

Relation between raised concentrations of fucose, sialic acid, and acute phase proteins in serum from patients with cancer: choosing suitable serum glycoprotein markers

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SUMMARY Serum concentrations of fucose, sialic acid, and eight acute phase proteins were measured in single specimens from patients with cancer in order to determine whether the raised concentrations of protein bound sugars commonly found in cancer correlate with increased concentrations of the acute phase proteins. Strong positive correlations were found only with α_1 -acid glycoprotein, α_1 -antitrypsin, and haptoglobins. Changes in protein bound sugars and acute phase proteins were also examined in relation to patients' disease states. Serum fucose was raised more often in patients with advanced disease than in those in whom the spread of the tumour was more restricted; increased sialic acid concentrations, however, were found with a similar frequency in both these groups. Combined use of fucose and sialic acid values gave a high degree of marker positivity which could be only slightly improved on by including measurement of acute phase proteins. The combined use of serum fucose and sialic acid concentrations may have value in monitoring patients with cancer: the sialic acid provides an index of the acute phase response and the fucose a measure of the tumour spread.

Serum fucose^{1,2} and sialic acid^{3,4} concentrations are often increased in patients with cancer, but there is no satisfactory explanation for these changes. There are many possibilities: increased glycosylation of serum glycoproteins; shedding of glycoproteins from the tumour cell surface; and increased concentrations of normal serum glycoproteins. The object of this study was to examine the possibility that increased concentrations of the so called acute phase proteins are responsible for the increased concentrations of fucose and sialic acid. Many acute phase proteins have raised values in patients with cancer,⁵ and some of these have a high carbohydrate content.⁶

Material and methods

Blood specimens were collected from 274 healthy

volunteers (79 men and 195 women; age range 18-65) who were attending blood donor sessions and 182 patients with cancer (35 men and 147 women; age range 14-75). The latter were either in hospital or attending outpatient clinics. Some were already receiving treatment and others had yet to start. Serum was separated by centrifugation and stored at -20°C until required for assay.

Table 1 gives details of the cancer group. The early subgroup consisted of women with positive cervical smears who were subsequently proved to have cervical intraepithelial neoplasia. The clinical subgroup consisted of patients with cancers that had arisen in various sites, who either had a small localised mass or successful treatment for more extensive disease. The advanced subgroup consisted of patients who either had an untreated extensive growth or progressive growth that was not responding to treatment; again, the cancers had arisen in various sites.

The concentrations of fucose, protein, and sialic

Table 1 Details of patients with cancer

| Primary tumour site | Patients' disease states (no of patients)* | | | |
|---------------------|--------------------------------------------|----------|----------|---------|
| | Early | Clinical | Advanced | Unknown |
| Breast | 0 | 7 | 11 | 3 |
| Cervix | 22 | 16 | 1 | 5 |
| Gastrointestinal | 0 | 1 | 7 | 5 |
| Lung | 0 | 0 | 5 | 0 |
| Lymphoid | 0 | 2 | 6 | 0 |
| Melanoma | 0 | 1 | 4 | 0 |
| Ovarian | 0 | 16 | 3 | 4 |
| Vagina/vulva | 0 | 0 | 2 | 5 |
| Miscellaneous | 0 | 7 | 12 | 1 |
| Unknown | 0 | 0 | 2 | 34 |

*See text for further information on definition of groups.

acid were determined in all the serum samples collected. The concentrations of eight acute phase proteins, α_1 -acid glycoprotein, α_1 -antitrypsin, caeruloplasmin, haemopexin, haptoglobins, α_2 -macroglobulin, prealbumin, and transferrin, were determined in 37 samples from the healthy group and 121 samples from the patients with cancer. Apart from excluding women taking oral contraceptives, specimens were chosen at random.

