

A Study of Select Blood-based Protein Biomarkers (Cytokines) for Breast Cancer Screening: BT Test[®] - Summary of Clinical Study and Results

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Test Description

The BT Test[®], which stands for Biomarker Translation Test, is a multivariate, non-invasive blood-based multi-protein biomarker analysis for the detection of breast cancer. The test was designed to differentiate invasive breast cancer (i.e. invasive breast cancer with and without concomitant DCIS) from non-cancer cohorts (i.e. healthy women or those with benign breast conditions).

Methodology:

Five hundred thirty two (532) female patients were prospectively recruited from nine (9) community-based medical facilities for this clinical study. Sixty-five (65) of these patients were later determined to be ineligible due to either limited specimen volume and/or specimen integrity concerns (e.g. sample hemolyzed). The clinical trial design consisted of a control group of women undergoing screening mammograms (HS, n=163) with a second group of women with abnormal screening or diagnostic mammograms scheduled for breast biopsy (final n=304). All screening patients had their blood drawn naïve to mammography and all biopsy women were drawn prior to the procedure. Final biopsy diagnostic assignments of invasive breast cancer positive (BC+, n=60) with and without concomitant DCIS (n=14, 46 respectively), or biopsy breast cancer negative (BC-, n=247) were made on the basis of tissue pathology reports. The invasive breast cancers consisted of infiltrating ductal carcinomas (n=50), infiltrating lobular carcinoma (n=5), or concomitant infiltrating ductal and lobular carcinoma (n=5). The biopsy breast cancer negative cohort served as a second control group.

Women were recruited between the ages of 35 and 75. Protocol specific exclusions were predicated on the potential to impact the protein biomarkers of interest, and included women who were pregnant, nursing, receiving chemo- or radiation therapy, patients with known autoimmune diseases and those taking antibiotics in the preceding two weeks. As a conservative measure, patients having had cancer in the previous ten years were also excluded from the study.

Clinical information about the subjects' medical history, lifestyle characteristics, and known breast cancer risk factors, including but not limited to age, number of pregnancies, age of menarche, breast feeding, hormonal therapy and family history of breast cancer was also collected. Both control groups and breast cancer positive groups had very similar demographic, risk and lifestyle characteristics. See Table 1 for select clinical trial patient collective data profile information.

Table 1: Select Subject Characteristics

Demographic Characteristics							
Cohort	Age	BMI	Hispanic	Native American	Asian	Black	White
BC+	54.7	27.9	5%	0%	0%	4%	93%
BC-	49.5	27.5	10%	0%	1%	3%	87%
HS	54.2	26.5	3%	2%	1%	2%	92%
Select Lifestyle Characteristics							
	Alcoholic Drinks per Week			Activity Level		Smoker	
	1-3	4-8	9-14	>14	Low	High	
BC+	42%	16%	4%	0%	11%	21%	19%
BC-	34%	13%	5%	1%	13%	23%	13%
HS	33%	15%	3%	1%	10%	25%	6%
Select Risk Factors							
	Age of Menarche	Birth Control Current	Birth Control Ever	FT Pregnancy	No. of FT Pregnancies	Breast Feeding	Avg No. of Months
BC+	13.0	4%	61%	74%	1.7	44%	7.4
BC-	12.7	4%	59%	76%	1.8	47%	6.3
HS	12.6	6%	59%	72%	1.7	40%	7.1
Family History of Breast Cancer							
	Family History	Mother	Sister	Grand-mother	Aunt	Cousin	Daughter
BC+	32%	12%	7%	4%	19%	11%	0%
BC-	40%	15%	4%	12%	17%	5%	0%
HS	59%	24%	7%	16%	16%	7%	1%

All specimens were analyzed for five cancer specific protein biomarkers (i.e. CEA, HGF, IL-8, IL-12_{p70/p40} and VEGF) using either ELISA or a multiplexed microbead assay while blinded to clinical information. Each of these analytes is well documented in the literature¹⁻⁴⁹ as being putatively associated with breast cancer, and are associated with the following immune system regulatory functions and activities; blood vessel growth, cell death, tumor growth, and inflammation and immune system responses to cancer.

