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## Comparison of Haptoglobin and Alpha<sub>1</sub>-Acid Glycoprotein Glycosylation in the Sera of Small Cell and Non-Small Cell Lung Cancer Patients

Porównanie glikozylacji haptoglobiny i α<sub>1</sub>-kwaśnej glikoproteiny w surowicy pacjentów z drobnokomórkowym i niedrobnokomórkowym rakiem płuca

### Authors' Contribution:

- A Study Design
- B Data Collection
- C Statistical Analysis
- D Data Interpretation
- E Manuscript Preparation
- F Literature Search
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### Summary

#### Introduction:

Cancer-related carbohydrate epitopes, which are regarded as potential diagnostic and prognostic biomarkers, are carried on the main acute phase proteins. It is not clear, however, if the glycosylation profile is similar in different glycoproteins, or it is protein specific to some extent. The aim of the study was to compare fucosylation, α<sub>2,3</sub> sialylation and expression of sialyl-Lewis<sup>x</sup> epitopes (sLe<sup>x</sup>) in the serum as a whole, AGP and haptoglobin of small cell (SCLC) and non-small cell lung cancer (NSCLC) patients with respect to healthy subjects as well as the cancer stage and its histological type.

#### Material and Methods:

Thirty-three NSCLC, 13 SCLC patients and 20 healthy volunteers were included in the study. Carbohydrate epitopes were detected by means of their reactivity with specific lectins and monoclonal anti-sLe<sup>x</sup> antibodies in direct or dual-ligand ELISA tests.

#### Results:

Significantly increased fucosylation was found in total serum in both cancer groups and in NSCLC haptoglobin. No difference was observed in SCLC haptoglobin or α<sub>1</sub>-acid glycoprotein in both cancer groups. Also α<sub>2,3</sub> sialylation was elevated in total serum, but not in α<sub>1</sub>-acid glycoprotein. This type of sialylation was undetectable in haptoglobin by means of MAA reactivity, in both healthy and cancer subjects. Complete sLe<sup>x</sup> antigens were overexpressed in total NSCLC serum and SCLC AGP, and their level was considerably lowered in cancer haptoglobin.

#### Discussion:

Typical acute phase proteins, haptoglobin and AGP, exhibit different glycosylation profiles in lung cancer. Alterations observed in haptoglobin reflected the disease process better than those in AGP. Comparison of haptoglobin and AGP glycosylation to that observed in total serum suggests that some efficient carriers of disease-altered glycoproteins still remain unidentified.

#### Keywords:

glycosylation • fucose • sialic acid • sialyl-Lewis<sup>x</sup> • small cell lung cancer • non-small cell lung cancer

**Full-text PDF:** <http://www.phmd.pl/fulltxt.php?ICID=1061788>**Word count:** 3244  
**Tables:** 2  
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**References:** 26**Author's address:** Mirosława Ferens-Sieczkowska, PhD, Department of Chemistry and Immunochemistry, Wrocław Medical University, Bujwida 44A, 51-345 Wrocław, Poland; e-mail: mirosława.ferens-sieczkowska@umed.wroc.pl**Abbreviations:** **sLe<sup>x</sup>** – sialyl-Lewis<sup>x</sup>; **AGP** –  $\alpha_1$ -acid glycoprotein; **SCLC** – small cell lung cancer; **NSCLC** – non-small cell lung cancer; **AAL** – *Aleuria aurantia* lectin; **MAA** – *Maackia amurensis* lectin; **SA** – sialic acid; **Hp** – haptoglobin; **TNM** – tumor node metastasis; **HSPC** – human serum protein calibrator; RT – room temperature.

## INTRODUCTION

Alterations in glycan structures are closely related to the mechanism of numerous diseases. Searching for cancer glycomarkers is a widely acceptable target in this field [6]. Glycan epitopes considered cancer-associated include Lewis-type tri- and tetrasaccharides, engaged in cancer cell extravasation and metastasis [13,19]. Sialyl-Le<sup>x</sup> (sLe<sup>x</sup>) epitopes were also found on circulating serum glycoproteins. Their elevated levels in cancer patients have often been reported. The role of natural competitive inhibitors of metastasis was postulated though not proved for such glycans [25].

Two monosaccharide residues are of crucial importance for sLe<sup>x/a</sup> epitopes: fucose and sialic acid (SA) [13, 19]. In a flexible glycosylation machinery, attachment of both monosaccharides may be independent one from the other, and produced structures may be incomplete then. Thus it seems interesting to consider fucosylation and sialylation independently, and observe their possible correlation with cancer, comparing it to the expression of complete sLe<sup>x</sup>.

