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Lipid-associated Sialic Acid Test for the Detection of Human Cancer[1]

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ABSTRACT

A rapid method for the measurement of serum and/or plasma, lipid-associated sialic acid levels has been developed. This test has been applied to 850 human sera of which 670 came from patients with nine categories of malignant disease, 80 from persons with benign disorders, and 100 from normal individuals. Lipid-associated sialic acid concentrations were found to be significantly increased ($p < 0.001$) in all groups of cancer patients as compared to both those with benign diseases and normal controls. Test sensitivity in the detection of cancer ranged from 77 to 97%. Specificity was, respectively, 81 and 93% for the benign and normal groups. In small samples of patients, no association between test values and tumor burden was found. This test compares favorably with the most widely used tumor marker test, that for carcinoembryonic antigen.

INTRODUCTION

Previous work (2, 5, 7, 13, 16, 25) has shown that ganglioside levels are increased in cancer cells and in the plasma membranes of such cells as compared to their normal counterparts. These increases are reflected in elevated ganglioside blood levels found in cancer-bearing hosts. Such increases in blood concentrations have until now been documented principally in animals (5, 25). However, they have also been found (26) among a small number of patients tested. Larger clinical studies have been hindered by the tedious nature of existing methods for measuring individual gangliosides in serum specimens. Recently, Katopodis and Stock (11, 12) developed a more rapid assay which measures LSA,[3] rather than individual serum ganglioside concentrations. Using this test, LSA concentrations have been measured in the serum and plasma of normal individuals, in those with benign diseases, and in large numbers of patients with malignant neoplasms. The results of this work are presented in this paper. They show consistent, highly significant increases of LSA levels in cancer patients and suggest that the LSA test may prove to be an important new clinical tool for the diagnosis of cancer and the management of patients with cancer.

MATERIALS AND METHODS

Sera Studied. All sera from persons with malignant diseases were secured from Memorial Hospital for Cancer and Allied Diseases, New York, N. Y., while sera from those with benign disorders came from Memorial Hospital and the Queens General Medical Center, Queens, N. Y. Normal control sera were obtained from the New York Blood Center. They represent samples drawn from persons screened by a physician and found to be in good health and, therefore, suitable as blood donors. Patient sera were drawn at the time of hospital admission. For patients with cancer, this meant that the stage of disease often remained to be determined. Follow-up in patients with breast and colon cancer provided sufficient information regarding tumor to permit correlation between LSA test result and extent of disease to be considered. To prepare sera for testing, blood samples were permitted to clot at room temperature for 20 min and then at 4° for 20 min, after which they were centrifuged at 2000 rpm in an International Model PRJ refrigerated centrifuge for 5 min. Sera were then stored at −70° in a Revco freezer until the time of testing. Also tested were plasma samples. To obtain plasma, blood samples were drawn into a Vacutainer tube containing EDTA. Such samples were centrifuged in the same manner as clotted blood. The plasma aliquots were stored at −20°. Comparison of test values obtained with frozen and fresh specimens showed generally similar values, and so the data obtained with either sample type have been pooled.

LSA Assay. The improved procedure as published recently (12) has the following steps. (*a*) To a screw-cap culture tube (13 x 100 mm) were added 150 μl distilled water with a 500-μl Hamilton syringe. To this tube, 44.7 μl of plasma (or serum) were transferred with a capillary pipet (Unopette; Becton, Dickinson & Co.). The contents were vortexed for 5 sec. The tube was transferred to crushed ice. (*b*) Three ml cold (4–5°) 2:1 (v/v) chloroform: methanol were added to the tube, and the mixture was vortexed for 30 sec. (*c*) To this mixture was added 0.5 ml cold distilled water, the tube was capped, and the contents were mixed by repeatedly inverting the tube for 30 sec. (*d*) After centrifuging the tube 5 min at room temperature at 2500 rpm, 1 ml of the upper layer was transferred into a 13- x 100-mm culture tube. (*e*) Fifty μl phosphotungstic acid solution (1 g/ml) were added, and after mixing the tube stood at room temperature for 5 min. (*f*) The tube was centrifuged for 5 min at 2500 rpm, and the supernatant was removed by suction. (*g*) One ml water was added, and the tube was vortexed until the precipitate was in suspension without grossly visible particles (about 1 min). (*h*) One ml of the resorcinol reagent was added, and the tube was mixed and placed in boiling water for exactly 15 min. (*i*) Immediately after the 15 min, the tube was transferred to an ice and water bath and left for 10 min. (*j*) To the ice-cold tube, 2 ml 85:15 (v/v) butyl acetate:*n*-butyl alcohol were added at room temperature, and the tube was vortexed and centrifuged for 5 min at 2500 rpm. (*k*) The extracted blue color was read at 580 nm. The amount of LSA was determined by use of a standard curve[4] developed from a standard sample of *n*-acetyl neuraminic acid (Sigma Chemical Co.) using the formula

