Elevated Serum Acute Phase Protein Levels as Predictors of Disseminated Breast Cancer

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Serum levels of four acute phase proteins, alpha$_1$-acid glycoprotein (AAGP), alpha$_1$-antitrypsin (AAT), haptoglobin (Hpt), and C3, were measured prior to biopsy in 38 women subsequently shown to have Stage I and II breast cancer and prior to treatment in 16 women with Stage IV disease. Sixty-one women with benign and 28 women with no breast disease served as controls. Mean serum levels of all four proteins were significantly elevated in women with stage IV disease as compared to women with Stage I or II disease or controls. Normal *versus* elevated levels for each protein were defined and AAGP was found to be the single most sensitive predictor of disseminated disease among the four. AAGP was elevated in 81.3% of Stage IV, 25% of Stage II, 14.3% of Stage I, and 12.4% of controls. Women with multiple proteins elevated were most likely to have advanced stage disease. Composite analysis of all four proteins using number of proteins abnormal or logistic regression analysis gave results similar to AAGP, both showing increasing numbers of proteins abnormal with increasing stage of breast cancer. These results indicate that measurement of serum acute phase proteins may be useful in initial staging of breast cancer patients and in following patients for indications of disseminated disease.

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ALTHOUGH no tumor-specific protein has yet been identified in the serum of women with breast cancer, we have reported lower concentrations of several normally occurring proteins including albumin, transferrin, IgG, and IgM.[16] In addition, others have reported elevations of acute phase serum proteins in breast cancer patients.[4–6,8–10,12] This report corroborates and extends those observations, documenting the relationship between altered acute phase serum protein patterns and the stage of breast cancer. Although these acute phase proteins may also be found elevated in patients with other malignancies or inflammatory diseases, their measurement does appear to be useful for evaluating patients with known breast cancer.

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Materials and Methods

Ninety-nine white women and one man presenting to the Maine Medical Center for biopsy of a breast lesion were enrolled in the study after informed consent. All were otherwise healthy by clinical evaluation, and none were pregnant. Blood was drawn for analysis prior to biopsy and pathologic diagnosis. Subsequently, 61 patients were diagnosed as having benign breast disease and 39 patients as having malignant disease. Those with malignant disease were staged according to the American Joint Committee on Staging criteria.[1] Twenty-one patients were classified as Stage I, 16 as Stage II, and one as Stage IV disease. Also studied was a group of 15 patients with known Stage IV breast cancer who were not yet treated for recurrence and whose serum was available from the serum bank maintained by our laboratory. In addition, 28 healthy white women with no history of breast disease, age-matched to the malignant disease patients, were enrolled. These disease-free women and the patients with benign lesions collectively served as controls. Clinical and pathologic information was obtained on all study subjects including age, menstrual status, obstetrical history, estrogen use, previous medical history, family history of breast disease, specific histologic classification, pathologic stage, and estrogen receptor status of malignant lesions. Further characterization

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TABLE 1. Mean Serum Acute Phase Protein Levels in Study Subjects

		AAGP		AAT		HPT		C3	
Class	No.	Mean	Sem	Mean	Sem	Mean	Sem	Mean	Sem
Control	89	57	2.7	188	4.8	93	4.9	136	3.8
Stage I	21	64	7.2	194	13.8	110	9.2	128	3.9
Stage II	16	64	7.9	203	12.5	133	12.0	137	10.7
Stage IV	16	123	15.4	300	35.7	184	26.0	176	9.4
P values									
Stage I & II *vs* control		n.s.		n.s.		0.005		n.s.	
Stage IV *vs* control		0.001		0.01		0.005		0.001	
Stage IV *vs* Stage I & II		0.005		0.05		0.05		0.001	

of the study population is detailed in our previous report.[16] Serum protein analyses were carried out by an automated nephelometric technique developed at our laboratory and described in detail elsewhere.[13,14] Each serum sample was analyzed for 14 individual serum proteins, among which were four acute phase proteins: alpha-acid glycoprotein (AAGP), alpha-antitrypsin (AAT), haptoglobin (Hpt), and C3. Statistical methods included logistic regression analysis.[3]

Results

The normal and benign disease study subjects had similar serum protein concentrations and were combined as a single control group. Table 1 shows mean acute phase protein values for the controls and for the women with various stages of breast cancer. Patients with Stage IV disease show well-defined rises in mean concentration of all four proteins, while women with Stage I and II breast cancer show significant elevations only in mean haptoglobin levels. In spite of the fact that AAGP, AAT, and C3 mean concentrations are not significantly increased in early stage disease, a consistent trend towards higher values can be seen for AAGP and AAT.