Serum fucose concentrations were measured using a fucose dehydrogenase method similar to that described elsewhere.⁷ Briefly, 0.1 ml of serum was incubated with 0.9 ml of 0.155 mol/l H_2SO_4 at 100°C for 1 h. The acidified serum was neutralised with saturated barium hydroxide to a total volume of 1.8 ml; 0.2 ml of this supernatant was added to

0.45 ml of 0.2 mmol/l Tris/HCl, pH 8.2, 0.25 ml of 0.8 mg/ml L-fucose dehydrogenase (Miles Laboratories), and 0.1 ml of 10 mmol/l nicotinamide adenine dinucleotide. The sample was left at room temperature for 60 min, and the fluorescence was then measured at 525 nm after excitation at 340 nm. The fucose content was estimated by comparison with aqueous fucose standards. All values were corrected using serum blanks in which the proteins were unhydrolysed. The sialic acid content of serum was determined using a periodate/thiobarbituric acid method.⁸ Before assay the protein bound sialic acid was released by hydrolysing 50 μ l of serum in 2 ml of 0.05 mol/l H_2SO_4 at 80°C for 1 h. No free sialic acid was detected in unhydrolysed sera, and all specimens were corrected for deoxyribose contami-

Table 2 Serum concentrations of fucose, sialic acid, and certain acute phase proteins in patients with cancer

| Substance assayed | Serum concentration | | | | | Percent increase in patients with cancer† |
|----------------------------------------|----------------------|----------|-----------|-----------------|--------------|-------------------------------------------|
| | Patients with cancer | | | Reference group | | |
| | Mean | $\pm SD$ | Range | Mean | Upper limit* | |
| Fucose (mg/g protein) (182) | 2.1 | 1.0 | 0.69-6.4 | 1.1 | 1.8 | 45.6 |
| Sialic acid (mg/g protein) (179) | 9.2 | 3.3 | 3.3-24.5 | 5.9 | 9.4 | 35.2 |
| Acute phase proteins (g/l serum) | | | | | | |
| α_1 -Acid glycoprotein (120) | 1.2 | 0.55 | 0.4-2.7 | 0.8 | 1.2 | 35.8 |
| Haptoglobins (120) | 2.7 | 1.6 | 0.38-8.3 | 1.6 | 3.3 | 29.2 |
| α_1 -Antitrypsin (120) | 3.4 | 1.5 | 1.0-7.9 | 2.8 | 4.0 | 25.0 |
| Haemopexin (121) | 0.94 | 0.17 | 0.26-1.5 | 0.99 | 1.3 | 6.7 |
| Caeruloplasmin (117) | 0.40 | 0.10 | 0.17-0.70 | 0.36 | 0.58 | 3.4 |
| α_2 -Macroglobulin (112) | 2.2 | 0.58 | 0.77-4.4 | 2.5 | 4.0 | 0.9 |
| Prealbumin (113) | 0.23 | 0.08 | 0.04-0.42 | 0.34 | 0.45 | 0 |
| Transferrin (121) | 2.6 | 0.71 | 0.69-4.5 | 3.7 | 4.8 | 0 |

Values given in parentheses indicate the number of sera assayed. Reference values for fucose, sialic acid, and acute phase proteins are based on 270, 261, and 37 estimations respectively.

*Upper 95% confidence limit.

†Percent raised above the given confidence limit.

Table 3 Correlations between protein bound sugars and the acute phase proteins in serum samples from patients with cancer

| Acute phase protein | No of pairs* | Correlation coefficient | |
|-------------------------------|--------------|-------------------------|-------------|
| | | Fucose | Sialic acid |
| α_1 -Acid glycoprotein | 118 | +0.60† | +0.65† |
| Haptoglobins | 120/118 | +0.47† | +0.53† |
| α_1 -Antitrypsin | 120/118 | +0.44† | +0.54† |
| Haemopexin | 120/118 | +0.31† | +0.38† |
| Caeruloplasmin | 115 | +0.26 | +0.17 |
| α_2 -Macroglobulin | 112 | -0.24 | -0.35† |
| Prealbumin | 113 | -0.45† | -0.47† |
| Transferrin | 120/118 | -0.38† | -0.50† |

*A single value indicates that the number of pairs were the same for each sugar.

†Indicates that correlations are statistically significant ($p < 0.025$).

nation. Serum concentrations of fucose and sialic acid are expressed as mg/g serum protein.

