Imaging Features of HER2 Overexpression in Breast Cancer: A Systematic Review and Meta-analysis


Abstract

Breast cancer imaging phenotype is diverse and may relate to molecular alterations driving cancer behavior. We systematically reviewed and meta-analyzed relations between breast cancer imaging features and human epidermal growth factor receptor type 2 (HER2) overexpression as a marker of breast cancer aggressiveness. We focused on established clinical imaging modalities [mammography, breast ultrasound, magnetic resonance imaging (MRI), and/or [18F]fluorodeoxyglucose positron emission tomography (18F-FDG PET)] and used MEDLINE and EMBASE searches through February 2013. Of 68 imaging features that could be pooled (85 articles, 23,255 cancers; random-effects meta-analysis), 11 significantly related to HER2 overexpression. Results based on five or more studies and robustness in subgroup analyses were as follows: the presence of microlcifications on mammography [pooled odds ratio (pOR), 3.14; 95% confidence interval (CI), 2.46–4.00] or ultrasound (mass-associated pOR, 2.95; 95% CI, 2.34–3.71), branching or fine linear microlcifications (pOR, 2.11; 95% CI, 1.07–4.14) or extremely dense breasts on mammography (pOR, 1.37; 95% CI, 1.07–1.76), and washout (pOR, 1.57; 95% CI, 1.11–2.21) or fast initial kinetics (pOR, 2.60; 95% CI, 1.43–4.73) on MRI all increased the chance of HER2 overexpression. Maximum [18F]fluorodeoxyglucose standardized uptake value (SUVmax) was higher upon HER2 overexpression (pooled mean difference, +0.76; 95% CI, 0.10–1.42). These results show that several imaging features relate to HER2 overexpression, lending credibility to the hypothesis that imaging phenotype reflects cancer behavior. This implies prognostic relevance, which is especially relevant as imaging is readily available during diagnostic work-up.

Introduction

Breast cancer is the most common type of cancer and the leading cause of cancer-related death in women worldwide (1). It is a heterogeneous disease, which can be appreciated by its diverse imaging appearance (2), its histologic and molecular classifications (3–5), and its correspondingly diverse disease course. One of the most clinically relevant molecular aberrations in breast cancer is overexpression of the human epidermal growth factor receptor type 2 (HER2). HER2 overexpression occurs in 15% to 25% of invasive breast cancers, and is associated with an intrinsic worse prognosis but good response to HER2-targeted therapies (6).

Some of the many effects on the cellular level of HER2 overexpression are increased cell proliferation, cell survival, mobility, and invasiveness, as well as neo-angiogenesis by increasing vascular endothelial growth factor production (7). These cellular processes and their clinical course provide evidence that HER2-overexpressing breast cancers behave distinctly from other breast cancers, which might drive macroscopic appearance and physiologic parameters. These phenomena may be potentially visible by clinical imaging modalities. As of now, the literature relating imaging features to HER2 overexpression in breast cancer is diverse, scattered over different scientific and clinical fields, and often based on small studies. The purpose of this study was to comprehensively review that literature and use meta-analysis techniques to formally quantify the relation between imaging features and HER2-positive breast cancer. We specifically focused on established clinical imaging modalities [mammography, breast ultrasound, magnetic resonance imaging (MRI), and [18F]fluorodeoxyglucose positron emission tomography (18F-FDG PET)].

Identification of imaging features related to HER2 overexpression in breast cancer could not only increase our biologic understanding, but may also have potential future clinical relevance. Existence of imaging features...
related to HER2 overexpression would for instance sug-
estic value of breast cancer imaging phenotype. Fur-
furthermore, such features may have relevance in iden-
tifying potential sampling error in cases in which HER2
status is based on tumor biopsies, as practiced for neoad-
juvant treatment indication.

Materials and Methods

Literature search and study selection

We performed a comprehensive systematic literature
search of MEDLINE and EMBASE on February 8, 2013
using synonyms for HER2 and the imaging modalities
of interest in combination with breast cancer (Fig. 1). The
search was without restrictions. After combining the
searches and duplicate removal, two researchers (S.G.
Elias and A. Adams) independently performed all selec-
tion and data-extraction steps. First, we assessed titles
and abstracts, excluding only articles deemed ineligible
for full-text evaluation by both researchers. Then, we
reviewed the full text of those remaining and subsequent-
ly excluded articles only upon consensus. We documen-
ted exclusion criteria as follows: (i) non-original data (e.g.,
reviews, editorials, and guidelines), (ii) preclinical studies
(e.g., animal or in vitro studies), (iii) case reports (i.e.,
studies including less than 10 patients), (iv) non-primary
breast cancer (e.g., imaging of breast cancer metastasis or
lymph nodes), (v) experimental breast imaging modalities
(e.g., optical mammography or breast elastography), (vi)
treatment evaluation studies, and (vii) no imaging fea-
tures described or evaluated. We then reviewed each
selected article’s references to identify any articles missed
by the original search. Finally, articles with insufficient
data for review (e.g., presenting only P values), or studies
that presented identical data on the same patients were
excluded (keeping the largest series). If unsure about
duplicate data, we contacted the authors. As magnetic
resonance spectroscopy (MRS) and diffusion-weighted
imaging (DWI) are not established clinical imaging
modalities but could be implemented rather easily given
the clinical availability of MRI scanners, we did not
exclude these modalities but show the results in the
Supplementary Results.

Data extraction

First, we extracted study characteristics [e.g., study size,
overall patient and tumor characteristics, HER2 assessment
methodology, imaging acquisition details, use of the Breast
Imaging-Reporting and Data System (BI-RADS), which
improves comparability (2), and, more specifically related
to the risk of bias, whether or not image assessment was
blinded for HER2 status and whether the selection of
patients may have introduced selection bias]. Then, we
documented the numerical results of each imaging feature
in relation to HER2 status [for categorical data by cross-
tabulating absolute numbers, for continuous data using
means and standard deviations (SD)]. If necessary, we
matched different descriptors (e.g., for mammography, we
combined studies describing "well-defined mass margins"
and "smooth mass margins" to the overall descriptor "cir-
cumscribed margins"; Supplementary Tables S1–S3), com-
bined groups [e.g., if features were compared between
HER2-positive, triple-negative, and estrogen receptor
(ER)–positive breast cancer, we combined the latter two
groups], or approximated study data (e.g., using percen-
tages and totals to derive absolute numbers). For group
combination of categorical data, we used summation fol-
lowing cross-tabulation. For group combination of contin-
uous data, we used inverse variance weighted pooling.
Approximation of means and SD was necessary for several
DWI and 18F-FDG PET studies that did not report these
parameters using methods as outlined elsewhere (8, 9). All
data-extraction steps were double-checked by two
researchers (S.G. Elias and A. Adams).

Meta-analysis

For pooling of study results, we used DerSimonian–
Laird random effects models to allow for between-study
heterogeneity. For imaging features reported as categori-
cal data, we estimated pooled odds ratios (pOR) to
describe the relation between that feature and the chance
of HER2 overexpression. All breast cancers not belong-
ing to the imaging feature category of interest served as
reference category in these analyses. For imaging fea-
tures reported as continuous data, we estimated pooled
mean differences between HER2-positive and -negative
breast cancer. We assessed between-study heterogeneity
by F statistics in combination with Cochran Q test for
heterogeneity [denoted as P(Q)], and evaluated the
impact of publication bias by inspecting funnel plot
asymmetry in combination with Egger tests. We used
forest plots to inspect individual study data and meta-
analysis results.

