Carbohydrate Research, 122 (1983) 295–303
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

RADIOIMMUNOASSAY OF A CANCER-RELATED GLYCOPROTEIN. CIRCULATING LEVELS

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(Received June 25th, 1982; accepted for publication in revised form, March 19th, 1983)

ABSTRACT

Circulating levels of (a) tumor-related glycoprotein(s) were determined by radioimmunoassay for a variety of patients and controls, and correlated with sialic acid concentration. Levels were highest in patients with metastatic disease and progressively declined to those with localized disease receiving therapy. Values for normal, adjuvant, and cured patients were significantly lower. Sialic acid concentrations correlated best for the metastatic group but not for the normals.

INTRODUCTION

The presence of a circulating glycoprotein in high association with malignancy has been reported[1]. Clinically relevant questions raised by this observation include those of specificity and sensitivity, as well as relation to disease burden. In the present study, the circulating levels of this glycoprotein were determined, for a variety of patients, by a competitive radioimmunoassay. Similarly, a general elevation of circulating glycoproteins has been described for a variety of diseases including cancer[2]. Primarily ascribed to acute-phase reactants, a common structural feature is the presence of sialyl groups in the saccharide chains of these glycoproteins; increases in circulating gangliosides have also been associated with malignant disease[3–5]. Thus, elevations in serum sialic acid have been correlated with the presence of tumors[6–11]. Accordingly, we assayed parallel aliquots of plasma for both total and perchloric acid-soluble sialic acid content.

EXPERIMENTAL

Blood was collected by vein puncture and cells removed by centrifugation. After treatment with perchloric acid, aliquots were analyzed for glycoprotein by radioimmunoassay, and for sialic acid by g.l.c.[12]. Separate aliquots of the plasma were analyzed directly for sialic acid. All blood samples were received in the labo-

0008-6215/83/$ 03.00

ratory in coded, numerical order. The clinical status of the donors was separately evaluated, and data were coordinated after all assays were completed.

Radioimmunoassay of the cancer-related glycoprotein was performed on aliquots (100 μL) of the neutralized, perchloric acid-soluble fraction of plasma[13]. At least two dilutions of each sample were assayed to ensure that binding was between 20 and 80% of control values. Samples with results outside of this range were assayed again at appropriate dilutions. Quantitative determination was obtained by reference to an antigen-competition curve described previously[13]. Since the experimental results were obtained over a period of several months, the competition standards were repeated at frequent intervals to ensure the applicability of the standard values.

Sialic acid analysis was performed directly on aliquots of plasma, as well as on the neutralized, perchloric acid-soluble fraction. Acid hydrolysis (50mM sulfuric acid, 80°, 45 min) was followed by chromatography as described by Silver *et al.*[12]. Quantitative determination was obtained by measurement of the peak height compared to that of standard samples.

Patients were divided into the following groups:

(1) Normal controls.

(2) Adjuvant, with no evidence of disease. Individuals with prior history of cancer who had been treated (*e.g.*, surgery, chemotherapy) and currently were asymptomatic.

(3) Cured. Patients with a prior history of cancer who had been free of clinical disease for at least five years.

(4) Inpatient comparison group. Patients with a variety of diagnoses, but not cancer.

(5) Local disease. Patients with residual or continuing malignancy who had received therapeutic treatment.

(6) Local disease. Patients with diagnosed malignancy but no evidence of metastasis; samples obtained prior to therapy.

(7) Metastatic disease. Patients with disseminated cancer receiving treatment, generally chemotherapy.

(8) Metastatic disease. Patients with disseminated disease; samples obtained prior to therapy.

RESULTS AND DISCUSSION

The circulating levels of glycoprotein in the various groups is summarized in Figs. 1 and 2. The normal set was well clustered with a very low incidence of false positives (defined as exceeding two standard deviations above the mean). There is a reasonably clear correpondence between disease burden and glycoprotein level. The highest average values were exhibited by patients with metastatic cancer pretherapy. The values successively declined to patients with localized disease receiving treatment. Adjuvant individuals who were asymptomatic, as well as cured pa-

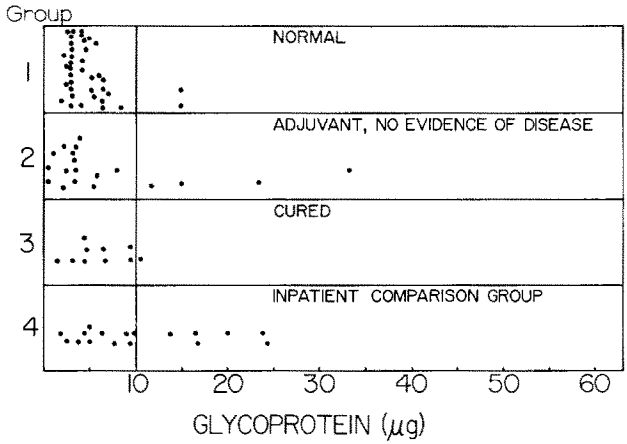

Fig. 1. Levels of circulating glycoprotein of non-cancer groups. The line at 10 μg of glycoprotein represents 2 standard deviations above the normal mean.