This trend can be seen more clearly by defining elevated (abnormal) *versus* normal concentrations for each of the four proteins on the basis of comparing intersecting frequency distribution patterns for the malignant and control groups. Table 2 defines cut-off values thus, derived for the four acute phase proteins and shows the percent of women in each group with individual values above the cut-off. Controls and Stage I subjects have generally similar rates of AAGP and Hpt elevations, while C3 and AAT vary. Stage II patients show a distinct trend towards higher value for each of the four proteins, and this is more clearly visible than in Table 1. Among the 16 Stage II women, 12 have lymph node metastases, and four are classified on the basis of size alone. Acute phase protein elevations are restricted in Stage II women to those with nodal metastases, but there are too few without nodal metastases to draw firm comparisons. A very high percentage of Stage IV patients show elevated concentrations of each of the four proteins.

A further analysis of the data can be made by tallying the number of acute phase proteins elevated in each individual study subject. Table 3 summarizes this. Individual study subjects with malignancy have multiple acute phase proteins elevated more often than controls, and this pattern becomes increasingly prevelant with increasing stage of disease. Only patients with Stage IV or nodal Stage II disease have all four proteins elevated, and, conversely, no patients with Stage IV disease have all four proteins in the normal range. Thus, increasing numbers of elevated serum acute phase proteins in a given individual indicate progressively increased risk of having disseminated breast cancer.

The composite analysis of acute phase proteins can be carried further by stepwise logistic regression analysis.[13] This technique first evaluates the four proteins in a stepwise manner and selects at each step the protein

TABLE 2. Percentage of Study Subjects with Elevated Acute Phase Protein Concentrations

Protein	MG/DL	Control (n = 89)	Stage I (n = 21)	Stage II (n = 16)	Stage IV (n = 16)
AAGP	>80	11 (12.4%)	3 (14.3%)	4 (25.0%)	13 (81.3%)
AAT	>240	5 (5.6%)	4 (19.1%)	3 (18.8%)	10 (62.5%)
HPT	>180	3 (3.4%)	1 (4.8%)	3 (18.8%)	8 (50.0%)
C3	>150	24 (27.0%)	1 (4.8%)	6 (37.5%)	12 (75.0%)

TABLE 3. Number of Elevated Acute Phase Proteins in Individual Study Subjects

# of proteins above defined cut-off	Control (n = 89)	Stage I (n = 21)	Stage II (n = 16)	Stage IV (n = 16)
4	0 (0.0%)	0 (0.0%)	1 (6.3%)	4 (25.0%)
3 or more	1 (1.1%)	1 (4.8%)	4 (25.0%)	9 (56.3%)
2 or more	7 (7.9%)	2 (9.5%)	5 (31.3%)	14 (87.5%)
1 or more	35 (39.3%)	6 (28.6%)	6 (37.5%)	16 (100.0%)
none	54 (60.7%)	15 (71.4%)	10 (62.5%)	0 (0.0%)

TABLE 4. Estimated Probability of Stage IV Breast Cancer*

Probability†	Control	Stage I	Stage II	Stage IV
1.00				2
0.95				1
0.90	1			1
0.85				
0.80	1		1	1
0.75	1		1	
0.70		1		2
0.65	1		1	2
0.60			1	
0.55	3			
0.50				1
0.45	2			
0.40	1			1
0.35		1		2
0.30	1	1		1
0.25	8	1	1	
0.20	6	1		
0.15	8	1	1	2
0.10	10	5	2	
0.05	33	7	6	
0.00	13	3	2	
Total	89	21	16	16

* Numbers represent study subjects at each interval, the dotted line arbitrarily demarcates high and low risk.

† Logarithmic regression analysis combining evaluation of four acute phase proteins in serum.

which adds most to the ability to discriminate Stage IV patients from Stage I and II patients. Each individual is then assigned a probability of having Stage IV disease. Table 4 displays these probabilities for all study subjects. At a probability level of 0.35, 11.2% of controls, 9.5% of Stage I patients, 25% of Stage II patients, and 81.2% of Stage IV patients would be classified as likely to have Stage IV disease. Table 5 compares this analytic approach with two others as predictors of disseminated breast cancer. In all three there is little difference between controls and women with Stage I disease, after which the percentage with positive results rise steadily. AAGP, logistic regression scoring and analysis using at least two proteins above the cut-off are equivalent in predictive value, both in keeping low the percentage of control and Stage I women labeled as positive and in identifying correctly women with disseminated disease. Prospective evaluation of patients using these analytic methods may subsequently show one superior to the others.