Besides pooling of data using all available studies, we
additionally performed meta-analyses in several prede-
fined study subgroups to evaluate possible sources of
between-study heterogeneity in the results: (i) excluding
studies with data on pure ductal carcinoma in situ (DCIS),
to focus on invasive cancer results only; (ii) excluding
studies selecting participants based on receptor expres-
sion (e.g., studies that excluded ER-positive breast can-
cer, thereby contrasting triple-negative to HER2 posi-
tive breast cancer) as this may threaten generalizability
of the results and/or might induce selection bias; (iii)
focusing on studies with a specific imaging acquisition
technique [analog vs. digital for mammography; studies
including 3T vs. only 1.5T field strength for MRI, as well
as higher spatial resolution MRI studies (i.e., sub-mm in
plane resolution and smaller than 2-mm slice
thickness)]; (iv) focusing on studies based on BI-RADS;
and (v) excluding studies for which we had to approx-
imate means and SDs.

We used R version 2.15.3 (R Foundation for Statistical
Computing, Vienna, Austria) for all analyses including
the packages rmeta and meta (10–12). We report pooled
estimates in combination with 95% confidence intervals.
(CI) and used two-sided P values of <0.05 for statistical significance. The report and conduct of this meta-analysis satisfies the PRISMA Statement (13).

Results

Literature search and study selection

Figure 1 shows an overview of the literature search and study selection process. The search yielded 1,673 unique articles, of which we excluded 1,469 based on title and abstract (83% because of non-original data, preclinical, or case reports), and 117 following full-text screening, of which 47 (40%) were (neo)adjuvant treatment studies, without correlation between imaging features and HER2 status. Reference cross-checking of the 87 eligible articles yielded seven additional articles not initially identified (2 non-indexed breast ultrasound articles, refs. 14, 15; 1 MRS, ref. 16; and 4 18F-FDG PET articles, refs. 17–20). We subsequently excluded five articles because of insufficient data (20–24), and four because of patient overlap (19, 25–27). Thus, we selected 85 articles for our review (14–18, 28–107), representing 81 unique patient populations, totaling

![Diagram](image-url)
Table 1. Characteristics of studies reporting on mammography, breast ultrasound, MRI, and $^{18}$F-FDG PET imaging features in relation to HER2 overexpression in breast cancer

<table>
<thead>
<tr>
<th>Ref., country, recruitment period</th>
<th>Patients</th>
<th>Breast cancers</th>
<th>HER2 assessment</th>
<th>Imaging acquisition and results</th>
<th>Readers</th>
<th>Imaging features described in review$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref., country, recruitment period</td>
<td>Recruitment</td>
<td>N</td>
<td>Age in years (range/SD)</td>
<td>N</td>
<td>Size in cm (range/SD)</td>
<td>Histology$^b$</td>
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<tr>
<td>Mamography</td>
<td>Agrawal et al. (28), Poland, 1992-2001</td>
<td>University hospital</td>
<td>184</td>
<td>57 (32-75)</td>
<td>184</td>
<td>NS (NS)</td>
</tr>
<tr>
<td></td>
<td>Aiello et al. (29), USA, 1988-1995</td>
<td>Breast cancer screening program</td>
<td>461</td>
<td>NS (NS)</td>
<td>461</td>
<td>NS (0.1-10)</td>
</tr>
<tr>
<td></td>
<td>Arora et al. (30), USA, 2005-2007</td>
<td>Cancer center; no prior breast cancer</td>
<td>1,323</td>
<td>56 (27-91)</td>
<td>1,323</td>
<td>1.5 (0.1-11)</td>
</tr>
<tr>
<td></td>
<td>Badra et al. (31), Greece, 1994-2004</td>
<td>University hospital; non-palpable screening-detected</td>
<td>75</td>
<td>56 (35-74)</td>
<td>75</td>
<td>NS (NS)</td>
</tr>
<tr>
<td></td>
<td>Cui et al. (32), China, 2009-2011</td>
<td>University hospital; triple-negative and triple-positive only</td>
<td>161</td>
<td>51 (27-80)</td>
<td>161</td>
<td>2.4 (NS)</td>
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<tr>
<td></td>
<td>Di Nubila et al. (33), Italy, 2000-2002</td>
<td>Cancer center; ≤ 35 y, no prior breast cancer</td>
<td>232</td>
<td>32 (23-30)</td>
<td>232</td>
<td>2.3 (NS)</td>
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<tr>
<td></td>
<td>Enache et al. (34), Romania, 2010-2011</td>
<td>General hospital; only ER$^c$</td>
<td>40</td>
<td>51 (25-88)</td>
<td>40</td>
<td>4.1 (0.6-10.7)</td>
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<tr>
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<td>Evans et al. (35), UK, 1978-1992</td>
<td>University hospital</td>
<td>126</td>
<td>NS (NS)</td>
<td>126</td>
<td>NS (NS)</td>
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<td></td>
<td>Facching et al. (36), Germany, 2001-2003</td>
<td>University hospital</td>
<td>423</td>
<td>60 (±1.3)</td>
<td>423</td>
<td>1.9 (1.4)</td>
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<td></td>
<td>Gajdos et al. (37), USA, 1993-1999</td>
<td>Tertiary care hospital</td>
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<td>59 (30-93)</td>
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<td>1.0 (0.1-3.0)</td>
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<td>Gu et al. (38), China, 2003-2006</td>
<td>University hospital</td>
<td>62</td>
<td>51 (31-79)</td>
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<td>NS (NS)</td>
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<td>Jiang et al. (39), China, 2008-2010</td>
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<td>63</td>
<td>49 (27-79)</td>
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<td></td>
<td>Kim et al. (40), South Korea, 2005-2008</td>
<td>Cancer center</td>
<td>151</td>
<td>46 (26-75)</td>
<td>151</td>
<td>NS (NS)</td>
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<tr>
<td></td>
<td>Ko et al. (41), South Korea, 2007-2008</td>
<td>Cancer center; only TN, ER$^+$ / HER2$^+$</td>
<td>239</td>
<td>52 (26-86)</td>
<td>239</td>
<td>2.3 (NS)</td>
</tr>
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</table>

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Table 1. Characteristics of studies reporting on mammography, breast ultrasound, MRI, and 18F-FDG PET imaging features in relation to HER2 overexpression in breast cancer (Cont’d)