Protein was determined by the biuret method.⁹ The serum protein concentrations for the healthy and cancer groups were 68.6 g/l (SD 4.8 g/l; $n = 269$) and 65.0 g/l (SD 7.8 g/l; $n = 182$) respectively.

Acute phase protein concentrations were estimated by radial immunodiffusion (Partigen Plates, Hoechst Pharmaceuticals). Standards were obtained from Hoechst and the results were expressed as g/l. The interassay coefficients of variation for the fucose, sialic acid, and acute phase protein assays were about 10%, 8%, and 5% respectively.

Results

Table 2 summarises the results of measurements of the serum concentrations of fucose, sialic acid, and the acute phase proteins in patients with cancer. The protein bound sugars were measured in more patients than the acute phase proteins. Using results of protein bound sugars only when acute phase protein values were available, however, made little difference to the data as presented. The mean values and the upper 95% confidence limits of the healthy group are also given in Table 1 for comparison. The upper reference limits found were similar to those previously quoted for acute phase proteins (Beringwerke, Hoechst Pharmaceuticals), fucose,¹ and sialic

acid.⁴ Only three of the acute phase proteins—namely, α_1 -acid glycoprotein, α_1 -antitrypsin, and haptoglobins—had appreciably raised concentrations and only the α_1 -acid glycoprotein was raised as often as the protein bound sugars. Increased fucose concentrations were the most common finding, occurring in about half of the patients with cancer.

Table 3 shows the correlations between the concentration of fucose or sialic acid and the concentrations of the different acute phase proteins in individual patients with cancer. The data fall into three groups: one group (α_1 -acid glycoprotein, α_1 -groups; one group (α_1 -acid glycoprotein, α_1 -antitrypsin, haptoglobins) in which the association is strongly positive; another group (prealbumin, transferrin), in which the association is strongly negative; and a third group (caeruloplasmin, haemopexin, α_2 -macroglobulin), in which the association is much weaker. Measurements on healthy serum samples did not indicate any strong correlations except in the case of haemopexin, which appeared to correlate strongly with both sugars (data not given).

Table 4 shows the degree of increase of the protein bound sugar in the patients with cancer according to patients' disease states. The number of patients in each subgroup with raised fucose concentrations increases with tumour progression, with a 70% increase in those with more advanced disease. On the other hand, the number of patients

Table 4 Effect of disease states of patients with cancer on the serum concentrations of fucose and sialic acid

| Patient group | Fucose | | Sialic acid | | Fucose or sialic acid raised (%)† |
|---------------|---------------------------|----------------|---------------------------|----------------|-----------------------------------|
| | Mean concentration (mg/g) | No raised† (%) | Mean concentration (mg/g) | No raised† (%) | |
| Early (22) | 1.5 ± 0.7 | 18.2 | 7.1 ± 1.5 | 9.1 | 27.3 |
| Clinical (50) | 2.1 ± 0.88 | 58.0* | 10.4 ± 3.7 | 54.0* | 82.0 |
| Advanced (53) | 2.8 ± 1.2 | 69.8 | 10.3 ± 3.6 | 52.8* | 73.6 |

Values in parentheses indicate the number of patients in each group. Mean ± standard deviation.

*Indicates a significant difference between the group marked and the respective combined marker group ($p < 0.025$; χ^2 test).

†Above the upper limit of the reference range.

Table 5 Fucose, sialic acid, and the acute phase proteins as combined markers of malignancy

| Parameters measured | No of patients with raised values | | |
|-----------------------------------------------------------------------------------------------|-----------------------------------|---------------|---------------|
| | Early (22) | Clinical (50) | Advanced (53) |
| Fucose | 4 | 29 | 37 |
| Fucose and sialic acid | 6 | 41 | 39 |
| Fucose, sialic acid, and α_1 -acid glycoprotein | 6 | 42 | 42 |
| Fucose, sialic acid, α_1 -acid glycoprotein, and haptoglobins | 7 | 42 | 43 |
| Fucose, sialic acid, α_1 -acid glycoprotein, haptoglobins, and α_1 -antitrypsin | 7 | 42 | 43 |

Figures in parentheses are the total number of patients in each group. Acute phase protein measurements were not available for 2, 2, and 7 patients in the early, clinical, and advanced groups respectively.

with raised sialic acid concentrations appears to be the same in the clinical and advanced subgroups. The frequency with which any one of the two sugars was raised in the clinical subgroup was significantly higher than when either sugar was considered singly (χ^2 test, $p < 0.025$; Table 4). No association was found between increased protein bound sugar values and the initial site of tumour growth (data not given).