Apparently the glycosylation of typical acute phase proteins,  $\alpha_1$ -acid glycoprotein (AGP) and haptoglobin (Hp), has been well documented. Variability of microheterogeneous variants of five AGP oligosaccharides was described, depending on the type and severity of the disease [5, 10, 25]. There is general agreement that glycosylation changes in cancer AGP resemble the chronic inflammation pattern [10], and concern mainly increased glycan branching and sLe<sup>x/a</sup> expression.

In Hp only the  $\beta$  subunit is glycosylated, bearing four N-oligosaccharides. Recently haptoglobin glycosylation was studied in prostate [9, 26], colon [21], liver [2], ovary [23, 24] and non-small cell lung [18] cancers. Altered glycosylation concerned fucosylation, both antennary, thus sLe<sup>x/a</sup> related, and localized in a core region of glycans. Altered branching and sialylation were also reported.

What is fairly surprising, direct comparison of Hp or AGP glycosylation profiles remains difficult, as these proteins

were usually studied independently, in different patient sets and with incompatible methodology. Some recent reports claim that both proteins are decorated with similar glycans, and their glycosylation profile reflects directly that present in the serum as a whole [1,4]. As it was not consistent with our experience, in this study we focused on the comparison of typical glycosylation features, i.e. fucosylation, sialylation and expression of sLe<sup>x</sup>, in the serum as a whole and two acute phase proteins, commonly treated as potential biomarkers, in the sera of small cell and non-small cell lung cancer patients.

## MATERIAL AND METHODS

### Clinical subjects

Forty-six lung cancer patients, treated in the Department of Pulmonary Diseases, Wrocław Medical University, were consecutively recruited to the study during a two-year period. Lung cancer was pathologically documented, and the disease stage was estimated with thoracic computed tomography. Upper abdominal metastatic disease was estimated with abdomen ultrasonography. Surgical tumor-node-metastasis (TNM) staging was done in non-small cell lung cancer (NSCLC) patients. All serum samples were collected before treatment (surgery or chemotherapy). The patients were divided into two distinct groups:

a) thirty-three patients were diagnosed with non-small cell lung cancer (NSCLC) at different stages of the disease development (Table 1). Nine patients had inoperable tumors at the time of diagnosis. During the course of our study 12 patients (35%) died after an average 11 months from the time of diagnosis. Histological examination proved 6 adenocarcinomas and 11 *ca. planoepitheliale*. The other cases represented undifferentiated cancer.

b) thirteen patients were diagnosed with small cell lung cancer (SCLC). Extensive disease with adrenal and hepatic metastases was confirmed for 8 of them. Four SCLC patients died during the course of the study (33%).

Table 1. Description of subjects with respect to the disease type and stage, protein, haptoglobin and AGP concentration

Group of subjects	Protein concentration [g/L]	Hp concentration [g/L]	AGP concentration [g/L]	Description of cancer subjects	
Control n=20	84.3±8.7	1.1±0.45	0.89±0.20	I°B	n=5 T2N0M0
NSCLC n=33	73.7±10	3.49±1.53 <sup>1</sup> p<0.0001	1.53±0.54 <sup>1</sup> p<0.0001 <sup>2</sup> p=0.035	II°B	n=2 n=1 T2N1M0 T3N0M0
				III°A	n=1 n=4 T1N2M0 T2N2M0
				III°B	n=4 n=6 T4N0M0 T4N2M0
				IV°	n=9 TxNxM1
				SCLC n=13	81.8±12.4
				ED	n=8

The protein, Hp and AGP concentration results are presented as a mean value±SD. Statistically relevant difference versus control group (1) and SCLC group (2) is given as p value calculated with Mann-Whitney test. LD – limited disease, ED – extended disease.

All experiments in this study were performed with respect to a control group established with serum samples of 20 healthy blood donors of generally good health and the results of routine blood tests within the physiological range. The informed consent of patients was obtained as well as the approval of the local Bioethical Committee.

**Protein, haptoglobin and α<sub>1</sub>-acid glycoprotein concentration**

Protein content in the samples was determined with the Lowry [17] method with bovine serum albumin (BSA) as a standard.

Goat antibodies against human haptoglobin and AGP were obtained in Wrocław Agriculture and Life Science University, purified according to the standard procedure [14], and applied for determination of haptoglobin and AGP concentrations in a radial immunodiffusion test [20]. Dako Human Serum Protein Calibrator (HSPC) was used as a standard. After 48 h diffusion and staining with Coomassie Brilliant Blue, diameters of precipitated rings were measured and the plot of Φ<sup>2</sup>=f(c) was used for calculation of the concentration.

