Mammaglobin as a Novel Breast Cancer Biomarker: Multigene Reverse Transcription-PCR Assay and Sandwich ELISA

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Background: The aim of this study was to examine the potential usefulness of a mammaglobin multigene reverse transcription-PCR (RT-PCR) assay and a mammaglobin sandwich ELISA as diagnostic tools in breast cancer.

Methods: We studied peripheral blood samples from 147 untreated Senegalese women with biopsy-confirmed breast cancer and gathered patient information regarding demographic, and clinical staging of disease. The samples were tested for mammaglobin and three breast cancer-associated gene transcripts by a multigene real-time RT-PCR assay and for serum mammaglobin protein by a sandwich ELISA assay.

Results: In 77% of the breast cancer blood samples, a positive signal was obtained in the multigene RT-PCR assay detecting mammaglobin and three complementary transcribed genes. Fifty samples from healthy female donors tested negative. Significant correlations were found between mammaglobin protein in serum, presence of mammaglobin mRNA-expressing cells in blood, stage of disease, and tumor size. Circulating mammaglobin protein was detected in 68% of the breast cancer sera, and was increased in 38% in comparison with a mixed control population. The RT-PCR assay and the ELISA for mammaglobin produced a combined sensitivity of 84% and specificity of 97%.

Conclusion: The ELISA and RT-PCR for mammaglobin and mammaglobin-producing cells could be valuable tools for diagnosis and prognosis of breast cancer. © 2004 American Association for Clinical Chemistry

The identification of sensitive and specific biomarkers for the detection of circulating breast cancer cells and for staging of breast cancer may be of considerable importance for clinical management of breast cancer and could provide an important tool for researchers. Previous studies have demonstrated the usefulness of reverse transcription-PCR (RT-PCR) based detection of mammaglobin, a homolog of the rat prostatic binding protein component 3 (1) and a member of the uteroglobin/clara cell protein family (secretoglobins) (2), for identification of disseminated breast cancer cells in blood, lymph nodes, and bone marrow (3–5). Mammaglobin, which is almost exclusively expressed in breast epithelial cells (6), is also overexpressed in a subset (70–80%) of primary and metastatic breast cancer tissues (7).

We recently developed a panel of four complementary expressed genes, mammaglobin, B305D, γ-aminobutyrate type A receptor π-subunit (GABA π), and B726P, to provide a panel with high sensitivity and specificity for detection of circulating breast cancer cells (8, 9). In our previous study, we evaluated this multigene RT-PCR assay in 27 primary breast cancer tissues, 50 lymph nodes infiltrated with metastatic breast cancer, and 27 non-breast cancer lymph node specimens (10). All primary breast tumors and metastatic breast cancer lymph nodes, but none of the control samples, demonstrated positive expression signals of mammaglobin, B305D, GABA π,

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Nonstandard abbreviations: RT-PCR, reverse transcription-PCR; GABA π, γ-aminobutyrate type A receptor π-subunit; and CEA, carcinoembryonic antigen.
and/or B726P. In the present study we focus on transcript detection of this gene panel in peripheral blood.

Detection of circulating biomarkers is important for diagnosis, prognosis, and monitoring of breast cancer patients. In addition to the classic biomarker CA15.3, newer prognostic and diagnostic circulating markers such as kallikreins 5 and 14 (11, 12) have recently been reported. Although little is known about detection of circulating mammaglobin protein at present, previous studies have suggested that detection of mammaglobin protein has potential as a biomarker for breast cancer (13, 14). Thus, in the present study we examined the relationship between peripheral blood-based detection of mammaglobin transcripts detected by a multigene RT-PCR assay and of mammaglobin protein concentrations in breast cancer patients and control individuals.

Materials and Methods

Patients
Beginning in February 2001, Senegalese women presenting to the Oncology Service at the Dantec Hospital of the University of Dakar with masses that were clinically diagnosed as breast cancer, and who had not undergone previous biopsy, surgery, or therapy, were invited to enroll in this study. Written informed consent was obtained in compliance with the Human Subjects Institutional Review Boards of the University of Washington and the University of Dakar. A 10-mL sample of blood was collected into EDTA Vacutainer Tubes and was immediately sent to the laboratory for processing. After collection of blood, a physical examination was performed, and tissue samples were obtained from the main tumor mass by needle core biopsy. Two adjacent needle core biopsies were obtained; one was placed into formalin for routine histologic processing and the other in RNA-later reagent (Ambion) for molecular studies. Chest x-rays and ultrasounds were performed to stage disease. In total, 197 patients were enrolled, of whom 147 (75%) were found to have biopsy-confirmed breast cancer. Serum samples from 142 patients with pathology-confirmed breast cancer were available for ELISA studies, and peripheral blood samples from 84 patients were available for RT-PCR testing for circulating breast cancer cells. Control blood samples from 142 patients with pathology-confirmed breast cancer were available for ELISA, and 100 cells/mL to establish detection limits of the assay.