<table>
<thead>
<tr>
<th>Ref., country, recruitment period</th>
<th>Recruitment</th>
<th>Age in years (range/SD)</th>
<th>N</th>
<th>Size in cm (range/SD)</th>
<th>Histology</th>
<th>HER2 assessment</th>
<th>Imaging acquisition and results</th>
<th>Imaging acquisition and results</th>
<th>Readers</th>
<th>Imaging features described in review</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuo et al. (42), Taiwan, 1999–2005</td>
<td>University hospital; stage IV disease, follow-up ≥ 1 y</td>
<td>998 57 (11)</td>
<td>998</td>
<td>NS (NS)</td>
<td>NOS</td>
<td>IHC BioGenex (CB11); FISH NOS</td>
<td>NS (NS)</td>
<td>NOS</td>
<td>1</td>
<td>NS</td>
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<tr>
<td>Li and Chen (43), China, 2005–2009</td>
<td>University hospital</td>
<td>110 52 (29–77)</td>
<td>110</td>
<td>NS (NS)</td>
<td></td>
<td>IHC NOS</td>
<td>49 (45)</td>
<td>NOS</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Ma et al. (44), USA, 1994–1998</td>
<td>Women’s Contraceptive and Reproductive Experiences Study</td>
<td>352 52 (29–77)</td>
<td>352</td>
<td>NS (NS)</td>
<td>NOS</td>
<td>IHC Dako (10H8)</td>
<td>62 (18)</td>
<td>NS</td>
<td>1</td>
<td>NS</td>
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<tr>
<td>Ma &amp;/C14 nsson et al. (45), Sweden, 1986–1995</td>
<td>Population-based; unifocal, ≤ 1.5 cm</td>
<td>361 60 (60)</td>
<td>361</td>
<td>NS (0.1–1.5)</td>
<td>NOS</td>
<td>IHC Dako (polyclonal)</td>
<td>142 (39)</td>
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<td>Yes</td>
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<td>Mun et al. (46), South Korea, 2007–2010</td>
<td>University hospital; screen-detected</td>
<td>213 50 (30–76)</td>
<td>213</td>
<td>2.4 (0.5–8.5)</td>
<td></td>
<td>IHC NOS; FISH NOS</td>
<td>77 (36)</td>
<td>NS</td>
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<tr>
<td>Palka et al. (47), Hungary, 2000–2003</td>
<td>University hospital; high-risk breast cancer adjuvant study</td>
<td>55 52 (33–70)</td>
<td>55</td>
<td>3.5 (NS)</td>
<td>NOS</td>
<td>IHC NOS</td>
<td>14 (25)</td>
<td>NS</td>
<td>1</td>
<td>Yes</td>
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<tr>
<td>Palka et al. (48), Hungary, 2004–2006</td>
<td>University hospital</td>
<td>560 58 (28–85)</td>
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<td>NS (NS)</td>
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<td>IHC NOS</td>
<td>85 (15)</td>
<td>NS</td>
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<td>Phipps et al. (49), USA, 1999–2008</td>
<td>BCSC; no history of breast cancer</td>
<td>7,281 NS (NS)</td>
<td>7,281</td>
<td>NS (NS)</td>
<td>IHC NOS; FISH NOS</td>
<td>172 (2)</td>
<td>NS</td>
<td>NOS</td>
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<td>Polan et al. (50), Spain, 1996–2005</td>
<td>Cancer Screening Program</td>
<td>834 NS (NS)</td>
<td>834</td>
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<td>111 (13)</td>
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<td>Seo et al. (51), USA, 2001–2003</td>
<td>University hospital</td>
<td>498 57 (24–95)</td>
<td>543</td>
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<td></td>
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<tr>
<td>Shin et al. (52), South Korea, 2005–2006</td>
<td>University hospital; node negative</td>
<td>710 49 (21–81)</td>
<td>715</td>
<td>NS (NS)</td>
<td></td>
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<td>165 (23)</td>
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<td>Sun et al. (53), China, 2004–2007</td>
<td>General hospital</td>
<td>144 49 (23–83)</td>
<td>144</td>
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<td></td>
<td>IHC Beijing Zhongshan Biotechnology (NS)</td>
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<td>Taneja et al. (54), UK, 1996–1997</td>
<td>University hospital</td>
<td>415 59 (27–70)</td>
<td>415</td>
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<td></td>
<td>IHC Dako (NS)</td>
<td>132 (32)</td>
<td>NS</td>
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<tr>
<td>Wang et al. (55), China, 2003–2006</td>
<td>University hospital</td>
<td>152 57 (27–82)</td>
<td>152</td>
<td>NS (NS)</td>
<td></td>
<td>IHC Dako (K2024)</td>
<td>73 (48)</td>
<td>NS</td>
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</table>

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Table 1. Characteristics of studies reporting on mammography, breast ultrasound, MRI, and 18F-FDG PET imaging features in relation to HER2 overexpression in breast cancer (Cont’d)

<table>
<thead>
<tr>
<th>Ref., country, recruitment period</th>
<th>Patients</th>
<th>Imaging acquisition and results</th>
<th>HER2 assessment</th>
<th>Histology</th>
<th>Positive</th>
<th>Imaging system (manufacturer)</th>
<th>Readers</th>
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<tr>
<td>Wang et al. (56), USA, 1997–2003</td>
<td>University hospital; only ER+</td>
<td>University hospital; only ER+</td>
<td>University hospital; only ER+</td>
<td>University hospital; only ER+</td>
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<td>General hospital</td>
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<tr>
<td>Wen et al. (58), China, 2010</td>
<td>University hospital</td>
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<tr>
<td>Yang et al. (60), USA, 1999–2005</td>
<td>Cancer center; premenopausal</td>
<td>Cancer center; premenopausal</td>
<td>Cancer center; premenopausal</td>
<td>Cancer center; premenopausal</td>
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<td>Au-Yong et al. (61), UK, 1996–1997</td>
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<td>Chen et al. (62), Taiwan, 2005–2009</td>
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<tr>
<td>Di Nublia et al. (33), Italy, 2000–2002</td>
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<td>University hospital</td>
<td>University hospital</td>
<td>University hospital</td>
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<tr>
<td>Kim et al. (63), South Korea, 2002–2006</td>
<td>Cancer center; only TN, ER+/PR+/HER2+</td>
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<thead>
<tr>
<th>Ref. country, recruitment period</th>
<th>Patients</th>
<th>Breast cancers</th>
<th>HER2 assessment</th>
<th>Imaging acquisition and results</th>
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<tr>
<td>Pang et al. (64), China, 2007-2008</td>
<td>University hospital</td>
<td>207</td>
<td>49° (25-79)</td>
<td>15.0 MHz</td>
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<tr>
<td>Shin et al. (62), South Korea, 2005-2006</td>
<td>University hospital; node negative</td>
<td>710</td>
<td>49° (21-81)</td>
<td>5.0 MHz</td>
</tr>
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<td>Wan et al. (65), USA, 1997-2003</td>
<td>University hospital; only ER\textsuperscript{+}</td>
<td>65 (30)</td>
<td>HDI-5000; IU-22 (Philips)</td>
<td>NS; NS</td>
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<tr>
<td>Pang et al. (64), China, 2007-2008</td>
<td>University hospital</td>
<td>151</td>
<td>1.0 (0.5-1.5)</td>
<td>1.5T MAGNETOM Verio (Siemens)</td>
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<tr>
<td>Agrawal et al. (66), USA, 2003-2005</td>
<td>University hospital</td>
<td>50</td>
<td>4.5° (1.7-11.8)</td>
<td>3T Achieva (Philips)</td>
</tr>
<tr>
<td>Bae et al. (67), USA, 2006-2006</td>
<td>University hospital; 1.5 cm only</td>
<td>66</td>
<td>51° (32-76)</td>
<td>1.5T MAGNETOM Sonata (Siemens)</td>
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<tr>
<td>Balzer et al. (68), Germany, 2005-2006</td>
<td>University hospital</td>
<td>128</td>
<td>60° (30-82)</td>
<td>1.5T MAGNETOM Symphony (Siemens)</td>
</tr>
<tr>
<td>Chang et al. (69), South Korea, 2004-2005</td>
<td>University hospital</td>
<td>136</td>
<td>48° (26-75)</td>
<td>1.5T MAGNETOM (Siemens)</td>
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<tr>
<td>Chen et al. (70), USA, 2006-2010</td>
<td>University hospital; neoadjuvant therapy</td>
<td>50</td>
<td>49° (28-82)</td>
<td>1.5T MAGNETOM Sonata (Siemens)</td>
</tr>
<tr>
<td>Choi et al. (71), South Korea, 2008-2010</td>
<td>University hospital</td>
<td>117</td>
<td>52° (29-81)</td>
<td>1.5T MAGNETOM Sonata (Siemens)</td>
</tr>
<tr>
<td>Costrini et al. (72), Italy, 2007-2011</td>
<td>University hospital</td>
<td>225</td>
<td>53° (12)</td>
<td>1.5T MAGNETOM (Siemens)</td>
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<tr>
<td>Fernández-Guinea et al. (73), Spain, 1999-2006</td>
<td>General hospital</td>
<td>68</td>
<td>56° (30-83)</td>
<td>1.5T MAGNETOM Symphony (Siemens)</td>
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<tr>
<td>Girardi et al. (74), Italy, 2006-2008</td>
<td>University hospital</td>
<td>72</td>
<td>54° (33-87)</td>
<td>1.5T MAGNETOM Symphony (Siemens)</td>
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</table>

