



Chapter 2

Biomarkers in Breast Cancer

Diagnostic Methods

Summary

- Chapter 2 of this report identifies 38 molecules, molecule combinations or image-based biomarkers with potential utility in the detection or diagnosis of breast cancer. For 27 (71%) of these, the authors calculated diagnostic sensitivity; for 27 (71%), diagnostic specificities are given and for 18 (47%), combined diagnostic sensitivities and specificities are indicated.
- These potential biomarkers were: [EDITED].
- Diagnostic biomarkers were identified in eight difference sources: blood (or serum), tumour tissue, nipple aspirate, breath, cancer cells, saliva, tear fluid and urine. Of these, blood and tumour tissue were indicated in 62% and 18% of cases, respectively. See Figure 2.1
- Diagnostic sensitivities of 90% or more were reported in 37% of cases, where this was calculated. In the case of an 80% or more sensitivity cut-off, the figure was 50%. Diagnostic specificities of 90% or more applied in 26% of cases and the equivalent specificity for a cut off of 80% or more, was 40%.
- Biomarker sources that offer minimally invasive methods were reported in 79% (n=30) of cases. These were blood (80%, n=24), nipple aspirate, breath, saliva, tear fluid and urine.
- Diagnostic biomarkers represented 11 different types or identities. These were: protein(s); analyte(s); DNA, gene(s); nucleic acids; spectroscopic parameters; glycans; histone modifications; Maillard product(s); metabolites and volatiles. See Figure 2.2

Breast Cancer Biomarkers, 2010

Figure 2.1 Source of biomarkers reported to have potential utility in the detection or diagnosis of breast cancer

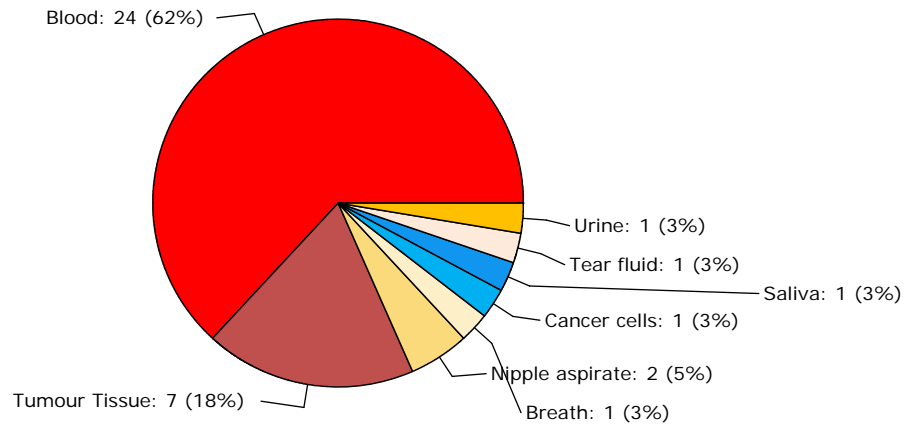
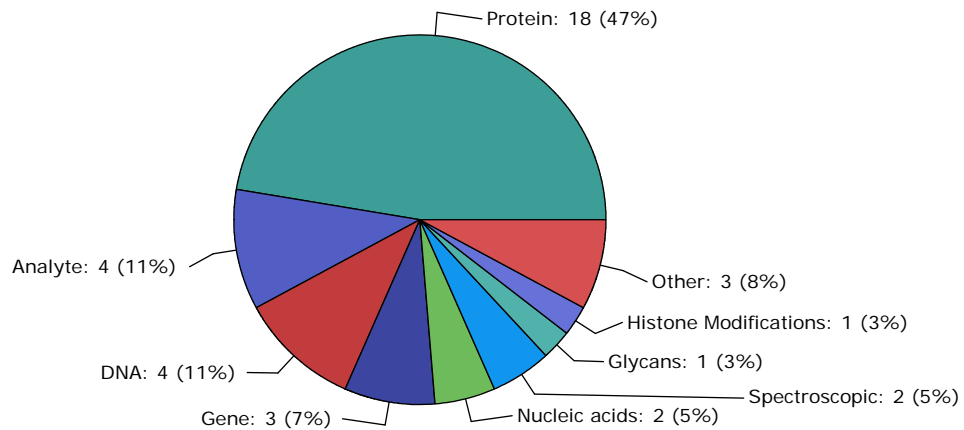


