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The Prognostic and Predicting Roles of Tumor-Infiltrating Lymphocytes in Breast Cancer: A Meta-Analysis

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Abstract: *Background*: The relationship between lymphocyte infiltrates (LIs) and breast cancer outcome remains controversial. We performed this meta-analysis to elucidate the relationship.

Methods: A literature search identified 21 eligible studies.

Results: 16,097 patients were included. Multivariate analyses data for patients with unspecified receptors status showed that rich LIs expression was associated with 52% (hazard ratio [HR] = 0.48; 95% confidence interval [CI], 0.30-0.77), and 29% (HR = 0.71; 95% CI, 0.63-0.80) reduction in the risk of relapse and death, respectively. In the neoadjuvant setting, rich LIs predicted a 28% increase in complete pathological response rate. The prognostic and predictive utility of rich LIs was restricted to patients with estrogen receptor negative (ER-) or triple negative disease. Only rich CD8+ T cells tumors demonstrated clinical utility.

Conclusion: LIs significantly correlated to outcome predominantly in ER- tumors. Integrating immunotherapy with conventional therapy may warrant future research in breast cancer.

Keywords: Breast cancer, lymphocyte, prediction, prognosis.

INTRODUCTION

Several studies suggest that the immune response of the host plays pivotal roles in tumorigenesis, tumor development, disease progression, and subsequent metastasis [1, 2]. Moreover, the intensity of tumoral immune response influences the efficacy of cancer therapy, and favorably affects the clinical outcome in several solid tumors [1, 3-5]. Conversely, the presence of certain T-cell subsets such as the expression of regulatory T cell-specific forkhead box transcription factor (FOXP3) have shown negative prognostic effect [6].

Lymphocyte infiltrates (LIs) in breast cancer is an intriguing phenomenon that predominates in aggressive breast cancers including estrogen receptor negative (ER-) tumors [7], high-grade tumors [8, 9], basal-like tumors [10], and BRCA1-associated cancers [11]. Also shown is the high expression of LIs in the medullary histological type [12], a subset with a known favorable prognosis.

Nevertheless, the literature concerning the characterization of LIs and their prognostic utility in breast cancer has been inconsistent. These findings could be explained by the substantial diversity in patient population, description of LIs, the immunological response involved, and the methods and criteria used to qualify the immune response [13].

To the best of our knowledge, no published meta-analysis has examined the clinical utility of LIs in patients with breast cancer. The lack of such data and the conflicting outcomes of reported studies have prompted the current meta-analysis.

METHODS

Search Strategy

Between January 1985 and April 2013, we identified studies of interest by first conducting an electronic literature search of the following databases: MEDLINE, EMBASE, and the Cochrane Library. We also searched for relevant abstracts in conference proceedings of the American Society of Clinical Oncology and the European Society for Medical Oncology.

We used exploded Medical Subject Heading terms or key words terms 'breast', 'cancer', and 'neoplasm'. The terms were combined with 'infiltrate', 'inflammatory', 'immune response', 'lymphocyte', 'B cell', 'T cell', 'CD20', 'CD3', CD4', and 'CD8'. In the second step, we combined these keywords using the Boolean operator 'and' with 'prognosis', 'prediction', and 'pathologic response'. In addition, we manually reviewed the reference lists of relevant studies to identify additional pertinent articles.

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Selection Criteria

We included all studies that met the following criteria: (i) published in English language between January 1985 and April 2013; (ii) included patients of any age and with any stage of breast cancer; (iii) investigated the prognostic and/or the predictive role of LIs or its immunohistochemistry (IHC) markers in breast cancer tumors; (iv) reported hazard ratio (HR) for relapse-free survival (RFS) or overall survival (OS), or reported the odds ratio (OR) for complete pathological response (pCR), or reported adequate data allowing such outcomes to be computed; and (v) published as original articles (no case reports, case series, reviews, comments, letters, or editorials). When two or more articles reported duplicate data, we included only the most recent data, the study with the longer follow-up, or the most relevant study. However, we included studies that have used the same data set but examined different LIs markers. We excluded studies that only examined the effects of therapy on LIs expression.

Data Extraction

Four authors (EI, MA, MMA, GAK) independently inspected each item identified by the search and applied the inclusion/exclusion criteria. All authors reviewed the articles completely and discussed the data intended for extraction.

We used a standardized Microsoft Excel sheet to abstract data for each study that met inclusion criteria. Extracted data included the following fields: first author's last name, publication year, brief study description, study design (prospective versus retrospective), disease stage (early versus metastatic), number of patients, median age, histology, receptors status, median follow-up, method used to determine LIs, anatomical location of LIs, and outcome measures. We extracted information concerning blinding versus open interpretation of LIs, however as most of the included studies were of retrospective nature, therefore a study quality framework was not utilized.

Outcome Measures

The outcome measures extracted or computed were the HRs and its 95% confidence interval (CI) for RFS or OS. We also extracted or calculated the OR for the pCR in neoadjuvant studies. Because studies have used different definitions for LIs, the HR or OR was considered as the risk ratio between tumors with rich versus those with no/low LIs expression.