**Direct lectin-ELISA**

Serum samples, loaded directly to the microplate surface, contained 800 ng of protein diluted with 15 mM phosphate buffered saline (150 mM), pH 7.4 (PBS) per well, thus saturating the microplate binding sites. Coating was performed for 12 h at room temperature (RT). Next, the plates were rinsed with PBS (3x 30 s, 200 µl/well) and still free binding sites were blocked with 5% BSA in PBS. After

extensive washing with 15 mM Tris-buffered saline (150 mM), pH 7.4, containing 0.05% Tween-20 (TBS-T), plates were incubated with biotinylated *Aleuria aurantia* or *Maackia amurensis* lectins (Vector Laboratories, 1 h, RT). The unbound lectin was washed out and the bound fraction was detected with Extravidin-alkaline phosphatase conjugate in TBS-T (Sigma-Aldrich Company, 1:20000, 30 min incubation, RT). Enzyme activity was measured with p-nitrophenyl phosphate (1 µg/ml) in 0.1 M diethylamine buffer, pH 9.6, containing 1 mM MgCl<sub>2</sub>, 200 µl/well. After 30 min the reaction was stopped with 50 ml of 1 M NaOH and the absorbance was measured at 405 nm, with the reference filter 630 nm, in a StatFax 2000 Microplate Reader.

**Direct anti-sLe<sup>x</sup> ELISA**

The plates coated as in the previous experiment were incubated with monoclonal mouse anti-sLe<sup>x</sup> IgM antibodies (Calbiochem, clone KM93, 1:1000) instead of lectin. Bound antibodies were detected with goat anti-mouse IgG/IgM immunoglobulin antibodies, alkaline phosphatase labeled (Chemicon, 1:5000). TBS-T containing 0.25% of BSA was used for all incubation and washing steps. Assay conditions were the same as in the previous experiments, but the enzyme reaction time was expanded to 1 h.

**Oxidation of anti-AGP antibodies**

Anti-AGP antibodies used as a capture agent in the next ELISA tests were deprived of their carbohydrate moiety to avoid further crossreactivity with sugar-recognizing ligand (anti-sLe<sup>x</sup> Ab or a lectin). Anti-AGP Ab were oxidized with an equal volume of 0.1 M sodium periodate in 0.1 M NaHCO<sub>3</sub>, pH 8.1, incubated for 1.5 h in the dark and dialysed to 0.1M NaHCO<sub>3</sub>, pH 9.2, to remove the excess of

the reagent [16]. *Vibrio cholerae* neuraminidase (Roche Molecular Biochemicals, Germany, 4 U/ml) was additionally used to ensure the sialic acid removal. The antibodies did not bind anti-sLe<sup>x</sup> Ab, AAL and MAA, as checked in dot-blot control experiments.

#### AAL, MAA and anti-sLe<sup>x</sup> reactivity of haptoglobin and AGP

ELISA tests with a capture ligand were applied to detect particular carbohydrate epitopes of haptoglobin and AGP. Microplates for haptoglobin capture were coated overnight with 0.5% horse methaemoglobin. After washing and blocking with BSA, the plate was loaded with serum samples containing exactly 2 µg of haptoglobin in 100 µl of TBS-T. Concentration of ligands as well as incubation conditions in the next steps were the same as in direct lectin- and anti-sLe<sup>x</sup>-ELISA. For the capture of AGP deglycosylated goat anti-AGP antibodies were applied (3 µg/well). Serum samples contained exactly 500 ng of AGP in 100 µl volume. In fucosylation analysis AAL dilution was increased to 1:7500, and the reaction time shortened to 15 min. The other ligand concentrations and incubation times remained unchanged.

#### Calculation of the results

To minimise the inter-experiment variations, which cannot be disregarded when a precisely defined standard of carbohydrate epitope is not accessible, in all experiments besides the control and cancer serum samples, HSPC was

loaded as a reference sample and used to calculate the results in arbitrary units. One unit of AAL/MAA/anti-sLe<sup>x</sup> reactivity (R) was defined as the ratio of the sample to HSPC optical density:  $R = OD_{\text{sample}} / OD_{\text{HSPC}}$ . In such an algorithm, the mean value of control samples should be close to 1. Thus values  $R > 1$  indicate higher and  $R < 1$  lower density of the examined glycoepitope than an average value in the normal serum.

#### Statistics

Basic statistical values (mean value and standard deviation) were calculated with Microsoft Excel software. The Statistica 9.0 package was used to analyse the significance of inter-group differences estimated with the Mann-Whitney test, and also to show the scatter of values within groups (box-and-whisker diagrams). A two-tailed *p* value of less than 0.05 was considered significant.