$$\text{LSA (mg/100 ml plasma)} = \frac{(x)\,(100{,}000\ \mu l)}{(y)\,(44.7\ \mu l)\,(1{,}000)}$$

where x = μg *n*-acetyl neuraminic acid read from standard curve for the sample, and y = 1 ml of supernatant divided by volume of entire supernatant. (In our experience, this has been 1.00/1.30.)

Statistical Analysis. A technique of exploratory data analysis, parallel schematic plots, was used initially to visualize the shapes of various distributions (27). The locations of several samples were com-

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[3] The abbreviations used are: LSA, lipid-associated sialic acid; CEA, carcinoembryonic antigen.

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[4] Standard curves and control plasma samples have been run each day to detect possible reagent or procedural errors.

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pared using the Kruskal-Wallis test (10). This reduced to the Wilcoxon test in the 2-sample case. Multiple comparisons were made, when appropriate, using Dunn's multiple comparison procedure (10). Whether LSA levels varied with age was investigated using Spearman's rank correlation (10). Lastly, the association between extent of disease and elevated LSA was investigated using Fisher's exact test. Because of the number of significance tests performed, it is likely that at least one null hypothesis was rejected when it was in fact true.

RESULTS

Samples from 850 persons were tested for LSA. These included 100 samples from normal individuals, 80 from patients with benign diseases, and 670 from patients with a variety of cancers.

Chart 1 presents a summary of LSA values in 4 of the test groups: normal controls; colon cancer; Hodgkin's disease; and lung cancer. The boxes in the 4 parallel schematic plots indicate the median, first, and third quartiles for each group. In addition, each datum value which lies below the first or beyond the third quartile by at least one interquartile range is indicated by a *dot*. These parallel schematic plots show unexpectedly large LSA values (outliers), which indicate that the data are not normally distributed, and so nonparametric methods of statistical analysis, such as the Kruskal-Wallis test (10), were used. Chart 1 also illustrates the considerable differences between LSA concentrations in the sera of normal individuals and individuals in the cancer groups.

Among the 100 normal individuals tested (Table 1), the median LSA value was 17.8 mg/100 ml, with values ranging between 13.8 and 16.0 mg/100 ml. Also tested as controls were sera from 2 groups of patients with benign disorders. The first group of 62 patients was hospitalized at Queens General Hospital and was affected principally by systemic medical problems. The patients were all acutely ill. The disorders in this patient group included acute gastrointestinal hemorrhage secondary principally to peptic ulcer disease (10); chronic alcoholism (11) (of these, 5 were admitted with cirrhosis of the liver); congestive heart failure (6); and diabetes (10). Other patients had a wide variety of benign diseases seen in an acute care general hospital ranging from asthma to cellulitis of the foot. The second group of 18 patients were patients at Memorial Hospital who had benign disease with findings that initially suggested the presence of cancer and the need for surgery. Among these were patients with fibroadenoma of the breast (3), leiomyomas of the uterus (2), laryngeal polyps, thyroid adenoma, and lipoma. The median LSA value for the Queens (medical) patients was 17.8 mg/100 ml and for the Memorial (surgical) patients was 17.5 mg/100 ml.

