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a systematic review

Kjaer, Ina Mathilde; Bechmann, Troels; Brandslund, Ivan; Madsen, Jonna Skov

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Review

Ina Mathilde Kjaer*, Troels Bechmann, Ivan Brandslund and Jonna Skov Madsen

Prognostic and predictive value of EGFR and EGFR-ligands in blood of breast cancer patients: a systematic review

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Abstract: Epidermal growth factor receptor (EGFR) serves as a co-target for dual/pan-EGFR-inhibitors in breast cancer. Findings suggest that EGFR and EGFR-ligands are involved in resistance towards certain breast cancer treatments. The aim is to explore the validity of EGFR and EGFR-ligands in blood as prognostic and predictive biomarkers in breast cancer. The systematic review was conducted in accordance to the PRISMA guidelines. Literature searches were conducted to identify publications exploring correlations between EGFR/EGFR-ligands in serum/plasma of breast cancer patients and prognostic/predictive outcome measures. Sixteen publications were eligible for inclusion. Twelve studies evaluated EGFR, whereas five studies evaluated one or more of the EGFR-ligands. Current evidence indicates associations between low baseline serum-EGFR and shorter survival or reduced response to treatment in patients with advanced breast cancer, especially in patients with estrogen and/or progesterone receptor positive tumors. The prognostic and predictive value of EGFR and EGFR-ligands in blood has only been investigated in highly selected subsets of breast cancer patients and most studies were small. This is the first systematic review evaluating the utility of EGFR and EGFR-ligands as predictive and prognostic biomarkers in blood in breast cancer. Further exploration in large well-designed studies is needed.

Keywords: breast cancer; epidermal growth factor receptor; epidermal growth factor receptor ligands; prediction; prognosis.

***Corresponding author: Ina Mathilde Kjaer,** MD, Department of Biochemistry and Immunology, Lillebaelt Hospital, Beriderbakken 4, DK-7100 Vejle, Denmark, Phone: +45 79406537,
E-mail: ina.mathilde.kjaer@rsyd.dk; and Department of Regional Health Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark
Troels Bechmann: Department of Oncology, Lillebaelt Hospital, Vejle, Denmark; and Department of Regional Health Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark
Ivan Brandslund and Jonna Skov Madsen: Department of Biochemistry and Immunology, Lillebaelt Hospital, Vejle, Denmark; and Department of Regional Health Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark

Introduction

Breast cancer is the most frequent cancer in women and the leading cause of cancer death in females worldwide [1]. Members of the epidermal growth factor (EGF) family of receptors and their ligands are implicated in the pathogenesis of many types of cancer, including breast cancer [2, 3]. Eleven ligands are known to the epidermal growth factor receptor (EGFR) family: EGF, betacellulin (BTC), amphiregulin (AREG), heparin-binding EGF-like growth factor (HB-EGF), transforming growth factor-α (TGFα), epiregulin (EREG), epigen (EPGN) and neuregulin 1-4 (NRG-1, NRG-2, NRG-3 and NRG-4). All ligands, except the neuregulins, are known to bind to the EGFR, also known as human epidermal growth factor receptor 1 (HER1), whereas none of the known ligands bind to human epidermal growth factor receptor 2 (HER2) [2]. Ligand binding induces activation of EGFR followed by a complex signaling process, resulting in activation of intracellular signaling pathways. A crosstalk between EGFR and HER2 is known to be crucial in the receptor signaling as the HER2-EGFR heterodimer exhibits augmented signaling activity [2, 4]. Furthermore, a crosstalk between the EGFR-family and the estrogen receptor (ER) signaling pathways is known, and may be implicated in resistance to endocrine therapy in breast cancer [5].

The prognostic and predictive role of HER2 in breast cancer has been intensely studied. The *HER2* gene is amplified and HER2 is overexpressed in 15% of breast cancer tumors and HER2-overexpression is associated with poor prognosis [6, 7]. Several targeted treatments are available for HER2-positive patients including both monoclonal antibodies and tyrosine kinase inhibitors. Both

trastuzumab and pertuzumab target the extracellular domain of HER2. Lapatinib is a dual EGFR/HER2-inhibitor and neratinib is a pan-EGFR-inhibitor, both targeting the intracellular receptor complex [8–12]. Moreover, the extracellular domain of HER2 can be detected in serum (S-HER2) and provides valuable information regarding detection of recurrence in HER2-positive patients and predicts response to trastuzumab [13–15]. EGFR and some EGFR-ligands are expressed differently in the breast cancer tissue in subsets of breast cancer patients, as well [16–19]. Furthermore, overexpression of EGFR and/or some EGFR-ligands in breast cancer tissue has been correlated to poor prognosis [16–19]. The extracellular domain of EGFR is shed to the circulation by proteolytic cleavage and can be measured in the blood as well as the EGFR-ligands. Several case-control studies report that levels of EGFR and EGFR-ligands in the blood of breast cancer patients are aberrant from levels in healthy controls [20–25].

A challenge in breast cancer treatment is the lack of treatment response and resistance to treatment and there are indications that EGFR and EGFR-ligands are involved in resistance to certain types of treatments including trastuzumab and anti-endocrine treatment [4, 5, 26]. Thus, there is a need for valid companion biomarkers in order to predict treatment response and provide early detection of treatment failure. EGFR or EGFR-ligands in the blood might be useful biomarkers in this context. The aim of this systematic review is to assemble the current evidence on the predictive and prognostic information provided by EGFR and EGFR-ligands in blood samples of breast cancer patients.

Methods

This systematic review was conducted in accordance to the Preferred Reporting Items for Systematic Review and Meta-analysis Protocols (PRISMA-P) 2015 Statement [27]. The study is registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration number: CRD42016039993 [28].

Eligibility criteria

Studies assessing plasma or serum levels of EGFR or any EGFR-ligand at any time during breast cancer diagnosis, treatment or follow-up were included. Studies of breast cancer patients at any age, with any subtype and any stage of disease, as well as studies including patients receiving any breast cancer treatment, were included. Only studies investigating protein expression of EGFR or EGFR-ligands were included, whereas studies regarding gene expression were excluded. Studies reporting any kind of predictive or prognostic outcome measures were included, as well as all study designs and all durations of follow-up. Only data reported in peer reviewed journals were included and there were no restrictions to publication language or year of publication.

