Evaluation of a Breast Cyst Fluid Protein Detectable in the Plasma of Breast Carcinoma Patients

DARROW E. HAAGENSEN, JR., M.D., PH.D., GWEN MAZOUJIAN, B.A., WALTER D. HOLDER, JR., M.D.
SVEN J. KISTER, M.D., SAMUEL A. WELLS, JR., M.D.

A radioimmunoassay has been developed for one of the major proteins isolated from human breast cyst fluid. Immunologically this protein is identical to a protein present in both human milk and saliva. Ninety-two normal women had plasma levels of this protein below 100 ng/ml (range 7–81 ng/ml; mean 31 ng/ml), and 85% had plasma levels below 50 ng/ml. More than one-half of the women with active gross cystic disease of the breast had plasma levels above 50 ng/ml. None of these women, however, had plasma levels above 150 ng/ml. A significant percentage of women with advanced breast carcinoma have been found to have plasma concentrations of this protein greater than 150 ng/ml.

From the Department of Surgery, Duke University Medical Center Durham, North Carolina and the Department of Surgery, Columbia University, College of Physicians and Surgeons, New York, New York

HUMAN BREAST gross cystic disease (GCD) has been identified by C. D. Haagensen as a specific disease entity separate from the complex of benign breast lesions usually classified as fibrocystic disease.[1] Gross cystic disease is a malady of premenopausal women which begins to occur after the age of 25, and regresses at the menopause unless the individual is taking hormonal medications. It often involves both breasts.

In patients with breast tumors with the clinical features of gross cysts, it is accepted practice to attempt aspiration, a procedure which proves the diagnosis if the characteristic turbid yellowish or brownish fluid is obtained and no palpable tumor remains. Cyst aspiration is also effective treatment because once a cyst has been aspirated it will usually not recur.

A long-term evaluation of more than 2,000 patients with GCD proved either by aspiration or biopsy has revealed that breast carcinoma developed at more than four times the frequency in normal women.[1] The breast carcinomas developed in GCD patients in an age range identical to patients without GCD.

These observations seem to indicate an association between breast carcinoma and gross cystic disease. To evaluate this possible relationship, we began in 1971 a biochemical study of the cyst fluid which Haagensen made available to us. Our biochemical and immunological analysis of this gross cystic disease fluid has resulted in the isolation of a specific glycoprotein.[2] This glycoprotein has an apparent molecular size of 70,000 by Sephadex gel chromatography. Acrylamide gel-sodium dodecyl sulfate analysis indicates a monomer size of approximately 15,000 daltons. By Ouchterlony immunodiffusion analysis, the protein demonstrates no immunological cross-identity with any normal human plasma proteins, but does demonstrate immunological identity with a protein present in both human milk and saliva. The concentration of this gross cystic disease fluid protein (GCDFP) is approximately 100-fold higher in GCD fluid than in either human milk or saliva. The lack of immunological cross-identity between the GCDFP and human plasma proteins by Ouchterlony analysis prompted the development of a radioimmunoassay (RIA) to determine if this glycoprotein could serve as an epithelial secretion marker detectable in the systemic circulation of patients with pathological conditions of the breast.

Materials and Methods

The isolation of the GCDFP and the development of the radioimmunoassay are described in detail elsewhere.[2] Gross cystic disease fluid was obtained by breast cyst aspiration from women under treatment for GCD at Columbia Presbyterian Medical Center. The GCD fluid was stored at −10° C.

Plasma samples (ethylenediamine tetraacetic acid) from patients with operable breast carcinoma or benign

Submitted for publication July 28, 1976.

Reprint requests: Darrow E. Haagensen, Jr., M.D., Ph.D., Box 3966, Duke University Medical Center, Durham, North Carolina 27710.

TABLE 1. *Gross Cystic Disease Fluid Protein Plasma Levels by RIA*

Patient Category	No. Cases	% GCDFP Plasma Levels: Above 50 ng/ml	% GCDFP Plasma Levels: Above 150 ng/ml
I. Normal women by physical examination			
No prior breast disease	92	14%	0%
II. "Normal" women blood donors	53	15%	0%
III. Women followed for GCD			
A. Cyst present	28	54%	0%
B. Cyst aspirated within two years	30	27%	0%
C. Cyst not aspirated within two years	32	6%	0%
IV. Women operated for benign breast disease			
A. Tissue specimen positive for GCD	38	42%	0%
B. Tissue specimen negative for GCD			
Adenofibroma	75	20%	0%
Fibrous disease	47	17%	0%
Microcystic disease	38	13%	0%
Other benign breast disease	26	8%	0%
V. Women followed after operation for benign breast disease (other than GCD)	21	5%	0%

breast disease were obtained prior to surgery. Plasma samples from clinic patients were obtained following breast examination. All plasma samples were stored at −10° C.