The Kruskal-Wallis test was used to compare the 2 patient groups and the normal group. No statistically significant difference was found, although the medical group had a noticeably greater spread in LSA values than the other 2 groups. LSA data for the 3 control groups are summarized in Table 1.

Measurements of LSA were then completed for individuals with cancer. Included were patients with 9 categories of cancer represented by samples of sizes between 13 and 196 patients. A summary of these data is given in Table 2. In all instances, the median LSA values for the cancer groups were significantly higher than the median LSA values for normal controls and for patients with benign disease. A Kruskal-Wallis test confirmed that the cancer group with the smallest median value (breast cancer) had a significantly higher median LSA value than the normal group ($\chi^2 = 86.03$, $df = 1$, $p < 0.001$). Similarly, the median LSA value for the breast cancer group was significantly higher than the median for the benign (medical and surgical combined) groups ($\chi^2 = 55.22$, $df = 1$, $p < 0.001$). Dunn's multiple comparison procedure confirmed that the median LSA value of each cancer group was significantly higher than the median LSA value for either the normal or benign group.

Further analysis using the Kruskal-Wallis test revealed that the median LSA values of the cancer groups themselves dif-

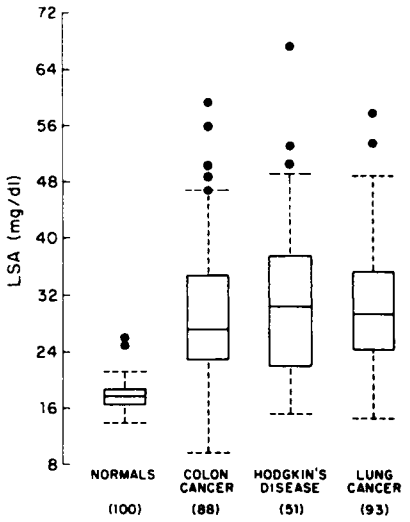

Chart 1. Parallel schematic plots: LSA in four groups. The median, first, and third quartiles of each of 4 of the test groups are indicated on a rectangle. Each datum which lies more than one interquartile range above the upper quartile or more than one interquartile range below the lower quartile is also plotted. The cancer groups appear to have both greater spread of LSA values and larger LSA values than do the normals.

Table 1

Summary of LSA values for controls

Type of control	Sample size	LSA (mg/100 ml)
Surgical (Memorial Hospital)	18	17.5[a] (12.5–22.0)[b]
Normals (nonpatients)	100	17.8 (13.8–26.0)
Medical (Queens General Hospital)	62	17.8 (5.0–34.3)
All	180	17.7 (5.0–34.3)

[a] Median value.
[b] Numbers in parentheses, range.

Table 2

Summary of LSA values for cancer patients

Type of cancer	Sample size	LSA (mg/100 ml)
Breast	111	24.3[a] (13.2–41.7)[b]
Melanoma	13	25.0 (19.7–52.7)
Colon-rectal	88	25.4 (8.8–59.3)
Miscellaneous	196	25.8 (13.0–80.0)
Lung	93	28.7 (14.0–57.8)
Hodgkin's disease	51	29.7 (14.9–67.4)
Sarcoma	37	29.8 (13.5–47.0)
Acute myelogenous leukemia	40	31.8 (11.0–90.0)
Chronic myelogenous leukemia	41	32.5 (12.7–77.0)
All	670	26.3 (8.8–90.0)

[a] Median value.
[b] Numbers in parentheses, range.

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fered significantly ($\chi^2 = 40.82$, $df = 8$, $p < 0.001$). By Dunn's multiple comparison procedure at the 0.05 overall significance level, the breast cancer median LSA value (24.3 mg/100 ml) was significantly lower than the miscellaneous cancer median LSA value (25.8 mg/ml) as well as significantly lower than the median LSA values for lung cancer, Hodgkin's disease, sarcoma, acute myelogenous leukemia, and chronic myelogenous leukemia. The breast cancer median LSA value was not significantly different from the colon cancer median LSA value (25.4/100 ml) or the melanoma median LSA value (25.0 mg/100 ml). No other median LSA values differed significantly from that of breast cancer by Dunn's multiple comparison procedure at the $\alpha = 0.05$ level. This multiple comparison procedure (10) is known to be conservative and declares relatively few of the medians to be significantly different from each other.