Sources and search strategy

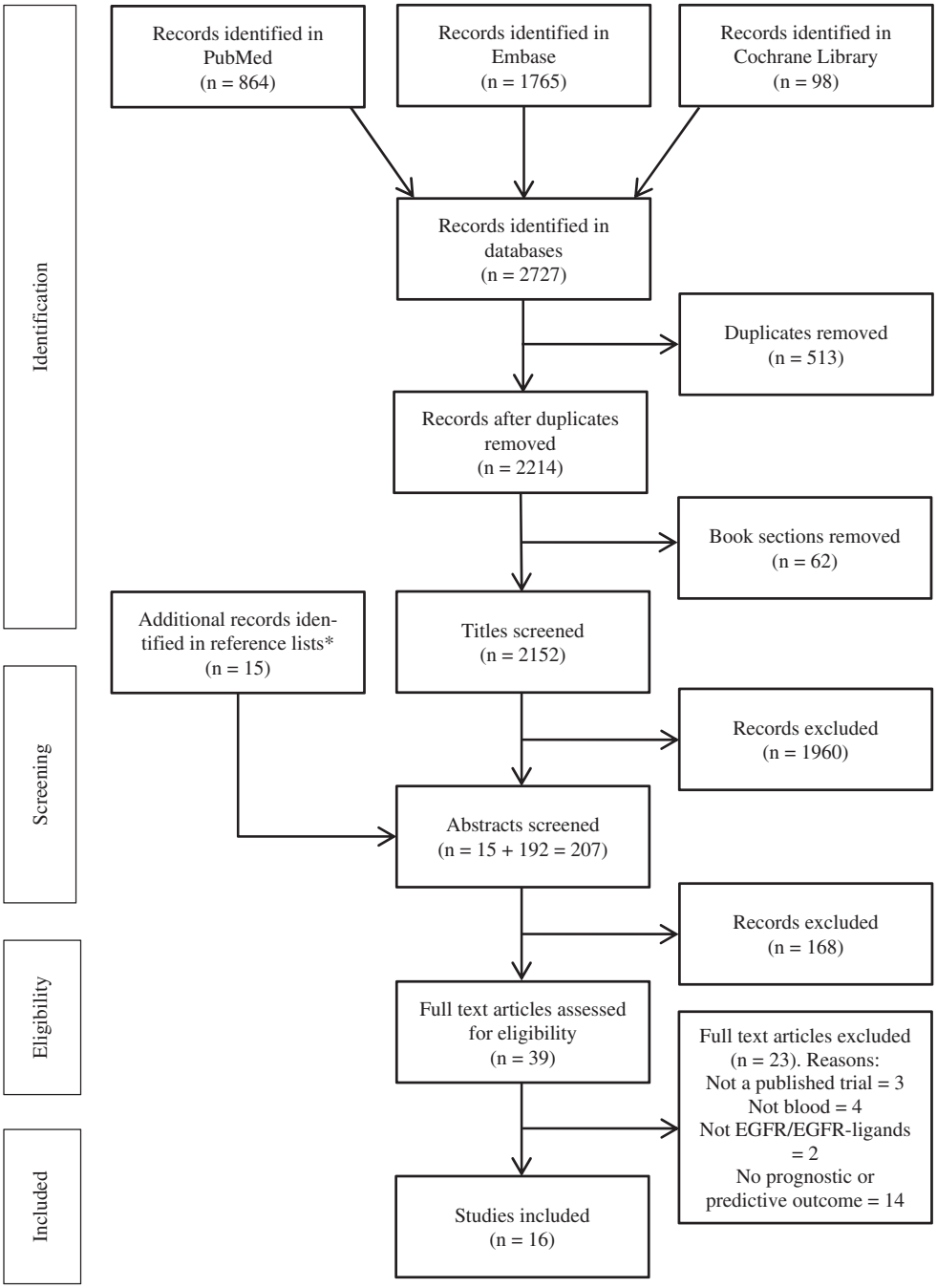
Studies were identified by searching PubMed (1946–2016.08.29), Cochrane Library (1994–2016.08.29) and EMBASE (1974–2016.08.29). The search strategies were developed in a cooperation of three reviewers (TB, JSM, IMK) and a Health Sciences Librarian with expertise in systematic review searches. Both index terms (e.g. MESH terms) and text words were included in the searches. To ensure high sensitivity in relation to identification of relevant studies, the final search-string was broad and thus a priori expected to have relatively low specificity. A draft of all searches can be found in the Supplemental Material 1. EndNote X7™ was used to identify and remove duplicates and book sections. To ensure literature saturation, relevant publications were identified in the reference lists of the included studies and relevant reviews (IMK), and included in the study selection process.

Study selection

The initial selection of studies was performed blinded and solely based on assessment of the title of the publication. It was performed independently by two reviewers (TB, IMK). If one or both reviewers assessed the titles as relevant for abstract screening, they were included. The same two reviewers (TB, IMK) then independently assessed the abstracts and publications were included for full text assessment if one or both reviewers assessed them to be potentially relevant. Two reviewers (TB, IMK) independently assessed full text reports and included studies meeting the eligibility criteria. Any disagreement or doubt was resolved by achieving consensus through discussion among three reviewers (TB, JSM, IMK). The selection process is illustrated in Figure 1.

Data extraction

Data were extracted from each of the included studies to a data extraction form (Supplemental Material 2) by one



* Reference lists of included studies and relevant reviews

Figure 1: Flow diagram illustrating the identification, screening and eligibility assessment of studies in accordance to Preferred Reporting Items for Systematic Review and Meta-analysis.

reviewer (IMK) and subsequently verified by two other reviewers (TB, JSM). Data regarding study design, population, size, country, year and follow-up were extracted as well as patient characteristics (age, gender, receptor status, TNM-stage, grade, BRCA-mutations and treatment). Type of blood specimen and the investigated EGFR-ligands and/or EGFR were registered as well as the outcome measures and the results of the studies. Discrepancies were solved by thorough examination of the publications and subsequent consensus (TB, JSM, IMK).

Bias assessment

The risk of bias was assessed by two reviewers (TB, IMK) for all included studies, using a tool for assessing bias in studies of prognostic factors ("QUIPS tool") [29]. Thus, for all included publications the risk of bias was assessed in six categories and the overall risk of potential bias was assessed. The six categories included study participation, study data available, prognostic factor measurement, outcome measurement, study confounding and statistical analysis and reporting. The risk of bias in each category was assessed as either high, moderate or low.

Data synthesis

Data synthesis was performed in collaboration with a statistician with expertise in meta-analysis in order to determine if the data were suitable for meta-analysis or if a narrative presentation would be preferred.

