Research Article

Systematic Review of the Use of Biomarkers in Early Breast Cancer Diagnosis

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Early detection of breast cancer improves prognosis. Unfortunately, in many instances patients' tumours are diagnosed following metastasis, thus making treatment more difficult and worsening their prognosis. The development of a specific biomarker for the early detection of breast cancer could potentially allow more women to be successfully treated yet at present there no validated biomarkers available. Only a few biomarkers (such as HER-2/neu, estrogen receptor, and progesterone receptor) have a limited utility for diagnosis and prognosis. Thus, there is a great need for new biomarkers for breast cancer. Here we review the current state of the art relative to the use of biomarkers in early breast cancer screening and diagnosis.

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Introduction

Worldwide breast cancer (BC) affects around 8 million women with the most common form, accounting for 70%, being estrogen-receptor positive (ER+)[1][2].

Currently, women with suspected breast cancer are referred for mammography screening (MS) and a subsequent biopsy for confirmation of the diagnosis. However, MS is not diagnostic and can result in up 80% false positives so that only around one in 4 women with an abnormal result will be found to have cancer after further inspection of abnormal areas with diagnostic mammography (DM) and eventually a biopsy[3][4]. Early detection of BC is associated with a better prognosis[5], with the 5-year survival for stage 1 BC being 90% but only 13% for stage 4[2]. However, in up to 5% of patients, when the disease is detected the cance has metastasised, which is associated with a worse prognosis[6].

The use of disease specific specific biomarkers offers the potential of earlier detection of BC and a timelier access to treatment which would improve prognosis, however, to date, no such validated biomarkers exist. Although current markers (such as HER2/neu, estrogen receptor (ER) and progesteron receptor (PR)) are useful prognostic tools that assist with disease management, they are not sufficiently discriminatory as although these markers are significantly raised in BC are also present in normal breast cells. Thus, there is a need for biomarkers which are only present in patients with BC[7] and not in healthy women or women suffering from other pathologies such as ovarian cancer[8], endometrial carcinoma[9], endometriosis[10], fibroids[11][12], cardiovascular disease[13], Alzheimer's disease[14] or colorectal cancer[15] where these markers maybe raised as well. Also, these markers may detect ER+ or HER2+ BC, but they can't neither detect triple-negative BC or distinguish between lymph node negative (LN-) or lymph node positive (LN+) or metastatic BC.

A growing number of early diagnostic biomarkers are currently under investigation whereas others are targeted at prediction of metastatic behaviour and selection of therapy[16], though at present none of these are used in clinical practice.

Here, we review the current data on biomarkers intended for the early detection of BC.

Methods

Searches were conducted using Pubmed, Scopus, CAB abstracts, Cochrane Library, Web of science core collection, NCRI cancer research database, Google Scholars, Embase and CINAHL, for articles published in the last 5 years.