#### RESULTS

##### Lectin and anti-sLe<sup>x</sup> reactivity

In lung cancer patients the content of haptoglobin and α<sub>1</sub>-acid glycoprotein in the serum was increased when compared to healthy controls (Table 1). This is a well-documented feature resulting from chronic inflammation accompanying the cancer [2, 9, 11].

Reactivity with fucose-specific AAL, sialic acid-specific MAA and anti-sLe<sup>x</sup> antibodies was analysed in total serum

Table 2. Reactivity of total serum protein, haptoglobin and AGP with AAL and MAA lectins and anti-sLe<sup>x</sup> antibodies

	reactivity	control	NSCLC	SCLC
Total serum	AAL	0.76±0.24	2.02±0.79 <sup>1</sup> <i>p</i> <0.0001 <sup>2</sup> <i>p</i> =0.0083	1.43±0.96 <sup>1</sup> <i>p</i> =0.0077
	MAA	0.94±0.15	1.32±0.33 <sup>1</sup> <i>p</i> <0.0001	1.18±0.18 <sup>1</sup> <i>p</i> =0.0017
	anti-sLe <sup>x</sup>	0.92±0.18	1.19±0.51 <sup>1</sup> <i>p</i> =0.039 <sup>2</sup> <i>p</i> =0.008	0.84±0.28
AGP	AAL	0.77±0.37	0.69±0.27	0.83±0.82
	MAA	1.31±0.56	1.86±1.98	1.73±1.17
	anti-sLe <sup>x</sup>	1.15±0.59	1.33±0.75	1.75±0.47 <sup>1</sup> <i>p</i> =0.013
Haptoglobin	AAL	1.17±1.04	2.55±1.64 <sup>1</sup> <i>p</i> =0.00034	1.7±1.25
	MAA	n.r.	n.r.	n.r.
	anti-sLe <sup>x</sup>	1.02±0.23	0.36±0.15 <sup>1</sup> <i>p</i> <0.0001	0.40±0.09 <sup>1</sup> <i>p</i> <0.0001

The results are shown as the mean value ±SD of the relative reactivity expressed in arbitrary units (defined in M&M section). Statistically relevant difference versus control group (1) and SCLC group (2) is given as *p* value calculated with Mann-Whitney test; n.r. – no reactivity

as well as in two acute phase proteins, AGP and haptoglobin, in patients diagnosed with lung cancer with respect to healthy controls. Mean values and standard deviations in groups are shown in Table 2, and the scatter of values in Figures 1-3.

As a general feature of cancer serum, an increase of AAL-reactivity, indicating elevated fucosylation, was observed in both SCLC and NSCLC patients (Fig. 1a). For both groups the alterations compared to healthy controls were statistically significant ( $p < 0.0001$  and  $p = 0.0077$  in Mann-Whitney test, for NSCLC and SCLC, respectively). Moreover, AAL reactivity distinguished SCLC from NSCLC samples, with  $p$  value equal to 0.0083. The increase of MAA reactivity in the sera of lung cancer patients (Fig. 1b) was also statistically significant ( $p < 0.0001$  for NSCLC and  $p = 0.0017$  for SCLC). Opposite to fucosylation, there was no difference in  $\alpha 2,3$  sialylation when NSCLC and SCLC samples were compared. Elevated expression of sLe<sup>x</sup> antigens was found only in NSCLC patients (Fig. 1c). This feature significantly distinguished the NSCLC group from both control and SCLC subjects ( $p = 0.039$  and  $p = 0.008$ , respectively).

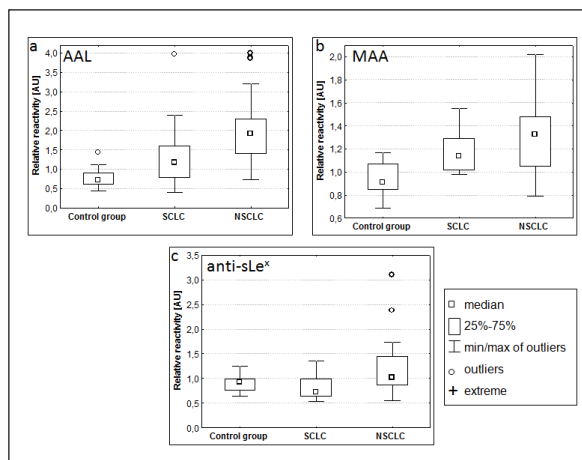


Fig. 1. Serum reactivity with lectins and anti-sLe<sup>x</sup> antibodies. Reactivity with *Aleuria aurantia* lectin, *Maackia amurensis* lectin and anti-sLe<sup>x</sup> antibodies was measured in total serum (800 ng of protein/well) by means of direct lectin or antibody ELISA

