

Current status of the prognostic molecular biomarkers in breast cancer: A systematic review (Review)

GORO KUTOMI, TORU MIZUGUCHI, FUKINO SATOMI, HIDEKI MAEDA,
HIROAKI SHIMA, YASUTOSHI KIMURA and KOICHI HIRATA

Department of Surgery, Surgical Oncology and Science, Sapporo Medical University, Sapporo, Hokkaido 060-8543, Japan

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Abstract. Biomarkers that facilitate the prediction of breast cancer prognosis can improve the quality of life in patients during the long period of illness and treatment. Particularly in recent years, with the advent of a more exhaustive analysis of genetic information and gene products, the molecular mechanisms at play during breast cancer have gradually become clearer. In the present study, a systematic review of the literature between 2009 and 2014 was conducted by searching for the keywords 'breast cancer', 'biomarkers', 'diagnosis', 'prognosis' and 'drug response' to clarify the present state of knowledge regarding biomarkers. In the final analysis, 16 studies on biomarkers for the breast cancer prognosis were retrieved. From these, 7 biomarkers in 9 studies were found to be strongly reliable predictors of prognosis and a further 7 biomarkers in 7 studies were poorly reliable. The use of these prognostic biomarkers should increase the options available for treatment algorithms.

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1. Introduction

Currently, breast cancer treatment is progressing on a daily basis, and is divided into subtypes based on the hormone

receptors human epidermal growth factor receptor type 2 (HER2) and Ki-67 (1). However, treatment algorithms do not necessarily result in satisfactory clinical outcomes. Therefore, to further improve the breast cancer prognosis, predictive factors are required in order to arrive at more accurate prognoses and improve treatment efficacy. If such biomarkers could be identified, it would be possible to provide appropriate treatments to relevant subjects, resulting in excellent clinical outcomes. To date, prognostic prediction has been based on older studies of morphological characteristics (2). More recent research has concentrated on molecular biomarkers (3). The present study reports the findings of a systematic review of prognosis for patients with breast cancer based on molecular biomarkers. The correlations between these biomarkers, prognosis and the treatment response may be useful for all breast cancer patients.

2. Literature search

A search of the PubMed database (National Center for Biotechnology Information, Bethesda, MD, USA) using the key words 'breast cancer,' 'biomarkers,' 'diagnosis,' 'prognosis' and 'drug response' retrieved 1,689 potential studies. Subsequent to filtering for studies involving humans and written in English, 76 studies were excluded. When the remaining reports were limited to the period between 2009 and 2014, an additional 688 were excluded. Of the remainder, 520 studies were excluded, as they did not contain the full text. Finally, the abstracts of 405 studies were evaluated and those that contained insufficient descriptions of diagnostic performance, prognosis and drug response were excluded, resulting in a total of 16 studies for analysis (Fig. 1).

3. Highly reliable biomarkers (Table I)

Retinoic acid receptor α (RARA). Approximately 1/3 of estrogen receptor α (ER α)-positive breast cancer patients treated with tamoxifen experienced a relapse of the disease (4). RARA is a potential biomarker for tamoxifen resistance (5). The anti-tumor properties of RARA can be explained in association with the interaction of the receptor with ER α and their joint genomic binding site (6). The association between ER α resistance and RARA resistance was confirmed using tamoxifen-susceptible and -resistant cell lines (MCF7 and

Correspondence to: Dr Goro Kutomi, Department of Surgery, Surgical Oncology and Science, Sapporo Medical University, S-1, W-16, Chuo, Sapporo, Hokkaido 060-8543, Japan
E-mail: kutomi@sapmed.ac.jp

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LCC2, respectively). The tamoxifen-resistant cells were found to express high levels of RARA (7).

Patients with ER α -positive breast cancer tumors with high internal levels of RARA protein that were treated with tamoxifen as adjuvant therapy exhibited shorter recurrence-free survival (RFS) than patients with low internal levels of RARA protein (7). Johansson *et al* (7) performed an investigation into serum RARA levels using ELISA, and found significantly higher RFS rates in patients with high RARA expression levels compared with patients with low levels: Hazard ratio (HR)=4.1; 95% confidence interval (CI)=1.55-11.0; P=0.0046. Therefore, RARA may potentially be a useful target of new treatment regimens and a biomarker to predict the effectiveness of tamoxifen adjuvant treatment in ER α -positive breast cancer.