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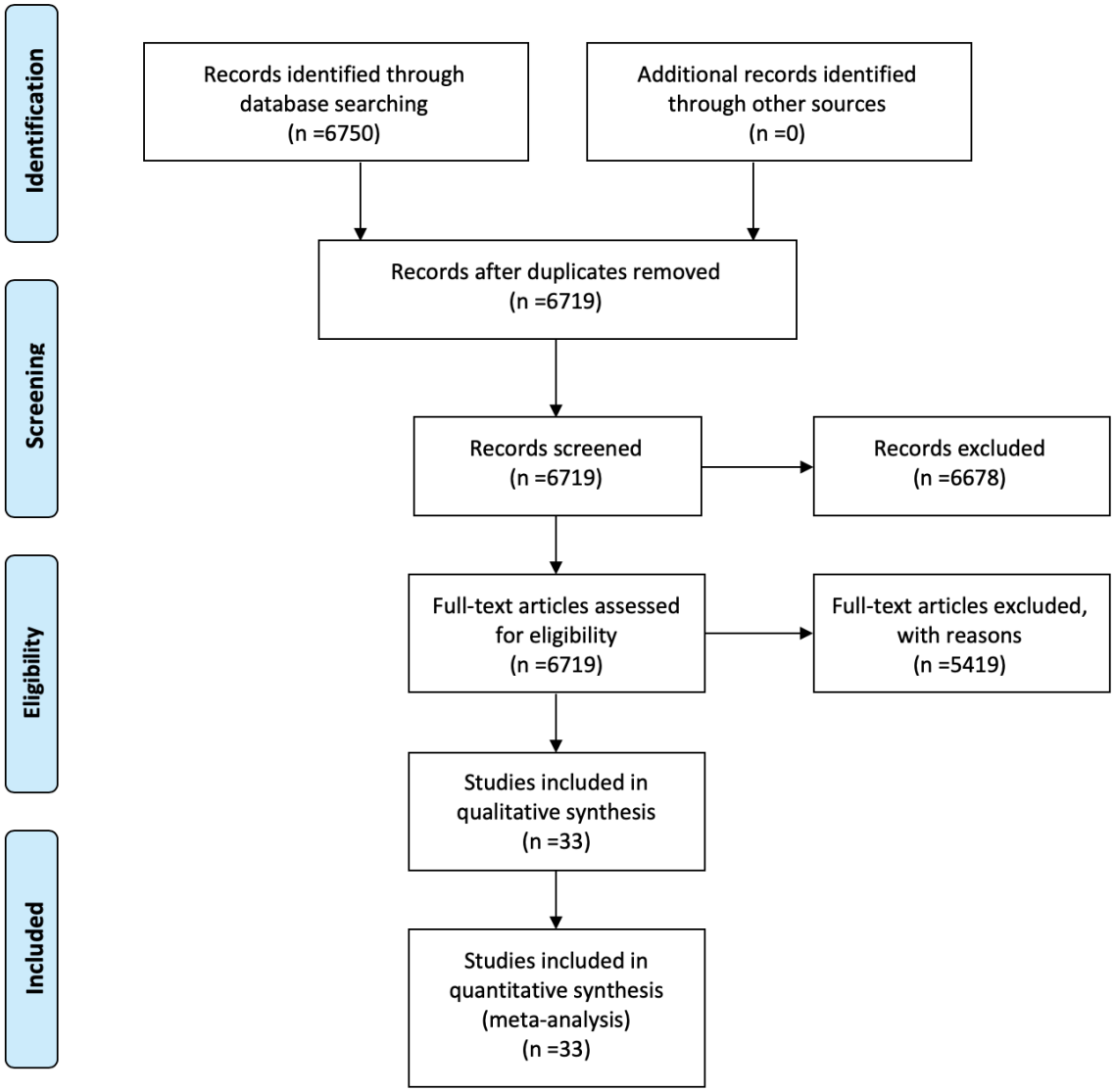

PRISMA 2009 Flow Diagram

Results and Discussion

As shown in the Prisma flow diagram, the search using the different databases and the criteria mentioned above yielded the articles mentioned in *Table 1*. Most of the developed biomarkers are proteins, RNA or DNA and a small proportion are based on tumor mechanical properties. Most came from studies in plasma/serum/blood and a minority from tissues, nipple fluid, urine or saliva. The biomarker which is closest to clinical use is the one described by Shimomura et al, 2016[17] but is not yet validated and is based on miRNA detection what makes difficult to develop a simple lateral flow assay because miRNA needs to be isolated, amplified and labelled prior to testing it in the lateral flow assay strip[18][19].

Also, the use of protein panels that detect multiple biomarkers may enhance sensitivity and specificity in a clinical setting[20], however none of the multiple biomarkers shown in *Table 1* was also yet validated.

In addition, most of the tests do not distinguish between LN- or LN- cancers or have been validated by the inclusion of advanced BC samples for comparison or specify the BC subtypes analysed what does not make possible to distinguish those cancers that are prone to progress and need treatment.

The ideal test would be specific for biomarkers that could be incorporated in a lateral flow assay and be developed to test a single drop of blood or other fluid directly into a testing strip, offering a fast and accurate diagnosis to allow a quick and adequate therapeutic intervention[21]. These tests would allow the patient

to be selected for auxiliary imaging based on the presence of specific biomarkers which would specifically indicate the presence of occult cancer in patients with a normal SM.

Data and software availability

All data underlying the results is available as part of the article.

Competing interests

Biomarkers, Ltd, a split -off Roma Laboratories, Ltd is a company dedicated to the development of biomarkers for cancer.

Grant information

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To University of Hull, University of York and Robert Gordon University libraries for use of their resources

