

ORIGINAL ARTICLE

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

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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.

It is well known that various carbohydrate antigens are expressed on the cell surface during tumour progression.^{1,2} Serum levels of the antigens are frequently raised in patients with malignancies, and detection of these antigens may have an adjunctive role to play in their diagnosis.³⁻⁷ Serum levels of the carbohydrate antigens are, however, also raised in some patients with non-malignant lung diseases such as idiopathic pulmonary fibrosis (IPF), bronchiectasis, and diffuse panbronchiolitis without coexistent malignancy.⁸⁻¹² IPF is defined as diffuse interstitial pulmonary fibrosis of unknown aetiology which has a chronic progression.¹³⁻¹⁶ In patients with IPF the incidence of lung cancer is much higher than that in the general population¹⁷ so, when increased serum levels of the antigen are seen in patients with IPF, further invasive and costly tests are often undertaken to rule out adenocarcinoma of the lung or other organs.

Among the carbohydrate antigens, sialyl Lewis X-i antigen has been used as a tumour marker for lung cancer, especially lung adenocarcinoma.³⁻⁷ However, raised serum levels of this antigen are observed in some patients with the abovementioned respiratory diseases.⁸⁻⁹ Recent studies have shown that, in addition to cancer cells, immature cells which undergo vigorous proliferation in the developing lung of the human embryo and fetus also express the antigen,¹⁸ and that the antigen is selectively expressed in the pulmonary epithelial cells that cover the remodelling alveolar septa in patients with IPF.⁷ Although increased serum levels of the antigen have been found not to be specific to patients with lung adenocarcinoma, there is as yet no specific test to differentiate lung adenocarcinoma from other pathophysiological conditions in which the antigen levels are increased.

Judging from their solubility in perchloric acid, Kannagi *et al* considered that core proteins of cancer associated mucin-type glycoproteins are highly heterogeneous and can be classified into several characteristic molecular species.⁵ In the present study we have evaluated the pattern of antigen expression in serum samples from patients with lung adenocarcinoma and compared it with those from patients with IPF

by Western blotting analysis in order to establish a specific test to differentiate lung adenocarcinoma from IPF, since IPF is often accompanied by raised serum levels of sialyl Lewis X-i antigen which generally increases further with disease progression.¹⁹

METHODS

Clinical and chemical samples

Seventy seven consecutive admissions were identified between January 1996 and December 1997 with diagnoses including 40 lung adenocarcinoma, 25 IPF, eight bronchiectasis, and four diffuse panbronchiolitis. All of the patients with lung adenocarcinoma had been confirmed by biopsy samples. The serum levels of sialyl Lewis X-i antigen were measured by radioimmunoassay using a commercial kit (Otsuka Assay Laboratories, Tokushima, Japan). Patients whose serum level of sialyl Lewis X-i antigen was <50 U/ml or those with acute infection were excluded, as were patients with lung adenocarcinoma with fibrotic changes on the chest CT scan. The diagnoses of IPF and diffuse panbronchiolitis were established by a combination of medical history, physical examination, laboratory tests, chest radiography, pulmonary function tests, arterial blood analyses, and the results of lung biopsy according to previously described criteria.^{13-16, 20, 21} A chest CT scan was performed on all patients with IPF, bronchiectasis, and diffuse panbronchiolitis to rule out malignant disease. Patients with non-malignant respiratory disease were followed up. Only patients followed up for 3 years who did not develop malignant disease at any site were included in the study. Serum samples of the patients with raised antigen levels (>50 U/ml) were stored at -30°C and analysed by Western blotting.

A monoclonal antibody against sialyl Lewis X-i antigen (FH-6) was given by Otsuka Assay Laboratories (Tokushima, Japan). Phosphate buffered saline (PBS) was purchased from Nissui Pharmaceutical (Tokyo, Japan) and bovine serum albumin (BSA) from Sigma (St Louis, MO, USA).

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

Patient no	Diagnosis	Age	Sex	Brinkman index	Serum sialyl Lewis X-i antigen (U/ml)
1	Lung adenocarcinoma	47	M	1500	100.0
2	Lung adenocarcinoma	77	F	0	140.0
3	Lung adenocarcinoma	50	M	1200	150.0
4	Lung adenocarcinoma	45	M	300	51.4
5	Lung adenocarcinoma	56	F	800	157.0
6	Lung adenocarcinoma	65	F	300	102.0
7	Lung adenocarcinoma	36	M	100	550.0
8	IPF	66	M	600	317.0
9	IPF	58	M	1000	145.3
10	IPF	74	M	1800	100.0
11	Lung adenocarcinoma	65	F	0	109.0
12	Lung adenocarcinoma	49	M	600	155.0
13	Lung adenocarcinoma	60	F	0	230.0
14	Lung adenocarcinoma	52	M	640	145.0
15	Lung adenocarcinoma	44	M	600	116.0
16	Lung adenocarcinoma	71	F	0	129.6
17	Lung adenocarcinoma	66	F	1600	136.0
18	Diffuse panbronchiolitis	67	F	0	111.0
19	IPF	57	M	400	282.0
20	IPF	49	M	675	62.0
21	IPF	55	F	0	66.4
22	Bronchiectasis	72	F	0	109.6
23	Diffuse panbronchiolitis	53	M	640	77.4

