>
<td>71</td>
<td>F</td>
<td>0</td>
<td>129.6</td>
</tr>
<tr>
<td>17</td>
<td>Lung adenocarcioma</td>
<td>66</td>
<td>F</td>
<td>1600</td>
<td>136.0</td>
</tr>
<tr>
<td>18</td>
<td>Diffuse panbronchiolitis</td>
<td>67</td>
<td>F</td>
<td>0</td>
<td>111.0</td>
</tr>
<tr>
<td>19</td>
<td>IPF</td>
<td>57</td>
<td>M</td>
<td>400</td>
<td>282.0</td>
</tr>
<tr>
<td>20</td>
<td>IPF</td>
<td>49</td>
<td>M</td>
<td>675</td>
<td>62.0</td>
</tr>
<tr>
<td>21</td>
<td>IPF</td>
<td>55</td>
<td>F</td>
<td>0</td>
<td>66.4</td>
</tr>
<tr>
<td>22</td>
<td>Bronchiectasis</td>
<td>72</td>
<td>F</td>
<td>0</td>
<td>109.6</td>
</tr>
<tr>
<td>23</td>
<td>Diffuse panbronchiolitis</td>
<td>53</td>
<td>M</td>
<td>640</td>
<td>77.4</td>
</tr>
</tbody>
</table>

IPF = idiopathic pulmonary fibrosis; Brinkman index = (number of cigarettes per day) × (years smoked).

Figure 1 Western blotting of serum samples of patients 1–10. Antigens were separated by SDS-PAGE on 6% polyacrylamide and electrophoretically transferred to nitrocellulose membranes. Details of electrophoretic and immunostaining procedures with FH-6 antibody are described in the text. Patient numbers are the same as in table 1. IPF=idiopathic pulmonary fibrosis.
times reveals several separate bands or diffuse smear-like
differential diagnosis we evaluated the pattern of expression
blotting analysis. In order to establish the specific test for a
that the pattern of expression would differ on Western
are not the same as those in patients with IPF, it is expected
proteins of the antigen in patients with lung adenocarcinoma
increased serum levels of the antigen are found in patients
nosis in patients with raised levels of the antigen. When
antigen are also increased in some patients with non-
Sialyl Lewis X-i antigen has been used as a tool for the clinical
positive likelihood ratio, and negative likelihood ratio in 20
19, and 20; figs 1 and 2), while in one patient (no 21 in fig 2)
with any particular pattern of sialyl Lewis X-i antigen.
each group of patients were variable and were not associated
with IPF; idiopathic pulmonary fibrosis.

Figure 2 Western blotting of serum samples of patients 11–23.
Antigens were separated by SDS-PAGE on 6% polyacrylamide and
electrophoretically transferred to nitrocellulose membranes. Details of
electrophoretic and immunostaining procedures with FH-6 antibody
are described in the text. Patient numbers are the same as in table 1.

DISCUSSION
Sialyl Lewis X-i antigen has been used as a tool for the clinical
assessment of lung cancers, especially lung adenocarcinoma.
However, the carbohydrate antigens including sialyl Lewis X-i
antigen are also increased in some patients with non-
malignant lung diseases such as IPF without coexistent malignancy.6·26 There is as yet no test to make a specific
diagnosis in patients with raised levels of the antigen. When
increased serum levels of the antigen are found in patients
with IPF without a suspicious mass on the chest CT scan, further
invasive and costly tests are often considered to rule out
adenocarcinoma originating from other organs. If the core
proteins of the antigen in patients with lung adenocarcinoma
are not the same as those in patients with IPF, it is expected
that the pattern of expression would differ on Western blotting
analysis. In order to establish the specific test for a
differential diagnosis we evaluated the pattern of expression
of the antigen in serum samples of lung adenocarcinoma and
compared it with that of IPF by Western blotting analysis.
Western blotting analysis for carbohydrate antigens some-
times reveals several separate bands or diffuse smear-like
bands.27·28 The variations in molecular size are now thought to
be due to length polymorphism of the gene encoding for
apomucin.29 Mucins are glycoproteins of high molecular
weight which contain a large amount of carbohydrate.
O-linked to protein through serine and threonine. Mucins exhibit abnormal carbohydrate epitopes or expose a large part
of their protein core, resulting in the appearance of different
peptide epitopes.30 In this study we showed clear heterogeneity
by Western blotting, both in the size and the pattern of
expression of the bands, between lung adenocarcinoma and
IPF. Our observation may be explained by the presence of dif-
f erent circulating core proteins in the serum of patients with
cancer and IPF, and we speculate that these various
glycosylated mucins originate from either cancer cells or non-
malignant cells. Thirteen of 14 serum samples from patients
with lung adenocarcinoma had a band at 120 or 130 kD, and
five of the six patients with IPF had molecular weight bands at
<97.4 kD. Two patients with diffuse panbronchiolitis had a
similar pattern to that observed in patients with IPF. The pat-
tern observed in these two diseases were apparently different
from those of lung adenocarcinoma. Higher molecular weight
bands (at 120 or 130 kD) may be related to malignancy and
smaller molecular weight bands (<97.4 kD) seem to be asso-
ciated with non-malignant disease. The pattern of sialyl Lewis
X-i antigen in one patient with bronchiectasis was similar to
that observed in lung adenocarcinoma. Since the serum level of
sialyl Lewis X-i antigen rarely exceeds 50 U/ml in patients
with bronchiectasis, we were unable to enroll more than one
patient into this study so the statistical power is very low.
Studies on larger populations are required to elucidate further
the biochemical difference or similarity in the core proteins
observed between non-malignant lung diseases and adeno-
carcinoma, although this study may form the basis. Other
tests such as gel filtration chromatography might be more
sensitive for detecting the antigen.

Our results suggest that Western blotting of sialyl Lewis X-i
antigen is of diagnostic value because of its high sensitivity
and specificity and its clinical usefulness in differentiating
lung adenocarcinoma from other non-malignant diseases
such as IPF or diffuse panbronchiolitis.

Authors’ affiliations
S Sato, H Ishikawa, M Ohtsuka, K Sekizawa, Division of Respiratory Medicine, Institute of Clinical Medicine, University of
Tsukuba, Japan
Y T Yamashita, Center for General Medicine, Ryukyu University Hospital, Japan

REFERENCES
1 Itzkowitz SH, Yamanaka M, Fukushima Y, et al. Immunohistochemical
comparison of Lea, monosialosyl Lea(CA19–9), and disialosyl Lea antigens
2 Itzkowitz SH, Yamanaka M, Montgomer SC, et al. Expression of Tr,
3 Gold P, Freedman SO. Specific carcinoma embryonic antigens of the human
characterization of human cancer-associated serum glycoprotein antigens
expressing fucosyl or sialylfucosyl type 2 chain polylactosamine. Cancer
7 Sato H, Ishikawa H, Kamma H, et al. Serum sialyl Lewis X-i antigen
levels in non-small cell lung cancer: correlation with distant metastasis
8 Sato H, Kamma H, Ogawa T, et al. Clinical significance of serum levels
of a carbohydrate antigen, sialyl SSEA1, in patients with fibrosing lung
carbohydrate antigens in patients with diffuse panbronchiolitis. Am Rev