Results

Literature search and study selection

The search identified 864 publications in PubMed, 1765 in Embase and 98 in Cochrane Library, a total of 2727 publications. After removal of duplicates (n = 513) and book sections (n = 62), 2152 publications were included for title screening. In the title screening 1960 publications were excluded leaving 192 publications for initial abstract screening. Additional 15 studies were identified through the reference lists of included papers and relevant reviews, and included in the abstract screening. In total 207 abstracts were screened and 168 publications were excluded after abstract screening. A total of 39 full text publications were assessed for eligibility and 23 were excluded due to the reasons outlined in Figure 1. Sixteen publications were included in the systematic review, of which only one was identified in the reference lists. Characteristics of the 16 included publications are shown in Table 1. A total of 12 studies evaluated EGFR and the results from these are shown in Table 2. Regarding the EGFR-ligands, AREG was evaluated in two studies, EGF in four studies and TGFα in two studies. Results are shown in Table 3. No studies investigating BTC, HB-EGF, EREG, EPGN or NRG-1-4 were identified. The biomarkers were analyzed in serum in all studies.

EGFR

S-EGFR was evaluated in relation to prognostic or predictive outcome measures in a total of 12 studies. Results are shown in Table 2. Of the 12 studies evaluating the predictive or prognostic value of S-EGFR, nine studies included patients with locally advanced or metastatic breast cancer [9, 20, 32, 33, 35, 36, 38, 40, 42], whereas two studies included patients with early breast cancer [37, 39]. In one study, patients with all clinical stages of disease were included and results were reported for the entire group of patients. Thus, in this study no specified information was provided regarding patients with metastatic breast cancer or early breast cancer [41].

S-EGFR in locally advanced or metastatic breast cancer

Results of the nine studies evaluating the predictive or prognostic value of S-EGFR in locally advanced or metastatic breast cancer are shown in Table 2. Overall, three of the nine studies reported significant correlations between low baseline S-EGFR and a shorter survival or reduced response to treatment in patients with locally advanced or metastatic breast cancer [20, 38, 40]. The remaining six of the nine studies including patients with locally advanced or metastatic breast cancer did not report significant correlations between S-EGFR and prognostic or predictive outcome measures [9, 32, 33, 35, 36, 42].

Predictive and prognostic value of S-EGFR in relation to tissue HER2-status

Only three of the nine studies evaluating the prognostic or predictive value of S-EGFR in patients with locally advanced or metastatic breast cancer evaluated the value in relation to HER2-status. These three studies included HER2-positive patients only [9, 33, 36]. All patients received either trastuzumab alone, or chemotherapy alone or in combination with either lapatinib or trastuzumab. No correlations between baseline S-EGFR and progression free survival (PFS), overall survival (OS), response rate (RR) or clinical benefit rate (CBR) were reported [9, 33, 36]. Out of the remaining six studies that did not evaluate correlations between S-EGFR and outcome in relation to HER2-status, three studies did not report HER2-status of the included patients [20, 35, 40] and three studies did report HER2-status but did not evaluate the correlation between S-EGFR and outcome in relation to HER2-status [32, 38, 42].

Table 1: Characteristics of included studies evaluating the prognostic or predictive value of EGFR or EGFR-ligands in blood of breast cancer patients.

Author, year Country	Study design	Number of centers	Number of patients in biomarker analysis (total number of patients)	Biomarkers evaluated[a]
Baselga et al. [30], 2014 Multicenter, international	Prospectively planned biomarker analysis in a randomized phase III trial	204 25 countries	714–727 (808)	AREG, EGF and TGFα
Bhatavdekar et al. [31], 1994 India	Retrospective	1	51 (69)	EGF
Cameron et al. [9], 2008 Multicenter, international	Prospectively collected samples in a randomized phase III trial	Multiple	367 (399)	EGFR
Gasparini et al. [32], 2005 Italy	Prospectively collected samples in a phase I study	1	14 (15)	EGFR
Hudelist et al. [33], 2006 Austria	Retrospective	1	33 (NR)	EGFR
Kim et al. [26], 2016 Korea	Retrospective analysis in prospectively collected samples	1	50 (124)	AREG
Kim et al. [34], 2013 Korea	Retrospective analysis in prospectively collected samples	1	50 (124)	EGF
Lafky et al. [35], 2005 Multicenter, USA	Retrospective analysis in samples collected in a randomized phase II trial	Multicenter	64 (93)	EGFR
Muller et al. [20], 2006 Germany	Retrospective analysis in samples collected in a randomized phase II trial	Multicenter	101 (NR)	EGFR
Rhee et al. [36], 2011 Korea	Retrospective biomarker analysis in prospectively collected samples	3	64 (126)	TGFα, EGF and EGFR
Rocca et al. [37], 2009 Italy	Retrospective biomarker analysis in prospectively collected samples	1	119 (NR)	EGFR
Sandri et al. [38], 2007 Italy	Retrospective analysis in a prospective phase III study	1	113 (178)	EGFR
Schippinger et al. [39], 2007 Austria	Retrospective	1	108 (150)	EGFR
Souder et al. [40], 2006 Multicenter, international	Retrospective analysis in a randomized phase III study	201 29 countries	535 (907)	EGFR
Tas et al. [41], 2015 Turkey	Prospective study in a heterogeneous study population	1	96 (96)	EGFR
Witzel et al. [42], 2006 Germany	Retrospective	1	76 (NR)	EGFR

[a]Biomarker analysis was performed in serum in all studies. AREG, amphiregulin; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; NR, not reported; TGFα, transforming growth factor-α.

Predictive and prognostic value of S-EGFR in relation to hormone receptor-status

Hormone receptor (HR)-status refers to the status of ER and the progesterone receptor (PR) in breast cancer tumors. The way the included studies defined HR-status varied. In the following, patients are referred to as having HR-positive tumors if one or both receptors were positive or if the status of both receptors was unknown. A total of three studies evaluated the prognostic or predictive value of S-EGFR in patients with locally advanced or metastatic breast cancer in relation to HR-status. Two of these studies (the study by Souder et al. [40] and the study by Lafky et al. [35]) included HR-positive patients only and evaluated S-EGFR at baseline before initiation of letrozole and/or tamoxifen. Souder et al. [40] reported that low baseline S-EGFR correlated to significantly reduced OS in HR-positive patients, whereas no correlations to time to progression (TTP), time to treatment failure (TTF), overall response rate (ORR) or CBR were found. Lafky et al. [35] found no correlation between baseline S-EGFR and PFS or OS in HR-positive patients. The third study evaluating the value of S-EGFR in relation to HR-status in locally advanced or metastatic breast cancer is the study by Muller et al. This study evaluated S-EGFR in patients receiving epirubicin and either paclitaxel or cyclophosphamide and found that patients with HR-positive tumors and low baseline S-EGFR had significantly lower median survival [20]. Six studies did report HR-status; however,

Table 2: The prognostic or predictive value of EGFR in blood of breast cancer patients.