Figure 2.2 Types of biomarkers reported to have potential utility in the detection or diagnosis of breast cancer



- Of the 38 reports on potential diagnostic breast cancer biomarkers, 28 (74%) referred non-specifically to "breast cancer", nine (18%) referred to metastatic breast cancer and three (8%) referred to early-stage breast cancer.
- Early-stage diagnostic biomarkers reported were [EDITED] See Tables 2.1-2.3.

Breast Cancer Biomarkers, 2010

Table 2.1 Biomarkers reported to have potential utility in the detection or diagnosis of breast cancer (name/identity, type, sensitivity (SEN), specificity (SPE), reference)

NAME/IDENTITY	TYPE	SEN	SPE	REF
Near IR	Spectroscopic	100	100	2.20
Filamin-A	Protein	97	68	2.4
Apolipoprotein C-I, C-terminal-truncated form of C3a and Complement Component C3a	Proteins	96	95	2.7
[EDITED]	Proteins	95	95	2.2
[EDITED]	Analytes	95	95	2.33
[EDITED]	Volatiles	94	85	2.36
EGFR5 or TOX methylation	Genes	92	92	2.26
[EDITED]	Proteins	92		2.34
[EDITED]	Spectroscopic	91	94	2.10
Circulating Nucleic Acids	Nucleic acids	90	95	2.1
Serum proteome panel	Analyte panel	88	78	2.15
[EDITED]	Protein	88		2.21
[EDITED]	Nucleoside panel	88	89	2.23
RASSF1A methylation	Gene	88		2.28
[EDITED]	Analytes	88	86	2.31
Oncoprotein HCCR-1	Protein	87		2.35
[EDITED]	Protein	85		2.3
[EDITED]	Protein	80		2.21
[EDITED]	Tumour antigens	80	100	2.24
Alpha 2-HS glycoprotein	Protein	79	90	2.17
[EDITED]	Histone Modifications	79		2.18

Breast Cancer Biomarkers, 2010

Table 2.1 (continued) Biomarkers reported to have potential utility in the detection or diagnosis of breast cancer (Name/identity, type, sensitivity (SEN), specificity (SPE), reference)

NAME/IDENTITY	TYPE	SEN	SPE	REF
Osteopontin	Gene	78		2.29
[EDITED]	Analyte panel	71	71	2.16
[EDITED]	Protein	69		2.3
[EDITED]	Protein	54	73	2.27
[EDITED]	Proteins	53	98	2.9
Circulating cell-free DNA	DNA	53	87	2.12
Biotinidase	Protein			2.5
Salivary metabolites	Metabolites			2.6
[EDITED]	Telomeres			2.8
[EDITED]	DNA			2.11
[EDITED]	DNA			2.13
[EDITED]	Proteins			2.14
ALCAM	Protein			2.19
[EDITED]	Glycans			2.22
[EDITED]	Maillard Product			2.25
[EDITED]	Protein			2.30
[EDITED]	Protein			2.32

Breast Cancer Biomarkers, 2010

Table 2.2 Biomarkers reported to have potential utility in the detection or diagnosis of breast cancer (name/identity, biomarker function (if known), reference)