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### Table 1. Characteristics of studies reporting on mammography, breast ultrasound, MRI, and $^{18}$F-FDG PET imaging features in relation to HER2 overexpression in breast cancer (Cont’d)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Recruitment Period</th>
<th>Breast Cancers</th>
<th>HER2 Assessment</th>
<th>Imaging Acquisition and Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gomez-Raposo et al. (74),</td>
<td>University hospital; neoadjuvant therapy</td>
<td>24 NS (NS)</td>
<td>4.4 (2.4-6.4)</td>
<td>3T Achieva (Philips); NS; NS; DCE</td>
</tr>
<tr>
<td>Spain, 2008-2011</td>
<td></td>
<td>24</td>
<td></td>
<td>NS; Blinded</td>
</tr>
<tr>
<td>Lee et al. (79),</td>
<td>University hospital; neoadjuvant therapy</td>
<td>194 NS (NS)</td>
<td>2.2 (0.1-3.5)</td>
<td>1.5T Signa (Philips); Tumor blood flow</td>
</tr>
<tr>
<td>South Korea, 2008-2009</td>
<td></td>
<td>194 NS (NS)</td>
<td></td>
<td>Blinded</td>
</tr>
<tr>
<td>Montemurro et al. (86),</td>
<td>University hospital; mammography occult-axillary metastasis</td>
<td>57 NS (NS)</td>
<td>2.0 (0.7-12.0)</td>
<td>1.5T Intera (Philips); Tumor blood flow</td>
</tr>
<tr>
<td>Italy, 2003-2005</td>
<td></td>
<td>57</td>
<td></td>
<td>Blinded</td>
</tr>
<tr>
<td>Montemurro et al. (86),</td>
<td>University hospital; mammography occult-axillary metastasis</td>
<td>75 NS (NS)</td>
<td>3.6 (0.7-12.0)</td>
<td>1.5T NOS (GE); NS; Blinded</td>
</tr>
<tr>
<td>Italy, 2003-2005</td>
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<td>75</td>
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### Table 1. Characteristics of studies reporting on mammography, breast ultrasound, MRI, and $^{18}$F-FDG PET imaging features in relation to HER2 overexpression in breast cancer (Cont’d)

<table>
<thead>
<tr>
<th>Ref., country, recruitment period</th>
<th>Patients</th>
<th>Breast cancers</th>
<th>HER2 assessment</th>
<th>Imaging acquisition and results</th>
<th>Readers</th>
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<tbody>
<tr>
<td>Recruitmen</td>
<td>N</td>
<td>Age in years (range/SD)</td>
<td>Size in cm (range/SD)</td>
<td>Histology*</td>
<td>Positive</td>
</tr>
<tr>
<td>N scrolls (87), Japan, 2007–2008</td>
<td>General hospital</td>
<td>35</td>
<td>58f (35–77)</td>
<td>35</td>
<td>1.6f (0.5–3.0)</td>
</tr>
<tr>
<td>Nakajo et al. (87),*</td>
<td>General hospital</td>
<td>35</td>
<td>58f (35–77)</td>
<td>35</td>
<td>1.6f (0.5–3.0)</td>
</tr>
<tr>
<td>Sah et al. (88), India, 2007–2011</td>
<td>University hospital</td>
<td>47</td>
<td>46f (35–75)</td>
<td>47</td>
<td>NS (NS)</td>
</tr>
<tr>
<td>Shin et al. (89), South Korea, 2008–2010</td>
<td>University hospital; ≥1 cm only</td>
<td>184</td>
<td>48f (28–72)</td>
<td>184</td>
<td>3.5f (1.0–10.0)</td>
</tr>
<tr>
<td>Szabó et al. (90), Sweden, NS</td>
<td>University hospital</td>
<td>61</td>
<td>47f (34–78)</td>
<td>60</td>
<td>2.4f (0.3–7.0)</td>
</tr>
<tr>
<td>Tozaki and Hiroi (91), Japan, 2008–2009</td>
<td>General hospital; ≥1.5–3.0 cm only</td>
<td>50</td>
<td>52f (30–80)</td>
<td>50</td>
<td>2.3f (1.5–3.0)</td>
</tr>
<tr>
<td>Youk et al. (92), South Korea, 2009–2011</td>
<td>University hospital</td>
<td>269</td>
<td>50f (26–83)</td>
<td>271</td>
<td>2.4f (0.5–10.0)</td>
</tr>
<tr>
<td>FDG-PET</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Choi et al. (70), South Korea, 2008–2010</td>
<td>University hospital</td>
<td>117</td>
<td>52f (29–81)</td>
<td>117</td>
<td>2.8f (2.2)</td>
</tr>
<tr>
<td>Garcia Vianello et al. (93), Spain, NS</td>
<td>General hospital; neoadjuvant therapy</td>
<td>60</td>
<td>53f (11.7)</td>
<td>60</td>
<td>4.3f (2.1)</td>
</tr>
<tr>
<td>Gi-Rendo et al. (17), Spain, 2000–2004</td>
<td>University hospital</td>
<td>251</td>
<td>51f (24–87)</td>
<td>251</td>
<td>2.3f (0.3–4.9)</td>
</tr>
<tr>
<td>Groheux et al. (94), France, 2006–2009</td>
<td>General hospital; only T2, T3 or T4</td>
<td>130</td>
<td>50f (30–81)</td>
<td>130</td>
<td>3.1f (2.1–14.0)</td>
</tr>
<tr>
<td>Heudelet et al. (95), France, 2008–2009</td>
<td>Cancer center</td>
<td>45</td>
<td>50f (26–85)</td>
<td>45</td>
<td>2.5f (0.8–9.0)</td>
</tr>
<tr>
<td>Humbert et al. (96), France, NS</td>
<td>Cancer center; large or locally advanced</td>
<td>115</td>
<td>51f (NS)</td>
<td>115</td>
<td>NS (NS)</td>
</tr>
</tbody>
</table>

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Table 1. Characteristics of studies reporting on mammography, breast ultrasound, MRI, and $^{18}$F-FDG PET imaging features in relation to HER2 overexpression in breast cancer (Cont’d)