Three coded groups of plasma samples were obtained by courtesy of Dr. Hans J. Hansen of Hoffman-La Roche. The first group consisted of 53 samples from "normal" women who were screened regularly for carcinoembryonic antigen (CEA). A specific history of benign breast disease in these women was not available. In the second group, 131 plasma samples from patients with carcinoma other than breast, were obtained. Also provided were 11 plasma samples from patients with chronic inflammatory disease (4 pancreatitis, 4 cirrhosis, 3 ulcerative colitis).

The gross cystic disease fluid protein levels in plasma are recorded as nanograms (ng) of GCDFP per ml of plasma. Duplicate assay variability of plasma samples was ±3%. All GCDFP plasma levels greater than 50 ng/ml were determined by analysis of 50 ul aliquots from dilutions of the plasma sample into bovine plasma (GIBCO). Linearity between two separate doubling dilutions was within ±7% in all samples analyzed.

Clinical determinations of the effectiveness of therapy for breast carcinoma were based on the clinically accepted standards presently in existence at Columbia Presbyterian Medical Center and Duke University Medical Center. The clinicians treating breast carcinoma patients were not aware of the GCDFP plasma levels at the time of treatment and conversely analysis of plasma samples was carried out on number coded samples without knowledge of patient status.

Results

The results of GCDFP plasma level determinations have been tabulated as a percentage of GCDFP plasma levels above 50 ng/ml and above 150 ng/ml. These two divisions were chosen empirically after initial analysis of the data.

The range of GCDFP plasma levels in 92 normal women (age range 24–72 years, mean of 50 years) varied from 7 ng/ml to 81 ng/ml with a mean of 31 ng/ml (Table 1, Category I). Eighty-six per cent of these normal women had GCDFP plasma levels below 50 ng/ml.

Analysis of 53 "normal" women blood donors resulted in a similar GCDFP plasma level profile with 85% of these women having GCDFP plasma levels below 50 ng/ml (Table 1, Category II).

Gross cystic disease patients have been divided into three sub-groupings for analysis (Table 1, Category III): A) a cyst aspirated at the time of the plasma sample; B) a recent history of cystic disease (cyst aspirated within the last two years), but no dominant palpable breast mass suggestive of a cyst at the time of the plasma sample; C) a history of cystic disease, but without a cyst aspirated within the previous two years. Of the patients in whom active gross cystic disease was present (Group A), 54% had GCDFP plasma levels above 50 ng/ml. However, none of these patients had levels above 150 ng/ml (highest level 132 ng/ml). The patients with less recent GCD (Groups B and C) had GCDFP plasma levels similar to normal women.

Two hundred and twenty-four women operated upon for benign breast disease at Columbia Presbyterian Medical Center were evaluated for plasma levels of GCDFP (Table 1, Category IV). Of the patients with a pathological diagnosis of GCD in the breast specimen (Category IV-A), 42% had GCDFP plasma levels above 50 ng/ml, the highest level being 104 ng/ml. Of the patients without evidence of GCD in the breast tissue specimen (Category IV-B), 16% had GCDFP plasma levels above 50 ng/ml with the highest level being 91 ng/ml (occurring in a patient with an adenofibroma).

Twenty-one women in followup after operation for benign breast disease (other than GCD) have been analyzed for GCDFP plasma levels. Only one of these women had a GCDFP plasma level over 50 ng/ml (Table 1, Category V).

One hundred and fifty-four patients with breast carcinoma whose disease was classified as Stage A or B according to the Columbia Clinical Classification[3] had preoperative plasma determinations of GCDFP. The patients have been stratified for analysis (Table 2, Category I) relative to the extent of axillary lymph node involvement with carcinoma. For the 0, 1–3, and 4–7 categories of axillary lymph node involvment with carcinoma, approximately 20% of these patients had GCDFP plasma levels above 50 ng/ml. For the category of 8 or more positive axillary lymph nodes, 41% of these patients had GCDFP plasma levels above 50 ng/ml. Totally, 37 of the 154 Stage A and B breast carcinoma patients had preoperative GCD plasma levels above 50 ng/ml. Nine of these 37 patients had the pathological demonstration of concurrent GCD in the resected breast.