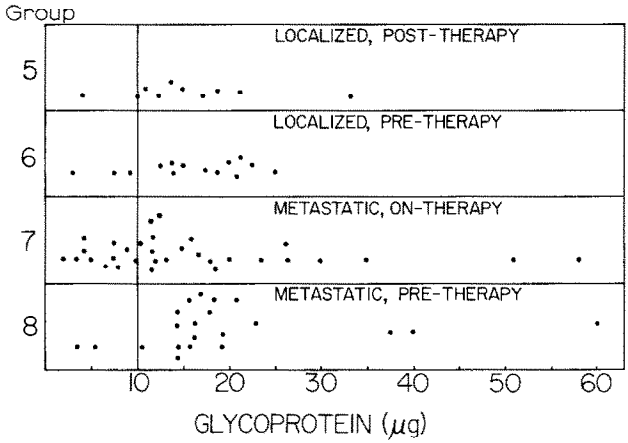

Fig. 2. Levels of circulating glycoprotein of patients with active cancer.

tients, had circulating glycoprotein levels not significantly different from those of the normal group (Fig. 3). Statistical evaluation of the data is summarized in Tables I and II.

The correlation between levels of sialic acid and those of glycoprotein is illustrated in Figs. 4–8. There is concurrence between either perchloric acid-soluble or total sialic acid, and glycoprotein levels for patients with metastatic disease. The lack of correlation between levels of sialic acid and glycoprotein for the normal group (Fig. 4) and the group with localized disease (Fig. 5) suggests that measurement of sialic acid alone may not be suitable for monitoring therapy. Comparison between total levels of sialic acid and glycoprotein for these groups failed to reveal any correspondence.

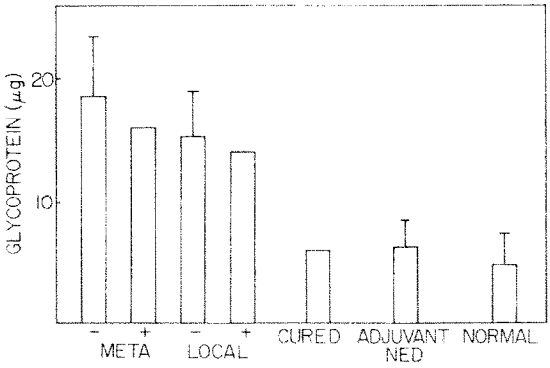

Fig. 3. Mean levels of glycoprotein for the various groups. The symbols (+)and (−) refer to patients receiving or not yet on therapy, respectively. The errors bars are for the composite set of metastatic, localized, or adjuvant and cured. Abbreviations: Meta, metastatic; Local, localized; and Ned, no evidence of disease.

In contrast, patients in the adjuvant or cured groups (Fig. 6) as well as those with metastatic disease (Fig. 7) showed a clear correlation between the measured parameters. The data for the inpatient comparison-group (Fig. 8) are inconclusive. One interpretation of these results is that, in local disease or as a result of normal

TABLE I

STATISTICAL EVALUATION OF GLYCOPROTEIN DETERMINATION DATA

Groups	*P value*	*Means*
Normal (1) *vs.* localized (5 and 6)	<0.0001	4.73 *vs.* 15.0
Normal (1) *vs.* metastatic (7 and 8)	<0.00001	4.73 *vs.* 17.1
Normal (1) *vs.* adjuvant (2)	<0.2	4.73 *vs.* 6.02
Normal (1) *vs.* cured (3)	<0.2	4.73 *vs.* 6.15
Inpatient (4) *vs.* localized (5 and 6)	<0.02	10 *vs.* 15.0
Inpatient (4) *vs.* metastatic (7 and 8)	<0.005	10 *vs.* 17.1

TABLE II

CIRCULATING GLYCOPROTEIN LEVELS IN PATIENT GROUPS

Group	*Mean level*
Normal (1)	4.73 ±2.73
Adjuvant (2)	6.02
Cured (3)	6.15
Inpatient comparison (4)	10.0
Localized, post-therapy (5)	14.1
Localized, pre-therapy (6)	15.3
Metastatic, on-therapy (7)	16.0
Metastatic, pre-therapy (8)	18.6

