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ORIGINAL REPORT

Estrogen- and Progesterone-Receptor Status in ECOG 2197: Comparison of Immunohistochemistry by Local and Central Laboratories and Quantitative Reverse Transcription Polymerase Chain Reaction by Central Laboratory

Sunil S. Badve, Frederick L. Baehner, Robert P. Gray, Barrett H. Childs, Tara Maddala, Mei-Lan Liu, Steve C. Rowley, Steven Shak, Edith D. Perez, Lawrence J. Shulman, Silvana Martino, Nancy E. Davidson, George W. Sledge, Lori J. Goldstein, and Joseph A. Sparano

From the Eastern Cooperative Oncology Group, Boston, MA; the University of California, San Francisco, San Francisco, CA; Genomic Health Inc, Redwood City, CA; sanofi-aventis, Bridgewater, NJ; North Central Cancer Treatment Group, Rochester, MN; Cancer and Leukemia Group B, Chicago, IL; and the Southwest Oncology Group, San Antonio, TX.

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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Corresponding author: Sunil Badve, MD, Indiana University School of Medicine, 350 West 11th St CPL-4050, Indianapolis, IN 46202; e-mail: sbadve@iupui.edu.

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ABSTRACT

Purpose

Central and local laboratory concordance for hormone receptor measurement is therapeutically important. This study compares estrogen receptor (ER) and progesterone receptor (PR) measured by local laboratory immunohistochemistry (IHC), central IHC, and central reverse-transcriptase polymerase chain reaction (RT-PCR) using a proprietary 21-gene assay.

Patients and Methods

A case-control sample of 776 breast cancer patients from Eastern Cooperative Oncology Group (ECOG) study E2197 was evaluated. Central IHC Allred score for ER and PR was obtained using tissue microarrays and 1D5 ER antibody and 636 PR antibody. Quantitative RT-PCR for ER and PR in whole sections was performed using the 21-gene assay.

Results

For ER, the concordance between local and central IHC was 90% (95% CI, 88% to 92%), between local IHC and central RT-PCR was 91% (95% CI, 89% to 93%), and between central IHC and central RT-PCR was 93% (95% CI, 91% to 95%). For PR, the concordance between local IHC and central IHC was 84% (95% CI, 82% to 87%), between local IHC and central RT-PCR was 88% (95% CI, 85% to 90%), and between central IHC and central RT-PCR was 90% (95% CI, 88% to 92%). Although concordance was high, IHC ER-negative cases that were RT-PCR positive were more common than IHC ER-positive cases that were RT-PCR negative. In ER-positive patients, ER expression by central IHC Allred score was marginally associated with recurrence ($P = .091$), and ER expression by central RT-PCR was significantly associated with recurrence ($P = .014$). However, recurrence score, which incorporates additional genes/pathways, was a highly significant predictor of recurrence ($P < .0001$).

Conclusion

There is a high degree of concordance among local IHC, central IHC, and central RT-PCR by the proprietary gene assay for ER and PR status. Although ER expression is marginally associated with relapse in ER-positive patients treated with chemohormonal therapy, recurrence score is a highly significant predictor of recurrence.

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INTRODUCTION

Breast cancer is a heterogeneous disease with a highly variable clinical course. The discovery of hormone receptor (HR)–positive breast carcinoma was of major prognostic and therapeutic import.[1] However, accurate assessment of the status of estrogen receptor (ER) and progesterone receptor (PR) remains problematic.

The quantitative ligand-binding assay (LBA) was supplanted in the 1990s by immunohistochemistry (IHC).[2,3] All methodologies are challenged by preanalytic and analytic variability. Preanalytic factors include fixation delay/type/duration, and protocol variations. Analytic factors include intra-/inter-observer variability and cut points with regard to percentage of cells stained and staining intensity.[4] This variability may lead to inappropriate treatment decisions.

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Quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) by Onco*type* DX (Genomic Health Inc [GHI], Redwood City, CA) assay quantifies gene expression using RNA extracted from fixed, paraffin-embedded tissue.[6] To evaluate the utility of standardized quantitative RT-PCR assessment of ER and PR by Onco*type* DX as an alternative to IHC, we compared local and central IHC and central quantitative RT-PCR measurement of ER and PR in breast carcinomas from 776 patients.