Following analysis, lab results were combined with patient specific clinical information (See Table 2), aggregated according to intended clinical utility (described below), randomized, and split into training sets (70% of subjects) and validation sets (30% of subjects). The training set was used to develop and optimize proprietary relational algorithms that combine and translate the analytical data along with the patient's medical profile to generate a BT Test Score and report (see "Discussion" section below for more details on BT Score[®] relevance).

The validation set was then used to test the validity of the refined BT Test algorithms and to test the accuracy of the BT Score on independent data.

Table 2: Collected Subject Data

Patient Medical History:

- Age _____ Height _____ ft _____ in Weight _____ lbs
- Age at first menstrual cycle _____
- What is your current menopausal status:
 - Pre-menopausal (still having periods)
 - Peri-menopausal (last period within last year)
 - Post-menopausal (last period over a year ago)
- Have you ever used hormonal birth control?
 - Yes No
 - Age at first use _____ Total # of yrs used _____
- Have you ever used estrogen replacement therapy?
 - Yes No
 - Age at first use _____ Total # of yrs used _____
- Number of Full Term Pregnancies: _____
Age at first pregnancy _____
- Total months breast feeding (all children) _____
- Check all relatives which have had breast cancer:
 - Grandmother Sister
 - Mother Aunt
 - Cousin Daughter
 - No Family History Unknown
- Please check your ethnic origin
 - Hispanic or Latino
 - Asian
 - American Indian or Native Alaskan
 - Native Hawaiian or Pacific Islander
 - Black/African American
 - White
- Do you have Osteoporosis? Yes No
- Do you smoke? Yes No
- How physically active are you?
 - Inactive Moderately active Very Active
- Have you had previous breast biopsies performed?
 - 0 1-3 3+
- How many alcoholic drinks do you consume per week?
 - 0-4 4-9 9-14 14+

Indications of Use:

The data generated in this study gives rise to two potential indications of use that were evaluated separately. First is the potential use of the BT Test as a screen for Breast Cancer in an asymptomatic population, either as a complement to, or as a potential replacement for a mammogram. Data for this application was limited to all Breast Cancer Positive subjects, all Healthy Screens and a randomly selected group Biopsy Breast Cancer Negative subjects (n=10% of screening population) to be more reflective of the intended clinical use population.

The second potential use of the BT Test is as a Biopsy Referral tool. For this indication, the data was limited to subjects who had been referred for a biopsy. This construct of the Test was designed to provide a “yes/no” decision as to the need for a biopsy with the purpose of insuring 100% of the patients with breast cancer would be referred for biopsy while reducing the large number (est. 0.6 – 1.2 million) of unnecessary biopsies performed each year.

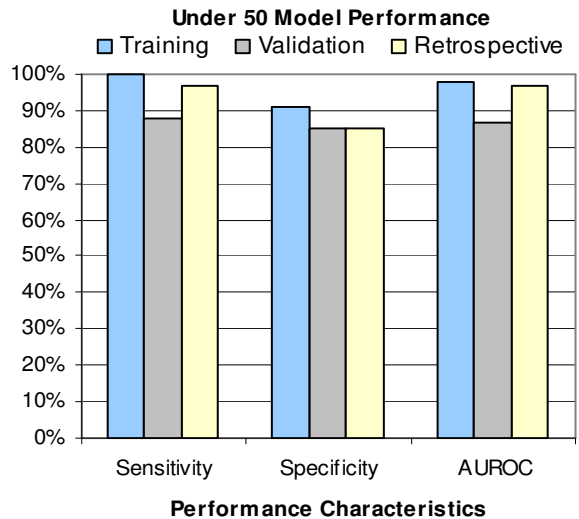
BT Test Screening Use:

In screening use, significant differences were found when the marker data was age stratified. As such, the data was evaluated independently for women under the age of 50, women between 50 and 60, and women 60 or older.