Table 5 illustrates the effect of combining the results for the sugar assays with those of α_1 -acid glycoprotein, α_1 -antitrypsin, and haptoglobins. The data for the sugars alone has already been given in Table 4. Inclusion of the acute phase protein measurements had little effect on combined marker increase.

Discussion

Current data suggest that increased concentrations of serum sialic acid in patients with cancer reflect an acute phase response. A strong correlation was noted between three of the acute phase proteins measured (α_1 -acid glycoprotein, α_1 -antitrypsin, and haptoglobins) and the concentrations of this sugar. Furthermore, using the reported sialic acid content of these proteins,⁶ it could be roughly estimated that the magnitude of their increase was sufficient to account for most of the extra serum sugar.

In a few patients with raised sialic acid concentrations α_1 -acid glycoprotein, α_1 -antitrypsin, and haptoglobins were not increased; in these cases the cause of the raised sialic acid value is unclear. It may be associated with a certain patient type or prognosis. We could not identify from our records any common clinical feature that correlated with the finding. We have limited details on follow up, however, and we intend to study this aspect more closely.

Two previous studies^{3,10} have attempted to examine the relations between serum sialic acid val-

ues and acute phase proteins in cancer. Bradley *et al*³ reported correlation coefficients with α_1 -acid glycoprotein, α_1 -antitrypsin, and haptoglobins of 0.95, 0.90, and 0.86 respectively. These are much higher than those found in the present study. This discrepancy may be explained by the fact that Bradley *et al* used a different method to measure sialic acid. Their method was non-specific and gave serum values that were much higher than those previously reported by other groups.^{2,4} Another study,¹⁰ which was limited to malignant melanomas, reported that sialic acid measurements correlated quite strongly with α_1 -acid glycoprotein but did not give a value for the correlation coefficient. No comment was made on the correlation between sialic acid and other acute phase proteins.

Although increased serum fucose concentrations in patients with cancer also correlate with increases in certain acute phase proteins, the correlation coefficients were less than those found for sialic acid. It seems doubtful whether the fucose and acute phase protein changes are directly related because the acute phase proteins concerned contain insufficient amounts of fucose to account for the raised serum value. The acute phase proteins may increase their fucose content in cancer; this possibility is being investigated. It is possible that other serum proteins—for example, antichymotrypsin and immunoglobulins—contribute to the increased fucose concentrations, but it seems unlikely. Either they are infrequently raised in cancer—for example, IgG³—or they are present in serum in too small amounts to make a significant contribution—for example, α_1 -antichymotrypsin.⁶

The tumour itself may also contribute to circulating fucose concentrations either by spontaneous release of glycoproteins as the mass grows or as a result of cell damage through host attack or treatment. A number of studies¹¹⁻¹⁵ have shown that malignant changes may be accompanied by increased expression of membrane associated,

fucose containing macromolecules. Furthermore, tumours can shed fucosyl containing components into their environment.¹⁶ Tumours may also contribute indirectly to serum fucose by promoting increased fucosylation of existing glycoproteins. Increased fucosyltransferase values have been noted in malignant tissues^{17, 18} and serum samples from patients with cancer.¹⁹

When the sugar data were classified according to patients' disease states we obtained further support for the idea that serum fucose and sialic acid are increased by different mechanisms. Not only were the patterns of increase for the two sugars different, but also for patients in whom the involvement of the tumour was limited either naturally or by treatment, the increase in fucose concentration often complemented the increase in sialic acid. The combined use of these two markers gave a high degree of positivity that could only be slightly improved on by including the acute phase protein measurements. To the best of our knowledge, the combined use of fucose and sialic acid for monitoring the effects of treatment has not been previously reported. Our results indicate that further investigations are necessary to assess the full potential of this approach.

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