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

Western blotting analysis

Electrophoresis and immunostaining procedures were performed as reported previously.²²⁻²⁴ 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 index²⁵), 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 poly-dispersed pattern which is usually observed in Western blotting analysis of glycoproteins.²⁶⁻²⁹

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 155.0 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

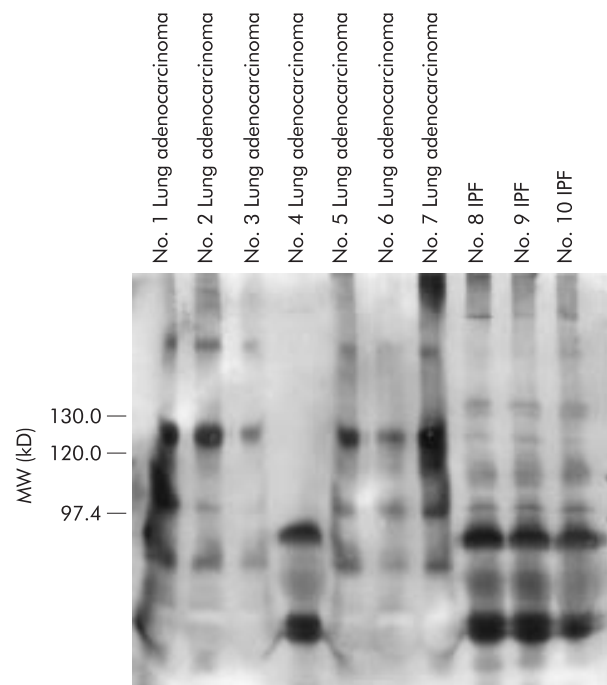


Figure 1 Western blotting of serum samples of patients 1–10. Antigens were separated by SDS-PAGE on 6% polyacrylamide and electrophoretically transferred to nitrocellulose membranes. Details of electrophoretic and immunostaining procedures with FH-6 antibody are described in the text. Patient numbers are the same as in table 1. IPF=idiopathic pulmonary fibrosis.

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,

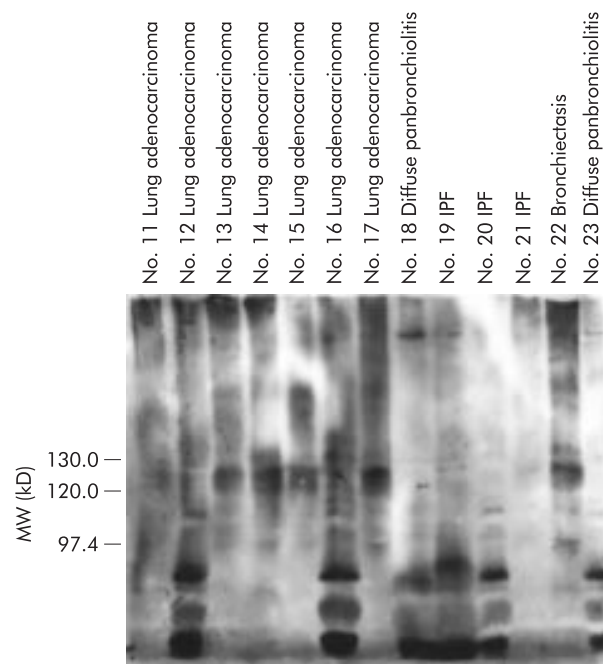


Figure 2 Western blotting of serum samples of patients 11–23. Antigens were separated by SDS-PAGE on 6% polyacrylamide and electrophoretically transferred to nitrocellulose membranes. Details of electrophoretic and immunostaining procedures with FH-6 antibody are described in the text. Patient numbers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

19, and 20; figs 1 and 2), while in one patient (no 21 in fig 2) with serum antigen levels of 66.4 U/ml no band was seen at this molecular level. The pattern of antigen expression with two clear bands at <97.4 kD and the absence of bands at 120 and 130 kD was diagnostic for IPF. The sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio in 20 patients with lung adenocarcinoma and IPF were 92.9%, 83.3%, 5.57, and 0.09, respectively.

In two patients with diffuse panbronchiolitis (nos 18 and 23, fig 2) the pattern was similar to that observed in patients with IPF with two clear bands at <97.4 kD and faint bands at 120 or 130 kD. However, in one patient with bronchiectasis (no 22, fig 2) the pattern of expression was similar to that found in patients with lung adenocarcinoma. The Brinkman indices in each group of patients were variable and were not associated with any particular pattern of sialyl Lewis X-i antigen.

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.^{8–12} 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 sometimes reveals several separate bands or diffuse smear-like

bands.^{26–29} The variations in molecular size are now thought to be due to length polymorphism of the gene encoding for apomucin.³⁰ 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.²⁷ 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 different 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 patterns 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 associated 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 adenocarcinoma, 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.

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