Differences in LSA values by sex were investigated. For each cancer group and control group, LSA values were compared for males and females using the Kruskal-Wallis test (10).

For each cancer group and control group, the rank correlation between age and LSA values was also calculated. Age and LSA were significantly (negatively) correlated in the lung cancer group ($r = -0.29$, $p = 0.004$) and significantly (positively) correlated in the benign group ($r = 0.25$, $p = 0.031$). No other rank correlations between LSA values and age were significant.

To further evaluate the LSA test, sensitivity (true positives) and specificity (true negatives) were considered. A cutoff value had to be set, so that LSA above that value would be considered positive, and LSA below that value would be considered negative. When data for normal individuals have a normal distribution, the cutoff is taken to be the mean plus 2 S.D., which would give 21.70 mg/100 ml using the LSA values for the normal individuals considered here. Since no assumption of normality of the data is made, the median plus the interquartile range, which is 20.15 mg/100 ml, may be used as the cutoff value. We selected a cutoff of 20 mg/100 ml because it is 20.15 mg/100 ml rounded off. If our cutoff was too low, this would be reflected by decreased specificity. If our cutoff was too high, this would be reflected by decreased sensitivity. Since the minimum of all of the specificities and sensitivities we calculated was 0.77, we consider the cutoff of 20 mg/100 ml to be adequate.

Sensitivity and specificity of the LSA test are considered in Tables 3 and 4. Using 20 mg/100 ml as the upper limit of normal, test sensitivity in those with cancer varied from 77 to 97%. As might be expected from the median test values, the test was least sensitive for breast cancer. In normal individuals, test specificity was 93%, so that only 7% of those tested were falsely positive. For patients with benign surgical disorders, the test was also 94% specific. Specificity was lower (77%) when considered for individuals with benign medical diseases.

A portion of the colon and breast carcinoma data was classified by extent of disease, which permitted the sensitivity and specificity of the LSA test in relation to tumor burden to be determined for these diseases. Taking 20 mg/100 ml as the upper limit of normal, the number of elevated values was compared pairwise for those with local, regional, or distant disease *versus* those with no disease or with benign disorders. Using Fisher's exact test, patients with local carcinoma of the breast (Table 5) showed highly significant differences when compared to either the normal or benign groups ($p < 0.001$). The sensitivity was 100%. The specificity was 93% for normals and 81% for those with benign diseases. When individuals whose breast carcinoma had metastasized to regional lymph nodes were considered and their test values compared to those of normal individuals or others with benign breast disease, again the difference in elevated values was highly significant ($p < 0.001$). For regional disease, the sensitivity was 96%. The findings are similar when comparisons of LSA values are made between individuals with extensive distant breast carcinoma and those who have no breast carcinoma or benign disease. The differences are significant with p values less than

Table 3

Specificity of the LSA test

LSA test specificity	*n*	LSA ≤ 20 (normal)	Specificity = true negative
Normals	100	93	0.93
Medical (Queens General Hospital)	62	48	0.77
Surgical (Memorial Hospital)	18	17	0.94

Table 4

Sensitivity of the LSA test

LSA test sensitivity	*n*	LSA > 20 (elevated)	Sensitivity = true positive
Breast carcinoma	111	86	0.77
Colon carcinoma	88	81	0.92
Hodgkin's disease	51	46	0.90
Leukemia			
Acute myelogenous leukemia	40	36	0.90
Chronic myelogenous leukemia	41	35	0.85
Lung carcinoma	93	82	0.88
Melanoma	13	12	0.92
Miscellaneous carcinoma	196	180	0.92
Sarcoma	37	36	0.97

Table 5

Extent of disease and the LSA test (breast cancer)