RNA extraction and cDNA synthesis

Needle core biopsies in RNAlater reagent were shipped to Seattle on liquid nitrogen. Tissue samples (10–30 mg each) were transferred into 1 mL of lysis buffer [Ambion Poly(A) Pure mRNA Purification Kit] and disrupted by agitation with 2 g of 1 mm zirconia beads (BioSpec Products Inc.) for 3 min in a MiniBeadBeater (MidWest Scientific) run on the highest setting. RNA was isolated according to the manufacturer’s protocol and eluted with 60 μL of elution buffer. Reverse transcription was performed for 1 h at 42 °C with oligo(dT) primers and 10 μL of Superscript (Invitrogen) in a final volume of 150 μL.

For peripheral blood cell samples, the tumor cell-enriched blood cell lysates were shipped to Seattle on liquid nitrogen and processed according to the manufacturer’s protocol (mRNA Isolation Kit; Roche). mRNA was eluted with 25 μL of nuclease-free H2O and reverse-transcribed into cDNA by use of oligo(dT) primers (Gibco) and 8 μL of Superscript Reverse Transcriptase (Gibco) in a final volume of 120 μL.

Multigene real-time RT-PCR

The specific primers and 6-carboxyfluorescein-labeled TaqMan® probes used to detect mRNA expression of mammaglobin, GABA\(\pi\), B305D, and B726P simultaneously are shown in Table 1. Primers were designed to cross intron–exon junctions to exclude genomic DNA from amplification. Expression was measured by quantitative real-time PCR with the ABI 7700 Prism™ sequence detection system (Applied Biosystems). Actin expression was measured in separate reactions as a quality control for blood cDNA samples. Specimens with actin expression <50 copies were excluded from analysis.

Fifty PCR cycles were performed with TaqMan 1000 Rxn PCR Core Reagents (part no. 430 4439; Applied Biosystems) and 0.0375 U/μL TaqGold, 1× Buffer A; 5 mM MgCl2, 0.2 mM each of dCTP, dATP, and dGTP; 0.4 mM dUTP; 0.01 U/μL AmpEraser UNG; 80 nL/μL glyc-erol; 0.5 nL/μL gelatin; and 0.1 nL/μL Tween 20. PCR conditions were 1 cycle at 50 °C for 2 min, 1 cycle at 95 °C for 10 min, and 50 cycles 95 °C for 15 s, 60 °C for 1 min, and 68 °C for 1 min. Multigene copy numbers were calculated by use of a calibration curve constructed with
TaqMan SDS analysis software from serial dilutions of four plasmids containing target gene cDNA sequences. Final copy numbers were determined as medians of triplicate reactions. Triplicate reactions were pooled, and a 20-μL aliquot was separated by agarose electrophoresis using a 4% E-Gel (Invitrogen). Gene identities were determined according to amplicon size.

mammaglobin elisa
Antibodies were generated against purified native mammaglobin protein complex (15). We coated 96-well microtiter plates overnight at 4 °C with the monoclonal antibody RO48 at 200 ng/well. The wells were then blocked by incubation with 50 g/L nonfat milk in phosphate-buffered saline for 2 h at room temperature. For constructing the calibration curve, different dilutions of purified native mammaglobin protein complex were prepared in normal human serum. Biotinylated RO28 (1 mg/L) was added and incubated at room temperature for 1 h. Plates were washed, and streptavidin-horseradish peroxidase was added and incubated for 30 min. Tetramethylbenzidine substrate was added for 15 min before the reaction was stopped with 0.05 mol/L sulfuric acid, and the absorbance was read at 450 nm. Each specimen was run in duplicate, and CVs were <10%.