PATIENTS AND METHODS

Tumor Samples and Patient Clinical Data

The Eastern Cooperative Oncology Group (ECOG) E2197 study was a prospective, randomized, clinical trial that included 2,872 assessable patients with HR-positive or -negative breast cancer and 0 to 3 positive lymph nodes.[7] The protocol specified treatment with four 3-week cycles of doxorubicin (60 mg/m^2) plus cyclophosphamide 600 mg/m^2 (AC) or docetaxel 60 mg/m^2 (AT), and hormonal therapy if HR positive. Median follow-up was 76 months. There was no relapse rate difference between treatment arms. A case-control sample of 776 patients who did (n = 179) or did not (n = 597) relapse were evaluated by the Onco*type* DX assay; all relapses were selected, and a subsample of nonrelapses were controls. Paraffin blocks were reviewed by ECOG and GHI to confirm the presence of greater than 5% carcinoma. Seven samples lacked invasive carcinoma in both tissue microarray (TMA) cores but were assayed by RT-PCR: these results were excluded from the concordance analysis. Histologic subtype and grade (revised Nottingham criteria) were assessed centrally from whole tissue sections by one pathologist (F.L.B.).

Local IHC

The local ER/PR assays were performed and interpreted using local protocols and cut points.

Central IHC

A total of 20 TMA blocks were constructed using two 1.0-mm cores per patient tumor.[8] Sections 4 μm in size from TMAs were immunostained using the DakoCytomation EnVision+ System (Dako, Carpinteria, CA). After deparaffinization, sections were rehydrated and endogenous peroxidase was blocked with 1% H_2O_2 in methanol. After pressure-induced epitope retrieval (Biocare Medical Decloaking Chamber, Concord CA; citrate buffer, pH 6.0), sections were incubated with anti-ER antibody (clone 1D5, dilution 1:100; Dako) or anti-PR antibody (clone 636, dilution 1:200; Dako) at room temperature. The reaction was visualized using the EnVision+ kit and 3,3′-diaminobenzidine as chromogen followed by light counterstaining with hematoxylin. Positive and negative controls were used in each staining run.

TMAs were reviewed centrally and scored visually by two pathologists (S.B. and F.L.B.), blinded to all clinicopathologic data, using the Allred score (AS), a method that semiquantitates the proportion of positive cells (scored on a 0 to 5 scale) and staining intensity (scored on a 0 to 3 scale), with a maximum score of 8 (AS > 2 was considered positive).[3]

Sample Preparation and Gene Expression Analysis Using Onco*type* DX

Gene expression was quantitated from whole tumor sections by Genomic Health using the Onco*type* DX assay.[9] RNA was extracted from three 10-μm whole sections or from six 10-μm sections when manual microdissection (MMD) was required. MMD of whole sections was performed when tumor was amenable to dissection and constituted less than 50% of the epithelia. After DNase I treatment, total RNA content was measured and absence of DNA contamination verified. Gene-specific reverse transcription was performed, followed by quantitative PCR (TaqMan) in 384-well plates using Prism 7900HT instruments (Applied Biosystems, Foster City, CA). The expression of 16 cancer-related genes, including ER (*ESR1*) and PR (*PGR*), were normalized relative to five reference genes (*ACTB*, *GAPDH*, *GUSB*, *RPLP*, and *TFRC*); reference normalized expression ranged from 0 to 15 units, where each 1-unit increase reflects approximately a two-fold increase in RNA. Classifications of ER and PR status were based on preestablished cutoff points, 6.5 and 5.5 units, established from three prior studies comparing IHC and RT-PCR.[10,11] Assay variability for each gene from instruments, operators, reagents and day-to-day variation was less than 0.5 units (standard deviation).

Table 1. Distribution of ER and PR Expression

Variable	No.	%
ER by central RT-PCR		
Negative (< 6.5)	317	41
6.5-< 7.5	31	4
7.5-< 8.5	51	7
8.5-< 9.5	127	16
9.5-< 10.5	143	18
10.5-< 11.5	87	11
11.5-< 12.5	20	3
PR by central RT-PCR		
Negative (< 5.5)	411	53
5.5-< 6.25	55	7
6.25-< 7.0	67	9
7.0-< 7.75	77	10
7.75-< 8.5	76	10
8.5-< 9.25	54	7
9.25-< 12.5	36	5
ER by central IHC, Allred Score		
Negative (0-2)	360	47
3	27	4
4	37	5
5	33	4
6	76	10
7	125	16
8	111	14
PR by central IHC, Allred Score		
Negative (0-2)	360	47
3	26	3
4	30	4
5	43	6
6	53	7
7	61	8
8	196	25

NOTE. Central RT-PCR, n = 776. Central IHC, n = 769.
Abbreviations: ER, estrogen receptor; RT-PCR, reverse-transcriptase polymerase chain reaction; PR, progesterone receptor; IHC, immunohistochemistry.