Statistical Analyses

The pooled estimates of the HR or OR and their CIs were the primary end points of the meta-analysis. We calculated unreported HR or OR and its 95% CI using the procedure proposed by Tierney *et al*. [14], that is based on the method reported by Parmar *et al*. [15] Where appropriate, we also used the built-in calculator of the Review Manager for Windows software version 5.2.3 to compute pertinent data (The Cochrane Collaboration, Oxford, UK). We added 0.5 to a cell frequency of zero to calculate the estimates. In studies that reported a univariate and a multivariate analysis for the same comparison, we only used the latter.

We assessed the heterogeneity of the results by inspecting the graphical presentations and by calculating a X^2 test of heterogeneity and the I^2 statistic of inconsistency [16, 17]. Statistically significant heterogeneity was defined as a X^2 P value less than 0.1 or an I^2 statistic greater than 50%. The pooled estimates of HR or OR, together with the associated 95% CI, were obtained using the DerSimonian and Laird random-effects model [18]. We performed meta-regression analysis to determine to what extent the effects of clinical variables could explain any demonstrated heterogeneity. The dependent variable was the lnHR or InOR, where appropriate, weighted for the inverse of variance to perform weighted least-square linear regression. We first conducted a univariate regression analysis for each relevant variable followed by a multivariate regression analysis including only variables found significant in the univariate analysis. We assumed the data to be missing at random, therefore, observed study characteristics were used to impute missing data by means of multiple imputations [19].

We performed subgroup analyses to assess the potential contributions of various clinicopathological variables to the main outcome. Studies that did not provide sufficient data to permit estimating relevant parameters in a subgroup analysis were excluded from that statistical pooling. Any comparison/analysis that was derived from a single study was not reported. A funnel plot estimating the precision of trials (plots of logarithm of the HR or OR against its inverse standard error) was examined for asymmetry to determine publication bias [20]. Publication bias was also quantified by the regression asymmetry test by Egger [20].

All statistical tests were two-sided. We used Comprehensive Meta-analysis Software for all pooled estimates (Biostat, version 2.2.064, Englewood New Jersey, USA). For meta-regression analyses, we used the SPSS statistical package (IBM SPSS Statistics for Windows, version 20.0., New York, USA).

RESULTS

We identified 437 potentially relevant articles (Fig. **1**). After exclusion of duplicate references, nonrelevant literature, and those that did not satisfy the inclusion criteria, 21 candidate articles were included. Tables **1** and **2** show the abstracted data of the included studies. Eighteen studies evaluated the prognostic utility of LIs on RFS and/or OS [13, 21-37], and 5 studies examined the value of LIs in predicting pCR [21, 34, 38-40] (two of those 5 studies also examined survival [21, 34].

Calabro *et al*. [22] used two data sets in a single report (155 breast tumor samples from the Medical University of Graz, and 1044 patients with invasive ductal carcinoma [IDC] from a publically available data set). Denkert *et al*. [38], classified their patients into two cohorts (i. e., training and validation sets (214, and 840 patients, respectively)).

There were 16,097 patients in the included studies with a median age of 55 years (95% CI, 49– 58.9 years) as abstracted from the studies' reported median age.