RESULTS

PATIENT CHARACTERISTICS
Histologic and cytologic review of biopsies confirmed the presence of breast cancer in 147 Senegalese patients. The mean age of the patients was 47.7 years (range, 13–77 years). Approximately one half (47%) were menopausal, and only 10 currently used any form of hormonal contraception. Mean gravidity was 5.8 (range, 0–19), and mean age of first pregnancy was 19.4 years. Few (3%) had any history of hormone replacement therapy, and 1% reported tobacco or alcohol use. Eleven women (7%) had a history of obesity. Most of these women had advanced disease and large tumors, with only 33% of tumors being 5 cm or less in size, whereas 43% were 10 cm or greater (Table 2). Evidence of nodal involvement was present in 92% of cases, and the vast majority (80%) of the cancers were stage III and higher.

GENE TRANSCRIPTS IN BLOOD AND TISSUE
To determine the sensitivity of the multigene RT-PCR assay, we added the breast cancer cell lines MDA-MB-415 and BT-474 to peripheral blood aliquots from a healthy volunteer (Fig. 1). Although no formal determination was made of the assay’s detection limit or limit of quantifica-
tion, multigene real-time expression signal was detected in this study at 1 cell/mL of blood for both cell lines. Similarly, mammaglobin real-time RT-PCR detected an expression signal at 1 cell/mL for MDA-MB-415 and at 100 cells/mL for BT-474.

Gene expression analysis of cells collected from blood of 84 women with breast cancer and 50 healthy female volunteers was performed with the multigene RT-PCR assay. Mammaglobin expression was detected in 61%, GABA\(\pi\) in 18%, B726P in 5%, and B305D in 8% of the blood samples. Overall, expression of one or more of these genes was present in 77% of samples from women with confirmed breast cancer but none of the 50 samples from women without cancer (Table 3).

We next examined the expression frequency of the four genes in corresponding breast cancer biopsies from 46 patients. Mammaglobin expression was detected in 85% (39 of 46) of cases (Table 3). GABA\(\pi\) was detected in 52% of the tumors, but only when mammaglobin was also present. In two other cases, biopsy samples were positive for B726P or B305D, but not for mammaglobin. Thus, 89% of tissues examined expressed at least one of the studied genes.

ASSOCIATIONS BETWEEN GENE TRANSCRIPTS AND TUMOR CHARACTERISTICS
We next examined the associations between detection of gene transcripts in peripheral blood and tumor characteristics (Table 4). Overall, among women with biopsy-confirmed breast cancer, detection in peripheral blood of any of the four genes included in the multigene assay was not significantly associated with increasing tumor size \((P = 0.4)\). Likewise, detection of mammaglobin transcripts alone was not associated with tumor size \((P = 0.4)\). However, detection of GABA\(\pi\) alone in peripheral blood...
was associated with tumor size \((P < 0.001, \text{test for trend})\) with no expression in patients with small tumors (2–5 cm; \(n = 30\)), expression in 14% of medium tumors (6–9 cm; \(n = 22\)), and expression in 40% of the largest tumors (≥10 cm; \(n = 30\)). In addition, GABA\(\pi\) was not detected in stage I, II, or IIIA cancers but was expressed in blood samples from 29% of those with stage IIIB or IV disease \((P = 0.004, \text{test for trend})\). Mammaglobin \((P = 0.07)\) and GABA\(\pi\) \((P = 0.05)\) were each marginally associated with increased nodal involvement; only 20% of those with NO disease were positive for mammaglobin compared with 60–70% of those with N1 or N2 disease. Similarly, 10% of women with N0 or N1 disease, compared with 29% of women with N2 disease, had GABA\(\pi\) detected in blood samples. Overall, detection of the four gene transcripts was associated with stage of disease \((P = 0.03)\), being present in 53% of those with stage II, 82% of those with stage III, and 87% of those with stage IV breast cancer.

Demographic and behavioral characteristics were also examined in association with detection of gene transcripts in peripheral blood. Detection of mammaglobin or other transcripts did not vary significantly with the age or gravidity of the patient; however, detection of GABA\(\pi\) transcripts was inversely associated with menopausal status, as 28% of premenopausal compared with 9% of postmenopausal women with breast cancer had GABA\(\pi\) transcripts detected in peripheral blood \((P = 0.02)\).

**ASSOCIATIONS BETWEEN SERUM MAMMAGLOBIN AND TUMOR CHARACTERISTICS**

Circulating mammaglobin protein was evaluated in sera from 142 women with confirmed breast cancer and was detected in 70% of the samples. In addition, sera from 53 Senegalese and 38 US women without breast cancer were available, and ROC curve analysis was used to determine the optimum (highest sum of sensitivity and specificity) mammaglobin protein concentration predictive of breast cancer. A concentration of 1.71 \(\mu\)g/L was established for positivity, giving a breast cancer-specific mammaglobin protein concentration that was positive in 38% of the 142 cancer sera samples but only in 3% of the 91 control sera.