Statistical Analysis

Because of the low overall relapse rate in E2197, a case-control design was used to select the study cohort, with relapses sampled more heavily than nonrelapses.[12] Patients were sampled separately within 16 strata formed by the cross-classifications of relapse, HR, axillary nodal, and treatment arm status.

Agreement measures between RT-PCR and IHC include overall concordance, calculated as the number of samples that agree divided by the total number of samples, and κ statistics (agreement adjusted for chance).[13] Exact 95% CIs for the concordance statistic were calculated with the *F* distribution method using SAS PROC FREQ (SAS Institute, Cary, NC).[14] Positive agreement was calculated as the number of samples positive by both assays divided by the number of samples positive by the IHC assay. Negative agreement was calculated similarly.

Cox proportional hazards regression using weighted pseudo-likelihood estimation examined the associations between ER expression (IHC and RT-PCR) and relapse-free interval (RFI).[15] RFI was defined as the time from entry on E2197 to the first evidence of breast cancer relapse, defined as invasive breast cancer in local, regional, or distant sites, including the ipsilateral breast, but excluding new primary breast cancers in the opposite breast. Follow-up for

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Table 2. Concordance of Central RT-PCR by Onco*type* DX and Central and Local IHC for ER Status

Measure	Central IHC+ No.	Central IHC+ %	Central IHC− No.	Central IHC− %	Total Onco*type* DX	Local IHC+ No.	Local IHC+ %	Local IHC− No.	Local IHC− %	Total Onco*type* DX
Central RT-PCR+	404	99	50	14	454	414	95	45	13	459
Central RT-PCR–	5	1	310	86	315	21	5	296	87	317
Total central IHC	409		360		769	435		341		776
Concordance, %			93					91		
95% CI			91% to 95%					89% to 93%		
Kappa, %			86					83		
95% CI			82% to 89%					79% to 87%		
Central IHC+						382	89	27	8	409
Central IHC−						48	11	312	92	360
Total local IHC						430		339		769
Concordance, %								90		
95% CI								88% to 92%		
Kappa, %								80		
95% CI								76% to 85%		

Abbreviations: RT-PCR, reverse-transcriptase polymerase chain reaction; IHC, immunohistochemistry; ER, estrogen receptor.

relapse was censored at the time of death without relapse, new primary cancer in the opposite breast, or at the time the patient was last evaluated for relapse. Five-year recurrence rates were estimated from the Cox proportional hazards regression models, on the basis of the empirical cumulative hazard estimate of the survival function. Associated 95% CIs were obtained by employing the normal theory approximation to the logarithm of the survival estimates. Analyses of time to relapse were weighted because inferences were for the full E2197 study population. All statistical tests were two sided, and $P < .05$ was considered significant.

RESULTS

Patient Characteristics

A total of 776 samples are reported in the analysis of local IHC and central RT-PCR and 769 samples in the analysis of central IHC. Seven samples were nonassessable for central IHC (lack of invasive tumor in both TMA cores). Distributions of patient characteristics and tumor characteristics are in Table A1 (online only).

Distribution of ER and PR

ER and PR by central IHC was bimodal (Table 1). Among ER-positive patients, ER and PR by central RT-PCR were both more normally distributed than by central IHC (58% of ER-positive patients and 63% of PR-positive patients had an AS of 7 or 8).

Concordance Between IHC and RT-PCR

ER concordance. Concordance was high between central IHC and central RT-PCR ER status (Table 2); positive agreement was 99% (95% CI, 98% to 100%), and negative agreement was 86% (95% CI, 82% to 89%). In the absence of a gold standard, the terms percentage of positive and percentage of negative agreement describe diagnostic accuracy as alternatives to the terms sensitivity and specificity as suggested by the US Food and Drug Administration (FDA).[16]

The two-by-two table for the local IHC versus central RT-PCR comparison is presented in Table 2. Positive agreement was 95% (95% CI, 93% to 97%). Negative agreement was 87% (95% CI, 83% to 90%).

Between 14% (central IHC) and 13% (local IHC) of samples classified as ER negative by IHC are ER positive by central RT-PCR, whereas 1% and 5% of RT-PCR–negative cases were ER positive by local and central IHC, respectively.

The distribution of AS by central IHC compared with central RT-PCR is shown in Figure 1. Although correlation exists between the measures, for the 14% of discordant cases where RT-PCR was positive and the central IHC assay by the AS was negative, the RT-PCR values were often high and not close to the 6.5 cutoff. For these discordant cases, RT-PCR measurements range from 6.5 to 10.4 units.

PR concordance. Concordance was high between central IHC and central RT-PCR PR status (Table 3); positive agreement was 85% (95% CI, 81% to 88%). Negative agreement was 96% (95% CI, 93% to 98%).