For women under 50, the calculated area under the receiver operating characteristic (AUROC) curve for the BT Test was 0.98 within the training set and 0.87 upon

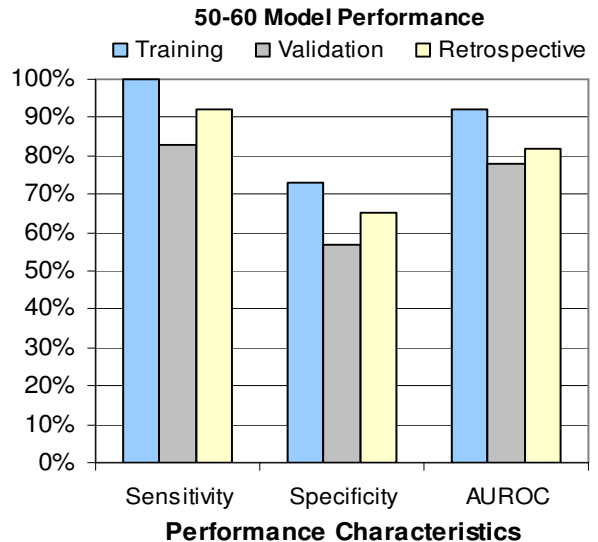
cross validation, as shown in Fig 1. The associated sensitivity of the test was 100% and specificity 91% within the training set and 88% and 85% within the cross validation set respectively.

Figure 1: BT Test Performance on Women under 50



For women between 50 – 60 years of age, the calculated area under the receiver operating characteristic (AUROC) curve for the BT Test was 0.95 within the training set and 0.78 upon cross validation, as shown in Fig 1. The associated sensitivity of the test was 100% and specificity 73% within the training set and 83% and 57% within the cross validation set respectively.

Figure 2: BT Test Performance on Women 50 - 60



For women 60 and older, the differentiation between breast cancer and controls significantly deteriorated, with marked decreases observed between training and validation performance.

For purposes of comparison, Figure 3 shows BT Test performance relative to GE's digital mammography PMA trials. Table 3 provides a summary of existing performance data on both full-field digital (FFDM) and screen-film mammography (SFM) modalities.

Figure 3: BT Test vs. Digital Mammography PMA Data

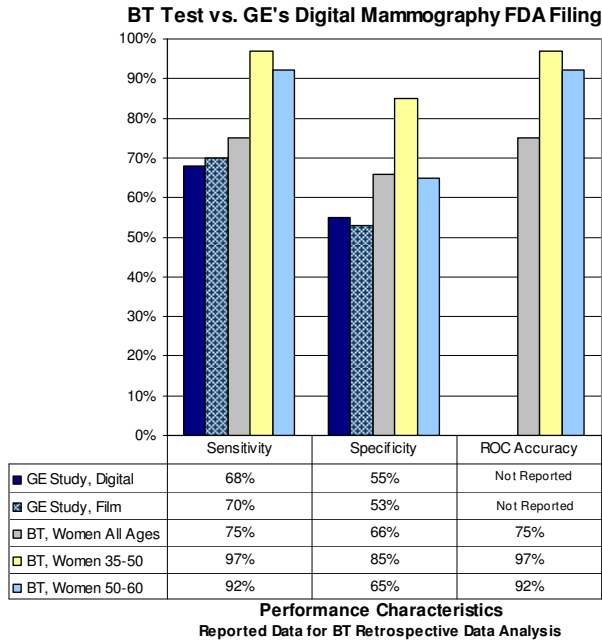


Table 3: American College of Radiology Imaging DMIST^{*,50} Mammography Report

Performance of Mammography* and BT Test			
Test Procedure (Women Under 50)	Area Under ROC	Sensitivity (%)	Specificity (%)
Film Mammography	0.69 (0.59 - 0.79)	51 (37 - 65)	92 (91.8 - 92.2)
Digital Mammography	0.84 (0.48 - 0.90)	78 (68 - 88)	90 (89 - 91)
BT Test**	0.97	0.97	0.85
Test Procedure (All Women)	Area Under ROC	Sensitivity (%)	Specificity (%)
Film Mammography	0.74 (0.70 - 0.78)	66 (60 - 72)	92 (91.8 - 92.2)
Digital Mammography	0.78 (0.74 - 0.82)	70 (64 - 76)	93 (91.8 - 92.2)
BT Test**	0.75	0.73	0.66