Publication	Patients					Sample points	Median follow-up, months	Cut-off, ng/mL	Results
	n	Stage of disease	HER2	HR	Treatment				
Studies including patients with all clinical stages of BC									
Tas et al. [41] 2015	96	All clinical stages	+: 32% –: 67% ?: 1%	ER: +: 71% –: 28% ?:1%	PR: +: 66% –: 33% ?: 1%	Heterogeneous In both the adjuvant and metastatic setting	Not well described	Mean: 19.4	103.5	– S-EGFR not correlated to 2-year survival or response
Studies including patients with locally advanced or metastatic BC									
Cameron et al. [9] 2008	367	Locally advanced or MBC	+	ER+ and/or PR+:47% ER– and PR–: 49% ?: 4%		Capecitabine or lapatinib + capecitabine	Baseline	NR		– S-EGFR as a log transformed continuous variable not correlated to PFS in either treatment group
Gasparini et al. [32] 2005	14	MBC	+:36% –:57% ?:7%	ER: +:47% –:40% ?:13%	PR: NR	Gefitinib + epirubicin	Baseline + at end of treatment	NR		– No correlations between pre- and post S-EGFR and OR
Hudelist et al. [33] 2006	33	MBC	+	ER: +:33% –:67%	PR: +:21% –:79%	Trastuzumab +/– chemotherapy	Baseline + sporadic afterwards	41.5	65	– S-EGFR not correlated to PFS, OS, response rate or CBR
Lafky et al. [35] 2005	64	MBC	NR	PR+/ER(+/–): 63% ER+/PR(–/?): 25% ?: 12%		Letrozole	Baseline, 1 month, 3 months	NR		– Significant decrease in percent change in log S-EGFR after 1 month and 3 months of letrozole compared with baseline S-EGFR. No difference in S-EGFR between 1 and 3 months – Baseline S-EGFR not correlated to PFS or OS (data not shown)
Muller et al. [20] 2006	101	MBC	NR	ER: –: 35% NR: 65%	PR: NR	Epirubicin + paclitaxel or epirubicin + cyclophosphamide	Baseline and after 3 courses of therapy (n = 39)	8.9	45	– Baseline S-EGFR not correlated to PFS, OS, median survival or response. No difference in PFS between treatment groups – Pts with ER+ tumors and ↓baseline S-EGFR had significantly lower median survival – S-EGFR significantly lower after three cycles of therapy than before. No association with response

Table 2 (continued)

Publication	Patients				Treatment	Sample points	Median follow-up, months	Cut-off, ng/mL	Results
	n	Stage of disease	HER2	HR					
Rhee et al. [36] 2011	64	Locally advanced or MBC	+	ER+ and/or PR+: 38% Remaining: NR	Lapatinib + capecitabine	Baseline	21		– S-EGFR not correlated to response
Sandri et al. [38] 2007	113	MBC	+: 11% –: 45% ?: 44%	ER: PR: +: 53% +: 40% –: 38% –: 49% NR: 9% NR:11%	Cyclophosphamid + methotrexate or same + thalidomide	Baseline and after 2 months treatment	NR	45	– No change in S-EGFR after 2 months in pts with response, stable disease or progressive disease – ↓baseline S-EGFR predicts reduced response at 2 months and 24 weeks – ↓baseline S-EGFR correlated with shorter PFS and OS. Not significant in the multivariate analysis
Souder et al. [40] 2006	535	Locally advanced, loco-regional recurrent or MBC	NR	ER+ and/or PR+: 66% ER– and PR–: <1% ?: 34%	Letrozole or tamoxifen, crossover at progression	Baseline before first-line hormone therapy	NR	44.1	– S-EGFR not correlated to TTP, TTF, ORR or CBR – ↓S-EGFR correlated to significantly reduced OS
Witzel et al. [42] 2006	76	MBC	+: 44% –: 56%	ER: PR: +: 67% NR –: 33%	Heterogeneous In the metastatic setting	Baseline at onset of MBC	18	45	– S-EGFR not correlated to OS, PFS or median survival
Studies including patients with early BC									
Rocca et al. [37] 2009	119	Early BC, no secondary lesions	+: 10% –: 63% ?: 27%	ER– and PR–: 19% ER+ and/or PR+: 81%	Surgery and adjuvant therapy	Before and after surgery	93		– Preoperative S-EGFR not associated with DFS (data not shown) – Significant 15% linear increase of event risk relative to 1 ng/mL decrease of S-EGFR – Pts with S-EGFR decrease (preoperative-postoperative) greater than or equal to the observed mean had a worse prognosis ($p = 0.05$) as compared to pts with a decrease lower than average
Schippinger et al. [39] 2007	108	Invasive primary BC	+: 17% –: 67% ?: 16%	ER+ and PR+: 50% ER+ or PR+: 19% ER– and PR–: 31%	Epirubicin alone or combined with docetaxel or paclitaxel or cyclophosphamide	Baseline before neoadjuvant therapy			– Baseline S-EGFR not correlated to histopathological response

BC, breast cancer; CBR, clinical benefit rate; DFS, disease free survival; EGFR, epidermal growth factor receptor; ER, estrogen receptor; HER2, human epidermal growth factor 2; HR, hormone receptor; MBC, metastatic breast cancer; NR, not reported; OR, overall response; ORR, overall objective tumor response rate; OS, overall survival; Pts, patients; PFS, progression free survival; PR, progesterone receptor; TTF, time to treatment failure; TTP, time to progression; ?, unknown; ↓, low; ↑, high.

Table 3: The prognostic or predictive value of EGFR-ligands in blood of breast cancer patients.