The two-by-two table for the local IHC versus central RT-PCR comparison is presented in Table 3. Positive agreement was 86% (95% CI, 81% to 89%), and negative agreement was 89% (95% CI, 86% to 92%).

HR concordance. HR positivity was defined as ER and/or PR positive. Concordance between local IHC, central IHC, and central RT-PCR HR status was high (Table 4). Between 8% (central IHC) and 12% (local IHC) of samples classified as HR negative by IHC are HR positive by RT-PCR; in contrast, between 6% (central IHC) and 7% (local IHC) of samples classified as HR negative by RT-PCR are HR positive by IHC.

ER Expression and Recurrence Rates

To determine the relationship between ER expression and recurrence risk, we performed the primary analysis on all study patients. A subset of patients were ER positive by local IHC, central IHC, and central RT-PCR, and were not known to have received hormonal therapy, either because hormone therapy was not given or because it was not known whether hormone therapy was given (32, 46, and 62, respectively; Table A2, online only); in a secondary analysis we excluded these patients (< 15% of the total).

Figure 2 shows the relationship between central IHC AS and central RT-PCR for ER, and 5-year recurrence rates for all patients (Fig 2A) and for the subset excluding ER-positive patients who didn't receive hormonal therapy (Fig 2B). Five-year recurrence rates are

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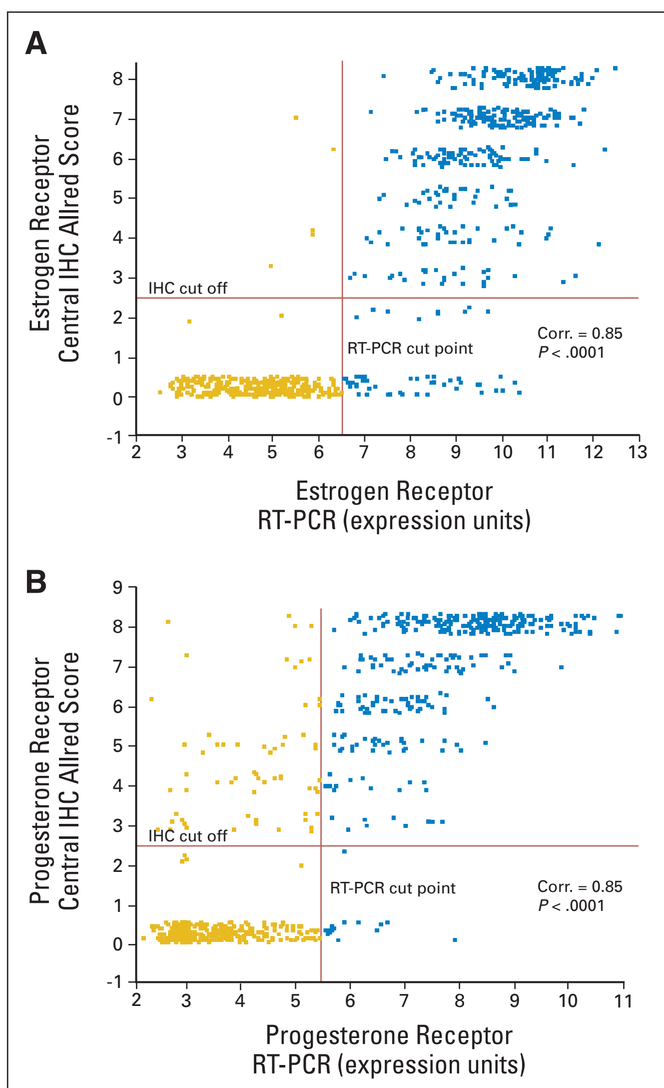

Fig 1. The distribution of expression by central IHC (Allred score) and expression by central RT-PCR using Onco*type* DX. (A) Estrogen receptor expression; (B) progesterone receptor expression. IHC, immunohistochemistry; RT-PCR, reverse-transcriptase polymerase chain reaction; corr, Spearman rank correlation.

similar for the subset of patients compared with the full analysis population. When all patients were included (ER negative and ER positive), ER expression by central IHC and by central RT-PCR was statistically significant. When only ER-positive patients were analyzed, ER expression by central IHC was marginally associated with recurrence ($P = .091$) and central RT-PCR was significantly related to recurrence ($P = .014$). The pattern of 5-year recurrence rates across the seven expression categories for central IHC and central RT-PCR in Figure 2 have similar shapes; however, the CIs for lower levels of ER by AS are larger than those for lower levels of ER by RT-PCR.