Having such a glance at total serum fucosylation and sialylation we focused on the comparison of these features in two acute phase proteins, commonly regarded as carriers of altered glycans attributed to the disease. In AGP, no statistically significant alterations in lectin reactivity, both AAL and MAA, were found (Fig. 2 a,b). Although alterations in fucose and  $\alpha 2,3$  sialic acid content were not relevant when measured by means of lectin reactivity, elevated expression of sLe<sup>x</sup> epitopes was measured with anti-sLe<sup>x</sup> antibodies (Fig. 2 c). The wide range of the two middle quartiles as well as frequent outlier values suggest significant ontogenetic variability of this feature, especially in NSCLC patients. Statistical significance of increased sLe<sup>x</sup> expression was observed only for SCLC versus the control group ( $p = 0.013$ , Table 2).

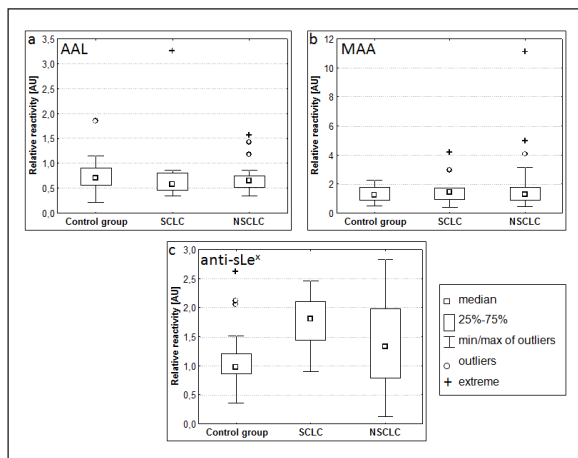


Fig. 2. AGP reactivity with lectins and anti-sLe<sup>x</sup> antibodies. Reactivity with *Aleuria aurantia* lectin, *Maackia amurensis* lectin and anti-sLe<sup>x</sup> antibodies was measured in AGP (500 ng/well) by means of dual ligand ELISA

Haptoglobin was MAA-unreactive, both in control and cancer samples. *Aleuria aurantia* reactivity of haptoglobin was increased in both groups of cancer patients (Fig. 3a), but it was statistically significant only for NSCLC subjects ( $p = 0.00034$ ). The reactivity of haptoglobin with anti-sLe<sup>x</sup> antibodies (Fig. 3b) was dramatically decreased in both SCLC and NSCLC samples when compared to healthy controls ( $p < 0.0001$ ). This finding was rather surprising, as sLe<sup>x</sup> increase is often regarded as a common feature of cancer cells as well as serum glycoproteins in cancer patients.

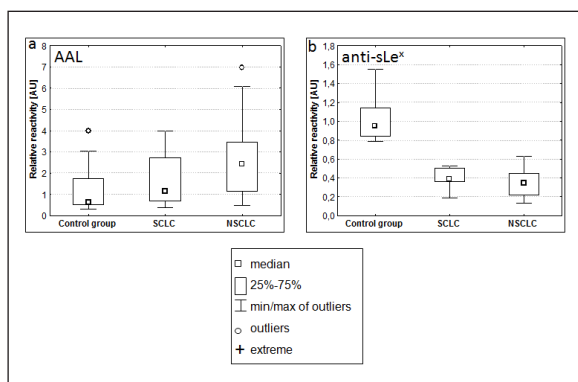


Fig. 3. Haptoglobin reactivity with AAL and anti-sLe<sup>x</sup> antibodies. Reactivity with *Aleuria aurantia* lectin and anti-sLe<sup>x</sup> antibodies was measured in haptoglobin (2  $\mu$ g/well) by means of dual ligand ELISA

### Comparison of NSCLC glycosylation with respect to cancer stage and histological type

To obtain further insight into the examined glycosylation parameters and their possible relation to the disease progress, we divided NSCLC patients ( $n = 33$ ) into three groups reflecting the disease staging according to TNM criteria. Eight samples were included in the group representing stage I+II, 16 cases as III, and 9 cases as IV (Table 1). AAL, MAA and anti-sLe<sup>x</sup> reactivity was then compared in total serum, AGP and Hp. The results are shown in Fig. 4. No trend line was