	Publication	Patients					Sample points	Median follow-up (months)	Cut-off	Results
		n	Stage of disease	HER2	Treatment	HR				
AREG	Kim et al. [26] 2016	50	MBC	+	Firstline trastuzumab + taxane	ER: +: 34% −: 66% PR: +: 18% −: 82%	Baseline	29.2	0.5 ng/mL	– ↑S-AREG correlated to significantly shorter PFS
	Baselga et al. [30] 2014	714	Locally recurrent, nonresectable or MBC	+	Pertuzumab + trastuzumab + docetaxel or placebo + trastuzumab + docetaxel	ER+ and/or PR+: 48% ER− and PR−: 51% ?: 1%	Baseline, week 9 and at disease progression	PFS: 19.3 OS: 30	Median (NR)	– S-AREG not correlated to progression – No difference between treatment arms in pts with PFS events and samples available at baseline, week 9 and at progression – S-AREG at baseline and week 9 were comparable in pts with and without PFS events
EGF	Kim et al. [34] 2013	50	MBC	+	Firstline trastuzumab +taxane	ER: +: 34% −: 66% PR: +: 18% −: 82%	Baseline	29.2	10 pg/mL	– ↑S-EGF correlated to significantly longer OS – S-EGF not correlated to PFS
	Rhee et al. [36] 2011	64	Locally advanced or MBC	+	Lapatinib + capecitabine	ER+ and/or PR+: 38% Remaining: NR	Baseline	21		– S-EGF not correlated to response
	Baselga et al. [30] 2014	727	Locally recurrent, nonresectable or MBC	+	Pertuzumab + trastuzumab + docetaxel or placebo + trastuzumab + docetaxel	ER+ and/or PR+:48% ER− and PR−: 51% ?: 1%	Baseline, week 9 and at disease progression	PFS: 19.3 OS: 30	Median (NR)	– S-EGF not correlated to progression – No difference between treatment arms in pts with PFS events and samples available at baseline, week 9 and at progression – S-EGF at baseline and week 9 were comparable in pts with and without PFS events
	Bhatavdekar et al. [31] 1994	51	Advanced	NR	NR	NR	Prior to surgery	Min. 2 years	NR	– S-EGF not correlated to 2-year survival (data not shown)
TGFα	Rhee et al. [36] 2011	64	Locally advanced or MBC	+	Lapatinib + capecitabine	ER+ and/or PR+: 38% Remaining: NR	Baseline	21	3.75 pg/mL	– ↓S-TGFα correlated to higher response rate – S-TGFα not correlated to TTP or OS
	Baselga et al. [30] 2014	721	Locally recurrent, nonresectable or MBC	+	Pertuzumab + trastuzumab + docetaxel or placebo + trastuzumab + docetaxel	ER+ and/or PR+:48% ER− and PR−: 51% ?: 1%	Baseline, week 9 and at disease progression	PFS: 19.3 OS: 30	Median (NR)	– S-TGFα not correlated to progression – No difference between treatment arms in pts with PFS events and samples available at baseline, week 9 and at progression – S-EGF at baseline and week 9 were comparable in pts with and without PFS events

Table 3 (continued)

	Publication	Patients					Sample points	Median follow-up (months)	Cut-off	Results
		n	Stage of disease	HER2	Treatment	HR				
BTC	No studies identified									
HB-EGF	No studies identified									
NRG	No studies identified									
EREG	No studies identified									
EPGN	No studies identified									

AREG, amphiregulin; BTC, betacellulin; EGF, epidermal growth factor; EPGN, epigen; ER, estrogen receptor; EREG, epiregulin; HB-EGF, heparin-binding EGF-like growth factor; HER2, human epidermal growth factor 2; HR, hormone receptor; MBC, metastatic breast cancer; NRG, neuregulin; NR, not reported; OS, overall survival; PFS, progression free survival; PR, progesterone receptor; TGFα, transforming growth factor-α; TTP, time to progression; ↓, low; ↑, high; ?, unknown.

the correlation between S-EGFR and outcome in relation to HR-status was not evaluated [9, 32, 33, 36, 38, 42].

S-EGFR in early breast cancer

Two studies evaluated the predictive or prognostic value of S-EGFR in early breast cancer. The results are shown in Table 2. Rocca et al. [37] evaluated S-EGFR in patients with early breast cancer before and after surgery and found no correlation between preoperative S-EGFR and disease free survival (DFS). However, a decrease in S-EGFR from pre-operative levels to post-operative levels greater than or equal to the observed mean was associated with worse DFS. Schippinger et al. [39] evaluated S-EGFR in patients with primary invasive breast cancer before neoadjuvant treatment with either epirubicine alone or in combination with either docetaxel, paclitaxel or cyclophosphamide and found no correlation between S-EGFR and histopathological response (histopathological response here serves as a surrogate outcome). Neither of the studies reported correlations between S-EGFR and outcome in relation to either HER2-status or HR-status.

EGFR-ligands

All five studies identified that examined the prognostic or predictive value of EGFR-ligands included patients with locally advanced or metastatic breast cancer, whereas no studies investigating the prognostic or predictive role of EGFR-ligands in early breast cancer were identified. Results are shown in Table 3. Furthermore, all studies that evaluated EGFR-ligands included patients with HER2-positive tumors only, except in one study in which HER2-status was unknown [31]. HR-status of the patients in the identified studies were either mixed or not reported as shown in Table 3.

Two of the five studies evaluating EGFR-ligands evaluated the value of S-AREG [26, 30]. One study found that patients with high S-AREG had significantly shorter PFS than patients with low S-AREG, whereas the other study found no correlation between S-AREG and either PFS or disease progression [26, 30].

A total of four studies evaluated the value of S-EGF [30, 31, 34, 36]. No correlations between S-EGF and either disease progression or PFS were found in one study [30], whereas another study reported that patients with high S-EGF had significantly longer OS compared to patients with low S-EGF, but no correlation to PFS was found [34]. One study reported that S-EGF did not identify patients

with better or worse 2-year survival [31] and one study reported that there was no association between S-EGF and response [36].

Two studies evaluated S-TGFα [30, 36]. One study found the RR in patients with low S-TGFα to be significantly higher than in patients with high S-TGFα, whereas no correlations between S-TGFα and either TTP or OS were found [36]. The other study found no correlations between S-TGFα and either disease progression or PFS [30].

S-EGFR/EGFR-ligands in relation to S-HER2

Several of the identified studies evaluated both S-HER2 and S-EGFR as prognostic or predictive biomarkers in breast cancer; however, only three of the identified studies reported a combined analysis of these two biomarkers [20, 40, 42]. Souder et al. found no added value from S-EGFR when compared to S-HER2 alone in relation to ORR, CBR, TTF and TTP. However, patients with decreased S-EGFR (<44.1 ng/mL) and normal S-HER2 (<15 ng/mL) had significantly reduced OS compared to patients with normal levels [40, 43]. Witzel et al. [42] and Muller et al. [20] evaluated the combination of S-EGFR and S-HER2 in metastatic breast cancer as well, and both studies reported a tendency of shorter survival in patients with high S-HER2 and low S-EGFR as compared to patients with normal levels of both markers. Regarding serum levels of EGFR-ligands, no studies evaluated the combination with S-HER2.

Bias assessment

Assessment of risk of bias can be found in Supplemental Material 3. Overall, the risk of bias in the included studies varied. A common and crucial risk of bias was lack in data reporting and inadequate description of study populations. The risk of confounding was not accounted for in several of the studies, i.e. HER2-status of the tumor. Outcome measures were well defined in most studies. Finally, several studies did not report either the coefficient of variation or the limit of detection of the biomarker analysis (Supplemental Material 3).