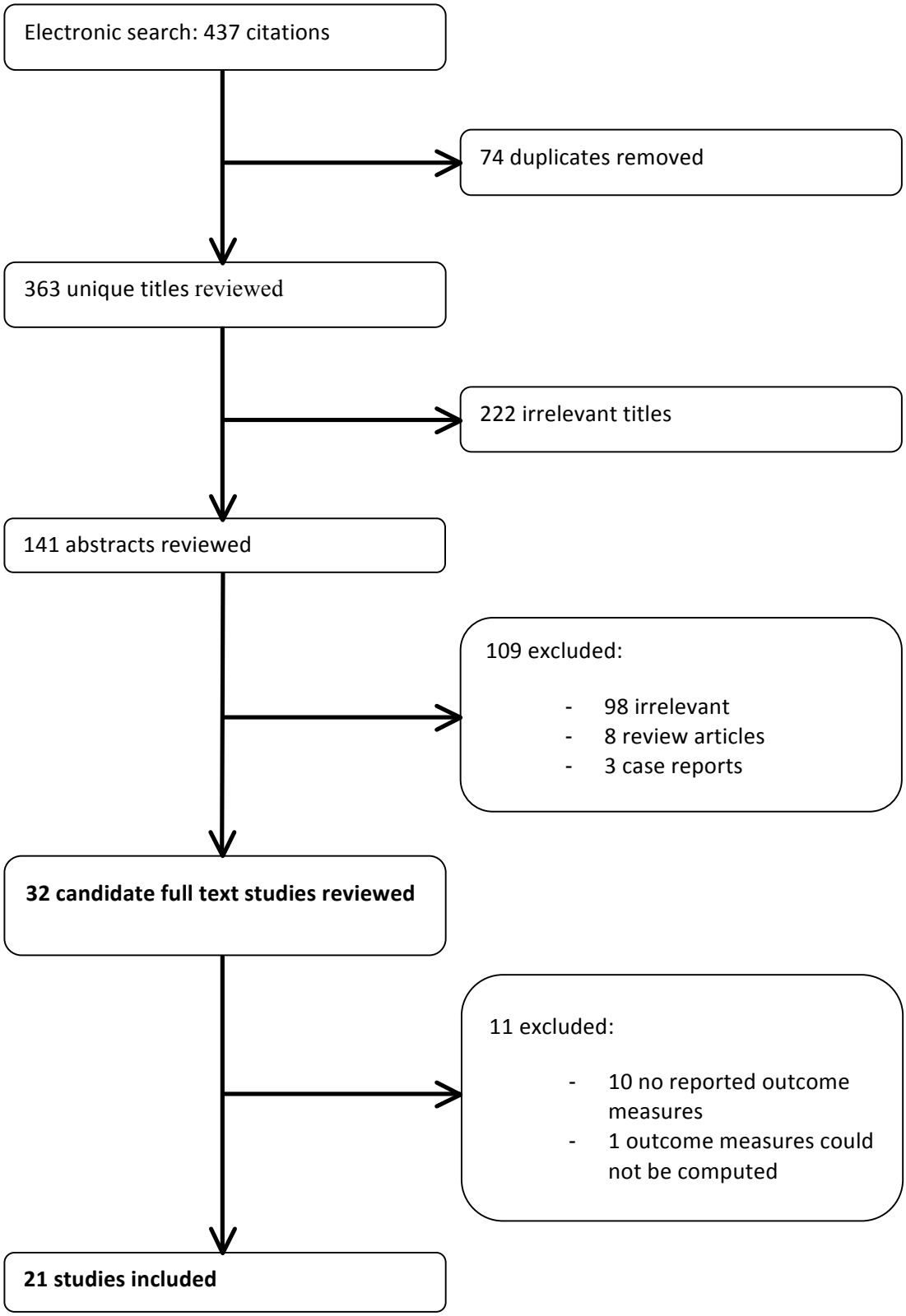

Fig. (1). Flowchart of literature search and the selection of the 21 included studies.

Approximately 100 patients had metastatic disease, while the remaining had non-metastatic or locally advanced breast cancer. In patients analyzed for survival outcome, the median percentage (95% CI) of patients with positive estrogen receptor (ER+), negative estrogen receptor (ER-), and positive HER2 (HER2+) were 65% (50-73%), 35% (27-50%), and 17% (13-27%), respectively. The corresponding percentages for patients in the neoadjuvant setting were 13% (0-68%), 87% (32-100%), and 32% (23-100%), respectively.

Table 1. Clinical characteristics of studies evaluating the effects of lymphocyte infiltrates on relapse-free and overall survival (16 studies).

Study	Patients Population & Design	Histology	No.	Median Age (Years)	Follow- Up Months	Receptors %			LI[a] Method	LI Location	LI	Blinding	Outcome
						ER+	ER-	HER2+					
Alexe 2007 [21]	NM[b], retrospective	NR[c]	31	NR	86	0	100	100	HE[d]	T[e]	LI-NS[f]	Yes	RFS[g]
Baker 2011 [13]	NM, retrospective	NR	1953	NR	63	76	24	NR	IHC[h]	T, S[i], TS[j]	CD8	NR/NC[k]	OS[l]
Calabro 2009 [22]	NM, retrospective	NR	155	59	87	60	40	NR	IHC	T	LI-NS	NR/NC	OS
Calabro 2009 [22]	NM, retrospective	NR	1044	54	NR	51	49	NR	IHC	T	LI-NS	NR/NC	OS
Camp 1996 [36]	NM, retrospective	NR	89	NR	43	NR	NR	NR	IHC	T	CD3	Yes	RFS
Eiro´ 2012 [23]	NM, prospective	IDC[m], 100%	102	NR	85	56	46	17	IHC	T	CD3 CD20	NR/NC	RFS
Kim 2012 [24]	NM, retrospective	NR	72	49	33.7	50	50	17	IHC	TS	CD8 FOXP3	NR/NC	RFS
Kreike 2007 [37]	NM, retrospective	IDC, 83%	97	NR	61.2	0	100	0	IHC	T	LI-NS	NR/NC	RFS
Lee 2006 [25]	NM, retrospective	IDC, 76% MBC[n], 0.4%	679	58	117.6	78	22	11	HE	T, TS	Li-NS	NR/NC	OS
Liu 2012 [26]	NM, retrospective	NR MBC, 1.7%	3403	58.9	151	70	30	13	IHC	T	CD8	Yes	OS
Loi 2013 [27]	NM, retrospective	NR	2009	49	96	81	19	22	HE	T, S, TS	LI-NS	Yes	RFS, OS
Ma 2012 [28]	NM, prospective	NR	81	NR	60	72	28	27	IHC	T	CD4 CD8 FOXP3 Ygama	Yes	RFS, OS
Mahmoud 2011 [29]	NM, retrospective	IDC, 59.5% MBC, 3%	1334	55	127	65	35	12	IHC	T, TS	CD8	NR/NC	OS
Mahmoud 2011 [30]	NM and metastatic, retrospective	NR	1445	55	128	73	27	13	IHC	T, TS	FOXP3	Yes	OS
Mohammed 2012 [31]	NM, retrospective	IDC, 100%	468	70% >50	165	65	35	17	HE	T	LI-NS	Yes	RFS, OS
Matkowski 2009 [32]	NM, retrospective	IDC, 100%	88	62	39	67	33	NR	IHC	T	CD4 CD8	NR/NC	OS
Rakha 2009 [33]	NM, retrospective	IDC, 49% MBC, 2%	1597	100% < 71	114	NR	NR	NR	HE	TS	LI-NS	NR/NC	RFS, OS
West 2011 [34]	NM, retrospective	IDC, 100%	255	66% ≥ 50	83	0	100	27	IHC	TS	LI-NS	NR/NC	RFS, OS
West 2013 [35]	NM, retrospective	IDC, 100%	144	80% ≥ 50	83	0	100	29	IHC	T	CD8 FOXP3	Yes	RFS, OS