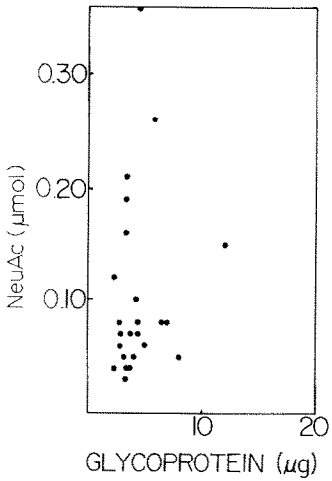

Fig. 4. Levels of sialic acid (perchloric acid-soluble) and glycoprotein in the serum of normal individuals. There is no significant correlation between the two data sets. The mean value for sialic acid is 0.088 ±0.04.

fluctuations, several complex carbohydrates increase in the circulation, thus accounting for changes in sialic acid not especially restricted to those components measured by the radioimmunoassay. As tumor burden increases, the proportion of sialic acid associated with the assayed glycoproteins increases. A recent study has directly compared levels of acid-soluble, bound sialic acid with levels of carcinoembryonic antigen in patients with colon cancer[14]. Although the latter compound is a recognized marker for this malignancy, the values for sialic acid were found to be at least as reliable, in a diagnostic sense, and complementary to a small degree. The

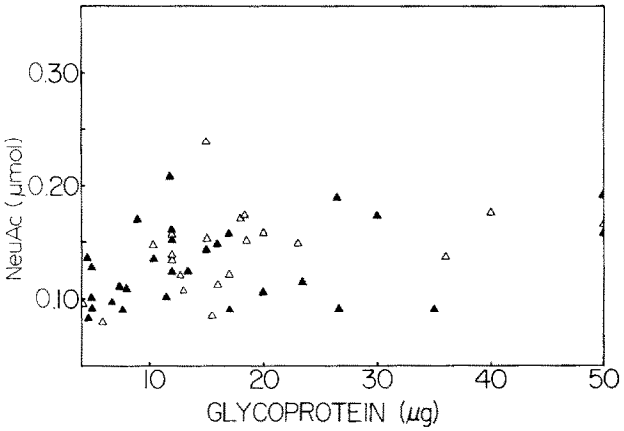

Fig. 5. Levels of sialic acid (perchloric acid-soluble) and glycoprotein in the serum of patients with localized disease; (△), prior to therapy, and (▲), post-therapy. There is no significant correlation between the two groups.

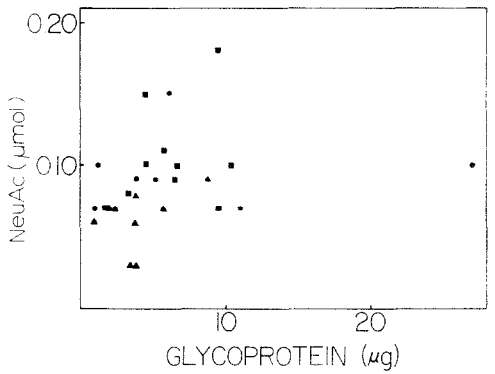

Fig. 6. Levels of sialic acid (perchloric acid-soluble) and glycoprotein in the serum of: (▲) adjuvant, (●) post-adjuvant, and (■) cured, patients; r = 0.777, $\alpha < 0.01$.

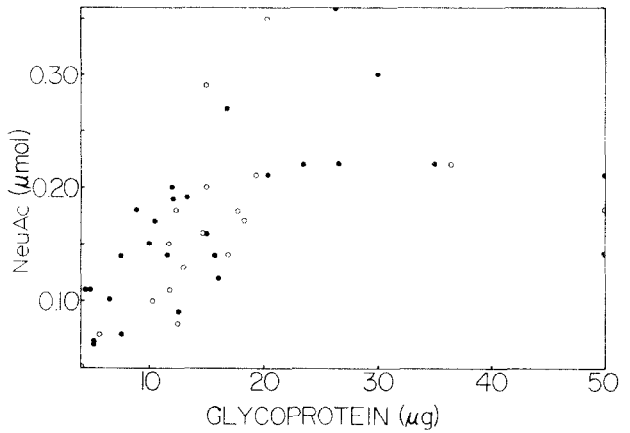

Fig. 7. Levels of sialic acid (perchloric acid-soluble) and glycoprotein in the serum of patients with metastatic disease; (●) prior to therapy, and (○) post- or on-therapy; r = 0.699, $\alpha < 0.01$.

studies in our laboratory show a reasonable correlation between sialic acid and the glycoprotein measured in the radioimmunoassay, with the latter less subject to short-term oscillations.

The significant differences between normal and either local or metastatic disease samples are quite marked (Tables I and II). The trends were for these levels of glycoprotein to diminish with successful therapy, since neither the adjuvant nor the cured group were measurably different from normal. The implication from this result is that at-risk adjuvant patients could be monitored by this technique for early recurrence of disease and, in addition, that glycoprotein levels may be a suitable index for assessing response to therapy.