Data synthesis

The 16 studies included in this systematic review showed considerable clinical heterogeneity both in relation to differences in study designs, outcome measures and in relation to differences in characteristics of study subjects, such as stage and subtype of breast cancer. In addition, the studies were heterogeneous when it comes to interventions both in relation to type of treatments and dosage and durations of treatments. Thus, the synthesis of the current evidence is narrative [44].

Discussion

Members of the EGFR-family are implicated in breast cancer pathogenesis and are important targets of breast cancer treatment [17]. Lack of response to treatment and resistance to treatment contributes to increased morbidity and mortality in breast cancer, and valid predictive biomarkers may improve this by providing personalized treatment. This is the first systematic review assembling current knowledge on serum or plasma levels of EGFR and EGFR-ligands as prognostic and predictive markers in breast cancer patients. Where prognostic markers help to predict patient outcome independent of treatment, predictive markers may help to identify patients who are likely to respond to a certain treatment or predict the effect of the treatment. Investigation of a pure prognostic marker would require a group of patients that did not receive any treatment, and, as non-treated patients are not included in any of the identified studies, pure prognostic information is not achieved. The information provided by many markers reflects a combination of prognostic and predictive information.

EGFR

The 12 studies evaluating the prognostic or predictive value of S-EGFR showed considerable variation in relation to study design, patient characteristics (disease stage, HER2-status, HR-status) and treatment regimens. In addition different cut-off levels for S-EGFR were used and the reasons for choice of cut-off varied between studies or were not explained. However, all significant correlations observed in advanced and metastatic breast cancer showed an overall association between low baseline S-EGFR and a shorter survival or reduced response to treatment [20, 38, 40]. Though several studies did not find the same significant trends, none of the identified studies reported opposing results. To our knowledge, no clear explanations for the observed correlations are known. It has, however, been hypothesized that as part of maintaining the proliferative activity, cancer cells with increased malignant potential may have a decreased proteolytic

cleavage of the extracellular part of EGFR and studies have reported that levels of S-EGFR are lower in metastatic breast cancer patients, as compared to healthy controls [20, 21]. The associations between low baseline S-EGFR and shorter survival or reduced response to treatment in metastatic breast cancer might also be a reflection of this. However, it is unknown if the correlations observed reflect prognostic information regarding malignity or predictive information regarding response to treatment. In the studies by Souder et al. [40] and Muller et al. [20], the correlations between low S-EGFR and reduced survival were found only in patients with HR-positive tumors/HR-status unknown who received anti-hormonal treatment and cytostatic treatment, respectively. This might indicate a possible prognostic or predictive role of S-EGFR, especially in HR-positive breast cancer patients. It is, however, unknown if the correlations might reflect associations between EGFR-expression and ER-expression in relation to prognosis in recurrent breast cancer [18, 45] or associations between EGFR-expression and response to treatment.

Overall, current evidence indicates that low S-EGFR in metastatic breast cancer is associated with shorter survival and reduced response to treatment, especially in HR-positive patients. However, findings are not consistent across studies and comprehensive aspects of the utility of S-EGFR as a predictive and prognostic marker in breast cancer remain to be uncovered.

EGFR-ligands

The prognostic and predictive value of S-AREG, S-EGF and S-TGFα in breast cancer has been investigated in several studies, whereas no studies evaluating the value of S-BTC, S-HB-EGF, S-NRG, S-EREG or S-EPGN were identified (Table 3). All studies regarding EGFR-ligands were performed in patients experiencing advanced or metastatic breast cancer and all the included patients were HER2-positive, except in one study where HER2-status was not reported. Baselga et al. did not find S-AREG, S-EGF or S-TGFα to be associated with PFS in any of the treatment groups. However, despite levels of the ligands in serum are known at baseline, week 9 and at progression, the possible information of a decrease or an increase in tumor marker levels were not evaluated [30]. Evaluation by cut-offs might limit the sensitivity of the tumor marker as compared to evaluation by changes in levels of the tumor markers from baseline [46]. Several other studies evaluated the value of S-AREG, S-EGF and S-TGFα as well [26, 31, 34, 36]. These studies, however, included only few patients and the results should be interpreted with caution. Overall, S-AREG, S-EGF and S-TGFα have only been partially investigated in highly selected subsets of breast cancer patients, whereas the prognostic and predictive value of the remaining EGFR-ligands has not yet been explored in a clinical setting. Evidence towards the importance of EGFR-ligands as biomarkers in breast cancer is provided by studies reporting aberrant expression of some EGFR-ligands in breast cancer tissue and associations between expression of EGFR-ligands in tissue and prognosis [16, 47]. Furthermore, several case-control studies show higher levels of EGFR-ligands in the blood of breast cancer patients as compared to healthy controls [22–25]. Thus, further studies are needed in order to clarify the role of EGFR-ligands as prognostic or predictive markers in breast cancer patients as well as for monitoring effect of treatment and/or resistance.

S-EGFR/EGFR-ligands in relation to S-HER2

A crosstalk between HER2 and EGFR is known to be crucial in the receptor signaling, and HER2 is the preferred heterodimerization partner of EGFR [4]. Furthermore, there are indications that EGFR and EGFR-ligands are involved in resistance to trastuzumab [4]. S-HER2 is known to provide valuable information regarding response to trastuzumab in HER2-positive breast cancer [13, 48]. Though S-HER2 is not the focus of this review, it is relevant to evaluate, if combined analysis of serum levels of HER2 and EGFR or EGFR-ligands provide additional clinical relevant information. Only three studies reported a combined analysis of both S-EGFR and S-HER2 in patients with metastatic breast cancer [20, 40, 42], whereas no studies reported a combined analysis of EGFR-ligands and S-HER2. Two of the studies [20, 42] that evaluated the combination of S-EGFR and S-HER2 reported a tendency of shorter survival in patients with high S-HER2 and low S-EGFR as compared to patients with normal levels of both markers. However, it is unclear whether the results of these two studies reflect the well examined effect of S-HER2 and/or S-EGFR since both studies reported only results of patients with normal levels of both markers as compared to patients with aberrant levels of both markers [20, 42]. Another study, however, reported that patients with decreased S-EGFR (<44.1 ng/mL) and normal S-HER2 (<15 ng/mL) had significantly reduced OS compared to patients with normal levels [40, 43]. Overall, there are indications that low S-EGFR may be associated with shortened survival in patients with normal levels of S-HER2. This might be partially explained by a resistance mechanism due to

a crosstalk between EGFR and ER in HR-positive patients receiving endocrine therapy. Further investigation of the combination of serum levels of HER2 and EGFR/EGFR-ligands is warranted.