<table>
<thead>
<tr>
<th>Ref., country, recruitment period</th>
<th>Patients</th>
<th>Ref.</th>
<th>General hospital</th>
<th>Recruitment</th>
<th>N</th>
<th>Age in years (range/SD)</th>
<th>Histology</th>
<th>HER2 assessment</th>
<th>Imaging features described in review&lt;sup&gt;1,8,9&lt;/sup&gt;</th>
<th>Readers</th>
<th>Imaging acquisition and results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iliea et al. (96), Japan, 2004-2005</td>
<td>General hospital</td>
<td>47</td>
<td>63&lt;sup&gt;a&lt;/sup&gt; (43-87)</td>
<td>47</td>
<td>2.1&lt;sup&gt;0&lt;/sup&gt; (0.9-11.9)</td>
<td>IDC Else DCIS</td>
<td>IHC Dako (HerceptTest)</td>
<td>□ □</td>
<td>14 (30)</td>
<td>Discovery LS (GE)</td>
<td>FOV; slice; 4.25 mm; res; 5.4 mm; fasting; 6 h; time; 55-60 min after 3.7 MBq/kg</td>
</tr>
<tr>
<td>Jin et al. (97), South Korea, 2006-2010</td>
<td>University hospital; neoadjuvant therapy no trastuzumab</td>
<td>273</td>
<td>44&lt;sup&gt;a&lt;/sup&gt; (23-78)</td>
<td>273</td>
<td>4.9&lt;sup&gt;a&lt;/sup&gt; (0.9-15.1)</td>
<td>IDC Else DCIS</td>
<td>IHC NOS</td>
<td>□ □</td>
<td>72 (26)</td>
<td>Discovery STE (GE); Biograph 16 or Biograph Truepoint 40 (Siemens)</td>
<td>FOV; slice; NS; res; 5.4 mm; fasting; 6 h; time; 50-60 min after 3.7 MBq/kg</td>
</tr>
<tr>
<td>Keam et al. (98), South Korea, 2006-2008</td>
<td>University hospital; neoadjuvant phase II trial</td>
<td>78</td>
<td>45&lt;sup&gt;a&lt;/sup&gt; (26-69)</td>
<td>78</td>
<td>4.5&lt;sup&gt;a&lt;/sup&gt; (2.0-11.0)</td>
<td>IDC Else DCIS</td>
<td>IHC NOS; FISH NOS</td>
<td>□ □</td>
<td>17 (22)</td>
<td>Gemini (Philips)</td>
<td>FOV; slice; NS; res; 5.4 mm; fasting; 6 h; time; 50-60 min after 3.7 MBq/kg</td>
</tr>
<tr>
<td>Kim et al. (99), South Korea, 2009-2010</td>
<td>University hospital; ≥1.0 cm</td>
<td>91</td>
<td>49&lt;sup&gt;a&lt;/sup&gt; (26-81)</td>
<td>91</td>
<td>2.2&lt;sup&gt;a&lt;/sup&gt; (1.0-6.5)</td>
<td>IDC Else DCIS</td>
<td>IHC Dako (NS); FISH NOS</td>
<td>□ □</td>
<td>17 (19)</td>
<td>Allegro (Philips)</td>
<td>FOV; slice; NS; res; 5.4 mm; fasting; 6 h; time; 50-60 min after 3.7 MBq/kg</td>
</tr>
<tr>
<td>Koelen et al. (100), the Netherlands, 2006-2011</td>
<td>Cancer center; neoadjuvant;&gt;3.0 cm and/or positive node</td>
<td>214</td>
<td>50&lt;sup&gt;a&lt;/sup&gt; (41-67)</td>
<td>214</td>
<td>NS (NS)</td>
<td>IDC Else DCIS</td>
<td>IHC NOS; CISH NOS</td>
<td>□ □</td>
<td>53 (25)</td>
<td>Gemini TF (Philips)</td>
<td>FOV; slice; NS; res; 5.4 mm; fasting; 6 h; time; 60 min; dose; 180-240 MBq</td>
</tr>
<tr>
<td>Main et al. (101), USA, NS</td>
<td>University hospital; &gt;0.5 cm</td>
<td>116</td>
<td>51&lt;sup&gt;a&lt;/sup&gt; (24-80)</td>
<td>116</td>
<td>NS (0.5-NS)</td>
<td>IDC Else DCIS</td>
<td>IHC Dako (HerceptTest)</td>
<td>□ □</td>
<td>20 (17)</td>
<td>Allegro (Philips)</td>
<td>FOV; slice; NS; res; 5.4 mm; fasting; 6 h; time; 60 min; dose; 2.5-5.2 MBq/kg</td>
</tr>
<tr>
<td>Nakspo et al. (97), Japan, 2007-2008</td>
<td>General hospital; &gt;5 mm</td>
<td>35</td>
<td>58&lt;sup&gt;a&lt;/sup&gt; (35-77)</td>
<td>35</td>
<td>1.6&lt;sup&gt;a&lt;/sup&gt; (0.5-3.0)</td>
<td>IDC Else DCIS</td>
<td>IHC Dako (HerceptTest)</td>
<td>□ □</td>
<td>7 (20)</td>
<td>Discovery STE (GE)</td>
<td>FOV; slice; NS; res; 5.1 mm; fasting; 6 h; time; 60 min; dose; 3.7 MBq/kg</td>
</tr>
<tr>
<td>Osborne et al. (102), USA, 2001-2004</td>
<td>Cancer center; &gt;6.0 cm and/or positive node</td>
<td>32</td>
<td>50&lt;sup&gt;a&lt;/sup&gt; (28-72)</td>
<td>32</td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt; (2.8-6.4)</td>
<td>IDC Else DCIS</td>
<td>IHC NOS; FISH NOS</td>
<td>□ □</td>
<td>13 (41)</td>
<td>Advance or Discovery LS (GE); Biograph (Siemens)</td>
<td>FOV; slice; NS; res; 5.1 mm; fasting; 6 h; time; 60 min; dose; 3.7 MBq/kg</td>
</tr>
<tr>
<td>Sanli et al. (103), Turkey, 2009-2011</td>
<td>University hospital</td>
<td>79</td>
<td>53&lt;sup&gt;a&lt;/sup&gt; (21-81)</td>
<td>79</td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt; (0.7-10.0)</td>
<td>IDC Else DCIS</td>
<td>IHC Thermoscientific (SP5)</td>
<td>□ □</td>
<td>28 (35)</td>
<td>NS</td>
<td>FOV; slice; NS; res; 5.4 mm; fasting; 6 h; time; 60 min; dose; 3.7 MBq/kg</td>
</tr>
<tr>
<td>Strover et al. (104), the Netherlands, 2007, NS</td>
<td>Cancer center; &gt;3.0 cm and/or positive node</td>
<td>37</td>
<td>49&lt;sup&gt;a&lt;/sup&gt; (30-68)</td>
<td>37</td>
<td>NS (NS)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>11 (30)</td>
<td>Gemini TF (Philips)</td>
<td>FOV; slice; NS; res; 5.4 mm; fasting; 6 h; time; 60 min; dose; 180-240 MBq</td>
</tr>
<tr>
<td>Tozaki and Hoshi (91), Japan, 2009-2009</td>
<td>General hospital; ≥1.5-3.0 cm only</td>
<td>50</td>
<td>52&lt;sup&gt;a&lt;/sup&gt; (30-86)</td>
<td>50</td>
<td>2.2&lt;sup&gt;a&lt;/sup&gt; (1.5-3.0)</td>
<td>IDC Else DCIS</td>
<td>IHC Dako (NS); FISH (PathVysion)</td>
<td>□ □</td>
<td>6 (12)</td>
<td>Discovery ST (GE)</td>
<td>FOV; 15.7-cm axial; slice; 3.3 mm; res; NS; fasting; NS; time; NS; dose; 4.3 MBq/kg</td>
</tr>
</tbody>
</table>

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Table 1. Characteristics of studies reporting on mammography, breast ultrasound, MRI, and 18F-FDG PET imaging features in relation to HER2 overexpression in breast cancer (Cont’d)