The inpatient-comparison group tended to show increases in the levels of both glycoprotein and sialic acid. Values in the normal range were exhibited by patients with cirrhosis and hepatitis, whereas the very highest glycoprotein values

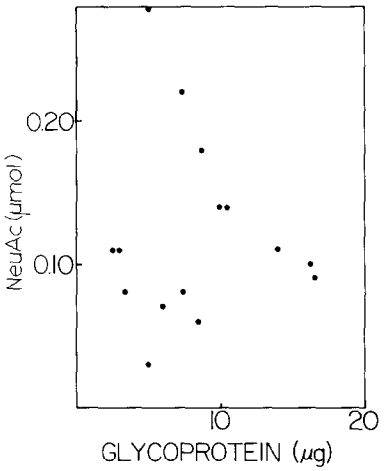

Fig. 8. Levels of sialic acid (perchloric acid-soluble) and glycoprotein in the serum of the inpatient-comparison group; $r = 0.536$, $0.01 < \alpha < 0.05$.

were observed for patients with systemic infections, and probably reflect the increases in the acute-phase reactant α_1-acid glycoprotein associated with such conditions.

A broad range of malignancies showed elevated glycoprotein values (Table III), but the ranges of glycoprotein concentrations did not reveal any specificity. The most common cancer-types, lung, mammary, and colon, all had similar values. Although this study was not sufficiently extensive to provide statistically relevant comparisons for all tumor types, it seems clear that mammary, lung, and gastroin-

TABLE III

GLYCOPROTEIN ASSAYS ON PATIENTS WITH DIAGNOSED MALIGNANCY

Diagnosis	*Samples*[a]			
	Total	*Negative*	±	*Positive*
Breast cancer	118	6	5	107
Lung cancer	70	1	3	66
Colon cancer	39	3	1	35
Melanoma	7	1		6
Lymphoma	17		1	16
Hodgkins disease	15	2		13
Others[b]	87	2	4	81
Total	353	15	14	324

[a]Positive is defined as exceeding two standard deviations above the normal mean (in excess of 10 μg); ± represents those values between one and two standard deviations above the normal mean. These data are composite and represent samples assayed over a two-year period, primarily from patients with metastatic disease. [b]Includes renal, rectal, prostate, hepatoma, pancreas, stomach, esophagus, bladder, larynx, thyroid, and common bile-duct diseases.

testinal cancer follow the overall trends and exhibit a good correlation between levels of circulating glycoprotein and tumor burden.

In order to assess the utility of the measurements just discussed as a predictor of disease state, an extended prospective study needs to be undertaken. Previous results from analysis of levels of sialic acid suggest that prognostic data of values may be obtained[15], but insufficient information is available at this time to permit broad generalizations to be drawn.

The association of glycoprotein levels with a wide variety of malignancies make it unlikely that the component(s) assayed can be identified with a specific tumor. Possible sources include the nonregulated cells, as well as modification of the synthetic profiles of normal cells by factors as yet undefined. Thus, the increased sialylation of normally circulating components may prolong their half-lives, and result in steadily rising plasma levels as a consequence of the altered balance between synthesis and clearance.

Several laboratories have shown that a glycoprotein having a solubility or antigenic properties (or both) like those of α_1-acid glycoprotein is biosynthesized by non-liver tisssues, both normal and malignant, including extracts prepared from a human-colon carcinoma grown in athymic mice[16,17]. Although the antibody employed in the studies described herein cross reacted with α_1-acid glycoprotein, the antigen employed for immunization can be distinguished from this component[13]. The increased levels of glycoprotein observed in this study, therefore, are likely to represent a composite of two or more entities with common determinants. If one such is present in substantial quantities, even large increases in the other(s) must be observed against a high-background level. Greater discrimination among the various isoforms is clearly dependent on higher antibody-selectivity which may be attained by monoclonal procedures. A number of other plasma proteins frequently elevated in disease states (those termed acute-phase reactants) were shown to be not reactive in the radioimmunoassay[13]. It should be noted that the major, perchloric acid-soluble glycoprotein normally present in the circulation is α_1-acid glycoprotein. This glycoprotein has been reported to interfere with lymphocyte mitogenesis and may play a role in the immune response of patients[18–21]. Elevations in the level of this component will result in concurrent rises in levels of sialic acid as α_1-acid glycoprotein is a major source of circulating sialic acid.

From the data presented herein, it seems clear that the measured glycoprotein levels are a broader index of tumor presence and burden than are levels of sialic acid alone. There seems to be little additional discrimination afforded by analysis for both glycoprotein and sialic acid.

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