One hundred and twenty-six breast carcinoma patients, operated upon for Columbia Clinical Classification Stage A and B breast carcinoma by Halsted radical mastectomy at Columbia Presbyterian Medical Center, have had GCDFP plasma level determinations postoperatively. Ninety-nine of the 126 patients had a preoperative GCDFP plasma level determination. Twenty-nine of these patients have had serial postoperative GCDFP plasma level determinations. A total of 162 followup GCDFP plasma level determinations have been performed on the 126 patients. The results of GCDFP plasma level determinations are tabulated (Table 2, Category II) relative to the clinical status of the followup patients.

One hundred and six of the patients had no evidence of recurrence of breast carcinoma at the time of analysis. Six of the 106 had GCDFP plasma values above 50 ng/ml. Three of these 6 patients were Stage B patients and had 4 or more axillary lymph nodes involved with carcinoma, thus falling into a high risk group for development of recurrence.

Three of the 126 patients have developed a primary breast carcinoma in the opposite breast. Two of the patients had GCDFP plasma levels above 50 ng/ml. One of these two patients is of interest relative to serial GCDFP plasma levels. This patient, age 75, at the time of initial operation for Stage B breast carcinoma had a GCDFP plasma level of 90 ng/ml. Three months after operation, the GCDFP plasma level was 58 ng/ml. Five months later, the GCDFP plasma level was 100 ng/ml, and a Stage A breast carcinoma was diagnosed in the remaining breast.

TABLE 2. *Gross Cystic Disease Fluid Protein Plasma Levels by RIA*

Patient Category (No. Cases)	% GCDFP Plasma Levels: Above 50 ng/ml	Above 150 ng/ml
I. Women operated for breast carcinoma Columbia clinical classification Stages A or B		
0 Axillary lymph nodes involved (84)	20%	0%
1–3 Axillary lymph nodes involved (36)	25%	0%
4–7 Axillary lymph nodes involved (12)	17%	0%
8+ Axillary lymph nodes involved (22)	41%	0%
II. Women in followup after operation for stage A or B breast carcinoma (Columbia patients)		
No evidence of recurrence at present (106)	6%	0%
Primary breast carcinoma developed in other breast (3)	66%	0%
Clinical evidence suspicious of recurrence (5)	20%	0%
Developed recurrence of breast carcinoma (12)	25%	25%
III. Women in followup after operation for breast carcinoma (Duke surgical oncology breast clinic patients)		
No evidence of recurrence at present (103)	30%	1%
Clinical evidence suspicious of recurrence (4)	75%	25%
Developed recurrence of breast carcinoma (6)	50%	17%
IV. Women with Stage C Breast carcinoma (8)	12%	0%
V. Women with Stage D Breast carcinoma (6)	50%	33%
VI. Women with postoperative local recurrence or distant metastases, or with initial distant metastases of breast carcinoma. All patients undergoing therapy		
Major anatomical site of metastases		
Local recurrence and soft tissue metastases (40)	35%	10%
Osseous metastases (40)	75%	60%
Hepatic metastases (24)	50%	33%
Pulmonary metastases (30)	57%	20%
Central nervous system metastases (4)	25%	0%

Eight months after operation for this second breast carcinoma, the patient's GCDFP plasma level was 11 ng/ml.

Five of the 126 patients have developed clinical evidence suspicious of recurrence, however, recurrence is undocumented. One of these 5 had a GCDFP plasma level above 50 ng/ml.

Twelve of the 126 followup patients have developed

TABLE 3. *Gross Cystic Disease Fluid Protein Plasma Levels by RIA*

Patient Category	No. Cases	% GCDFP Plasma Levels Above 50 ng/ml	% GCDFP Plasma Levels Above 150 ng/ml
I. Carcinoma other than breast			
Lung*	50	16%	0%
Colon†	26	11%	0%
Pancreas	5	0%	0%
Stomach	4	0%	0%
Kidney	5	0%	0%
Ovary	17	0%	0%
Uterus	7	0%	0%
Cervix	9	11%	0%
Melanoma	8	0%	0%
II. Chronic inflammatory diseases	11	18%	0%
III. Renal diseases on hemodialysis	14	29%	0%

* 64% have CEA >5 ng/ml.
† 73% have CEA >5 ng/ml.

clinical recurrence of breast carcinoma. Three of these 12 patients have developed increasing GCDFP plasma levels above 150 ng/ml (160, 365, 1500 ng/ml).