Perspectives

In recent years, HER2-targeted treatments have radically improved the prognosis for patients with HER2-positive tumors. As dual and pan-targeted therapies against the EGFR-system are now continuously implemented in breast cancer treatment, it is highly desirable to identify markers or a combination of markers that might predict response or resistance to the treatments. In lung cancer and colorectal cancer, EGFR-targeted treatments have an established therapeutic role [49, 50] and there are indications, that levels of EGFR and some of the EGFR-ligands in blood can provide valuable information regarding prediction of treatment outcome or prognosis in certain groups of patients [51–55]. In breast cancer, important aspects of the clinical implications of EGFR and EGFR-ligands in blood remain to be explored.

Conclusions

In conclusion, the evidence indicates associations between low S-EGFR and shorter survival and reduced response to certain treatments in metastatic breast cancer, especially in HR-positive patients. The utility of both S-EGFR and serum levels of EGFR-ligands as predictive and prognostic markers in breast cancer has only been investigated in highly selected subsets of breast cancer patients, so extensive aspects remain to be uncovered. Based on the current knowledge, it is not possible to make recommendations neither for nor against clinical application. Large well-planned studies are needed in order to clarify and validate the clinical impact of EGFR and EGFR-ligands in the clinical management of breast cancer patients.

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References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–86.
2. Roskoski R, Jr. The ErbB/HER family of protein-tyrosine kinases and cancer. Pharmacol Res 2014;79:34–74.
3. Mendelsohn J, Baselga J. Epidermal growth factor receptor targeting in cancer. Semin Oncol 2006;33:369–85.
4. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 2005;5:341–54.
5. Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. Annu Rev Med 2011;62:233–47.
6. Koninki K, Tanner M, Auvinen A, Isola J. HER-2 positive breast cancer: decreasing proportion but stable incidence in Finnish population from 1982 to 2005. Breast Cancer Res 2009;11:R37.
7. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177–82.
8. Swain SM, Baselga J, Kim SB, Ro J, Semiglazov V, Campone M, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. N Engl J Med 2015;372:724–34.
9. Cameron D, Casey M, Press M, Lindquist D, Pienkowski T, Romieu CG, et al. A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: updated efficacy and biomarker analyses. Breast Cancer Res Treat 2008;112:533–43.
10. Chan A, Delaloge S, Holmes FA, Moy B, Iwata H, Harvey VJ, et al. Neratinib after trastuzumab-based adjuvant therapy in patients with HER2-positive breast cancer (ExteNET): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2016;17:367–77.
11. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–92.
12. Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Press M, et al. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med 2011;365:1273–83.

13. Petersen ER, Sorensen PD, Jakobsen EH, Madsen JS, Brandslund I. Serum HER-2 predicts response and resistance to trastuzumab treatment in breast cancer. Clin Chem Lab Med 2013;51:1483–92.
14. Sorensen PD, Jakobsen EH, Madsen JS, Petersen EB, Andersen RF, Ostergaard B, et al. Serum HER-2: sensitivity, specificity, and predictive values for detecting metastatic recurrence in breast cancer patients. J Cancer Res Clin Oncol 2013;139:1005–13.
15. Kjaer IM, Brandslund I. Clinical use of S-HER2 in breast cancer for detecting metastatic recurrence and monitoring effect of trastuzumab treatment. LaboratoriumsMedizin 2014;38:325–31.
16. Olsen DA, Bechmann T, Ostergaard B, Wamberg PA, Jakobsen EH, Brandslund I. Increased concentrations of growth factors and activation of the EGFR system in breast cancer. Clin Chem Lab Med 2012;50:1809–18.
17. Eccles SA. The epidermal growth factor receptor/Erb-B/HER family in normal and malignant breast biology. Int J Dev Biol 2011;55:685–96.
18. Tsutsui S, Kataoka A, Ohno S, Murakami S, Kinoshita J, Hachitanda Y. Prognostic and predictive value of epidermal growth factor receptor in recurrent breast cancer. Clin Cancer Res 2002;8:3454–60.
19. Tsutsui S, Ohno S, Murakami S, Hachitanda Y, Oda S. Prognostic value of epidermal growth factor receptor (EGFR) and its relationship to the estrogen receptor status in 1029 patients with breast cancer. Breast Cancer Res Treat 2002;71:67–75.
20. Muller V, Witzel I, Pantel K, Krenkel S, Luck HJ, Neumann R, et al. Prognostic and predictive impact of soluble epidermal growth factor receptor (sEGFR) protein in the serum of patients treated with chemotherapy for metastatic breast cancer. Anticancer Res 2006;26:1479–87.
21. Asgeirsson KS, Agrawal A, Allen C, Hitch A, Ellis IO, Chapman C, et al. Serum epidermal growth factor receptor and HER2 expression in primary and metastatic breast cancer patients. Breast Cancer Res 2007;9:R75.
22. Kim BK, Lee JW, Park PJ, Shin YS, Lee WY, Lee KA, et al. The multiplex bead array approach to identifying serum biomarkers associated with breast cancer. Breast Cancer Res 2009;11:R22.
23. Shetty P, Patil VS, Mohan R, D'Souza LC, Bargale A, Patil BR, et al. Annexin A2 and its downstream IL-6 and HB-EGF as secretory biomarkers in the differential diagnosis of Her-2 negative breast cancer. Ann Clin Biochem 2017;54:463–71.
24. Chakrabarty S, Huang S, Moskal TL, Fritsche HA, Jr. Elevated serum levels of transforming growth factor-alpha in breast cancer patients. Cancer Lett 1994;79:157–60.
25. Vlaicu P, Mertins P, Mayr T, Widschwendter P, Ataseven B, Hogel B, et al. Monocytes/macrophages support mammary tumor invasivity by co-secreting lineage-specific EGFR ligands and a STAT3 activator. BMC Cancer 2013;13:197.
26. Kim JW, Kim DK, Min A, Lee KH, Nam HJ, Kim JH, et al. Amphiregulin confers trastuzumab resistance via AKT and ERK activation in HER2-positive breast cancer. J Cancer Res Clin Oncol 2016;142:157–65.
27. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev 2015;4:1.
28. Registration of the protocol in the International Prospective Register of Systematic Reviews (PROSPERO). Title: Prognostic and predictive value of EGFR and EGFR-ligands in the blood of breast cancer patients: a systematic review . Available from: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=39993.
29. Hayden JA, van der Windt DA, Cartwright JL, Cote P, Bombardier C. Assessing bias in studies of prognostic factors. Ann Intern Med 2013;158:280–6.
30. Baselga J, Cortes J, Im SA, Clark E, Ross G, Kiermaier A, et al. Biomarker analyses in CLEOPATRA: a phase III, placebo-controlled study of pertuzumab in human epidermal growth factor receptor 2-positive, first-line metastatic breast cancer. J Clin Oncol 2014;32:3753–61.
31. Bhatavdekar JM, Patel DD, Karelia NH, Vora HH, Ghosh N, Shah NG, et al. Tumor markers in patients with advanced breast cancer as prognosticators: a preliminary study. Breast Cancer Res Treat 1994;30:293–7.
32. Gasparini G, Sarmiento R, Amici S, Longo R, Gattuso D, Zancan M, et al. Gefitinib (ZD1839) combined with weekly epirubicin in patients with metastatic breast cancer: a phase I study with biological correlate. Ann Oncol 2005;16:1867–73.
33. Hudelist G, Kostler WJ, Gschwantler-Kaulich D, Czerwenka K, Kubista E, Muller R, et al. Serum EGFR levels and efficacy of trastuzumab-based therapy in patients with metastatic breast cancer. Eur J Cancer 2006;42:186–92.
34. Kim JW, Kim JH, Im SA, Lee KH, Kim JS, Kim TY, et al. Serum epidermal growth factor is associated with prognosis and hormone receptor status in patients with HER2-positive metastatic breast cancer treated with first-line trastuzumab plus taxane chemotherapy. Cancer Chemother Pharmacol 2013;72:1023–9.
35. Lafky JM, Baron AT, Cora EM, Hillman DW, Suman VJ, Perez EA, et al. Serum soluble epidermal growth factor receptor concentrations decrease in postmenopausal metastatic breast cancer patients treated with letrozole. Cancer Res 2005;65:3059–62.
36. Rhee J, Han SW, Cha Y, Ham HS, Kim HP, Oh DY, et al. High serum TGF-alpha predicts poor response to lapatinib and capecitabine in HER2-positive breast cancer. Breast Cancer Res Treat 2011;125:107–14.
37. Rocca A, Cancello G, Bagnardi V, Sandri MT, Torrisi R, Zorzino L, et al. Perioperative serum VEGF and extracellular domains of EGFR and HER2 in early breast cancer. Anticancer Res 2009;29:5111–9.
38. Sandri MT, Johansson HA, Zorzino L, Salvatici M, Passerini R, Maisonneuve P, et al. Serum EGFR and serum HER-2/neu are useful predictive and prognostic markers in metastatic breast cancer patients treated with metronomic chemotherapy. Cancer 2007;110:509–17.
39. Schippinger W, Dandachi N, Regitnig P, Hofmann G, Balic M, Neumann R, et al. The predictive value of EGFR and HER-2/neu in tumor tissue and serum for response to anthracycline-based neoadjuvant chemotherapy of breast cancer. Am J Clin Pathol 2007;128:630–7.
40. Souder C, Leitzel K, Ali SM, Demers L, Evans DB, Chaudri-Ross HA, et al. Serum epidermal growth factor receptor/HER-2 predicts poor survival in patients with metastatic breast cancer. Cancer 2006;107:2337–45.
41. Tas F, Bilgin E, Karabulut S, Duranyildiz D. Clinical significance of serum epidermal growth factor receptor (EGFR) levels in patients with breast cancer. Cytokine 2015;71:66–70.
42. Witzel I, Thomssen C, Krenkel S, Wilczak W, Bubenheim M, Pantel K, et al. Clinical utility of determination of HER-2/neu