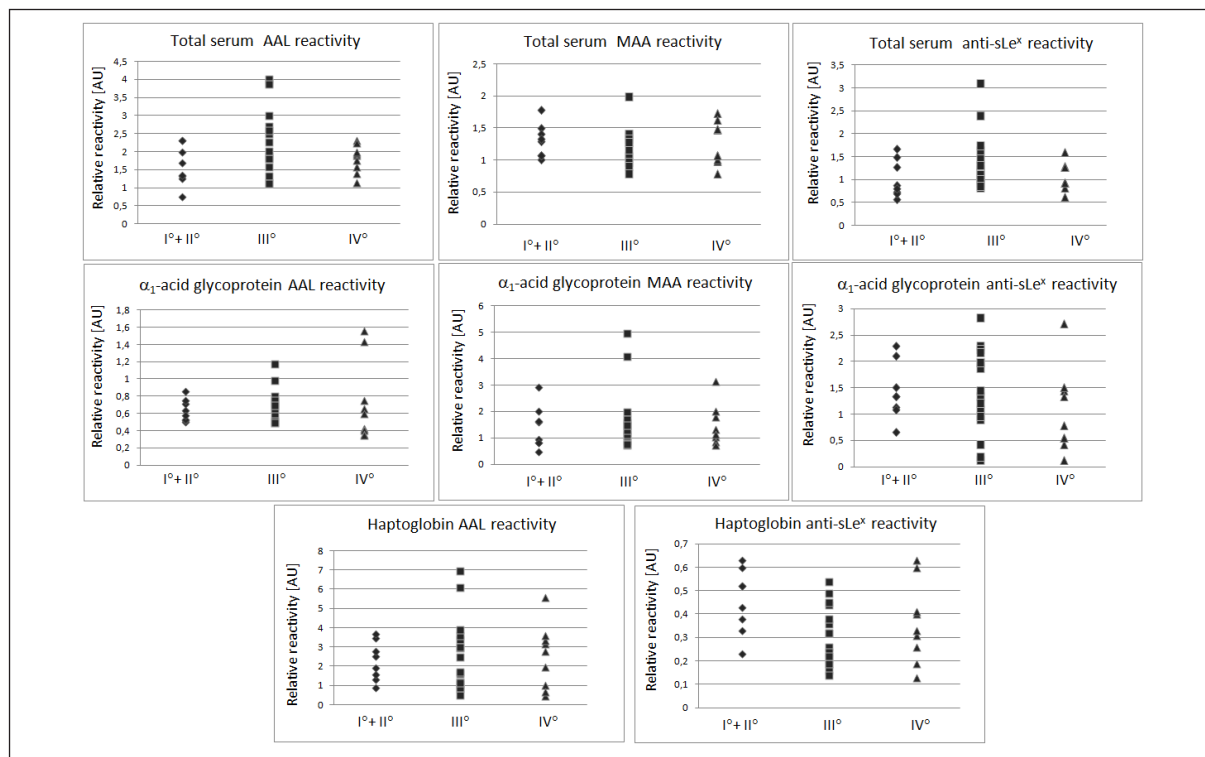


Fig. 4. Lectin and anti-sLeX reactivity in NSCLC patients sera with respect to the disease stage. NSCLC patients were divided into groups reflecting the cancer stage (I°+II°, III°, IV°) to compare the reactivity with AAL, MAA and anti-sLeX within the groups.

noticed in any case, suggesting that altered glycosylation in glycoproteins of lung cancer patients does not reflect the disease progress. This could also suggest that glycosylation changes appear relatively early in cancer disease and do not increase with the disease progress to a measurable extent.

For 17 NSCLC samples histological type of cancer was defined, i.e. adenocarcinoma (n=6) and ca. planoepitheliale (n=11). Also the comparison of glycoepitope reactivity within these groups (Fig. 5) did not suggest any histological relationship in AAL, MAA and anti-sLe<sup>x</sup> reactivity in total serum, AGP and Hp.

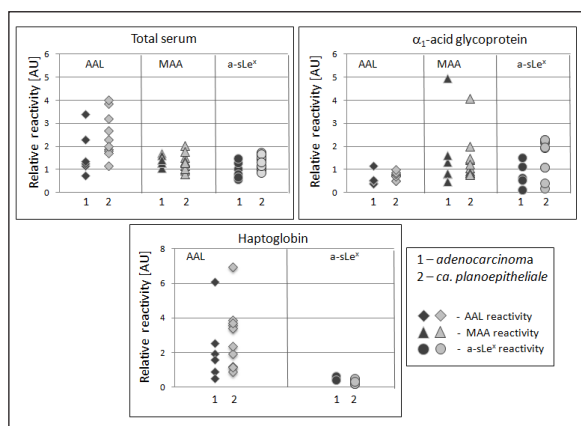


Fig. 5. Lectin and anti-sLeX reactivity in NSCLC patients sera with respect to the histological type of cancer. Expression of glycan epitopes was compared in histologically diagnosed adenocarcinoma and ca. planoepitheliale