NAME/IDENTITY	FUNCTION (IF KNOWN)	REF
[EDITED]		2.1
[EDITED]		2.2
[EDITED]	[EDITED]	2.3
[EDITED]	[EDITED]	2.3
Filamin-A	Filamin A is a cross-linking and scaffold protein	2.4
Biotinidase	Biotinidase is an enzyme	2.5
[EDITED]		2.6
Apolipoprotein C-I, C-terminal-truncated form of C3a and complement component C3a	Apolipoprotein C-I functions with lipoproteins; C-terminal-truncated form of C3a and complement component C3a are involved in immune response	2.7
[EDITED]	[EDITED].	2.8
[EDITED]	[EDITED]	2.9
[EDITED]		2.10
[EDITED]		2.11
[EDITED]		2.12
[EDITED]		2.13
[EDITED]	[EDITED]	2.14
[EDITED]		2.15
[EDITED]		2.16
Alpha 2-HS glycoprotein	Alpha 2-HS glycoprotein is a glycoprotein	2.17
[EDITED]c		2.18

Breast Cancer Biomarkers, 2010

Table 2.2 (Continued) Biomarkers reported to have potential utility in the detection or diagnosis of breast cancer (name/identity, biomarker function (if known), reference)

NAME/IDENTITY	FUNCTION (IF KNOWN)	REF
ALCAM	ALCAM is activated leukocyte cell adhesion molecule; the CD166 protein/antigen	2.19
[EDITED]		2.20
[EDITED]	[EDITED]	2.21
[EDITED]	[EDITED]	2.21
[EDITED]		2.22
[EDITED]		2.23
[EDITED]		2.24
[EDITED]	[EDITED]	2.25
[EDITED]		2.26
[EDITED]	[EDITED]	2.27
[EDITED]	[EDITED]	2.28
[EDITED]	[EDITED]	2.29
[EDITED]		2.3
[EDITED]		2.31
[EDITED]	[EDITED].	2.32
[EDITED]		2.33
[EDITED]		2.34
[EDITED]	[EDITED]	2.35
[EDITED]		2.36

Breast Cancer Biomarkers, 2010

Table 2.3 Biomarkers reported to have potential utility in the detection or diagnosis of breast cancer (name/identity, opportunity, reference)

NAME/IDENTITY	OPPORTUNITY	REF
[EDITED]	MID BC	2.1
[EDITED]	MID MBC	2.2
[EDITED]	MID BC	2.3
[EDITED]	MID MBC	2.3
[EDITED]	MID BC	2.4
[EDITED]	MID BC	2.5
[EDITED]	MID BC	2.6
[EDITED]	MID BC	2.7

MID BC = minimally invasive diagnostic for breast cancer; DBC = diagnostic for breast cancer; MID EBC = minimally invasive diagnostic for breast cancer

Breast Cancer Biomarkers, 2010

Table 2.3 (Continued) Biomarkers reported to have potential utility in the detection or diagnosis of breast cancer (name/identity, opportunity, reference)

NAME/IDENTITY	OPPORTUNITY	REF
[EDITED]	MID BC	2.1
[EDITED]	DBC	2.8
[EDITED]	MID BC	2.9
[EDITED]	DBC	2.10
[EDITED]	MID EBC	2.11
[EDITED]	MID BC	2.12
[EDITED]	MID BC	2.13
[EDITED]	MID MBC	2.14
[EDITED]	MID EBC	2.15

MID BC = minimally invasive diagnostic for breast cancer; DBC = diagnostic for breast cancer; MID EBC = minimally invasive diagnostic for breast cancer

Breast Cancer Biomarkers, 2010

Table 2.3 (Continued) Biomarkers reported to have potential utility in the detection or diagnosis of breast cancer (name/identity, opportunity, reference)

NAME/IDENTITY	OPPORTUNITY	REF
[EDITED]	MID BC	2.1
[EDITED]	MID BC	2.16
[EDITED]	MID BC	2.17
[EDITED]	MID BC	2.18
[EDITED]	MID BC	2.19
[EDITED]	DBC	2.20
[EDITED]	DBC	2.21
[EDITED]	MID BC	2.21
[EDITED]	MID BC	2.22