Supplementary Material

Table 1

	Database	Ref. (doi Article/Project)	Authors	Year	A. Study design						B. Validation	C. Application population	
					Q1	Q2	Q3	Q4	Q5	Q6	Q1	Q1	
	Pubmed	10.3390/ijms18040878	Coy JF	2017	?	no	?	Blood	phagocytosed intracellular biomarkers	Yes	No	no	
	Pubmed	10.18632/oncotarget.9561	Moon et al.	2016	ER+, TN, HER2+	no	yes	Plasma	Protein (fibronectin)	no	No	yes	
	Pubmed	10.1016/j.cca.2016.08.003	Parisi et al.	2016	?	no	yes	Blood	CTCs/ ctDNA/ methylation	Yes	No	no	
	Pubmed	10.1111/cas.12880	Shimomura et al.	2016	?	no	yes	Serum	miRNA	Yes	No	no	
	Pubmed	10.1371/journal.pone.0122106	Garczyk et al.	2015	?	yes	yes	Serum	Protein (AGR2/3)	Yes	No	yes	
	Pubmed	10.1186/1471-2407-14-176	Khan et al.	2014	?	no	no	Serum	Protein (survivin)	Yes	No	yes	
	Google Scholar	10.1186/s12885-015-1414-7	Zelig et al	2015	ER+, TN, HER2+	no	no	Blood	PBMCs+ plasma (total biochemical composition)	Yes	No	no	
	NCRI	CA188991	Zhong, Wenwan/ USA	2019	?	?	?	Serum	miRNAs	Yes	N/A	no	
	NCRI	BC130865	Johnston, Stephen/ USA	2017	ER+, TN, HER2+	yes	yes	Serum	immunosignatures	Yes	N/A	no	
	NCRI	CA214172	Paulovich, Amanda G/ USA	2019	?	?	no	Plasma	Protein	Yes	N/A	yes	
	NCRI	CA188678	Hill, Deirdre/USA	2016	?	?	no	Tissue	Protein	no	N/A	no	
	NCRI	CA214183	Marks, Jeffrey R/	2021	?	?	no	Blood	Protein	Yes	N/A	yes	

			USA										
	NCRI	CA168925	Greene, Mark I/ USA	2017	?	?	?	Serum	Autoantibodies/ EA3	Yes	N/A	yes	
	NCRI	10.1158/1078-0432 / CA194024	Park, Ben H/ USA	2014/2021	ER+	yes	no	Plasma	DNA	Yes	No	no	
	NCRI	CA199996	Keely, Patricia J/ USA	2020	?	?	no	Tissue	collagen features	Yes	N/A	no	
	NCRI	CA179144	Mangone, Marco / USA	2016	?	?	no	Tissue	miRNAs	Yes	N/A	no	
	NCRI	BC142144	Pitteri, Sharon / USA	2018	?	?	no	breast interstitial fluid	Protein	Yes	N/A	no	
	NCRI	IN-17-009	Ebert, Martin/ Australia	2018	?	?	no	Tissue	ELFIS	Yes	N/A	no	
	NCRI	CA193067	Hall, Adam Roger / USA	2017	?	?	?	?	hydroxymethylation/g DNA	?	N/A	no	
	NCRI	10.1200/JCO.2016.70.8594 / CA155289	Willmann, Juergen Karl/ USA	2017/ 2016	?	?	no	Tissue	Ultrasound	Yes	No	no	
	NCRI	BC150233	Won, Chang Hee / USA	2019	?	?	no	Tissue	tumor mechanical properties	Yes	N/A	no	
	CINAHL	10.1158/1078-0432.CCR-08-2319	Suzuki et al	2009	ER+, TN, HER2+	yes	no	Tissue	Protein acetylation/ HDAC	no	No	no	
	SCOPUS	10.1126/science.aar3247	Cohen et al	2018	?	no	no	Blood	proteins/ cell-free DNA	Yes	No	no	
	SCOPUS	10.1016/j.clbc.2017.05.004	Lourenco et al	2017	?	no	no	Serum	Protein array	Yes	No	no	
	SCOPUS	10.1016%2fj.critrevonc.2016.12.009	Porto-Mascarenhas et al	2017	?	?	?	Saliva	Proteins	Yes	No	yes	
	SCOPUS	10.1007%2fs13277-016-5190-z&partnerID=40	Li et al	2016	ER+, TN, HER2+	yes	no	Plasma	cf DNA methylation	no	No	no	
	SCOPUS	10.1177%2f0003702815620127	Depciuch et al	2016	TN	no	no	Tissue	Raman Spectroscopy and Infrared Spectroscopy	no	No	no	
	SCOPUS	10.1007%2fs10549-015-3431-2	Zhang et al	2015	?	?	?	Serum	HOTAIR (DNA)	Yes	No	no	
	SCOPUS	10.1371/journal.pone.0141876	Beretov et al	2015	ER+	yes	yes	Urine	Proteins	Yes	No	no	