Among the 142 women with confirmed breast cancer, mammaglobin serum protein >1.71 \(\mu\)g/L was marginally associated with increasing tumor size \((P = 0.09, \text{test for trend})\), but not with lymph node involvement \((P = 0.5)\). In addition, mammaglobin protein >1.71 \(\mu\)g/L was marginally associated with increasing clinical stage of disease \((P = 0.10, \text{test for trend})\), with only 29% of samples from women with stage I or II cancer being positive for mammaglobin protein compared with 31% of those with stage IIIA, 34% of those with stage IIIB, and 48% of those with stage IV disease (Table 4). None of the US controls and only three of the Senegal control sera had increased serum mammaglobin.

Interestingly, among those with mammaglobin serum protein above the concentration selected as the upper limit of normal (>1.71 \(\mu\)g/L; \(n = 54\)), mammaglobin serum concentration was predictive of disease severity. Mean natural log-transformed mammaglobin serum concentrations in clinical stages I to IIIB were all between 0.9 and 1.4 \(\mu\)g/L, but increased to 2.3 \(\mu\)g/L in stage IV disease \((P = 0.02, \text{ANOVA}; \text{Fig. 2})\). Similarly, mammaglobin serum concentrations were strongly associated with increasing tumor size \((P = 0.001, \text{ANOVA})\); the mean natural log-transformed mammaglobin serum concentration in tumors <10 cm was 1.2 \(\mu\)g/L compared with 2.3 \(\mu\)g/L in tumors 10 cm or larger.

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**Table 4. Detection of breast cancer markers by clinical tumor characteristics.**

<table>
<thead>
<tr>
<th>Clinical stage(^b)</th>
<th>MG* PCR (n = 84)</th>
<th>GABA(\pi) PCR (n = 84)</th>
<th>B726 PCR (n = 84)</th>
<th>B305 PCR (n = 84)</th>
<th>Multigene PCR (n = 84)</th>
<th>MG protein &gt;1.71 (\mu)g/L (n = 142)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumor size(^b)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–5 cm</td>
<td>17/30 (57%)</td>
<td>0/30 (0%)</td>
<td>2/30 (7%)</td>
<td>2/30 (7%)</td>
<td>21/30 (70%)</td>
<td>14/47 (30%)</td>
</tr>
<tr>
<td>6–9 cm</td>
<td>16/22 (73%)</td>
<td>3/22 (14%)</td>
<td>1/22 (5%)</td>
<td>1/22 (5%)</td>
<td>19/22 (86%)</td>
<td>12/31 (39%)</td>
</tr>
<tr>
<td>≥10 cm</td>
<td>17/30 (57%)</td>
<td>12/30 (40%)</td>
<td>1/30 (3%)</td>
<td>4/30 (13%)</td>
<td>24/30 (80%)</td>
<td>28/61 (46%)</td>
</tr>
<tr>
<td><strong>Lymph nodes(^b)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LN0</td>
<td>2/10 (20%)</td>
<td>1/10 (10%)</td>
<td>2/10 (20%)</td>
<td>1/10 (10%)</td>
<td>6/10 (60%)</td>
<td>6/12 (50%)</td>
</tr>
<tr>
<td>LN1</td>
<td>28/42 (67%)</td>
<td>4/42 (10%)</td>
<td>1/42 (2%)</td>
<td>1/42 (2%)</td>
<td>32/42 (76%)</td>
<td>26/69 (38%)</td>
</tr>
<tr>
<td>LN2/3</td>
<td>20/31 (65%)</td>
<td>9/31 (29%)</td>
<td>0/31 (0%)</td>
<td>5/31 (16%)</td>
<td>26/31 (84%)</td>
<td>22/60 (37%)</td>
</tr>
<tr>
<td><strong>Clinical stage(^b)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1/1 (100%)</td>
<td>0/1 (0%)</td>
<td>0/1 (0%)</td>
<td>0/1 (0%)</td>
<td>1/1 (100%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>II</td>
<td>1/4 (25%)</td>
<td>0/4 (0%)</td>
<td>0/4 (0%)</td>
<td>0/4 (0%)</td>
<td>1/4 (25%)</td>
<td>3/6 (50%)</td>
</tr>
<tr>
<td>III</td>
<td>9/15 (60%)</td>
<td>0/15 (0%)</td>
<td>0/15 (0%)</td>
<td>0/15 (0%)</td>
<td>9/15 (60%)</td>
<td>6/24 (25%)</td>
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<tr>
<td>IIIA</td>
<td>10/14 (71%)</td>
<td>1/14 (7%)</td>
<td>1/14 (7%)</td>
<td>1/14 (7%)</td>
<td>13/14 (93%)</td>
<td>5/16 (31%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>13/19 (68%)</td>
<td>4/19 (21%)</td>
<td>0/19 (0%)</td>
<td>0/19 (0%)</td>
<td>14/19 (74%)</td>
<td>14/41 (34%)</td>
</tr>
<tr>
<td>IV</td>
<td>16/30 (53%)</td>
<td>9/30 (30%)</td>
<td>2/30 (7%)</td>
<td>6/30 (20%)</td>
<td>26/30 (87%)</td>
<td>25/52 (48%)</td>
</tr>
</tbody>
</table>