* Mammography Data Source: Full-Field Digital Mammography, Tech Evaluation Center, Assessment Program, Volume 20, No.16, February 2006. Results based on both digital and film mammography for over 42,500 women. Released NEJM, Sept. 16, 2005, Pisano et al. 2005a,b., American College of Radiology Imaging Network's (ACRIN's) Digit
** Retrospective results shown here

BT Test Biopsy Referral Use:

As within the screening population, significant differences were found between the analytical results of younger women (<50) and older women (50 and over), giving rise to separate data analysis.

For women under 50, models were generated and cross validated that were able to refer all cancer cases on to biopsy, while preventing a reasonable percentage of unnecessary biopsies. As shown on Table 4, the training model referred 100% of cancers at cut-points of 0.175-0.275 while preventing a maximum of 33% of unnecessary biopsies. The cross validation shows 100% of cancers being referred at the same cut-points while preventing a maximum of 44% of unnecessary biopsies.

Table 4: BT Biopsy Referral Test Performance (Condensed)

	Cut-Point	0	0.075	0.175	0.275	0.3	0.425
Training Set	Sens	100.0%	100.0%	100.0%	100.0%	0.0%	0.0%
	Spec	31.7%	31.7%	31.7%	31.7%	100.0%	100.0%
Validation Set	Sens	100.0%	100.0%	100.0%	100.0%	0.0%	0.0%
	Spec	44.8%	44.8%	44.8%	44.8%	100.0%	100.0%

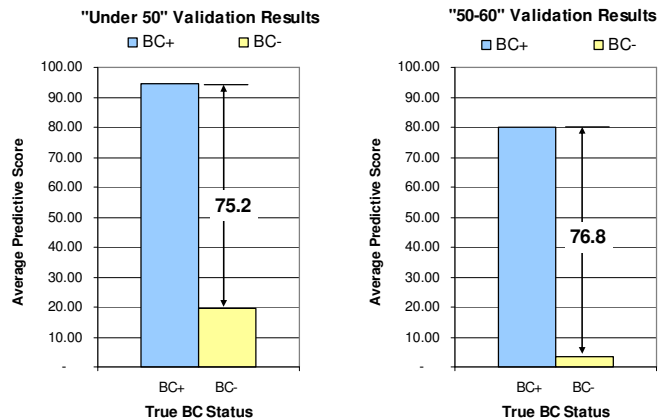
For women over 50, the biopsy referral indication did not produce statistically meaningful results.

Discussion:

The clinical trial data allowed for the development and cross validation of algorithms for both screening and biopsy referral indications of use. Within this enriched population screening study, age stratified analysis increased the BT Test's ability to differentiate invasive breast cancer cohorts from non-breast cancer cohorts.

This is particularly true for women under 50, where the cross validation data achieved a very strong 0.87 AUROC. In clinical practice, BT Test Scores above 60 can be considered abnormal suggesting an increased likelihood of breast cancer (see Figure 4 for an example of validated BT Scores for breast cancer positive and negative women).

Figure 4: Example of Predictive BT Scores



When used in conjunction with exiting imaging modalities and best practices for breast cancer screening, the results of the BT Test has the ability to assist healthcare practitioners in their efforts to detect breast cancer earlier when intervention is most successful and survivability is over 96%.

Within the Biopsy Referral portion of the study, the changes to the BT Test's clinical utility algorithm indicates that it may be possible to reduce the large number of unnecessary biopsy performed each year in the U.S. by at least 25-30%, benefiting patients (i.e. reducing unneeded surgeries and potential complications) and reducing costs.

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