Aromatase expression. Aromatase expression by breast cancer cells has been shown to influence the effectiveness of endocrine treatments for breast cancer (8,9). Ellis *et al* (10) conducted a study using a sample from a clinical trial comparing tamoxifen and letrozole as neoadjuvant endocrine therapies, and found that aromatase expression levels in tumor and somatic cells was correlated with treatment-induced changes in Ki-67, RFS, and breast cancer-specific survival (BCSS) (11-13).

Aromatase expression was correlated with a smaller tumor size (P=0.01), a higher Allred score of estrogen receptor (P=0.006) (14) and lower Ki-67 levels (P=0.003). In addition, aromatase expression by tumor cells was a significant prognostic factor of the independent variables RFS (HR=2.3; 95% CI=1.2-4.6; P=0.01) and BCSS (HR=3.76; 95% CI=1.4-10.0; P=0.008) (15). The aforementioned data supports the use of aromatase blockers as the first choice treatment for post-menopausal, hormone-positive breast cancer.

Osteopontin. Osteopontin is a secreted extracellular matrix adhesion protein associated with tumor cell invasion and metastasis (16,17). Pang *et al* (18) examined the clinical and pathological effects of the adhesion molecules osteopontin-c, E-cadherin and β -catenin in breast cancer, and found higher expression levels of all the aforementioned adhesion molecules in breast cancer compared with normal tissue. The expression of osteopontin-c was associated with lymph node metastasis, and higher tumor node-metastasis classification (19) and histological grade (19). In addition, high expression levels of osteopontin-c have been correlated with tumor recurrence and metastasis, as well as triple negative subtypes, which are predictive factors of the independent variables disease-free survival (DFS; HR=3.094; 95% CI, 1.229-7.789; P=0.016) and overall survival (OS; HR=2.558; 95% CI, 1.048-6.243; P=0.039) (20). Therefore, the development of treatments targeting osteopontin-c may be beneficial for the treatment of breast cancer.

Ki-67. Ohno *et al* (21) examined the role of Ki-67 as a predictive biomarker of treatment response in a randomized, multicenter study to compare the effectiveness of docetaxel subsequent to treatment with fluorouracil/epirubicin/cyclophosphamide with or without capecitabine, in patients with operable breast cancer. The endpoint was the rate of pathological complete response (pCR). Analysis of hormone receptors and the Ki-67

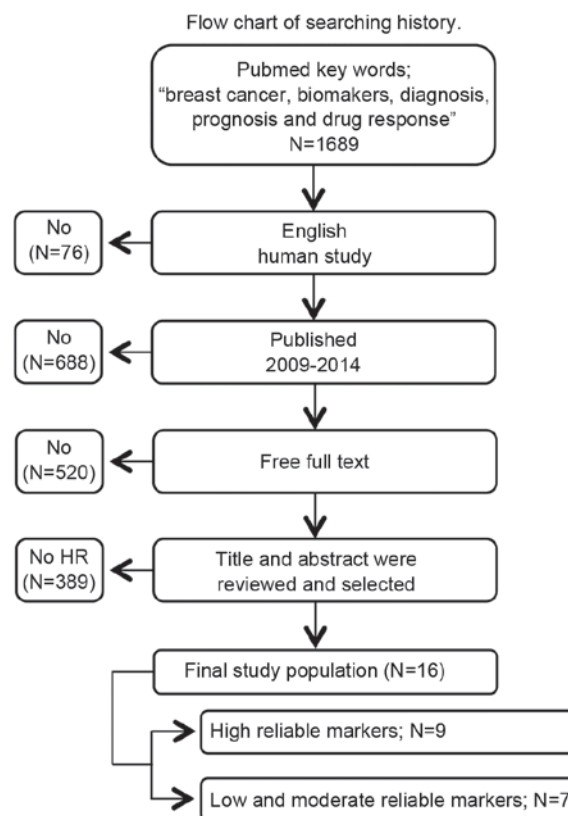


Figure 1. Flow chart of search history. PubMed searches were conducted. A total of 16 studies were selected according to the inclusion criteria of the present study.

labeling index (Ki-67LI) by multivariate logistic regression analysis identified Ki-67 as an independent prognostic factor (HR=2.718; 95% CI=1.331-5.549; P=0.0061). In addition, the aforementioned results also suggest that the Ki-67LI prior to treatment was a predictor for the response to preoperative docetaxel treatment and preoperative capecitabine treatment in early-stage breast cancer.