[a]lymphocyte infiltrates; [b]non-metastatic; [c]not reported; [d]hematoxylin-eosin; [e]tumor; [f]lymphocyte infiltrates non-specified; [h]immunohistochemistry; [i]stromal; [j]intratumoral and stromal; [k]not reported or not clear; [l]overall survival; [m]invasive ductal carcinoma; [n]medullary carcinoma.

For studies reporting on histological subtypes, most patients had IDC (ranging from 59.5% to 100%), while medullary breast cancers were present in approximately 232 patients including 132 reported by Rakha *et al*. [33]. The median follow-up, when reported, was 96.5 months (95% CI, 63–127 months). Only three studies were prospective [23, 28, 39], whereas in 9 studies, assessors of LIs were blinded from patients' outcome [21, 26, 27, 30, 31, 35, 36, 38]. In the remaining 10 studies, blinding was not reported or it was not clear if was implemented.

Risk of Bias

The funnel plots for studies that tested the effects of LIs on RFS or OS showed no asymmetry and the Egger linear regression tests were not significant, indicating no evidence of significant publication bias (2-sided P values, 0.81 and

Table 2. Clinical characteristics of studies evaluating the effects of lymphocyte infiltrates on complete pathological response rate (5 studies).

Study	Patients Population & Design	Histology	No.	Median Age (Years)	ER+	ER-	Her2+	LI[a] Method	LI Location	LI	Blinding	Overall pCR%[b]
Alexe 2007 [21]	NM[c], retrospective	NR[d]	13	NR	0	100	100	HE[e]	T[f]	LI-NS[g]	Yes	15
Denkert 2010 [38]	NM, retrospective	NR	1058	NR	NR	NR	NR	IHC[h]	T, TS[i]	LI-NS[j]	Yes	13
Ono 2012 [39]	NM, prospective	NR	180	52	26	74	23	IHC	S[k]	CD4	NR/NC[l]	32
West 2011 [34]	NM, retrospective	IDC[m], 100%	113	50% ≥ 50	0	100	27	IHC	TS	LI-NS	NR/NC	43
Yamaguchi 2012 [40]	NM, retrospective	NR	68	60% ≥ 50	68	32	38	IHC	TS	LI-NS	NR/NC	24

[a]lymphocyte infiltrates; [b]complete pathological response percentage; [c]non-metastatic; [d]not reported; [e]hematoxylin-eosin; [f]tumor; [g]lymphocyte infiltrates non-specified; [h]immunohistochemistry; [i]intratumoral and stromal; [j]lymphocyte infiltrates non-specified; [k]stromal; [l]not reported or not clear; [m]invasive ductal carcinoma.

0.16, respectively). On the contrary, the funnel plot for studies that assessed the predictive value of LIs on pCR showed asymmetry and the Egger linear regression test was significant, indicating publication bias (2-sided P value = 0.026).

Analysis of Pooled Estimates

In most of, we found heterogeneity upon using the fixed-effects models (data not shown). Therefore, we based all analyses on random-effects models as described in the methodology section.

ANALYSIS OF RFS (TABLE 3)

Multivariate Analysis

We first examined the utility of any detention used for rich LIs to prognosticate RFS for all patients regardless of their tumor receptors status (ER-not specified, ER-NS), only using data that were based on multivariate analysis in the original studies. Data for this pooled measure were abstracted or computed from seven studies (Table **3**), and displayed that rich LIs was associated with a 52% reduction in the risk of relapse (HR = 0.48; 95% CI, 0.30-0.77, P = 0.0025).

LIs, Not Specified (LIs-NS)

Table **3** shows that among patients with rich LIs-NS, the prognostic benefit was apparent when data for ER- patients were pooled with a relapse risk reduction of 75% (HR = 0.25; 95% CI, 0.13-0.47; P <0.0001). For patients with triple-negative tumors, we demonstrated a 73% reduction in the risk of relapse. However, enough data were not available to examine the effect of LIs-NS in ER+ tumors.

Other LIs Markers

The analysis showed that rich CD8+ tumor versus less was associated with 88% relapse risk reduction (HR = 0.12; 95% CI, 0.04-0.32; P <0.0001) for ER-NS tumors. Data for CD8+ T cells in ER- tumors were only available from the study of West *et al*. [35]. It in the latter study, CD8+ rich predicted **a** 42% reduction in breast cancer relapse (data not shown). On the other hand, CD3+ rich was not found to be a prognostic variable. Likewise, analysis **of** FOXP3 rich tumors failed to show a prognostic value in ER-NS disease (HR = 1.08; 95% CI, 0.67-1.73; P = 0.76).