MID BC = minimally invasive diagnostic for breast cancer; DBC = diagnostic for breast cancer; MID EBC = minimally invasive diagnostic for breast cancer

Breast Cancer Biomarkers, 2010

Table 2.3 (Continued) Biomarkers reported to have potential utility in the detection or diagnosis of breast cancer (name, opportunity, reference)

NAME/IDENTITY	OPPORTUNITY	REF
[EDITED]	MID BC	2.1
[EDITED]	MID BC	2.23
[EDITED]	MID BC	2.24
[EDITED]	DBC	2.25
[EDITED]	BCB	2.26
[EDITED]	MID MBC	2.27
[EDITED]	MID MBC	2.28
[EDITED]	MID BC	2.29
[EDITED]	MID BC	2.3

MID BC = minimally invasive diagnostic for breast cancer; DBC = diagnostic for breast cancer; MID EBC = minimally invasive diagnostic for breast cancer

Breast Cancer Biomarkers, 2010

Table 2.3 (Continued) Biomarkers reported to have potential utility in the detection or diagnosis of breast cancer (name/identity, opportunity, reference)

NAME/IDENTITY	OPPORTUNITY	REF
[EDITED]	MID BC	2.1
[EDITED]	MID BC	2.31
[EDITED]	MID BC	2.32
[EDITED]	MID BC	2.33
[EDITED]	MID BC	2.34
[EDITED]	MID BC	2.35
[EDITED]	MID BC	2.36

MID BC = minimally invasive diagnostic for breast cancer; DBC = diagnostic for breast cancer; MID EBC = minimally invasive diagnostic for breast cancer

Breast Cancer Biomarkers, 2010

Table 2.4 Biomarkers identified in the diagnosis of breast cancer
(name/identity of biomarker, development stage, biomarker source, reference)

NAME/IDENTITY	STAGE	SOURCE	REF
[EDITED]	Early Clinical	Biopsy	2.8
[EDITED]	Early Clinical	Biopsy	2.10
[EDITED]	Early Clinical	Biopsy	2.18
[EDITED]	Research	Biopsy	2.20
[EDITED]	Early Clinical	Biopsy	2.21
[EDITED]	Early Clinical	Biopsy	2.25
[EDITED]	Early Clinical	Biopsy	2.29
[EDITED]	Early Clinical	Blood	2.1
[EDITED]	Early Clinical	Blood	2.2
[EDITED]	Early Clinical	Blood	2.3
[EDITED]	Early Clinical	Blood	2.3
[EDITED]	Early Clinical	Blood	2.4
[EDITED]	Research	Blood	2.5
[EDITED]	Early Clinical	Blood	2.7
[EDITED]	Early Clinical	Blood	2.9
[EDITED]	Early Clinical	Blood	2.11
[EDITED]	Early Clinical	Blood	2.12
[EDITED]	Research	Blood	2.13

Breast Cancer Biomarkers, 2010

Table 2.4 Biomarkers identified in the diagnosis of breast cancer
(name/identity of biomarker, development stage, biomarker source, reference)

NAME/IDENTITY	STAGE	SOURCE	REF
[EDITED]	Early Clinical	Blood	2.15
[EDITED]	Early Clinical	Blood	2.21
[EDITED]	Early Clinical	Blood	2.24
[EDITED]	Early Clinical	Blood	2.33
[EDITED]	Research	Breath	2.36
[EDITED]	Research	Cancer cells	2.26
[EDITED]	Early Clinical	Nipple aspirate	2.3
[EDITED]	Early Clinical	Nipple aspirate	2.34
[EDITED]	Early Clinical	Saliva	2.6
[EDITED]	Early Clinical	Serum	2.14
[EDITED]	Early Clinical	Serum	2.17
[EDITED]	Research	Serum	2.19
[EDITED]	Early Clinical	Serum	2.22
[EDITED]	Early Clinical	Serum	2.27
[EDITED]	Early Clinical	Serum	2.28
[EDITED]	Early Clinical	Serum	2.31
[EDITED]	Early Clinical	Serum	2.32
[EDITED]	Early Clinical	Serum	2.35