TABLE 5. Predictive Value of Three Analytic Approaches for Identifying Disseminated Breast Cancer

	% of women with positive results			
	Control	Stage I	Stage II	Stage IV
AAGP > 80 mg/dL	12.4	14.3	25.0	81.3
Logistic regression score > .35	11.2	4.8	25.0	81.3
At least two proteins above cut-off	7.9	9.5	31.3	87.5

No clinical or pathologic parameters (including age, menstrual status, and estrogen receptor status) other than stage of malignancy influence the individual acute phase protein concentrations or composite analyses in this study.

Discussion

The etiology of acute phase protein elevations in patients with regional or disseminated breast cancer is not known. Among the four acute phase proteins analyzed in the current study, AAGP is the most reliable indicator of disseminated breast cancer. By itself, it compares favorably as a predictor of risk with the two other analytic techniques utilizing multiple protein measurements. Individually, AAT also is a quite sensitive indicator of disseminated breast cancer, albeit to a lesser extent than AAGP. This lesser sensitivity might, at least in part, be due to the MZ variant phenotype found in one of 25 individuals and associated with lower serum AAT concentrations. Hpt, whose frequency of elevation with Stage IV disease as opposed to controls is somewhat lower than AAT, also is known to have phenotypic variants associated with lower serum concentrations.[7,9] C3 levels are elevated in a high percentage of Stage IV patients, but a relatively high percentage of controls also show elevations. The lower rate of C3 elevations in Stage I patients cannot presently be explained.

Published studies of acute phase proteins in breast cancer have generally reported results consistent with ours and are summarized in Table 6. Similar findings have also been reported for a number of other solid tumors including carcinoma of the bowel, prostate, bladder, cervix, head and neck, as well as both Hodgkin's and non-Hodgkin's lymphomas.[7,17] Our study expands upon previous work with individual acute phase proteins by ranking them according to predictive value and analyzing them compositely as well as singly.

The biologic significance of elevated serum acute phase proteins in malignancy is not known. Elevations of acute phase proteins do, however, appear to be associated with suppression of cellular immunity. Baskies *et al.*[2] reported a correlation between elevated levels of serum haptoglobin and alpha-acid glycoprotein and decreased *in vitro* lymphocyte reactivity to phytohemagglutinin and *in vivo* delayed hypersensitivity to dini-

TABLE 6. Acute Phase Proteins in Breast Cancer: Comparison of Current Study to Previous Reports

Ref No	Proteins	Stage of malignancy	Controls	Results
Current	AAT AAGP Hpt C3	I, II, IV	B/N	All proteins significantly increased in M *vs* B/N; IV *vs* I & II No significant difference I & II *vs* B/N
2	AAT AAGP Hpt	I, II, IV	B/N	All proteins significantly increased, IV *vs* B/N; IV *vs* I & II No significant difference I & II *vs* B/N
3	AAT AAGP Hpt	I-IV		AAGP increased in advanced stage
5	AAT AAGP Hpt	Preop I & II	B	No significant difference I & II *vs* B *
6	Hpt	I, II, III	B/N	Significant increase IV *vs* I & II No significant difference I & II *vs* B +
7	AAT AAGP	I-IV	B	AAT significantly increased IV *vs* I & II AAGP significantly increased M *vs* B IV *vs* I & II
8	Hpt	I, II, IV	B/N	Significantly increased IV *vs* I & II No significant difference I & II *vs* B/M

* Increased AAT levels three months postoperatively associated with higher rate of progressive disease.

† Increased Hpt levels in I and II carried increased risk recurrence.
M: Malignant; B: Benign; N: Normal.

trochlorobenzene in 147 preoperative patients with various solid malignancies as compared to 58 normal controls. They reported that the elevation of acute phase proteins was even more closely related to the degree of immunosuppression than to tumor extent.

Measurement of serum acute phase proteins in breast cancer may have clinical value. Although analysis of acute phase proteins does not help in distinguishing patients with malignant disease from those with benign breast disease, it may prove valuable for following cancer patients and identifying those who are developing recurrent disease. Preoperative measurement of serum acute phase proteins also could be useful in identifying those patients who may already have disseminated disease and who might benefit from further preoperative staging evaluation. The yield of positive preoperative bone scans consistent with metastatic disease is only 2% for Stage I patients and 7% for Stage II patients but may be as high as 58% for Stage III patients.[11,15] According to data analyzed in the current study, a woman with breast cancer having two acute phase protein concentrations elevated simultaneously in the serum is at significant risk for disseminated disease, and this risk becomes even greater if three or four are elevated. Because the skeleton is the commonest site of breast cancer metastases, the usefulness and cost-benefit ratio of bone scans might be greatly improved by performing preoperative scans only on those Stage I and II patients who also have elevated acute phase proteins. This approach has already proven effective in more efficiently evaluating patients with prostatic cancer.[17]

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