	Local	Normal	Local	Benign (all)	
Positive (LSA > 20)	12	7	12	15	
Negative (LSA ≤ 20)	0	93	0	65	
Fisher's exact test *p*	< 0.001		Fisher's exact test *p*		< 0.001
Sensitivity = 12/12	= 1.000		Sensitivity = 12/12		= 1.000
S.E. of sensitivity	< 0.001		S.E. of sensitivity		< 0.001
Specificity = 93/100	= 0.930		Specificity = 65/80		= 0.813
S.E. of specificity	= 0.026		S.E. of specificity		= 0.044
Misclassified = 7/112	= 0.063		Misclassified = 15/92		= 0.163
	Regional	Normal	Regional	Benign (all)	
Positive (LSA > 20)	23	7	23	15	
Negative (LSA ≤ 20)	1	93	1	65	
Fisher's exact test *p*	< 0.001		Fisher's exact test *p*		< 0.001
Sensitivity = 23/24	= 0.958		Sensitivity = 23/24		= 0.958
S.E. of sensitivity	= 0.041		S.E. of sensitivity		= 0.041
Specificity = 93/100	= 0.930		Specificity = 65/80		= 0.813
S.E. of specificity	= 0.026		S.E. of specificity		= 0.044
Misclassified = 8/124	= 0.065		Misclassified = 16/104		= 0.154
	Distant	Normal	Distant	Benign (all)	
Positive (LSA > 20)	6	7	6	15	
Negative (LSA ≤ 20)	1	93	1	65	
Fisher's exact test *p*	< 0.001		Fisher's exact test *p*		= 0.001
Sensitivity = 6/7	= 0.857		Sensitivity = 6/7		= 0.857
S.E. of sensitivity	= 0.132		S.E. of sensitivity		= 0.132
Specificity = 93/100	= 0.930		Specificity = 65/80		= 0.813
S.E. of specificity	= 0.026		S.E. of specificity		= 0.044
Misclassified = 8/107	= 0.075		Misclassified = 16/87		= 0.184

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0.001. LSA test sensitivity for those with distant metastases is 86%. Using the Kruskal-Wallis test, it was found that the LSA values for persons with local, regional, or distant disease were not significantly different, although the sample sizes were too small for this negative result to be conclusive.

Turning to carcinoma of the colon (Table 6), differences in the frequency of elevated LSA values also are highly significant when comparison is made pairwise between individuals with local, regional, or distant disease and controls who have benign disorders or who are free of disease (Fisher's exact test, $p < 0.001$). In these groups, sensitivity was 82, 100, and 100%, respectively, for those with colon cancer. Specificity was 81% for persons with benign disease and 93% for normal individuals. Again with small samples, no significant differences were found in the LSA values of those with local, regional, and distant disease.

Table 6

Extent of disease and the LSA test (colon cancer)

	Local	Normal	Local	Benign (all)	
Positive (LSA > 20)	9	7	9	15	
Negative (LSA ≤ 20)	2	93	2	65	
Fisher's exact test p	< 0.001		Fisher's exact test p		< 0.001
Sensitivity = 9/11	= 0.818		Sensitivity = 9/11		= 0.818
S.E. of sensitivity	= 0.116		S.E. of sensitivity		= 0.116
Specificity = 93/100	= 0.930		Specificity = 65/80		= 0.813
S.E. of specificity	= 0.026		S.E. of specificity		= 0.044
Misclassified = 9/111	= 0.081		Misclassified = 17/91		= 0.187
	Regional	**Normal**	**Regional**	**Benign (all)**	
Positive (LSA > 20)	5	7	5	15	
Negative (LSA ≤ 20)	0	93	0	65	
Fisher's exact test p	< 0.001		Fisher's exact test p		< 0.001
Sensitivity = 5/5	= 1.000		Sensitivity = 5/5		= 1.000
S.E. of sensitivity	< 0.001		S.E. of sensitivity		< 0.001
Specificity = 93/100	= 0.930		Specificity = 65/80		= 0.813
S.E. of specificity	= 0.255		S.E. of specificity		= 0.044
Misclassified = 7/105	= 0.067		Misclassified = 15/85		= 0.176
	Distant	**Normal**	**Distant**	**Benign (all)**	
Positive (LSA > 20)	7	7	7	15	
Negative (LSA ≤ 20)	0	93	0	65	
Fisher's exact test p	< 0.001		Fisher's exact test p		< 0.001
Sensitivity = 7/7	= 1.000		Sensitivity = 7/7		= 1.000
S.E. of sensitivity	< 0.001		S.E. of sensitivity		< 0.001
Specificity = 93/100	= 0.930		Specificity = 65/80		= 0.813
S.E. of specificity	= 0.026		S.E. of specificity		= 0.044
Misclassified = 7/107	= 0.065		Misclassified = 15/87		= 0.172