Denkert *et al* (22) obtained 1,166 breast cancer bioassay specimens from a large-scale cohort study established to investigate neoadjuvant treatment (the GeparTrio trial) and evaluated pre-treatment Ki-67 levels by immunohistochemical analysis. The study used the standardized, 3-endpoint, cut-off algorithm (pCR, DFS and OS) (23). The Ki-67 index and preoperative chemotherapy variables were divided into 3 subgroups each: ≤ 15 , 15.1-35 and $\geq 35\%$, and pCR rates were 4.2, 12.8 and 29.0%, respectively (P<0.0005). The HR for prognosis also increased in response to Ki-67 (HR=1, 3.32 and 9.20, respectively), indicating that Ki-67 is a prognostic predictor for hormone receptor-positive, but not triple-negative, breast cancer. The aforementioned findings regarding Ki-67 may provide important information for the development of other quantitative biomarkers.

DeCensi *et al* (24) examined postoperative remission and prognosis in response to Ki-67 in early stage ER α -positive breast cancer patients treated with tamoxifen for 4 weeks as a short-term neo-adjuvant therapy and reported that post-treatment levels of Ki-67 in the second (14-19%), third (20-29%), and top ($\geq 30\%$) quartiles had recurrence HRs of 2.92 (95% CI, 0.95-8.96), 4.37 (1.56-12.25) and 6.05 (2.07-17.65),

Table I. List of biomarkers that presented ≥ 2 risk ratios compared with the control populations (high reliable markers).

Authors	Biomarker	Endpoint	Condition	Result			No.	Sample	Periods	(Refs.)
				HR	95% CI					
Johansson <i>et al</i>	RARA	RFS	Low RARA High RARA	1.0 4.1	- 1.55-11.0 (P=0.046)		72	Blood	1991-1996	(7)
Ellis <i>et al</i>	Aromatase	BCSS	Absent Present	1.0 3.76	- 1.4-10.0 (P=0.008)		169	Biopsy	1998-1999	(15)
Pang <i>et al</i>	Osteopontin	DFS	Low	1.0	-		200	Biopsy	2002	(18)
		OS	High Low	3.094 1.0	1.229-7.789 (P=0.016) -					
			High	2.558	1.048-6.243 (P=0.039)					
Ohno <i>et al</i>	Ki-67	pCR	<10 ≥ 10	1.0 2.718	- 1.331-5.549 (P=0.0061)		504	Biopsy	2002-2004	(21)
Denkert <i>et al</i>	Ki-67	pCR	0-15% 15.1-35% >35%	1.0 3.3 9.2	- - (P=0.00005)		1,166	Biopsy	2002-2005	(22)
DeCensi <i>et al</i>	Ki-67	OS	Pre-tamoxifen <20 ≥ 20 Post-tamoxifen <20 ≥ 20	1.0 3.66 1.0 5.50	- 0.81-16.47 (P=0.128) - 1.26-23.16 (P=0.006)		86	Biopsy	2004-2007	(24)
Tsang <i>et al</i>	CEACAM6	OS	CEACAM6 (-) CEACAM6 (+)	1.0 4.107	- 1.478-11.417 (P=0.007)		312	Biopsy	2003-2009	(28)
Cizkova <i>et al</i>	PIK3CA	DFS	Wild type Mutant type	1.0 4.26	- 1.4-13 (P=0.0063)		80	Biopsy	2007	(31)
Zhu <i>et al</i>	TIMP-	DFS	Low High	1.0 3.085	- 1.096-8.680 (P=0.033)		99	Biopsy	2008	(36)
		OS	Low High	1.0 12.907	- 1.499-111.2 (P=0.020)					

RARA, Retinoic acid receptor α ; RFS, recurrence-free survival; BCSS, breast cancer-specific survival; pCR, pathological complete response; CEACAM6, Carcinoembryonic antigen-related cell adhesion molecule 6; PIK3CA, Phosphatidylinositol 4,5-bisphosphate 3-kinase; catalytic subunit α ; TIMP-1, Tissue inhibitor of metalloproteinases-1; DFS, disease free survival; OS, overall survival.

respectively, compared with those in the bottom quartile ($<14\%$; $P=0.001$). The mortality risks were 5.5-fold higher when Ki-67 levels were $\geq 20\%$ (95% CI=1.26-23.16; $P<0.006$) when compared to those with Ki-67 levels $<20\%$ ($P=0.006$). The authors concluded that the level of Ki-67 subsequent to short-term neoadjuvant tamoxifen is a good predictor of RFS and OS, supporting the use of Ki-67 as a surrogate biomarker to personalize adjuvant treatment and to cost-effectively screen novel drugs.

Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6). CEACAM6 is a human carcinoembryonic antigen that functions as a multi-functional regulatory protein and is overexpressed in various cell processes associated with cancer (25,26). CEACAM6 expression in atypical ductal hyperplasia has been suggested to serve an important role in the development of breast cancer (27). CEACAM6 has also been associated with invasive and treatment-resistant breast cancer (28). However, in a large-scale cohort study, CEACAM6 expression in luminal breast cancer exhibited no effect on OS or correlation with prognosis, although an association between CEACAM6 expression and prognosis in breast cancer over-expressing HER2 was revealed, as the high expression group tended to exhibit poorer OS (28). The aforementioned results appear to indicate that treatment is required for breast cancer patients with HER2 overexpression and the presence of CEACAM6.

Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α (PIK3CA). PIK3CA is a cancer gene coding for 1 of 2 phosphoinositide 3-kinase (PI3 K) subunits (29), which is a gain-of-function mutation in certain types of cancer, and is present in 20-40% patients with breast cancer (30). Cizkova *et al* (31) identified PIK3CA mutations in 17 (21.3%) tumors among 80 HER2-positive patients treated with trastuzumab for 1 year. Patients exhibiting wild-type PIK3CA demonstrated an improved DFS compared with patients exhibiting the PIK3CA mutations. The prognosis for HER2-positive patients with PIK3CA mutations treated with trastuzumab was significantly worse than for patients exhibiting the wild-type variation, which is considered to occur since the PI3K/protein kinase B pathway is adversely affected by PIK3CA mutations, resulting in the lower efficacy of trastuzumab. Thus, the detection of PIK3CA mutations is only required in HER2-positive patients.

Tissue inhibitor of metalloproteinases-1 (TIMP-1). Paclitaxel is the first chemotherapy treatment of choice for patients with lymph node metastasis (32,33). However, there are currently no biomarkers to predict susceptibility to chemotherapy. TIMP-1 has been shown to protect cells from apoptosis (34). A previous epidemiological study demonstrated an association between high levels of TIMP-1 and reduced responsiveness to cyclophosphamide/methotrexate/5-fluorouracil and anthracycline-based chemotherapy regimens (35).

In a retrospective study of 99 breast cancer patients, Zhu *et al* (36) reported a correlation between TIMP-1 expression levels in primary tumors and improved responsiveness to paclitaxel-based chemotherapy. Kaplan-Meier survival analysis revealed that patients with high TIMP-1 levels had

poorer 5-year DFS than those with lower TIMP-1 levels (71.1 vs. 88.5%, respectively; $P=0.020$). The 5-year OS was also lower (78.9 and 96.7%, respectively, $P=0.004$). The responsiveness to paclitaxel-based chemotherapy was significantly worse when the TIMP-1 expression levels were high. The aforementioned findings indicate that TIMP-1 may be a useful predictive biomarker for chemotherapy resistance.

4. Low and moderately reliable biomarkers (Table II)

Ferritin light chain (FTL). Ferritin is a ubiquitous iron-binding protein. In vertebrates, there are 2 types of apoferritin, which are assembled from 24 subunits including light and heavy chain types. The ratio between the ferritin heavy chain and FTL can vary greatly, depending on the tissue type and cellular conditions (37). The increase in ferritin from different cancer tissue samples exhibited a close correlation with disease onset (38). Ricolleau *et al* (39) investigated the utility of FTL as a prognostic marker for lymph node metastasis-positive breast cancer and determined an FTL cut-off level in tumors of 2.4. The high FTL level group had a significantly lower metastasis-free survival rate, indicating that FTL was an independent prognostic marker (HR=1.30; 95% CI=1.10-1.50; $P=0.001$) (40).