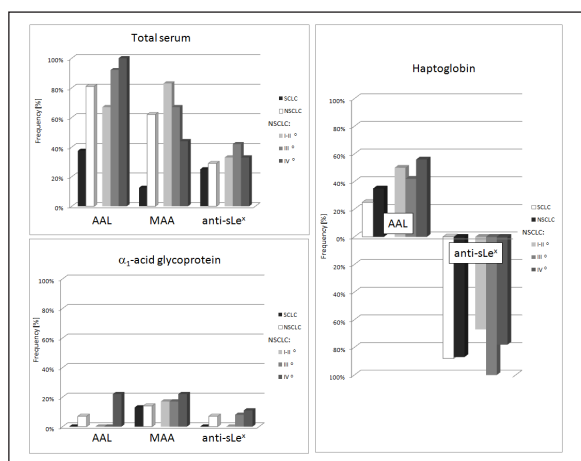
### Frequency of altered glycosylation in cancer samples

Based on the glycosylation parameters obtained for the control group we calculated the range of normal values as the mean value ± 2SD. According to the parameters in normal statistical distribution, such a range comprises 95.5% of values of the general population. Indeed, in the control group, values exceeding this range were observed for 1 case in serum AAL reactivity, 1 case of Hp AAL reactivity, 1 case of AGP anti-sLe<sup>x</sup> reactivity and 1 case of Hp anti-sLe<sup>x</sup> reactivity. In the latter case, however, the reactivity was elevated, opposite to cancer samples. This range of normal values was used for calculation of the frequency of out-of-the-range values in cancer samples. In a statistical approach this is equal to the sensitivity of the test. The percentage of such cases is shown in Figure 6, comparing SCLC and NSCLC groups as well as NSCLC subgroups according to the cancer stage.

Glycosylation of α<sub>1</sub>-acid glycoprotein was found to be the least suitable parameter for estimation of the disease occurrence and progress. Most of the cancer cases were within normal values and the number of cases outside of the range fluctuated from 0 to 20%.

In total serum glycoproteins the expression of sLe<sup>x</sup> epitopes seemed less relevant (20% for SCLC and 30-40% for NSCLC outside of the range) than fucosylation and sialyla-





**Fig. 6.** Frequency of cases exceeding the normal values of AAL, MAA and anti-sLe<sup>x</sup> reactivity in the sera of SCLC and NSCLC patients. The normal range of AAL, MAA and anti-sLe<sup>x</sup> reactivity was calculated as the mean value ± 2SD of control subjects reactivity

tion solely. The number of cases with the lectin-reactivity increased above the cut-off value was higher for NSCLC (60-96% for AAL and 40-80% for MAA) than SCLC (38% for AAL and 10% for MAA). In this case the frequency seems to be slightly related to the cancer stage: the frequency of elevated fucosylation increases with the progress of cancer and, in contrast, the frequency of increased α<sub>2,3</sub> sialylation seems to be lower in the cases of advanced cancer.

Overfucosylated haptoglobin was observed in only 20% of SCLC patients and 40-60% of NSCLC cases. In conclusion, the most striking observation was the lowering of haptoglobin anti-sLe<sup>x</sup> reactivity, as values below the normal range were observed in 90% of SCLC and 94 % of NSCLC cases. No relation to the disease progress was observed in this case.

## DISCUSSION

Diagnostic and prognostic potential of carbohydrate biomarkers for cancer is continually believed hopeful [3, 4, 11]. Although glycosylation studies are frequent, general conclusions attributing altered structures to the pathological process are hard to reach, and the challenge results from the enormous diversity of glycan isoforms.

The precise pattern of oligosaccharides may be obtained by means of mass spectrometry and chromatographic methods. A drawback of such research is often the small number of samples subjected to the study, thus neglecting the inter-personal variability. The alternative strategy of glycan analysis is then based on the interaction of oligosaccharides with specific ligands, antibodies or lectins. Although not defining detailed oligosaccharide structure, such data may reflect the possible contribution of glycans in the events *in vivo*. This approach facilitates the estimation of differences closely related to the disease. Unfortunately, the results obtained by means of different strategies are often inconsistent. The other con-

trovery concerns the question whether different glycoproteins in the studied clinical material present a similar glycosylation profile, or the pattern of glycans may be protein-specific.