<table>
<thead>
<tr>
<th>Patients</th>
<th>Breast cancers</th>
<th>HER2 assessment</th>
<th>Imaging acquisition and results</th>
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<tr>
<td></td>
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<td>Imaging features</td>
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<td>Ret., country, recruitment period</td>
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<td>Age in years (range/SD)</td>
<td>NS</td>
</tr>
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<td>Ueda et al. (105), Japan, 2005 – 2007</td>
<td>145</td>
<td>54 a (34–81)</td>
<td>NS</td>
</tr>
<tr>
<td>Ueda et al. (106), Japan, 2007 – 2008</td>
<td>11</td>
<td>74 a (39–90)</td>
<td>NS</td>
</tr>
<tr>
<td>Wang et al. (107), USA, 2007 – 2009</td>
<td>98</td>
<td>53 a (13)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Notes:**
- **Imaging features**
  - ArDi: Architectural distortion
  - BrDe: Breast density
  - Cal: Calcifications
  - Dis: Distribution
  - FoAs: Focal asymmetry
  - Mar: Margin of mass
  - Mas: Mass
  - Mor: Morphology
  - Oth: Other
  - Sha: Shape of mass
  - SkTh: Skin thickening
  - Features annotation mammography: ArDi, Architectural distortion; BrDe, Breast density; Cal, Calcification; Dis, Distribution; FoAs, Focal asymmetry; Mar, Margin of mass; Mas, Mass; Mor, Morphology; Oth, Other; Sha, Shape of mass.
  - Features annotation breast ultrasound: Cal, Calcification; EcPa, Echo pattern of mass; Hal, Echogenic halo as lesion boundary; Mas, Mass; Ori, Orientation of mass; PAF, Posterior acoustic features of mass; Sha, Shape of mass.
  - Features annotation MRI: ADC, Apparent diffusion coefficient; Cho, Choline peak; EnRa, Enhancement ratio; Fis, Fisher score; InEn, Internal enhancement; KiCu, Kinetic curve; Mar, Margin of mass; Mas, Mass; MuFo, Multifocality; Oth, Other; Sha, Shape of mass; TtP, Time to peak enhancement; T2Si, T2 signal intensity.

**Abbreviations:**
- BCSC, Breast Cancer Surveillance Consortium
- CISH, Chromogenic in situ hybridization
- FOV, Field of view
- GE, General Electric
- IDC, Invasive ductal carcinoma
- IMS, Internazionale Medico Scientifica
- N, number
- NOS, Not otherwise specified
- NS, Not stated
- PR, Progesterone receptor
- TN, Triple-negative
- UK, United Kingdom
- USA, United States of America

**Symbols:**
- *, Present
- #, Absent
- #&, IHC1 +
- &&, IHC2 +
- &&&, IHC3 +
- **, IHC3 + or (IHC2 + and FISH +).

**Mean:**
- $\bar{x}$

**Median:**
- $\tilde{x}$

**Interquartile range:**
- IQR

**Underlined features are according to BI-RADS.**
23,159 patients with 23,255 breast cancers including 4,213 HER2-positives. Four populations gave rise to two separate publications on different imaging features and/or modalities (16, 54, 61, 66, 70, 75, 81, 104). A total of 33 articles reported on mammography (28–60), 12 on breast ultrasound (14, 15, 28, 33, 41, 52, 56, 61–65), 28 on MRI (16, 66–92), and 20 on 18F-FDG PET (17, 18, 70, 87, 91, 93–107). Of these, eight articles reported on two imaging modalities: five on both breast ultrasound and mammography (28, 33, 41, 52, 56), and three on both 18F-FDG PET and DWI (70, 87), or MRS (91). Table 1 shows the characteristics of the selected studies. Most articles exclusively studied invasive breast cancer (67%), followed by both invasive breast cancer and DCIS (25%). A few studies exclusively studied DCIS (7%) or were not clear whether they studied only invasive cancer, DCIS, or both (1%). The threshold for HER2 overexpression was variable between studies, with 32% using the established clinical standard [i.e., immunohistochemistry (IHC) result of 3+, or IHC result of 2+ with gene-amplification], 27% using only IHC 3+, 29% using another threshold/method, and 12% not reporting this item. Studies reporting on MRI or 18F-FDG PET more often used the clinical standard (39% and 40%, respectively) than mammography (21%) or breast ultrasound articles (25%). Whether image assessment was blinded for HER2 status was not reported in 60% of articles. In 8% of the articles, a specific breast cancer molecular subtype (predominantly ER-positive breast cancer) was excluded in their analysis (2 MRI, 2 ultrasound, and 5 mammography studies). Study results were based on BI-RADS in 64% of mammography, 36% of ultrasound, and 65% of MRI studies (excluding studies focused on non-BI-RADS imaging features such as apparent diffusion coefficient).

The results of the meta-analyses are given below. A narrative review of imaging features that were assessed in relation to HER2 overexpression in individual studies but that could not be meta-analyzed as data were too limited can be found in the Supplementary Results. Forest plots of all individual meta-analysis results are shown in Supplementary Figs. S1–S67.

**Mammography imaging features in relation to HER2 overexpression in breast cancer**

**Study characteristics.** The 33 articles reporting on mammography features included a total of 17,745 breast cancers (40–7,281 per study), of which 2,559 were HER2-positive (14%; range, 2%–54%). The average or median reported patient age per study varied between 32 and 63 years, and tumor size between 1.0 and 4.1 cm, although the majority did not report size. Four studies investigated only DCIS, and 19 only invasive cancer. Seven studies reported to exclusively use digital mammography, four studies both analog and digital, and 14 only analog mammography. Five studies preselected patients based on receptor expression (Table 1).

**Meta-analysis.** Figure 2 shows the meta-analysis results of the evaluated mammography imaging features. Five features were significantly associated with HER2 overexpression, and two reached borderline significance. The presence of microcalcifications strongly increased the chance of HER2 overexpression (pOR, 3.14; 95% CI, 2.46–4.00; P < 0.001; 23 studies), which was not dependent on whether or not a mass was associated with such microcalcifications. When microcalcifications were present, especially branching or fine linear morphology increased the chance of HER2 overexpression (POR, 2.11; 95% CI, 1.07–4.14; P = 0.03; 6 studies), but distribution patterns of microcalcifications were not associated. Breast density also increased the chance of HER2 overexpression. In particular, BI-RADS breast density category 4 (extremely dense) showed a pOR of 1.37 (95% CI, 1.07–1.76; P = 0.01; 9 studies of which 3 were follow-up studies). Finally, a mammographic high suspicion for malignancy increased the chance of HER2 overexpression (pOR, 1.95; 95% CI, 1.22–3.10; P < 0.01; 3 studies), but circumscribed mass margins decreased the chance of HER2 overexpression by 63% (pOR, 0.37; 95% CI, 0.14–0.94; P = 0.04; 8 studies). The presence of a mass decreased the chance of HER2 overexpression with borderline significance (pOR, 0.67; 95% CI, 0.44–1.01; P = 0.06; 13 studies), and skin thickening borderline significantly increased its chance (pOR, 1.49; 95% CI, 0.95–2.34; P = 0.08; 2 studies).