A second group of breast carcinoma patients evaluated postoperatively consisted of 113 patients who came initially to the Duke Surgical Oncology Breast Clinic with no evidence of recurrent disease (Table 2, Category III). A total of 350 followup GCDFP plasma level determinations have been performed on the 113 patients with 71 of the 113 patients having serial determinations. One hundred and three of the 113 patients have no evidence of carcinoma recurrence. Plasma levels of GCDFP above 50 ng/ml were detected in 30 of these 103 patients and 5 of the 30 patients had GCDFP plasma levels above 100 ng/ml with one patient being above 150 ng/ml.

The patient with a GCDFP plasma level above 150 ng/ml is 64 years old and had a left modified mastectomy in May 1975 with metastases to 8 of 16 axillary lymph nodes. The patient also underwent a right simple mastectomy at the same time for "prophylaxis." No carcinoma was found in the right breast specimen. One month after surgery the patient was placed on a year of adjuvant chemotherapy consisting of oral Cytoxan, Methotrexate, and 5-Fluorouracil. The initial GCDFP plasma level, determined 6 months after surgery, was 47 ng/ml. Over the next 8 months 5 serial GCDFP plasma levels have been measured revealing sequential values of 81, 150, 285, and 570 ng/ml. The patient is still asymptomatic for breast carcinoma recurrence and is being examined at two month intervals.

Four of the 113 patients have developed clinical evidence suspicious of recurrence, however, recurrence is undocumented. Three of the 4 patients have GCDFP plasma levels above 50 ng/ml and one of the three has progressed in GCDFP plasma levels from 140 to 410 ng/ml. This patient is 78 years of age and has developed bone pain in the lower back and hips which by bone scan and skeletal x-rays is consistant with osteoarthritis.

Six of the 113 patients have developed recurrence of breast carcinoma during the course of followup. Three of the 6 patients have had serially increasing GCDFP plasma levels. One of the 6 patients had developed a GCDFP plasma level above 150 ng/ml before the detection of carcinoma recurrence.

Eight Stage C breast carcinoma patients were evaluated preoperatively. Seven of the 8 had GCDFP plasma levels below 50 ng/ml with the one exception being 53 ng/ml (Table 2, Category IV).

Two of 6 Stage D breast carcinoma patients who were not operated upon and who were awaiting therapy had GCDFP plasma levels above 150 ng/ml (Table 2, Category V). One was 850 ng/ml and the other was 160 ng/ml. None of the 6 was thought to have distant metastases by standard clinical criteria at the time of evaluation.

One hundred and thirty-eight patients with postoperative local recurrence or distant metastases, or with initial distant metastases of breast carcinoma have been evaluated for GCDFP plasma levels (Table 2, Category VI). Eighty-seven of the 138 patients have been followed serially with a total of 500 GCDFP plasma level determinations. All patients were receiving hormone, radiation, or chemical therapy when the plasma samples were obtained. Of the 138 patients, 42 had GCDFP plasma levels above 150 ng/ml. The 138 patients have been stratified relative to the major anatomical site of metastatic involvement (Table 2, Category VI). This stratification revealed GCDFP plasma levels above 150 ng/ml in 60% of patients with osseous metastases, 33% of patients with hepatic metastases, 20% of patients with pulmonary metastases, and 10% of patients with local recurrence or soft tissue metastases. GCDFP plasma levels above 300 ng/ml (10-fold above the mean value for normal women) were present in 28 of the metastatic breast carcinoma patients and 11 of these patients had GCDFP plasma levels above 1000 ng/ml. Of the 42 metastatic breast carcinoma patients with GCDFP plasma levels above 150 ng/ml, 25 of the patients were 55 years of age or older.

Of 131 plasma samples from patients with carcinoma other than breast, all demonstrated GCDFP plasma levels below 100 ng/ml (Table 3, Category I). The

precise clinical stage of carcinoma in each of these patients was not available; however, most of these patients were listed in the Hoffman-La Roche files as metastatic patients, and CEA values in patients with colonic or pulmonary carcinoma ranged up to 8000 ng/ml.