Anatomical Location for LIs Interpretation

Analysis of the location of LIs (intratumoral *vs* stromal *vs* both intratumoral and stromal) showed that LIs prognosticated DFS for ER-NS, ER-, and HER2- tumors. However, in ER+ tumors, rich LIs did not prognosticate RFS regardless of the location of expression (Table **3**).

ANALYSIS OF OS (TABLE 4)

Similarly, we first examined the utility of any definition used to qualify rich LIs to prognosticate OS for all patients regardless of their tumor receptors status (ER-NS), only using data derived from multivariate analyses. Data for this pooled measure were abstracted or computed from five studies, showing that rich LIs were associated with **a** 29% decrease in mortality (HR = 0.71; 95% CI, 0.63-0.80; P <0.0001).

LIs-NS

Table **4** shows that among patients with rich LIs-NS and ER-NS tumors, those with tumor rich LIs-NS demonstrated **a** 55% reduction in the risk of death compared with those with less LIs-NS expression (HR = 0.45; 95% CI, 0.23-0.87; P = 0.018). The prognostic benefit for ER- tumor only showed a trend with a reduction of death of 73% (HR = 0.27; 95% CI, 0.07-1.03; P = 0.055). On the other hand, analysis of ER+ tumor showed that LIs-NS was not prognostic.

Other LIs Markers

Rich CD8+ versus less was associated with **a** death risk reduction of 24% for ER-NS tumors combined (HR = 0.76; 95% CI, 0.63-0.92; P = 0.0042), 44% for ER- tumors (HR = 0.56; 95% CI, 0.39-0.81; P = 0.0019), but was not significant for ER+ tumors. The role of rich CD4+ T cells was also found to be not significant. Similar to its lack of a prognostic value in RFS analysis, the presence of FOXP3 rich tumor could not prognosticate OS in ER-NS or ER- tumors.

Table 3. Pooled analysis of hazard ratios of the effects of lymphocyte infiltrates on relapse-free survival (random effects models).

Lymphocyte Infiltrates	Receptors	Study	HR[a] and 95% CI[b]			P Value	Model HR and 95% CI			P value
			HR	Lower	Upper		HR	Lower	Upper	
All LIs[c]	ER-NS[d]	Kim 2012 [24]	1.00	0.61	1.63	1.00	0.48	0.30	0.77	0.0025
		Loi 2013 [27]	0.30	0.11	0.81	0.0181				
		Ma 2012 [28]	0.04	0.01	0.16	<0.0001				
		Mohammed 2012 [31]	0.31	0.13	0.74	0.0083				
		Rakha 2009 [33]	0.67	0.53	0.85	0.0009				
		West 2013 [35]	0.65	0.40	1.06	0.0811				
		Camp 1996 [36]	0.75	0.26	2.16	0.5943				
		Rakha 2009 [33]	0.67	0.53	0.85	0.0009				
LIs-NS[e]	ER-	Alexe 2007 [21]	0.19	0.04	0.90	0.0359	0.25	0.13	0.47	<0.0001
		Kreike [37]	0.24	0.09	0.62	0.0035				
		Loi 2013 [27]	0.30	0.11	0.81	0.0181				
LIs-NS	TN	Kreike [37]	0.24	0.09	0.62	0.0035	0.27	0.13	0.53	0.0002
		Loi 2013 [27]	0.30	0.11	0.81	0.0181				
CD8+	ER-NS	Kim 2012 [24]	0.36	0.09	1.46	0.1517	0.12	0.04	0.32	<0.0001
		Ma 2012 [28]	0.04	0.01	0.16	0.0000				
CD3+	ER-NS	Eiro´ 2012 [23]	0.81	0.53	1.23	0.3265	0.80	0.54	1.19	0.27
		Camp 1996 [36]	0.75	0.26	2.16	0.5943				
FOXP3	ER-NS	Kim 2012 [24]	1.00	0.61	1.63	1.0000	1.08	0.67	1.73	0.76
		Ma 2012 [28]	3.08	0.49	19.26	0.2291				
Location	Receptors									
Tumor	ER+ and ER-		0.64	0.50	0.80	0.0001	0.94	0.91	0.97	<0.0001
Stromal			0.94	0.88	1.00	0.0631				
Tumor and stromal			0.68	0.58	0.78	<0.0001				
Tumor	ER-		0.50	0.35	0.73	0.0003	0.31	0.15	0.62	0.001
Stroma			0.85	0.76	0.93	0.0010				
Tumor and stromal			0.30	0.15	0.62	0.0011				
Tumor	ER+		1.10	0.93	1.31	0.2799	1.01	0.96	1.06	0.78
Stromal			1.00	0.95	1.05	1.0000				
Tumor and stromal			0.89	0.44	1.80	0.7457				
Tumor	HER2-		0.80	0.69	0.93	0.0033	0.46	0.24	0.90	0.023
Stromal			0.87	0.80	0.94	0.0003				
Tumor and stromal			0.51	0.32	0.82	0.0227				
Method	Receptors									
Hematoxylin-eosin	All receptors		0.80	0.71	0.90	<0.0001	0.64	0.54	0.77	<0.0001
Immunohistochemistry	All receptors		0.64	0.54	0.77	<0.0001				

[a]hazard ratio ; [b]confidence interval; [c]lymphocyte infiltrates; [d]estrogen receptors not specified; [e]lymphocyte infiltrates non-specified.