**Sources of heterogeneity in meta-analysis results.** Of above features, the presence of a mass, microcalcifications, and circumscribed margins showed marked between-study heterogeneity. Restriction of the meta-analysis to studies including invasive cancers only did not explain this heterogeneity and results were largely similar, although the finding for branching or fine linear microcalcifications did not hold anymore (pOR, 0.58; 95% CI, 0.05–7.07; P = 0.67; 3 studies), and round/oval mass shape now decreased the chance of HER2 overexpression (pOR, 0.47; 95% CI, 0.28–0.78; P < 0.01; 4 studies). After excluding studies that preselected on receptor expression, findings were predominantly similar to the overall results, but circumscribed mass margins were no longer related to HER2 overexpression (pOR, 0.66; 95% CI, 0.24–1.84; P = 0.43; 4 studies), thus partly explaining the between-study heterogeneity. In turn, indistinct mass margins became more strongly associated with HER2 overexpression (pOR, 1.48; 95% CI, 1.00–2.20; P = 0.05; 5 studies). When we considered studies using only digital or only analog mammography, findings were also similar to the overall results. Studies based on BI-RADS confirmed the results for microcalcifications, breast density, and level of suspicion, but the associations for the presence of a mass, circumscribed margins, and branching or fine linear calcification morphology became weaker (Supplementary Figs. S68–S72).

**Breast ultrasound imaging features in relation to HER2 overexpression in breast cancer**

**Study characteristics.** The 12 articles reporting on breast ultrasound features included a total of 2,741 breast
cancers (32–715 per study), of which 944 were HER2-positive (34%; range, 23%–82%). The average or median reported patient age per study varied between 32 and 57 years, and tumor size between 2.1 and 2.6 cm (half did not report size). Nine studies investigated invasive cancer only, the remainder invasive cancer in combination with DCIS. Two studies preselected on receptor expression (Table 1).

### Meta-analysis

Figure 3 shows the breast ultrasound meta-analysis results. Three features were significantly associated with HER2 overexpression, and three reached borderline significance. The presence of a mass on ultrasound decreased the chance of HER2 overexpression (pOR, 0.40; 95% CI, 0.23–0.69; P < 0.001; 4 studies), and the presence of microcalcifications increased its chance (pOR, 2.45; 95% CI, 1.27–4.72; P < 0.01; 2 studies), also

---

<table>
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<th>Imaging feature</th>
<th>No. of studies</th>
<th>No. of cancers</th>
<th>No. of HER2+</th>
<th>DerSimonian–Laird random effects model</th>
<th>Heterogeneity</th>
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<td>Round or oval</td>
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<td>1,093</td>
<td>249</td>
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<td>Lobular</td>
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<td>Regional</td>
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<td>10,440</td>
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<td>Level of suspicion for malignancy</td>
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<td>1,414</td>
<td>320</td>
<td>1.96 (1.22–3.10)</td>
<td>&lt; 0.01*</td>
<td>0.39 0.19</td>
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</table>

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**Risk of HER2 overexpression in breast cancer**

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Figure 2. Meta-analysis of mammography imaging features in relation to HER2 overexpression in breast cancer. The respective reference categories consist of all breast cancers within each study not belonging to the category of interest. The Egger test for publication bias can only be performed if more than two studies are available for meta-analysis. If less than two studies reported on a particular feature, no meta-analysis can be performed. Statistical significance (i.e., two-sided P < 0.05) is marked with *. Error bars depict 95% CIs.
when associated with a mass (pOR, 2.95; 95% CI, 2.34–3.71; P < 0.001; 7 studies). High suspicion for malignancy on ultrasound significantly increased the chance of HER2 overexpression (pOR, 5.21; 95% CI, 1.14–23.73; P = 0.03; 2 studies). Irregular shaped masses increased the chance of HER2 overexpression with borderline significance (pOR, 1.26; 95% CI, 0.99–1.60; P = 0.06; 5 studies), as did hypo-echoic echo patterns (pOR, 2.13; 95% CI, 0.91–4.99; P = 0.08; 3 studies). Circumscribed mass margins also showed a trend toward a decreased chance of HER2 overexpression (pOR, 0.66; 95% CI, 0.43–1.02; P = 0.06; 4 studies).

**Sources of heterogeneity in meta-analysis results.** Restricting the meta-analysis to studies that only investigated invasive cancer did not materially change the overall results. Excluding two studies that preselected on receptor expression also did not change the results substantially, albeit the finding for circumscribed margins became less pronounced (pOR, 0.80; 95% CI, 0.50–1.27; P = 0.33; 2 studies). Studies based on BI-RADS confirmed the overall results for the presence of a mass and microcalcifications. pORs for circumscribed margin and hypo-echoic echo pattern were similar but did not reach statistical significance in this subgroup, but irregular shape became significant (pOR, 1.31; 95% CI, 1.02–1.69; P = 0.04; 4 studies; Supplementary Figs. S73–S75).

**MRI features in relation to HER2 overexpression in breast cancer**

**Study characteristics.** The 28 articles reporting on MRI features included a total of 2,783 breast cancers (17–271 per study), of which 766 were HER2-positive (28%; range, 12%–71%). The average or median reported patient age per study varied between 46 and 60 years, and tumor size between 1.5 and 5.0 cm. Two studies investigated DCIS only and 21 studies only invasive cancers.
Two studies exclusively used 3T, five both 1.5 and 3T, and 20 exclusively 1.5T systems, and one study did not provide this information. All studies used dedicated breast coils, and none preselected on molecular subtype (Table 1). Dynamic contrast enhancement (DCE)-MRI imaging protocols are shown in Supplementary Table S4.

**Meta-analysis.** Figure 4 shows the DCE-MRI meta-analysis results. Overall, two DCE-MRI imaging features were significantly related to HER2 overexpression, and three showed borderline statistical significance. Fast initial kinetics showed a strong positive association with HER2 overexpression (i.e., peak enhancement at first scan: pOR, 2.60; 95% CI, 1.43–4.73; \( P < 0.01 \); 5 studies), followed by washout-type kinetic curves (pOR, 1.57; 95% CI, 1.11–2.17; \( P = 0.01 \); 12 studies). In line with the latter, persistent-type kinetic curves showed a trend toward a lower chance of HER2 overexpression (pOR, 0.60; 95% CI, 0.34–1.06; \( P = 0.08 \); 9 studies). Four other articles reported on kinetic data as well, but analyzed and presented these data in such a way that we were not able to match these with descriptors used in other studies, which prevented us from including these results in the meta-analysis. Baltzer and colleagues used a semi-automated method for kinetic curve assessment and did not find an association between HER2 status and initial enhancement, washout rate, peak enhancement, or time to peak, nor between HER2 status and percentage persistent, plateau, washout, or strong enhancement (67). Kim and colleagues did not find a relation between HER2 status and initial slope, peak enhancement, time to peak enhancement, or washout ratio (76). Lee and colleagues found no relation between initial slope and HER2 status (79), and Youk and colleagues found no relation between early-phase percentage enhancement and HER2 status (92). Again following
18F-FDG uptake in relation to HER2 overexpression in breast cancer

Study characteristics. The 20 articles reporting on 18F-FDG uptake included a total of 2,027 breast cancers (12-273 per study), of which 567 were HER2-positive (28%; range, 12%-46%). The average or median reported patient age per study varied between 44 to 74 years, and tumor size between 1.6 and 5.1 cm. No study included only DCIS, five included invasive cancer and DCIS, and 15 only invasive cancer. Two studies selected on receptor expression: one study only included ER-positive breast cancer (106), and one excluded patients with HER2-positive disease who received trastuzumab within a neoadjuvant setting (97). All studies used whole-body PET or PET/CT, except one that used dedicated positron emission mammography (PEM; Table 1; ref. 107).