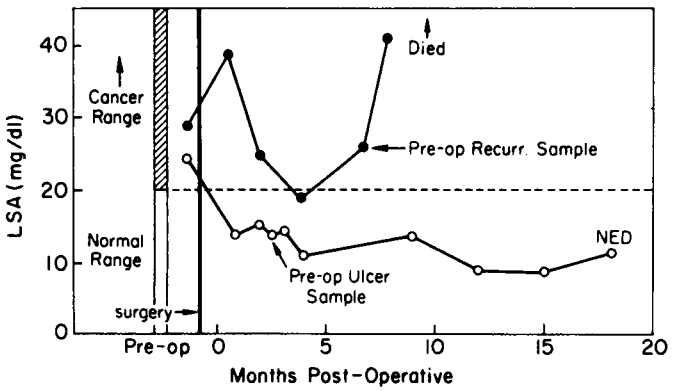

Chart 2. Changes in LSA levels during the course of disease in 2 patients with sarcoma. Both patients had elevated LSA values preoperatively at the time of diagnosis. The patient who is free of disease had a fall in LSA levels to normal after surgery. They remain low. In the other case, LSA levels fell transiently postoperatively but then rose sharply as tumor growth progressed. *NED*, no evidence of disease.

Prospective studies of changes in LSA values during the course of disease are underway. Preliminary data are encouraging as illustrated by the 2 patients with sarcoma whose serial LSA test results are plotted in Chart 2. In the patient who has remained disease free, LSA values fell to normal shortly after the tumor was resected at surgery. Another patient who died of his disease had a transient fall in serum LSA concentration on recovering from surgery but still generally maintained above normal LSA levels. On recurrence of clinical disease, the LSA values began to rise and reached their highest level shortly before the patient died.

DISCUSSION

This analysis of clinical data currently available for the LSA test confirms our previous observations (8, 12) which have indicated that LSA levels are significantly increased in the sera of cancer patients and that the LSA test may prove to be of clinical value. While increases are frequently modest, the demarcation between normal and abnormal levels is sufficiently sharp so that with a 20 mg/100 ml cutoff, test specificity in normals and a surgical patient group was found to be, respectively, 93 and 94% (false positives, 6 to 7%). Fortunately, age and sex seem to play a minor role, if any, in determining serum LSA concentrations. The nature of the tumor is important, however, so that in breast cancer, elevations are more modest, and test sensitivity is lower. In most forms of cancer, sensitivity is surprisingly high. It is 90% or above in 6 of the 9 cancer patient groups tested.

Test specificity and sensitivity data both look favorable when compared to those of the most widely used immunodiagnostic test, CEA. In a randomly selected group of normal persons, the CEA test is positive in 5 to 10% of those tested (6, 23) depending on the assay used (specificity, 90 to 95%). In those with benign diseases, CEA test specificity falls to approximately 65% (6), and it may be as low as 30% (1, 6) if patients with benign gastrointestinal disorders are tested. Sensitivity of the CEA test in colon cancer has been reported to be 49 to 78% (23). In a preliminary study, Dnistrian and Schwartz (4) compared the sensitivity of the LSA and CEA tests in patients with 7 forms of cancer. LSA levels were increased in 12 of 18 patients with colon cancer, and CEA levels were increased in 16 of 18 of the same patients. On the other hand, none of the 12 patients with melanoma tested and only 5 of 18 Hodgkin's disease patients had positive CEA tests, while with the LSA tests, 10 of the same 12 melanoma patients tested had abnormal values, as did 17 of 18 patients with Hodgkin's disease.