Anatomical Location for LIs Interpretation

Analysis of the location of LIs showed that rich LIs prognosticated OS for ER-NS tumors, with the presence of rich LIs in both tumor and stroma. For ER- tumors, rich LIs was associated with a lower risk of death if expressed in the tumors. However, for ER- or ER+ tumors analyzed separately, rich LIs expression was not significant, regardless of its location of expression. For HER2- tumors, rich LIs presence, either in the tumor or in the stroma, was prognostically significant.

Table 4. Pooled analysis of hazard ratios of the effects of lymphocyte infiltrates on overall survival (random effects models).

Lymphocyte Infiltrates	Receptors	Study	HR[a] and 95% CI			P Value	Model HR and 95% CI[b]			P Value
			HR	Lower	Upper		HR	Lower	Upper	
All LIs[c]	ER-NS[d]	Lee 2006 [25]	0.43	0.24	0.77	0.0045	0.71	0.63	0.80	<0.0001
		Liu 2012 [26]	0.79	0.68	0.91	0.0015				
		Ma 2012	3.34	1.21	9.23	0.0201				
		Mahmoud 2011 [29]	0.55	0.38	0.78	0.0009				
		Rakha 2009 [33]	0.57	0.44	0.74	0.0000				
LIs-NS[e]	ER-NS	Lee 2006 [25]	0.43	0.24	0.77	0.0045	0.45	0.23	0.87	0.0184
		Loi 2013 [27]	1.00	0.88	1.14	1.0000				
		Mohammed 2012 [31]	0.13	0.07	0.24	<0.0001				
		Rakha 2009 [33]	0.57	0.44	0.74	0.0045				
LIs-NS	ER-	Calabro 2009 [22]	0.67	0.53	0.85	0.0012	0.27	0.07	1.03	0.055
		Loi 2013 [27]	0.29	0.09	0.92	0.0360				
		Mohammed 2012 [31]	0.03	0.00	0.28	0.0021				
LIs-NS	ER +	Calabro 2009 [22]	1.15	0.94	1.40	0.1690	0.76	0.47	1.21	0.25
		Loi 2013 [27]	1.10	1.00	1.21	0.0500				
		Mohammed 2012 [31]	0.15	0.07	0.33	<0.0001				
CD4	ER-NS	Ma 2012 [28]	1.20	0.47	3.08	0.7043	1.00	0.95	1.05	0.98
		Matkowski 2009 [32]	1.00	0.95	1.05	1.0000				
CD8	ER-NS	Baker 2011 [13]	0.88	0.77	1.01	0.0676	0.76	0.63	0.92	0.0042
		Liu 2012 [26]	0.79	0.68	0.91	0.0015				
		Ma 2012 [28]	0.32	0.13	0.79	0.0133				
		Mahmoud 2011 [29]	0.55	0.38	0.78	0.0009				
		Matkowski 2009 [32]	1.00	0.97	1.03	1.0000				
CD8	ER-	Baker 2011 [13]	0.72	0.58	0.88	0.0020	0.56	0.39	0.81	0.0019
		Liu 2012 [26]	0.48	0.34	0.67	<0.0001				
		West 2011 [34]	0.36	0.15	0.85	0.0201				
CD8	ER+	Baker 2011 [13]	1.16	0.97	1.38	0.1029	1.01	0.74	1.36	0.96
		Liu 2012 [26]	0.85	0.66	1.10	0.2204				
FOXP3	ER-NS	Ma 2012 [28]	3.05	0.95	9.78	0.0607	1.65	0.86	3.17	0.13
		Mahmoud 2011 [30]	1.38	1.13	1.68	0.0015				
	ER -	Mahmoud 2011 [30]	1.00	0.98	1.02	1.0000	1.00	0.98	1.02	1.0000
		West 2013 [35]	1.00	0.97	1.03	1.0000				
Location	Receptors	West 2013 [35]	1.00	0.97	1.03	1.0000				
Tumor	ER+ and ER-		0.93	0.82	1.04	0.1908	0.80	0.69	0.94	0.005
Stromal			1.01	0.91	1.12	0.8555				
Tumor and stromal			0.80	0.69	0.93	0.0049				
Tumor	ER-		0.58	0.48	0.71	0.0000	0.79	0.58	1.07	0.13
Stromal			0.85	0.72	1.00	0.0539				
Tumor and stromal			0.79	0.58	1.07	0.1299				
Tumor	ER+		0.98	0.80	1.18	0.8049	1.14	1.01	2.16	0.051
Stromal			1.04	0.89	1.20	0.6378				
Tumor and stroma			1.23	0.92	1.64	0.1587				
Tumor	HER2 -		0.59	0.46	0.76	<0.0001	0.51	0.31	0.92	0.024
Stromal			0.85	0.78	0.93	0.0003				
Method	Receptors		0.57	0.44	0.72	<0.0001				
Hematoxylin-eosin	All receptors	HE	0.69	0.61	0.79	<0.0001	0.74	0.66	0.84	<0.0001
Immunohistochemistry	All receptors	IHC	0.89	0.86	0.93	<0.0001				

[a]hazard ratio ; [b]confidence interval; [c]lymphocyte infiltrates; [d]estrogen receptors not specified; [e]lymphocyte infiltrates non-specified.