Eleven patients with chronic inflammatory disease (4 pancreatitis, 4 cirrhosis, 3 ulcerative colitis) had GCDFP plasma levels below 100 ng/ml. Only two of the patients had levels above 50 ng/ml (52 and 54 ng/ml) (Table 3, Category II).

Fourteen patients on hemodialysis for kidney failure had GCDFP plasma levels below 100 ng/ml (Table 3, Category III).

Twenty-eight of the 87 metastatic breast carcinoma patients who have been followed serially have developed GCDFP plasma levels above 150 ng/ml. The results of serial GCDFP plasma levels in these 28 patients have correlated with the clinical response to therapy. In several specific cases, GCDFP levels have provided an earlier and more reliable indicator of treatment response or failure than standard clinical, laboratory, or roentgenographic determinants. Three case reports illustrating this point are presented.

Case Reports

Case 1. Duke Study No. D-9 (Fig. 1). This patient is a 51-year-old Caucasian multiparous woman who came initially to the Duke University Medical Center in February 1975 with a right breast mass and a palpable ipsilaterial supraclavicular lymph node. She had mild hypertension treated with methyldopa. Her menstrual periods had been regular until three months prior to her visit. She was admitted to the hospital in March 1975 where histological evaluation of biopsy material from both the breast mass and the supraclavicular lymph node revealed adenocarcinoma of the breast. In April 1975, she underwent a bilateral oophorectomy. Within three months enlarged firm lymph nodes became palpable in the right cervical area. The patient at this time had a normal liver scan, normal skeletal survey, normal chest x-ray, and her blood chemistries (specifically alkaline phosphatase, SGOT, and calcium) were within normal limits. A bone scan, however, indicated some increased tracer activity in the occipital region of the skull. The patient was started on combination chemotherapy in October 1975 consisting of oral Cytoxan, Methotrexate and 5-Fluorouracil (CMF). She did not tolerate this drug regimen well as her course was complicated by nausea, vomiting, and persistent leukopenia. In March 1976, because of disease progression, her drug regimen was modified to intravenous 5-Fluorouracil and Methotrexate, with oral Cytoxan. At present, the patient has multiple bone lesions and has soft tissue evidence of widespread metastatic disease. Figure 1 depicts the serial GCDFP plasma levels for this patient over the last 7 month period of time (October 23, 1975 to May 26, 1976).

Case 2. Duke Study No. D-64 (Fig. 2). The patient, a 62-year-old woman, had a right radical mastectomy for carcinoma of the breast in October 1974. This was followed by postoperative local irradiation therapy. Four months later, she came to the Duke University Medical Center with a palpable left supraclavicular lymph node which on biopsy showed metastatic adenocarcinoma.