Urokinase-type plasminogen activator (uPA) and plasminogen activator type 1 inhibitor (PAI-1). uPA, as a tumor-associated proteolytic factor, and PAI-1 serve important roles in tumor invasion and metastasis (41), and cell signaling, adhesion, migration and proliferation (42). In the final Chemo-N0 trial for the validation of The American Society of Clinical Oncology-recommended biomarkers (1993-1998; $n=647$; 12 centers; median follow-up period 113 months; range between 5 and 167 months), high uPA/PAI-1 levels were correlated with significantly lower DFS among breast cancer patients who did not receive adjuvant treatment (HR=1.84; 95% CI=1.1-3.0; $P=0.017$) and OS (HR=1.84; 95% CI=1.1-3.1; $P=0.02$). uPA/PAI-1 was also identified as a prognostic factor for breast cancer in other studies (43).

C-reactive protein (CRP). Serum CRP is a marker of acute inflammatory response and is considered to be a prognostic indicator in breast cancer (44,45). The Women's Healthy Eating and Living study was a randomized comparative study examining the effect of a diet high in vegetables and low in fat on the prevention of premature mortality in women diagnosed with breast cancer. Serum protein analysis of 2,023 of 3,088 eligible women showed that acute inflammation (CRP ≥ 10 mg/l) was markedly suppressed by a high vegetable/low fat diet, resulting in improved long-term survival (46). Although the anti-oncological effects remain unclear, CRP was identified as an independent biomarker for prognosis of survival in breast cancer (HR=1.96; 95% CI=1.22-3.13).

Chromosome 17 centromere enumeration probe (Ch17CEP). Chromosome 17 is the second densest chromosome in the human genome and codes for several genes, including BRCA1 and HER2 with important roles in breast cancer, as well as the housekeeping DNA repair genes TP53, RAD51C and RAD52B (47). Ch17 centromeric region duplication (Ch17CEP) is closely associated with HER2 amplification (48). Ch17CEP

Table II. List of biomarkers that presented <2 risk ratios compared to the control populations (low and moderate reliable markers).

Authors	Biomarker	Endpoint	Result			No.	Sample	Periods	(Refs.)
			Condition	HR	95% CI				
Jézéquel <i>et al</i>	FTL	MFS	<2.4	1.0	-	198	Biopsy	1980-2000	(40)
			>2.4	1.3	1.10-1.50 (P=0.001)				
Harbeck <i>et al</i>	uPA/PAI-1	DFS	Low	1.0	-	409	Blood	1993-1998	(43)
			High	1.84	1.1-3.0 (P=0.017)				
		OS	Low	1.0	-				
			High	1.85	1.1-3.1 (P=0.02)				
Villaseñor <i>et al</i>	CRP	OS	<1 mg/l	1.0	-	2,919	Blood	1995-2000	(46)
			≥10 mg/l	1.96	1.22-3.13 (P<0.001)				
Earl <i>et al</i>	Ch17CEP	OS	Normal	1.0	-	1,762	Biopsy	1996-2001	(50)
			Duplication	0.80	0.68-0.95 (P=0.009)				
Moreno-Aspitia <i>et al</i>	sHER2	DFS	<15	1.0	-	2,318	Blood	2004-2005	(55)
			≥15	2.36	1.19-4.70 (P=0.01)				
Sun <i>et al</i>	MAD1L1	OS	Low	1.0	-	461	Biopsy	2006	(57)
			High	1.825	-(P=0.027)				
Hartmann <i>et al</i>	PITX2P2 Methylation	TDM	TDM	1.66	1.21-2.28 (P=0.002)	241	Biopsy	NA	(66)

MFS, metastasis-free survival; uPA/PAI-1, urokinase-type plasminogen activator and plasminogen activator type 1 inhibitor; CRP, C-reactive protein; Ch17CEP, Chromosome 17 centromere enumeration probe; sHER2, soluble human epidermal growth factor receptor 2; MAD1L1, mitotic arrest deficient like 1; PITX2P2, methylation of paired-like homeodomain 2; TDM, time to distant metastasis; DFS, disease free survival; OS, overall survival.

overlap is also a powerful marker for genome instability in breast cancer and is correlated with susceptibility to chemotherapy (48,49). In novel endovascular access trial/BR9601 clinical trials, prognostic factors were analyzed and categorized according to breast cancer subtype. Although numerous factors were not associated with subtype, Ch17CEP overlap was an independent prognostic factor for DFS and OS for all subtypes. Ch17CEP overlap was identified as a prognostic biomarker for breast cancer treated with cyclophosphamide, methotrexate and fluorouracil therapy in combination with epirubicin (HR=0.80; 95% CI=0.68-0.95; P=0.009) (50).