Meta-analysis. After mean and SD approximation for eight studies (18, 70, 87, 94, 100, 102–104), we first meta-analyzed all 20 18F-FDG PET studies. A higher mean maximum standardized uptake value (SUV_max) in HER2-positive compared with HER2-negative breast cancer was found (pooled random effects mean difference, 0.36; 95% CI, −0.24–0.96; P = 0.24), with substantial between-study heterogeneity [I^2 = 0.47; P(Q) = 0.011]. Meta-analysis of 12 studies that straightforwardly reported means and SDs did increase the observed effect slightly (pooled random effects mean difference, 0.45; 95% CI, −0.29–1.19; P = 0.23), but still with substantial heterogeneity [I^2 = 0.45; P(Q) = 0.047]. A similar effect was observed when restricting the studies to the 18 without receptor expression selection [mean SUV_max, difference, 0.53; 95% CI, −0.08–1.14; P = 0.09; I^2 = 0.42; P(Q) = 0.034].

Meta-analysis of 10 studies reporting means and SDs and without preselection on receptor expression, likely representing the best evidence, resulted in a substantial decrease of between-study heterogeneity [I^2 = 0.20; P(Q) = 0.26], and showed that HER2-positive breast cancer has a statistically significant 0.76 higher mean SUV_max than HER2-negative breast cancer (95% CI, 0.10–1.42; P = 0.025; refs. 17, 91, 93, 95, 96, 98, 99, 101, 105, 107).

Publication bias
Visual inspection of funnel plot asymmetry in combination with Egger tests generally led to a low suspicion for publication bias, albeit the number of studies was sometimes too low for proper evaluation (Supplementary Figs. S81–S147).

Discussion
We comprehensively reviewed the literature relating imaging features to HER2 overexpression in breast cancer, and pooled the results for 68 imaging features using data from 23,255 breast cancers including 4,213 HER2-positive cancers. We found 11 imaging features to be significantly related to HER2 overexpression following meta-analysis: the presence of microcalcifications on mammography or ultrasound, branching or fine linear microcalcification morphology or extremely dense breast tissue on mammography, high overall suspicion for malignancy on mammography or ultrasound, washout and fast initial kinetics on DCE-MRI, and higher 18F-FDG uptake were all associated with increased chance of HER2 overexpression, whereas the presence of a mass on ultrasound and circumscribed mass margins on mammography significantly decreased that chance.

Which exact pathophysiological mechanisms may underlie these observations is speculative. The presence of microcalcifications indicating HER2 overexpression could be explained by the fact that DCIS, which may be a component of invasive cancer, is frequently HER2-positive and often shows microcalcifications (108–110). Alternatively, poorly differentiated tumors more often show central necrosis and rapid growth, resulting in deposition of microcalcifications along the ductal structures, and the finding could thus also be a reflection of the more aggressive nature of HER2-positive invasive cancers (109). Correlation between the presence of a mass (significant on ultrasound but also corroborated by the mammography and MRI findings) and decreased chance of HER2 overexpression is also likely a reflection of DCIS (less often presenting as a mass and more frequently HER2-positive; ref. 108). The aggressive nature of HER2-positive cancers in terms of invasiveness, cell mobility, and increased neangiogenesis (7) may further explain the relation with rapid kinetics and increased glucose metabolism as well as an overall high suspicion for malignancy. With regard to breast density, our results could indicate that dense breast tissue harbors a microenvironment more prone to the development of aggressive cancers, or could just merely be a reflection of younger women having both denser breasts and more frequently HER2-positive breast cancer (111, 112). The overall observation that...
circumscribed margins on mammography relates to less chance of HER2 overexpression may have been caused by an overrepresentation of triple-negative disease in several studies that selected patients based on receptor expression (mainly excluding ER-positive disease). Circumscribed margins, an otherwise benign feature, has been implicated as a triple-negative disease marker (113). Excluding these studies from the analysis weakened the relation between circumscribed margins and HER2 overexpression, suggesting that this feature is indeed more of a marker of triple-negative disease. A similar effect was observed for circumscribed mass margins on ultrasound.

To appreciate these findings, some strength and limitations of our meta-analysis need to be addressed. Our literature search was comprehensive and subsequent reference cross-checking of selected articles yielded only three additional indexed articles initially missed by the search. Then, the major strength of meta-analyses in general is that they increase the power to detect associations, and can identify sources of between-study heterogeneity in results. Nevertheless, a meta-analysis depends on individual study quality. Although we did not formally use a quality assessment tool, we evaluated several QUADAS-2–related study quality aspects relevant to our aims (114). For instance, we documented whether imaging assessment was performed blinded for HER2 status, which was true for 39% of articles but not reported in 60%. Most importantly, many articles ill-reported HER2 assessment methods and used variable thresholds, with only 32% of studies using the clinically established cutoff. Although this would thwart interpretation if the aim would be direct clinical application, this review focused on the biology between imaging and HER2 expression that may be visible over various HER2 threshold levels. Nevertheless, the results from this meta-analysis reflect univariable associations only, as individual studies did not adjust their results for potential confounders, such as lesion size or histologic breast cancer subtype, thus precluding solid causal inference.

The results of our meta-analysis show that imaging features relate to HER2 overexpression in breast cancer, which has several implications for future research relevant to clinical care. The diagnostic performance of breast imaging modalities may for instance improve by taking HER2 and other molecular subtypes into account when further developing rules for interpretation, resulting in "imaging signatures" specific for molecular breast cancer subtypes that are likely to outperform general rules of interpretation relating to breast cancer at large. Such imaging signatures may lead to improved detection of more aggressive breast cancer subtypes and may raise thresholds for recall and biopsy by identification of indolent disease. For example, the presence of microcalcifications with a linear/branching morphology on mammography may warrant biopsy as the chance of being consequential cancers could be high, while microcalcifications with other morphology could be actively surveyed. Also, in an era with increased use of neoadjuvant chemotherapy in which targeted therapy indication is based on pretreatment biopsies, imaging signatures predictive of HER2 overexpression may further help identify women with high risk of biopsy sampling error due to tumor heterogeneity. Finally, and perhaps most importantly, the relation between imaging features and cancer behavior may have prognostic relevance. Current clinical decisions pertaining to breast cancer treatment are based on established clinicopathologic prognostic information. Although this clinicopathologic information is pivotal for contemporary breast cancer care, the evaluation of prognostic models based on such information show that there is substantial room for improvement. For instance, the discriminative performance of such models expressed by the c-index typically is 0.70 to 0.75, with 0.5 being useless and 1.0 showing perfect discrimination (115, 116). Imaging features may thus further improve breast cancer prognostication beyond mere lesion size, and large studies are needed to establish their added value. This is especially compelling as imaging is readily available during standard diagnostic work-up for breast cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
The authors thank Youji He of the Southeast University (Nanjing, Jiangsu, China) for her support in interpretation of the articles written in Chinese.

Grant Support
This work was supported by a Dutch Cancer Society KWF research fellowship (UU 2010-4893 to S.G. Elias), and a René Vogels Foundation travel grant (to S.G. Elias).

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Received November 8, 2013; revised January 15, 2014; accepted April 21, 2014; published OnlineFirst May 7, 2014.

References


48. Mansson E, Bergkvist L, Christenson G, Persson C, Winterberg F. Mammographic casting-type calcifications is not a prognostic factor


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Published OnlineFirst May 7, 2014; DOI: 10.1158/1055-9965.EPI-13-1170

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*Cancer Epidemiol Biomarkers Prev* 2014;23:1464-1483. Published OnlineFirst May 7, 2014.

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