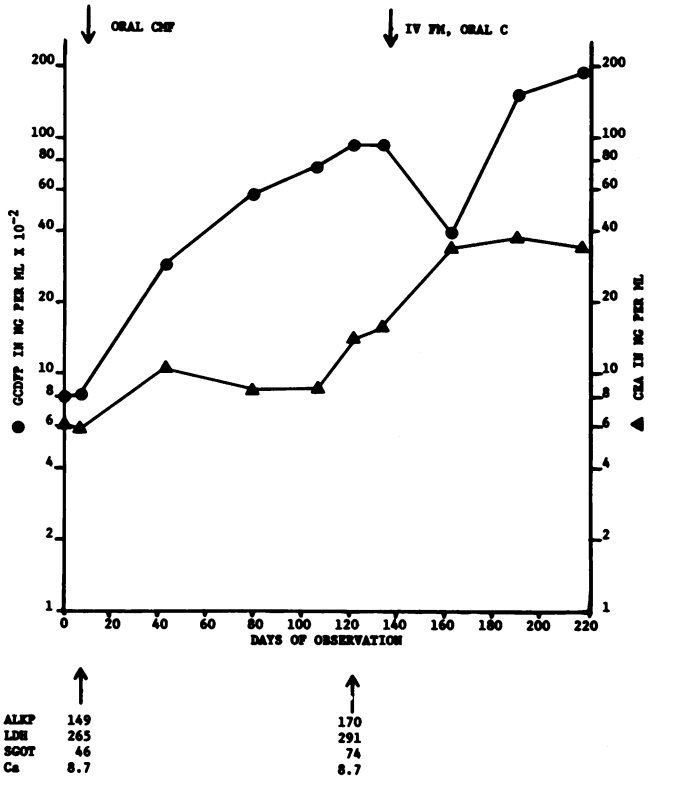

FIG. 1. Day 0 of observation is October 23, 1975. Initiation of therapy protocols is depicted by vertical arrows at the top of the figure. C = Cytoxan. M = Methotrexate. F = 5-Fluorouracil. Serum chemistry values are given at specific points (arrows) below the figure. ALKP = Alkaline Phosphatase (normal range 30–110 units). LDH = Lactic Dehydrogenase (normal range 90–200 units). SGOT = Serum Glutamic Oxaloacetic Transaminase (normal range 10–50 units). Ca = Calcium (normal range 8.5–10.5 mg/100 ml). Serial GCDFP plasma levels = ●. Serial CEA (Carcinoembryonic antigen) plasma levels = ▲ (normal range 0–5 ng/ml).

The patient was started on combination chemotherapy consisting of oral Cytoxan, Methotrexate, and 5-Fluorouracil. Two weeks after the initiation of chemotherapy, she developed leukopenia, and the drug therapy regimen was reduced. It was completely stopped in May 1975 when her white blood cell count reached 1500. By July 1975, the patient had a normal white blood cell count and was again put on a chemotherapeutic regimen as before. Within three months, however, there was a new palpable left supraclavicular lymph node, and a biopsy of this lymph node revealed metastatic adenocarcinoma. Estrogen receptor analysis of this tissue revealed 180 femtomoles per mg of tissue of 8 Svedberg estrogen receptor protein. The patient was put on a combination of Aminoglutethimide and Dexamethasone. Within one month, the Dexamethasone was replaced by Hydrocortisone. Over the next two months, the patient seemed to show some improvement, however, by late January 1976 her bone scan showed progression, and the Aminoglutethimide and Hydrocortisone were stopped. At this time, she was started again on combination chemotherapy consisting of Adriamycin, 5-Fluorouracil, and Cytoxan (AFC). Unfortunately, she has shown disease progression on this regimen. Figure 2 depicts the levels of GCDFP during her treatment course over the last 6 months (October 2, 1975 to March 31, 1976).

Case 3. Duke Study No. D-78 (Fig. 3). The patient, a 54-year-old Caucasian woman underwent a right radical mastectomy

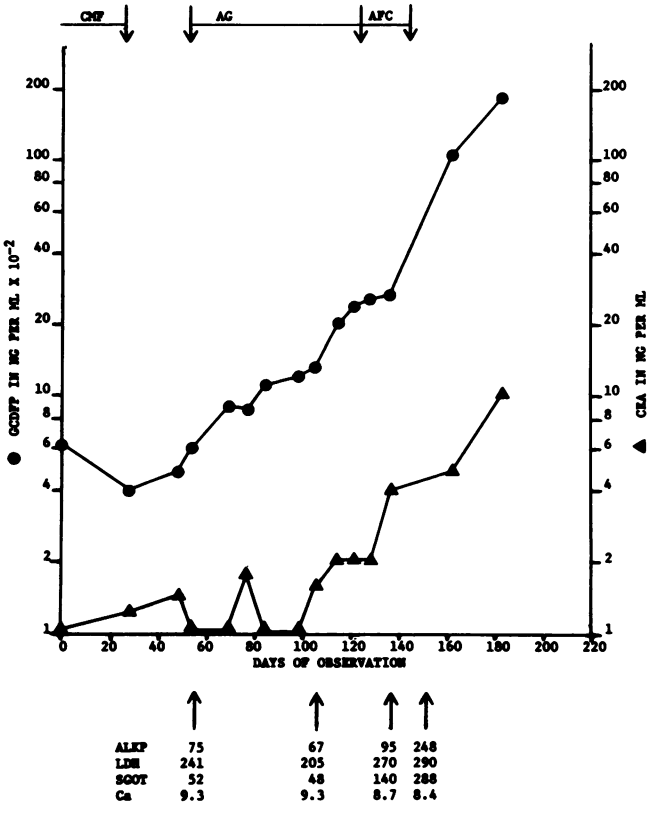

FIG. 2. Day 0 of observation is October 2, 1975. Initiation or termination of therapy protocols is depicted by vertical arrows at the top of the figure. C = Cytoxan. M = Methotrexate. F = 5-Fluorouracil. AG = Aminoglutethimide. A = Adriamycin. Serum chemistry values are given at specific points (arrows) below the figure. ALKP = Alkaline Phosphatase. LDH = Lactic Dehydrogenase. SGOT = Serum Glutamic Oxaloacetic Transaminase. Ca = Calcium. Serial GCDFP plasma levels = ●. Serial CEA plasma levels = ▲.