\(^a\) MG, mammaglobin.

\(^b\) Missing data: tumor size \((n = 2 \text{ for } \text{PCR}; n = 3 \text{ for } \text{protein}); \text{lymph nodes } \((n = 1)\); \text{clinical stage } \((n = 1 \text{ for } \text{PCR}; n = 2 \text{ for } \text{protein})\).

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DETECTION OF MAMMAGLOBIN PROTEIN AND/OR GENE TRANSCRIPTS IN PERIPHERAL BLOOD SAMPLES
Blood samples from 79 women with breast cancer were analyzed by the multigene RT-PCR assay and for mammaglobin protein by ELISA. In these women, 84% had either increased mammaglobin protein or detectable multigene transcripts. Three patient samples had increased mammaglobin protein concentrations but were negative for mammaglobin mRNA, whereas 17 samples were positive for mammaglobin mRNA but mammaglobin serum protein was <1.71 μg/L. More than one half (59.5%) of the breast cancer blood samples had mammaglobin transcripts detected, 14 more samples had gene transcripts from one or more of B305D, GABA_τ, and B726P detected, and an additional 5 samples had increased mammaglobin protein as quantified by ELISA.

The serum mammaglobin protein concentration was also associated with the detection of mammaglobin transcripts in blood by RT-PCR. In mammaglobin PCR-negative blood samples, the mean ELISA value for circulating protein was 1.8 μg/L compared with a mean ELISA value of 18.5 μg/L in mammaglobin PCR-positive samples (P = 0.01, t-test). Serum mammaglobin concentrations were also correlated to the log(copy numbers) of the multigene RT-PCR assay (P = 0.02).

Discussion
Development of biomarkers for detection and staging of breast cancer is of importance for clinical management of the disease. Several molecules, including carcinoembryonic antigen (CEA) and mucin-type markers (e.g., the MUC-1 gene and its glycoprotein antigens CA15.3 and CA27.29), have been used as biomarkers for metastatic breast cancer. Before-treatment sensitivities of the commonly used circulating tumor markers for breast cancer, CEA and CA15.3, have been reported as 12% (17) with an increase to 40% in breast cancer patients with recurrence. Several studies have reported the detection of circulating tumor cells by cytokeratins, CEA, and MUC-1 RT-PCR (3,18), but application of these assays has been hampered by lack of specificity (19–21).

In this study, we examined the expression of mammaglobin, B305D, GABA_τ, and B726P and the increase in mammaglobin protein concentrations in peripheral blood samples from breast cancer patients. For a cell-addition experiment in blood of a healthy volunteer, two breast cancer cell lines were used. MDA-MB-415 cells express ~100-fold higher concentrations of mammaglobin mRNA than BT-474 (data not shown). The multigene RT-PCR was able to detect both cell lines at 1 cell/mL of peripheral blood. The mammaglobin single RT-PCR assay could also detect the MDA-MB-415 cell line at 1 cell/mL, but the lower detection limit for BT-474 cells was 100 cells/mL. These results demonstrate a lower detection limit for the multigene RT-PCR, in particular for breast tumor cells with low mammaglobin gene expression.

The study population consisted of a large number of women with untreated breast cancer, almost all of whom had breast cancer that had already metastasized to regional lymph nodes. Given this, it was anticipated that these women had a high likelihood of having circulating tumor cells present. Moreover, the study population evaluated is probably not representative of newly diagnosed breast cancer patients in the US. Nonetheless, using a single 10-mL blood sample obtained before physical examination and biopsy, we detected at least one of the four breast cancer-associated transcripts and/or increased mammaglobin protein in 84% of women with breast cancer. Analysis of multiple samples collected over a 24-h period, as required for optimal detection of bacteremia by blood culture, might have boosted sensitivity even further.