and EGFR fragments in serum of patients with metastatic breast cancer. Int J Biol Markers 2006;21:131–40.
43. Lipton A, Ali SM, Leitzel K, Demers L, Harvey HA, Chaudri-Ross HA, et al. Serum HER-2/neu and response to the aromatase inhibitor letrozole versus tamoxifen. J Clin Oncol 2003;21:1967–72.
44. Thompson SG. Why sources of heterogeneity in meta-analysis should be investigated. Br Med J 1994;309:1351–5.
45. Klijn JG, Berns PM, Schmitz PI, Foekens JA. The clinical significance of epidermal growth factor receptor (EGF-R) in human breast cancer: a review on 5232 patients. Endocr Rev 1992;13:3–17.
46. Di Gioia D, Stieber P, Schmidt GP, Nagel D, Heinemann V, Baur-Melnyk A. Early detection of metastatic disease in asymptomatic breast cancer patients with whole-body imaging and defined tumour marker increase. Br J Cancer 2015;112:809–18.
47. McIntyre E, Blackburn E, Brown PJ, Johnson CG, Gullick WJ. The complete family of epidermal growth factor receptors and their ligands are co-ordinately expressed in breast cancer. Breast Cancer Res Treat 2010;122:105–10.
48. Witzel I, Loibl S, von Minckwitz G, Mundhenke C, Huober J, Hanusch C, et al. Monitoring serum HER2 levels during neoadjuvant trastuzumab treatment within the GeparQuattro trial. Breast Cancer Res Treat 2010;123:437–45.
49. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239–46.
50. Van Cutsem E, Lenz HJ, Kohne CH, Heinemann V, Tejpar S, Melezinek I, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. J Clin Oncol 2015;33:692–700.
51. Takahashi N, Yamada Y, Furuta K, Honma Y, Iwasa S, Takashima A, et al. Serum levels of hepatocyte growth factor and epiregulin are associated with the prognosis on anti-EGFR antibody treatment in KRAS wild-type metastatic colorectal cancer. Br J Cancer 2014;110:2716–27.
52. Ma BB, Chan SL, Ho WM, Lau W, Mo F, Hui EP, et al. Intermittent versus continuous erlotinib with concomitant modified "XELOX" (q3W) in first-line treatment of metastatic colorectal cancer: correlation with serum amphiregulin and transforming growth factor alpha. Cancer 2013;119:4145–53.
53. Addison CL, Ding K, Zhao H, Le Maitre A, Goss GD, Seymour L, et al. Plasma transforming growth factor alpha and amphiregulin protein levels in NCIC Clinical Trials Group BR.21. J Clin Oncol 2010;28:5247–56.
54. Ishikawa N, Daigo Y, Takano A, Taniwaki M, Kato T, Hayama S, et al. Increases of amphiregulin and transforming growth factor-alpha in serum as predictors of poor response to gefitinib among patients with advanced non-small cell lung cancers. Cancer Res 2005;65:9176–84.
55. Gregorc V, Ceresoli GL, Floriani I, Spreafico A, Bencardino KB, Ludovini V, et al. Effects of gefitinib on serum epidermal growth factor receptor and HER2 in patients with advanced non-small cell lung cancer. Clin Cancer Res 2004;10:6006–12.

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