in November 1972. Thirteen axillary lymph nodes were positive for tumor at the time. She came to the Duke University Medical Center in January 1975 with a skin recurrence of the right anterior chest wall. Combination chemotherapy consisting of oral Cytoxan, Methotrexate, and 5-Fluorouracil was begun, and the patient showed a marked improvement over the next 4 months. By May 1975, her bone scan was suspicious for metastatic involvement of the ribs, and by September 1975, she had hip pain, and a bone scan and skeletal X-rays indicated metastatic disease in the left pelvis and femur. She had also developed induration, redness, and peau d'orange changes in the left breast. The combination chemotherapy was stopped and the patient was begun on Calusterone 50 mg four times a day. In October 1975 the patient was admitted to the hospital and a biopsy of the left breast demonstrated adenocarcinoma. Estrogen receptor assay of the biopsy tissue was negative for estrogen receptor protein. However, it was felt that the Calusterone medication might have accounted for this. The patient was started on Aminoglutethimide and Dexamethasone, and within one month the Dexamethasone was replaced by Hydrocortisone. Shortly after beginning this drug regimen, the patient showed marked improvement with regression of the metastatic disease in the skin and left breast. The left breast became much softer, and all tumor deposits on the anterior chest wall disappeared. The bone scan and skeletal survey remained essentially unchanged until April 1976 when progression was demonstrated on the bone scan, the skeletal X-rays, and chest X-ray. Also, on physical examination, the soft tissue metastases in the anterior chest wall were returning. Figure 3 depicts the GCDFP levels of this patient over the last 6 months of her treatment course (October 9, 1975 to April 22, 1976).

Discussion

Our studies demonstrate that a unique protein isolated from GCD fluid can be found in varying concentrations in the plasma of patients with breast carcinoma (highest level was 30,000 ng/ml). However, this protein is also present in low concentrations (5–50 ng/ml) in the plasma of normal women. It is, therefore, not representative of an antigen specific for breast carcinoma. There is present in milk and saliva of normal women an immunologically related material to the isolated GCDFP. This finding suggests that GCDFP represents an epithelial secretory cell product. The elevated levels of this protein found in the plasma of patients with metastatic breast carcinoma appear to reflect an active release of this material by the malignant cells into the blood stream. In this regard, it is interesting to note that although the cysts of GCD contain extremely high levels of this protein (1–5 mg/ml), the plasma from GCD patients demonstrates at most only a twofold elevation above the range

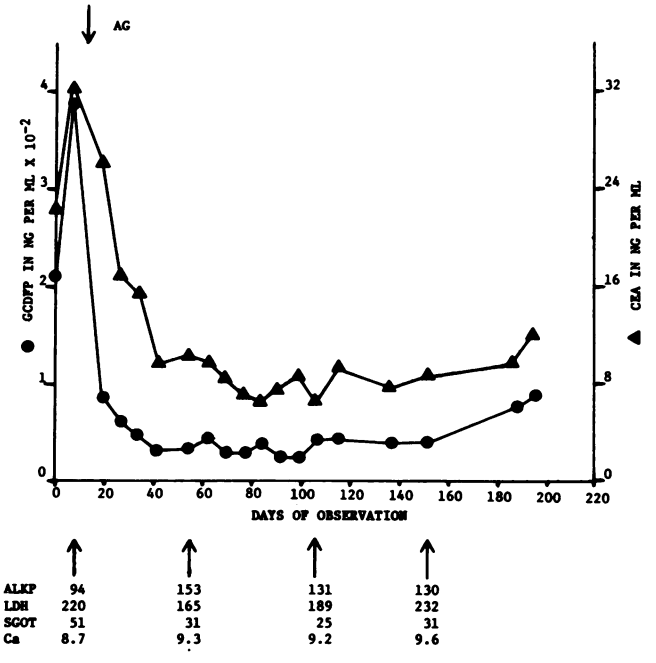

FIG. 3. Day 0 of observation is October 9, 1975. Initiation of therapy protocol is depicted by a vertical arrow at the top of the figure. AG = Aminoglutethimide. Serum chemistry values are given at specific points (arrows) below the figure. ALKP = Alkaline Phosphatase. LDH = Lactic Dehydrogenase. SGOT = Serum Glutamic Oxaloacetic Transminase. Ca = Calcium. Serial GCDFP plasma levels = ●. Serial CEA plasma levels = ▲.

of values found in "normal" women. The protein appears, therefore, to be a good marker for epithelial disorganization of secretory function which is associated with breast carcinoma.