Analysis Based on Methods of LIs Interpretation

Table **3** shows that designating LIs rich tumors using either hematoxylin and eosin-stained sections (HE) or IHC methods significantly prognosticated RFS with a lower HR for the IHC method (0.64 *vs* 0.80, respectively). Similarly, qualifying LIs rich tumors using HE or IHC methods prognosticated OS, albeit, with a lower HR associated with HE method (Table **4**).

ANALYSIS OF pCR (TABLES 5)

Of 1432 patients in the neoadjuvant setting, the median pCR was 24% (95% CI, 13-43%).

We computed the utility of any classification for rich LIs to predict pCR for all patients regardless their tumor receptor status using data that used multivariate analyses, and it was found that rich LIs was associated with a 28% increase in the pCR rate (OR = 1.28; 95% CI, 1.16-1.42; P <0.0001).

Table **5** also shows that among patients with rich LIs-NS, and ER-NS tumors, those with tumors that demonstrated rich LIs-NS had a 27% higher pCR rate (OR = 1.27; 95% CI, 1.14-1.40; P = <0.0001). Furthermore, among patients with ER- tumors, the presence of rich LIs-NS was associated with an almost seven-fold increase in the pCR rate (OR = 6.60; 95% CI, 2.27-19.16; P = 0.0005). On the other hand, rich LIs-NS did not predict pCR in HER2+ tumors and there were no sufficient data pertinent to ER+ tumors.

META-REGRESSION ANALYSES (TABLE 6)

To explain heterogeneity in the pooled estimates, we carried out a series of meta-regression analyses. The dependent variable was the lnHR or InOR, weighted for the inverse of variance to perform weighted least-square linear regression. We first conducted a univariate regression analysis including the following variables: median age, study size, median follow-up, receptors status, LIs groups, location of LIs, HE versus IHC for LIs interpretation, retrospective versus prospective design, and blinding versus open/unclear LIs interpretation.

For analysis of RFS, we demonstrated that larger study size was associated with higher HR, while higher percentage of ER- tumors was inversely associated with HR. For OS analysis, retrospective analysis was associated with lower HR, while the use of IHC was associated with higher HR. Table **6** also shows that the heterogeneity in pCR rates was partially explained by a positive relationship between the percentage of ER- tumors and OR.

Table 5. Pooled analysis of odds ratios of the effects of lymphocyte infiltrates on complete pathological response (random effects models).

LI	Receptors	Study	HR[a] and 95% CI			P Value	Model HR and 95% CI[b]			P value
			HR	Lower	Upper		HR	Lower	Upper	
LIs-NS[c]	ER-NS[d]	Denkert 2010 [38]	1.38	1.07	1.77	0.0115	1.27	1.14	1.40	<0.0001
		Denkert 2010 [38]	1.21	1.08	1.35	0.0008				
		Yamaguchi 2012 [40]	4.70	2.20	10.05	0.0001				
		West 2011 [34]	1.21	0.52	2.81	0.6578				
LIs-NS	ER-	Alexe 2007 [21]	8.33	0.32	216.35	0.20	6.60	2.27	19.16	0.0005
		West 2011 [34]	6.42	2.08	19.82	0.0012				
LIs-NS	HER2+	Alexe 2007 [21]	8.33	0.32	216.35	0.2019	1.64	0.79	3.44	0.19
		Ono 2012 [39]	3.72	0.67	20.63	0.1330				
		West 2011 [34]	1.21	0.52	2.81	0.6578				

[a]hazard ratio ; [b] confidence interval; [c] lymphocyte infiltrates non-specified; [d] estrogen receptors not specified.

Table 6. Results of the multivariate meta-regression analyses (random effects models).

Model	Model R^2	Covariates	Meta-Regression β coefficient (SE)	*P* Value
Relapse-free survival				
Pooled hazard ratio	0.37	Study size (large *vs* small)	0.00 (0.00)	0.001
		Percentage of ER- tumors	-0.025 (0.002)	0.004
Overall survival				
Pooled hazard ratio	0.51	Retrospective *vs* prospective study	-0.46 (0.14)	<0.0001
		IHC *vs* HE	0.95 (0.07)	<0.0001
Complete pathological response				
Pooled odds ratio	0.52	Percentage of ER- tumors	1.45 (0.33)	<0.0001

DISCUSSION

The present meta-analysis, including a large cohort of 16,097 patients reported from 21 studies, provided quantitative estimates of the prognostic and predictive values of LIs on breast cancer outcome. Using data that were based on multivariate analyses in the original studies for patients with unspecified receptors status showed that rich LIs was associated with 52% and 29% reduction in the risk of relapse and death, respectively. Moreover, rich LIs predicted a 28% increase in the pCR rate.