	SCOPUS	10.7785%2ftcrt.2012.500325	Liu et al	2015	?	?	?	Tissue	Resonance Raman and Raman spectroscopy	Yes	No	no	y
	CAB abstracts	10.1007/s10549-016-3796-x	Murphy et al	2016	Potential study	Potential study	Potential study	Breast milk	Several different components	Yes	N/A	yes	y
	CAB abstracts	10.1002/elps.201700123	Aslebagh et al	2018	?	?	?	Breast milk	Proteins	Yes	No	yes	y
	CAB abstracts	10.1002/jcla.21555	Santillán-Benítez et al	2013	?	?	?	BMI, leptin, leptin/adiponectin (L/A) ratio and CA 15-3	BMI, leptin, leptin/adiponectin (L/A) ratio and CA 15-3	Yes	No	yes	y
	EMBASE	10.1186/1471-2407-13-569	Grimm et al	2013	?	?	?	Blood	phagocytosed intracellular biomarkers	Yes	No	yes	y

Supplementary Material
SM1: PRISMA flow diagram
SM2: STANDARD TOOL
A- Study design
1. Breast cancer subtypes analysed?
2. Distinguishes lymph node (LN) negative (-) and LN positive (+) breast cancer or precancerous lesions?
3. Control samples include healthy volunteers and patients with other conditions rather than breast cancer and advanced breast cancer patients?
4. Sample type?
5. Biomarker type?
6. Do the results fit with other evidence?
B- Validation
1. Is precision, sensitivity, accuracy, sensitivity, specific and power described and enough to validate the test?
C- Application on population
1. Can a test kit device be developed and commercialized?
2. Can a laboratory assay be developed?
SM3: Details of the information presented on the Prisma diagram
Pubmed
1. breast neoplasms/ last 5 years – 65119 articles

2. biomarkers AND early diagnosis AND liquid biopsy – 23 articles, selected 6
3. biomarkers AND early detection of cancer AND liquid biopsy – 23 articles, discarded 21 duplicates, discarded the other 2
Cochrane Library
1. breast neoplasms/ last 5 years – 10299 articles
2. biomarkers AND early diagnosis AND liquid biopsy 0 articles
3. biomarkers AND early detection of cancer AND liquid biopsy – 0 articles
Google Scholar
1. breast neoplasms/ last 5 years by date – 413 results
2. biomarkers AND early diagnosis AND liquid biopsy by relevance - 170 results (the result is approximately, it depends very much if you select by date or relevance and come many, many papers who do not have nothing to do) – selected 1
3. biomarkers AND early detection of cancer AND liquid biopsy by relevance –81 results, selected 1 but discarded as duplicate
NCRI cancer research database
Early detection, diagnosis and prognosis/ Breast cancer/ Research – 660 entries, selected 14 projects
CINAHL
1. breast neoplasms/ last 5 years by date – 24,916 entries
2. biomarkers AND early diagnosis AND liquid biopsy – 1 result 1 but discarded as duplicate
3. biomarkers AND early detection of cancer AND liquid biopsy - 3 results, selected one from reference list, discarded 2 as one was a review about a previously selected paper and the other one was about prognosis and treatment response
Web of Science Core Collection
1. breast neoplasms/ last 5 years by date – 509 entries
2. biomarkers AND early diagnosis AND liquid biopsy – 0 results

3. biomarkers AND early detection of cancer AND liquid biopsy - 0 results
Scopus
1. breast neoplasms/ last 5 years by date – 84915 entries
2. biomarkers AND early diagnosis AND liquid biopsy – 281 results, selected 13 manuscripts and discarded 5 duplicates
3. biomarkers AND early detection of cancer AND liquid biopsy – 8 results, selected 2 and discarded as duplicates
CAB abstracts
1. breast neoplasms/ last 5 years by date – 5961 results
2. biomarkers AND early diagnosis AND liquid biopsy, – 1279 results, selected 1,
3. biomarkers AND early detection of cancer AND liquid biopsy - 4221results, selected 4, discarded 2 including 1 duplicate
EMBASE
1. breast neoplasms/ last 5 years by date / biomarkers AND early diagnosis AND liquid biopsy – 76 results – none selected
2. breast neoplasms/ last 5 years by date / biomarkers AND early detection of cancer AND liquid biopsy- 13 results – selected 1 (conference publication) and extracted correspondent publication
SM4: Search strategy
Search terms were as follows:
1. breast neoplasms, 2. biomarkers AND early diagnosis AND liquid biopsy or 3. biomarkers AND early detection of cancer AND liquid biopsy. For the NCRI cancer research database we used as common scientific outline: Early detection, diagnosis and prognosis/ Breast cancer and selected breast cancer. Duplicates were discarded.
Inclusion and exclusion criteria
Abstracts were reviewed and only studies that focused on the identification of early breast cancer biomarkers were included. Articles which focused on the development of prognostic or treatment response biomarkers were excluded.
Data extraction, synthesis and outcomes

Full-text of the relevant articles were obtained and assessed using a standard tool as described in supplementary material and summarized in table 1 (23)

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