The concept of using breast epithelial secretory proteins as plasma markers for breast carcinoma has been under investigation by other groups.[4,5] Both studies have analyzed milk proteins as potential markers. The casien RIA[4] is reported as a useful marker for breast carcinoma, however, the lactalbumin RIA[5] does not appear to be helpful in detecting breast carcinoma.

Our use of a specific gross cystic disease fluid protein for an RIA differs from the above approach of using milk proteins. Gross cystic disease appears to be linked epidemiologically with breast carcinoma and the synthesis of GCD fluid occurs under rather "normal" physiological conditions. Thus, GCD protein synthesis could hypothetically be more closely associated with breast carcinoma than milk protein synthesis. The results of our analysis do in fact indicate that a significant proportion of breast carcinomas synthesize and release the GCDFP protein.

The usefulness of single GCDFP plasma level determinations in patients with early breast carcinoma appears limited at present. Although a significant proportion of early breast carcinoma patients had GCDFP plasma levels between 50 and 150 ng/ml, this range of values overlaps with patients having GCD. For breast carcinoma patients above 55 years of age (not expected to have GCD), the GCDFP plasma levels between 50 and 150 ng/ml may have a greater diagnostic significance. This point awaits accrual of more data.

Serial measurements of GCDFP plasma levels appear to have diagnostic usefulness in monitoring for recurrence of breast carcinoma after surgery. In this regard, the initial recurrence of breast carcinoma after surgery in 6 of 18 patients was detected by increasing GCDFP plasma levels. Four of the 18 patients had developed GCDFP plasma levels above 150 ng/ml prior to initiation of therapy for metastases. Of 107 breast carcinoma patients in followup after surgery at the Duke Surgical Oncology Clinic who are without demonstrable recurrence, 7 patients have developed GCDFP plasma levels above 100 ng/ml and two of these patients have increased to above 150 ng/ml (410 and 570 ng/ml). Continued followup of this patient group is necessary before criteria can be firmly established relative to GCDFP plasma levels demonstrating breast carcinoma recurrence. It does appear that serial measurements of GCDFP plasma levels initiated prior to surgery for breast carcinoma and continued at regular followup intervals will be an effective method of detecting breast carcinoma recurrence in certain individuals.

The GCDFP plasma level measurements appear at present most useful in advanced breast carcinoma where the GCDFP plasma levels are elevated in a significant proportion of the patients. In this regard, 28 metastatic breast carcinoma patients known to have an elevated GCDFP plasma level were followed during therapy by serial plasma samples. The GCDFP plasma levels correlated well with the progression or regression of the metastases. Plasma concentrations of GCDFP, therefore, appear to be a useful method for monitoring the clinical response of these patients to therapeutic regimens.

Acknowledgments

Both academic and financial support for the development of the GCDFP assay were derived from Dr. Cushman D. Haagensen. The GCDFP analysis was aided by expert technical assistance from Mark Swanenburg, B.S., June Panick, R.N., Richard Meshulam, B.S., Jean Gates, B.S., and Emma Smart, R.N. Support for patient studies was obtained in part from NCI Contract No. NCI-CB-53851-37 and Grant No. RR-30 from the General Clinical Resources Center Program of the Division of Research Resources, NIH.

References

1. Haagensen, C. D.: Diseases of the Breast, 2nd edition, Philadelphia, W. B. Saunders Co., 1971: pp. 155–176.
2. Haagensen, D. E., Mazoujian, G., Dilley, W. G., et al.: Breast Gross Cystic Disease Fluid Analysis. I. Isolation and Radioimmunoassay for a Major Component Protein. (in preparation).
3. Haagensen, C. D.: The Choice of Treatment for Operable Carcinoma of the Breast, Surgery, 76:685–714, 1974.
4. Hendrick, J. D. and Franchimont, P.: Radioimmunoassay of Casein in the Serum of Normal Subjects and of Patients with Various Malignancies. Europ. J. Cancer, 10:725–730, 1974.
5. Kleinberg, D. L.: Human Lactalbumin: Measurement in Serum and in Breast Cancer Organ Cultures by RIA. Science, 190:276–278, 1975.