Detailed structural investigations of serum glycome as a whole concerned breast, ovarian and lung cancers [1,3,23,24]. Elevated content of antennary sLe<sup>x</sup>-related fucose in trisialylated glycans was reported in breast [1] and ovarian [23, 24] cancers, in some cases reflecting the disease progress [1]. Biantennary glycans with core fucose were decreased in lung [3], while they were elevated and dominant in prostate cancer [22]. The general level of fucosylation and sialylation was not estimated in these studies. In our study high expression of antennary-fucosylated sLe<sup>x</sup> epitopes was found only in NSCLC but not in SCLC samples. Lectin-measured fucose overexpression seemed to be higher than that of sLe<sup>x</sup>, suggesting the significant content of the core linked monosaccharide, which is strongly preferred by AAL. Beside overexpression of sLe<sup>x</sup>, which is related to α<sub>2,3</sub> linked sialic acid, some authors have reported the cancer-related increase of SNA-reactive, α<sub>2,6</sub> bound sialic acid [2, 18, 21]. This type of linkage was not analysed in our study, but both SA types may be overexpressed as a result of increased glycan branching.

In lung cancer AGP, only increase in sLe<sup>x</sup> was found to be disease-related, similarly to the other cancer types [1,3,24]. Elevated MAA reactivity accompanied this feature only in some (15-20%) cases. In our study AGP fucosylation was unchanged. Overfucosylation of this glycoprotein was, however, reported in gastric, colorectal, hepatic and pancreatic cancers by means of AAL reactivity studies [11], and in structural analysis of ovarian cancer and lymphoma [12].

Although increased fucosylation is a common feature in cancer haptoglobin [2,3,9,21,23,26], the type of linkage and localization of fucose residues remain an open question. Antennary fucose, a part of the sLe<sup>x</sup> epitope, was reported in ovarian and breast cancers [1, 23, 24]. Arnold et al. [3] reported sLe<sup>x</sup> epitopes in lung cancer haptoglobin. In prostate cancer Hp was overfucosylated, while fucosylated glycans were hardly detected in haptoglobin of normal subjects. The latter is similar to our earlier results [8]. AAL reactivity was elevated in many, though not all samples of prostate cancer and prostate benign disease. In our study haptoglobin fucosylation was significantly increased only in SCLC patients. This was, however, accompanied by a significant and consistent reduction of sLe<sup>x</sup> epitope expression, in both cancer types. This suggests that fucose is located in a core region of glycans. Low Hp reactivity with anti-sLe<sup>x</sup> and anti-sLe<sup>a</sup> antibodies, close to negative controls, was described in healthy and colon cancer samples by Park *et al.* [21]. Yoon *et al.* [26] reported elevated reactivity with anti-sLe<sup>x</sup> antibodies in 3 out of 11 samples of prostate cancer, while it was decreased in seven. In that study increased AAL reactivity was accompanied by diminished sLe<sup>x</sup> expression, at least

in some cases. We made a similar observation earlier in haptoglobin isolated from ascites fluids of ovarian cancer patients [7], thus suggesting that the majority of fucose residues are localized in a core region of oligosaccharides. Lack of Hp MAA reactivity, presented here, may be due to a generally low amount of  $\alpha$ 2,3 linked SA in this glycoprotein [8].

Statistical estimation of the frequency of significantly altered expression of glycans in cancer patients showed poor specificity (statistical type II error) in all measured parameters of AGP, serum sLe<sup>x</sup> and Hp fucosylation, as the frequency of abnormal values did not exceed 50% of cases. The most satisfactory specificity was obtained for haptoglobin sLe<sup>x</sup> expression. On the other hand, high frequency of increased AAL and MAA reactivity in total serum encourages further search for glycoproteins effi-

ciently carrying altered oligosaccharides. In the previous report we suggested the significant contribution of low molecular weight (26 and 29 kDa) glycoproteins in cancer serum fucosylation [15].

The results of the current study lead to a conclusion that both typical acute phase glycoproteins present different glycosylation profiles with regard to fucosylation, sialylation and sLe<sup>x</sup> expression in the same groups of lung cancer patients. Haptoglobin glycosylation profile is related to lung cancer to a higher extent than the AGP one. The latter is rather similar to the alterations typically observed in chronic inflammation, while the lowered reactivity of lung cancer Hp with anti-sLe<sup>x</sup> antibodies enables clear discrimination of cancer from normal samples. Thus Hp glycans are worth further detailed examination as a potential source of lung cancer glyco-biomarkers.

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