Although mammaglobin tissue expression has been shown in ~80% of breast cancers, previous studies using blood samples from patients reported detection of mammaglobin transcript in 25–54% of those with and in 10–25% of patients without metastatic breast cancer (3,18,22–26). In this study, we found mammaglobin transcripts in 61% of single blood samples from breast cancer patients. This increased rate of detection may be related to the fact that the patients examined were untreated, whereas many of the women examined in previous studies had undergone chemotherapy. Treatment may lower the number of detected circulating tumor cells. As mentioned above, because mammaglobin is not expressed in all breast cancers, we developed and recently reported on three complementary expressed genes that, when used in combination with mammaglobin, provided increased sensitivity for identification of disseminated breast tumor cells in lymph node specimens (10). In the present study, the addition of these three transcripts increased blood-based detection of circulating cells from 61% to 77% of women with breast cancer. It is possible
that obtaining additional samples may further increase the number of patients positive for breast cancer.

The sensitivity of the multigene assay was increased with increasing cancer stage, but detection of the mammaglobin transcript alone was only marginally associated with increased nodal involvement and not with other tumor or patient characteristics. Our findings agree with a recent study by Lin et al. (27), which evaluated the correlation between mammaglobin expression in peripheral blood and known prognostic factors for breast cancer patients. Whereas mammaglobin mRNA expression was frequently shown to be increased in patients with unfavorable prognostic factors (tumor size and disease stage), no significant differences could be confirmed. The same group also reported that mammaglobin mRNA detection combined with CEA or CA15.3 increased the sensitivity from 54% of 33 metastatic breast cancer patients to 81% and 90%, respectively, suggesting that mammaglobin mRNA may be a useful adjunct to existing serum markers.

We found that GABA$_\pi$ transcripts were more often present in breast cancer patients with larger tumors, nodal involvement, and advanced overall tumor stage. Interestingly, GABA$_\pi$ expression was higher in women who were premenopausal. These findings demonstrate a possible application of this marker to monitor disease progression and treatment efficacy in premenopausal patients in particular. Surprisingly, expression of B726P and B305D was detected only in small subsets of breast cancer blood specimens (5% and 8%, respectively). To confirm the expression of these target genes in the primary breast tumors, we analyzed biopsy tissue samples in a subset (n = 47) of patients. The percentages of tumors expressing mammaglobin (85%) and GABA$_\pi$ (53%) were consistent with earlier findings (8, 9), but B726P and B305D were expressed in only 6% of the tumor biopsies tested. Previously, we reported B726P and B305D overexpression in 40–50% and 60–70% of primary and metastatic breast cancer specimens, respectively (10), which has been confirmed by others for B726P (28). Specific characteristics of the breast tumors or patients (e.g., median age, later stage, ethnicity) could be the reason for a different gene expression profile. This would argue that B726P and B305D might exhibit different detection sensitivities in other patient populations. We are currently analyzing samples from US breast cancer patients with different ethnicities, age groups, and early stages of disease to confirm the application of this assay and its components.

Increasing serum concentrations of mammaglobin protein were associated with increasing clinical stage and tumor size. In addition, we found a significant correlation between mammaglobin serum protein and mammaglobin expression detected in the blood. Whereas circulating mammaglobin protein was detected in 70% of the samples from breast cancer patients, only 38% were considered increased compared with the control group. The cutoff value of 1.71 μg/L was established by use of a Senegalese and a US cohort of normal sera samples. The mean mammaglobin concentration in the Senegal normal sera (0.37 μg/L) was significantly higher than the mean concentration in US samples (0.10 μg/L). This finding might reflect a population difference regarding parity, nursing, and undiagnosed benign or malignant breast diseases. All of these conditions could affect the amount of mammaglobin protein released into the blood stream because mammaglobin expression is associated with mammary ductal proliferation (29). Detailed studies in different healthy populations need to be performed to identify conditions that could influence the serum mammaglobin protein concentration. Circulating mammaglobin protein may also be a good marker to monitor treatment and detect disease relapse. Moreover, the detection of serum mammaglobin protein could be combined with other markers, e.g., CEA, CA15.3, and circulating antibodies against tumor-specific epitopes, to further increase detection sensitivity.

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References