In various subgroup analyses, it was evident that the prognostic and predictive utility of rich LIs was almost restricted to patients with ER- tumors. For instance, rich LIs-NS predicted a 75% and a 73% reduction in the risk of relapse and death, respectively, in patients harboring ER- tumors or having triple negative disease, while no such advantage was seen among those with ER+ tumors. More impressive, was the seven-fold increase in pCR rate associated with rich LIs-NS among patients with ER- tumors. While, it is well known that LIs have been shown to predominate in ER- tumors [7], the biological process that may explain the preferential prognostic benefit of rich LIs for ER- but not for ER+ tumors is unclear.

In clinical practice, significant heterogeneity in treatment response may occur in tumors with identical clinicopathological characteristics [41], therefore, it may not be utterly unexpected for two distinctively different breast cancer subtypes to respond differently to immune stimulation. Therefore, the clinical utility of LIs appears to be subtype-specific and varies depending on the histologic characteristics of breast cancer. Even within a seemingly uniform cohort of 186 ER- breast tumors, there was a demonstrated heterogeneity in clinical outcomes, apparently related to the variability in the expression levels of immune response pathway genes [42].

LIs demonstrated a prognostic utility for HER-2- but not for HER2+ tumors. The exact elucidation for this observation was not clear due to the limited data that reported HER2+ disease. This may likely be related to the fact that LIs in HER2+ tumors have a preponderance of macrophages, whereas LIs in HER2- tumors are composed mostly of T cells [43].

Of all other lymphocyte markers, tumors expressing excess CD8+ T cells showed relapse and death risk reduction of 88% and 24%, respectively. This prognostic utility was shown among patients with ER- and ER+ tumors when grouped together. The prognostic value of rich CD8+ is in keeping with the fact that most LIs in breast cancer are CD8+ T cells [7, 44] and considered as cytotoxic effectors able to contribute to the better clinical outcome associated with their overexpression [45, 46]. On the contrary, neither FOXP3 expression, nor rich CD3+ or rich CD4+ demonstrated prognostic utility.

Analysis of the location of designating rich LIs expression prognosticated RFS for ER-NS, ER-, or HER2- tumors regardless of the location of expression. However, it appears that intratumoral expression is more valuable and the results were more consistent. Although there were some numerical differences in the estimate of pooled HRs using either HE or IHC for qualifying LIs, both methods significantly prognosticated RFS and OS.

The present meta-analysis has several limitations. First, some of the included studies had several quality and design shortcomings. For example, only three studies were prospective, and in only nine, the assessors of LIs were blinded from patients' outcomes. Such open interpretation may have its own bias attributable to knowing the clinical outcome. Yet, the consistent patterns of the pooled measures may suggest that the effect of unblended interpretation was minimal. Second, while testing for publication bias was not significant for studies examining the prognostic clinical utility of rich LIs, the predictive advantage of rich LIs in the neoadjuvant setting demonstrated significant publication bias. Nevertheless, this limitation represents the scarcity of the current available evidence.

Third, different studies have used different qualification criteria to determine LIs. Nonetheless, grouping methods into either HE- or IHC-based clearly showed that both methods were significantly able to prognosticate RFS and OS. Moreover, the large population of patients included in this meta-analysis probably minimized such diversity.

Fourth, the current meta-analysis could not address the potential interactions between the clinical utility of rich LIs and other relevant factors such as tumor grade, nodal status, or adjuvant therapy. Unfortunately, the included studies did not provide sufficient therapy details to permit such analysis. In our meta-regression analyses, the limitation of the available data in the original reports was restrictive as we were only able to model the following variables: median age, study size, median follow-up, receptors status, LIs groups, location of LIs, HE versus IHC for LIs interpretation, retrospective versus prospective design, and blinding versus open/unclear LIs interpretation.

Fifth, inherent to meta-analyses, the included population from individual studies demonstrated clinicopathological differences. Nonetheless, almost all patients had non-metastatic or locally advanced disease; IDC was the most common histologic subtype; and the included studies had patients with comparable median ages. To explore heterogeneity, we conducted a series of meta-regression analyses to assess the impact of several explanatory variables that may have contributed to the statistical heterogeneity. The meta-regression for RFS showed that study size was positively associated with HR, while the percentage of ER- tumors showed **an** inverse association. For OS analysis, retrospective analysis was associated with **a** lower HR, while the use of IHC was associated with a higher HR. In the neoadjuvant setting, a positive association was shown between the percentage of ER- tumors and OR.

CONCLUSION

In conclusion, our meta-analysis that included a large population of patients reported from 21 studies showed that LIs significantly prognosticated RFS and OS and successfully predicted the pCR rate. We demonstrated the clinical utility mainly among patients with ER- tumors, regardless of the use of LIs-NS or CD8+ T cell markers to qualify LIs. On the other hand, no apparent clinical value was demonstrated among patients with ER+ tumors. The

clinical utility of rich LIs should serve as an impetus for future clinical trials designed to integrate novel immune therapy with conventional therapeutic modalities among patients with breast cancer. Future research may also help identifying those who are most likely to benefit from immune-modulating therapies.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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