Of interest as well is the high sensitivity found for the LSA test in those with local and regional carcinomas of the breast and colon. Should these findings remain unchanged as the sizes of the groups studied increase, it would speak well for the diagnostic and monitoring value of the test.

Increases in the levels of sialic acid-associated substances in the sera of cancer patients have been noted by others. The association of glycoproteins with cancer has been well documented and reviewed (21). Sialic acid is a structural component of most glycoproteins. The 2 leading diagnostic markers of human cancer, CEA and α-fetoprotein, are both glycoproteins. In 1958, Winzler (28) reported significant elevations in protein-

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bound sialic acid levels in patients with cancer. Similar increases were noticed in patients with inflammatory disorders. MacBeth and Bekesi (18) in 1962 noted high total sialic acid concentrations in the sera of patients with cancer in their analysis of a study of plasma glycoproteins. They found that levels were increased among those who had distant metastatic tumor. About 10 years later, Brozmanova and Skrovina (3) studied serum sialic acid concentrations in individuals with bone tumors. They observed significant elevations among sarcoma patients as compared to those with benign neoplasms. In more recent studies, serum sialic acid concentrations have been found elevated in persons with breast carcinoma and malignant melanoma. Other investigations (14, 17, 19) have shown sialic acid levels to be associated with stage of disease, recurrence of disease, extent of metastases, and total tumor burden. Some laboratories have found that sialic acid levels are also increased in those with chronic or degenerative diseases, as is CEA (24).

Typical of the more recent work performed with sialic acid measurements is that reported by Silver *et al.* (24) for malignant melanoma. He compared the prevalence of elevated sialic acid levels in patients with (*a*) no evidence of disease following surgery, (*b*) minimal tumor burden, and (*c*) advanced metastatic disease. Using a calculated upper limit of normal of 2.3 μmol/ml, elevated levels were found, respectively, in 6, 31, and 95% of the 3 study groups. While sialic acid levels were also increased, although to a lesser extent, in those with rheumatoid arthritis, these investigators felt that sialic acid measurement should be useful in detecting the presence of recurrent, occult but still sizable quantities of tumor or assessing total tumor burden. Similarly, a positive association with stage of human breast cancer was shown by Hogan-Ryan *et al.* (9). This correlation was stronger with sialic acid than it was with either CEA or the erythrocyte sedimentation rate. Mrochek *et al.* (20) have measured protein-bound sialic acid levels in women with breast cancer. They report consistent increases occurring in those with progressive tumor and consistent decreases of such sialic acid measurements in patients whose disease is responding to therapy.

In regard to glycolipids, observations of Skipski *et al.* (26) and reports by Silver *et al.* (24) of elevated sialic acid levels in malignant melanoma patients stimulated Kloppel *et al.* (15) in 1977 to measure total levels of serum sialic acid glycolipids in mice bearing transplantable mammary carcinomas. They found levels to be 2.5 times those in normal mice. They also used the same assay to measure sialic acid glycolipid concentrations in human breast cancer patients and normal controls. A 2-fold increase in ganglioside levels was found. In 1978, Portoukalian *et al.* (22) also found ganglioside levels to be high in the plasma of patients with malignant melanoma. Full exploitation of these observations was hindered by the relatively tedious methods needed to measure serum gangliosides. The LSA test overcame this problem and has made larger scale clinical trials possible.

The high specificity and sensitivity of the LSA tests determined in the current study make further clinical evaluation desirable. It is necessary now to undertake the more extensive clinical studies which will show whether the LSA test (*a*) provides significant new prognostic information; (*b*) can be used following treatment to establish disease-free status; and (*c*) will serve as a reliable indicator of recurrence, with sufficient lead time to permit helpful therapeutic intervention. This work is under way.

Perhaps the most intriguing question is why ganglioside levels are increased in cancer patients. This may only be a reflection of tumor breakdown. On the other hand, it may have more fundamental implications, since as integral parts of the cell membrane, the gangliosides may play a central role in the process of cell-to-cell recognition and in the regulation of feedback growth inhibition that results from cell-to-cell contact. Encouraging further work along these lines will be an important contribution of this new test.

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