Soluble human epidermal growth factor receptor 2 (sHER2). HER2 is a 185-kDa protein arising from the intracellular, transmembrane and extracellular domains (ECD) (51). The ECD is occasionally spliced by metalloprotease, resulting in the release of sHER2 into the peripheral circulation (52). sHER2 is an important biomarker for HER2-positive breast cancer at any stage (53). In the N9831 adjuvant breast cancer trials, early stage HER2-positivity was identified as a biomarker of clinical outcome and disease progression (54). Moreno-Aspitia *et al* (55) evaluated sHER2 levels at the time of relapse using a sample from the N9831 clinical trial and found that DFS was lower in patients with sHER2 levels >15 ng/ml, compared with patients with lower levels of sHER2 (HR=2.36; 95% CI=1.19-4.70; P=0.01). Therefore, in early stage HER2-positive breast cancer, sHER2 was found to be a suitable prognostic biomarker for relapse, and survival in relapsed patients.

Mitotic arrest deficient like 1 (MAD1L1). MAD1L1 is a checkpoint gene associated with chromosomal instability. Abnormalities in MAD1L1 have been observed in a number of cancer types, including colon and lung cancer (56). Sun *et al* (57) analyzed MAD1L1 expression in breast cancer tissues from 461 patients and normal breast tissue to identify correlations between MAD1L1 expression and clinical pathological characteristics. The results of the aforementioned study revealed that OS was particularly worse in the high MAD1L1 expression group (HR=1.825; 95% CI=1.073-3.107; P=0.027). In patients with high nuclear MAD1L1 expression subsequent to taxol treatment, prognosis was poorer in the non-treated patients (P=0.026). Nuclear MAD1L1 expression therefore appears to have enhanced the treatment resistance and affected the prognosis of breast cancer, demonstrating that MAD1L1-positive breast cancer was not susceptible to taxol treatment.

Methylation of paired-like homeodomain 2 (PITX2P2). C-phosphate-G islands located within the gene regulatory site are associated with the suppression of gene expression. The methylation of DNA dinucleotides in this gene is a common early event subsequent to the onset of cancer (58-60). Methylation patterns specific to tumor subtypes, including breast cancer, are reportedly associated with clinical outcomes (61-63). Several studies reported that PITX2 DNA methylation was associated with a high risk of relapse in lymph node metastasis-positive, hormone receptor-positive breast cancer patients undergoing whole body adjuvant tamoxifen therapy (64,65). In a cohort

study of 241 lymph node metastasis-positive breast cancer patients with a history of anthracycline treatment, PITX2P2 methylation (a subtype of PITX2 methylation) was associated with an increased long-term relapse (HR=1.66; 95% CI=1.21-2.28; P=0.002) and reduced survival rates (HR=1.47; 95% CI=1.11-1.96; P=0.0084) (66).

5. Conclusions

In the present review of studies between 2009 and 2014, biomarkers were grouped according to reliability (high, medium, or low). A total of 3 studies were retrieved from the literature that classified Ki-67 as a high reliability biomarker. The utility of Ki-67 as a biomarker has been re-evaluated in the present study.

Of the high reliability biomarkers referred to in the 9 studies included in the present review, 0 were assessed by bioassays and only 1 mentioned biomarker measurement in peripheral blood. Although the evaluation of proteins in peripheral blood is relatively simple, in 2013, Johansson (7) reported that the identification of biomarkers is difficult, as measurements of biomarkers require too much quantification data. Therefore, the development of biomarkers from peripheral blood presents a challenge for future studies.

Although a number of molecules were identified in the present review, other markers, such as hormone receptors, were not widely evaluated. The ratio of ER- α /ER- β expression, β III-tubulin and thyroid-stimulating hormone were identified as oncological indicators in breast cancer. Thus, additional studies of the correlations between the aforementioned biomarkers and prognosis and treatment response may be useful.

This study hypothesizes that the most important biomarkers of breast cancer are found in the blood, and that RARA, uPA/PAI-1, CRP, sHER2 are good biomarkers in routine examination. The authors highlight RARA in particular as an important biomarker in breast cancer. Future studies on biomarkers are likely to progress the understanding of the topic. For all biomarkers, reliability is important, but for the development of useful biomarkers, cost and ease of monitoring are crucial considerations.

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