Breast Cancer Biomarkers, 2010

Table 2.4 Biomarkers identified in the diagnosis of breast cancer
(name/identity of biomarker, development stage, biomarker source, reference)

NAME/IDENTITY	STAGE	SOURCE	REF
[EDITED]	Early Clinical	Tear fluid	2.16
[EDITED]	Early Clinical	Urine	2.23

2.3 Nucleic Acids

Beck and co-workers^{2.1} have reported on circulating nucleic acids (CNA) in the serum of breast cancer patients. Serum CNA of 38 women with ductal carcinoma was extracted and sequenced, and compared with healthy controls. Subsequently, data from 26 patients with stages II to IV tumours and 67 healthy female controls were used as a training set providing a five-parameter model. Applying the model to a small group of patients with tumour stage I resulted in diagnostic sensitivities and specificities of 90% and 95%, respectively. The identification of specific circulating nucleic acid sequences may, therefore, offer useful non-invasive diagnostic potential.

2.6 Filamin-A

Alper and co-workers^{2.4} have reported on a study of the protein filamin-A in cancer patients and controls. Circulating filamin-A was measured in patient plasma samples using an ELISA assay. This study involved 134 patients with brain, breast, or ovarian cancer, 15 patients with active arthritis, and 76 healthy controls. Circulating filamin-A was detected in the plasma of 109 of 143 patients with breast cancer and primary brain tumours. Analysis of these study findings found that filamin-A showed 89.5% sensitivity and 97.8% specificity for glioblastoma at a cut-off of 21.0 ng/mL. Plasma levels of filamin-A (at a cut-off of 36.0 ng/mL) had a 96.7% sensitivity and a 67.8% specificity for metastatic breast cancer.

Filamin-A levels were found to be elevated in malignant breast or brain tissues, but not in control tissues. Filamin-A was found to be localised in the lysosomes of breast cancer cells, but not in normal human mammary epithelial cells. These findings indicate that filamin-A is a specific and sensitive marker for patients with astrocytoma or metastatic breast cancer.

2.7 Biotinidase

Studies of the proteomes from six breast cancer patients and six normal, healthy women by Kang^{2,5} and colleagues revealed 155 proteins, of which 33 proteins showed changes by more than 1.5-fold between the cancer patients and healthy controls. Of these, five proteins were selected for follow-up, including a1-acid glycoprotein 2, monocyte differentiation antigen CD14, biotinidase (BTD), and glutathione peroxidase 3. Using a blind set of plasma samples from 21 breast cancer patients and 21 normal healthy controls, BTD was significantly down-regulated (Wilcoxon rank-sum test, $p = 0.002$). These studies indicate that BTG may be a potential blood biomarkers for the detection of breast cancer.

2.12 Differential spectroscopy

Kukreti and colleagues^{2,10} have recently reported studies aimed at developing a self-referencing differential spectroscopy (SRDS), near-infrared method to identify breast cancer biomarkers. This method was tested with 60 subjects of which 17 patients had benign breast tumours, 22 had malignant breast tumours and 21 were controls. The retrospective use of this SRDS methods gave a sensitivity of 91% [20 of 22]; specificity, 94% [17 of 18]; positive predictive value, 95% [20 of 21]; and negative predictive value, 89% [17 of 19]). This method, therefore, offers biomarkers which correlate well with metastatic breast cancer.

2.19 Alpha 2-HS glycoprotein Antibody

In studies to identify serum biomarkers in breast cancer patients, Yi and colleagues^{2,17} carried out a stepwise purification of serum proteins, in an effort to detect the presence of antibodies that react with urinary proteins. These studies lead to the identification of the protein alpha 2-HS glycoprotein (AHSB) as a tumour antigen in the urine. Subsequently, blood samples were collected from 81 women diagnosed with breast cancer before surgery, and 73 female healthy controls. In antibody studies of AHSB, one-dimensional Western blot analysis detected the AHSB autoantibody in 64 of 81 breast cancer patients (selectivity = 79.1%) and in 7 of 73 controls (specificity = 90.4%). These data suggest that

AHSG and anti-AHSG autoantibody may be useful serum biomarkers for breast cancer diagnosis.

2.21 ALCAM

Kulasingam and co-workers^{2.19} have reported breast cancer biomarker studies of activated leukocyte cell adhesion molecule (ALCAM), together with carbohydrate antigen 15-3 (CA15-3) and carcinoembryonic antigen (CEA). These three proteins were measured in 100 healthy women, 50 healthy men and 150 breast carcinoma patients. The authors reported that serum ALCAM allows breast cancer patients to be differentiated from healthy controls and further studies are in progress.

2.22 Near IR Spectroscopy

Kukreti and co-workers^{2.20} have reported on newly discovered optical biomarkers using a double-differential spectroscopic analysis method for near-infrared (NIR, 650-1000 nm). In a pilot study to investigate 12 cancer patients and healthy controls, this methods gave 100% sensitivity and 100% specificity for the identification of breast cancer.

2.28 EGFR5 or TOX Genes

Abnormal methylation patterns are commonly seen in cancer, and Chung and co-workers^{2.26} have reported on the identification of methylation patters to differentiate colon, prostate and breast cancer cells from controls. This research included the study of 17 hypermethylation gene targets in cancer cells.

These studies show that SLC16A12, GALR2, TOX, SPOCK2, EGFR5 and DPYS are candidate biomarkers for breast cancer (methylation range 33%-79%), and, by combining EGFR5 or TOX hypermethylation, sensitivities and specificities in differentiating breast cancer cells from controls were 92% and 92%, respectively. Screening for these cancer specific hypermethylation patterns may offer new techniques for detecting and diagnosing breast cancer.

2.29 HER-2

In studies^{2,27} of serum HER-2 protein in breast cancer patients and the HER-2 Ile655Val single nucleotide polymorphism of the gene, 56 consecutive patients with primary breast, together with 45 healthy women, were evaluated. It was found that serum HER-2 levels were increased in breast cancer patients compared to controls, and a cut-off of 1.98 ng/ml identified breast patients with a sensitivity of 54% and a specificity of 73%.

Elevated serum HER-2 levels were linked to lymphovascular invasion ($p=0.022$), poor differentiation ($p=0.011$), advanced clinical stages ($p=0.001$) and lymph node metastasis ($p=0.011$). It was also found that the HER-2 Ile655Val SNP, Ile-Val and Val-Val genotypes showed significant serum HER-2 elevation compared to homozygous Ile-Ile (both $p<0.001$). These studies show serum HER-2 could be a useful as a biomarker for breast cancer diagnosis and disease progression.

2.31 Osteopontin

The metastasis-associated gene osteopontin shows alternative splicing patterns to give osteopontin-a, osteopontin-b and osteopontin-c. In studies by Mirza and co-workers^{2,29}, was found to be selectively expressed in invasive, but not in noninvasive, cancer cells. In their studies, they found that osteopontin-c was present in 16 of 20 breast cancers (80%), but was undetectable in 22 normal control specimens. Of the breast cancers, 43 of 56 biopsies (77%) stained positive for osteopontin-c and increased from grade 1 to grade 3. Overall, this variant was present in 78% of breast cancers, 36% of surrounding tissues and 0% of normal tissues. Additionally, osteopontin-c is associated with a higher proportion of breast cancers than oestrogen receptor (ER), progesterone receptor or HER2 and when used with ER and HER2 predicts grade 2-3 breast cancer. Osteopontin c may, therefore, have potential as a diagnostic and prognostic marker for breast cancer.