For comparison, the relationship between the Onco*type* DX recurrence score (RS) and recurrence risk in the subset of ER-positive patients (both node-negative and node-positive patients) was determined (Fig 3). RS was a significant predictor of recurrence ($P < .0001$) and was particularly effective at identifying low-risk patients, as noted by the narrow 95% CI for recurrence rate in patients with RS less than 18. Consistent with prior studies, RS was a significant predictor of recurrence ($P = .0016$) in the subset of node-negative patients (n = 188).[9]

ER Expression and RS

The distribution of RS by ER expression (central IHC and central RT-PCR) was explored in ER-positive patients (Fig 4A). Although correlation between RS and ER expression was modest (correlation = −0.22 for central IHC and −0.47 central RT-PCR), there were cases with high ER expression and high RS. Similar results were obtained for node-negative and node-positive patients analyzed separately (data not shown).

Tumor Grade and RS

The distribution of RS by tumor grade was explored in ER-positive patients by both central and local HC tumor grade assessment (n = 404) (Fig 4B). Although there was a strong association overall between RS and tumor grade ($P < .0001$ for both local and central grade), there were discrepancies. Modest agreement in assessment of histologic grade among the local and central pathologists was observed (concordance = 67% [95% CI, 63% to 70%]; weighted κ = 46%; Table A3, online only).

DISCUSSION

Accurate and precise measurement of HR status is of therapeutic importance because hormonal therapy reduces the relative risk of recurrence by more than 50% in breast cancer patients with hormone-sensitive tumors.[1,17,18] New methodologies for HR quantitation should be rigorously compared with the current measurement standard. In this study, there was a high level of concordance for ER, PR, and HR status between local and central IHC and central RT-PCR using the Onco*type* DX assay (Table 2). The similar concordance between IHC and RT-PCR, when compared with that observed between the local and central IHC, demonstrates that quantitative RT-PCR using the Onco*type* DX assay is an alternative method for determination of HR status.

IHC assessment of ER and PR is recommended by the American Society of Clinical Oncology to guide breast cancer treatment.[19] Unfortunately, interlaboratory variability with IHC assessment of ER/PR is relatively high in clinical practice. Rhodes et al[20] showed that 80% of laboratories demonstrated ER positivity in medium-/high-expressing tumors, but only 37% demonstrated ER positivity in low-expressing tumors.

There is diversity in IHC methodology. Protocol differences such as the use of antigen retrieval and the type of antigen retrieval such as pressure steam, used in this study, versus ambient steam, can contribute to variability.[20] Chromogen enhancers may also be used to increase sensitivity of IHC assays (these were not employed in this study). Antibody differences are important. In a comparison of two ER antibodies, 1D5 and SP1, 8% of tamoxifen-treated patients who were SP1 positive but 1D5 negative were noted to have better clinical outcomes. A recent IHC/RT-PCR comparison study of 607 patients using SP1 showed 96% concordance between SP1 and RT-PCR by Onco*type*.[27] Another possible source of bias in this study was the use of two 1.0-mm cores (total area = 3.14 mm^2) for HR assessment by IHC. Although this theoretically may introduce sampling bias, it is highly

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Table 3. Concordance of Central RT-PCR by Onco*type* DX and Central and Local IHC for PR Status

Measure	Central IHC+ No.	Central IHC+ %	Central IHC− No.	Central IHC− %	Total Onco*type* DX	Local IHC+ No.	Local IHC+ %	Local IHC− No.	Local IHC− %	Total Oncotype DX
Central RT-PCR+	347	85	15	4	362	322	86	43	11	365
Central RT-PCR–	62	15	345	96	407	54	14	357	89	411
Total central IHC	409		360		769	376		400		776
Concordance, %			90					88		
95% CI			88% to 92%					85% to 90%		
Kappa, %			80					75		
95% CI			76% to 84%					70% to 80%		
Central IHC+						330	89	79	20	409
Central IHC−						41	11	319	80	360
Total local IHC						371		398		776
Concordance, %								84		
95% CI								82% to 87%		
Kappa, %								69		
95% CI								64% to 74%		

Abbreviations: RT-PCR, reverse-transcriptase polymerase chain reaction; IHC, immunohistochemistry; PR, progesterone receptor.

unlikely given that many groups have reported that three 0.6-mm cores (total area = 1.13 mm^2) are highly concordant with whole tumor sections.

Just as all IHC assays are not the same, nor are all RT-PCR assays the same. Differences in RNA extraction, reverse transcription, PCR protocols and machines, primer/probe selection, and reagent manufacturing can contribute to assay variability. Central lab measurement of ER and PR expression by RT-PCR using Onco*type* DX employs a large number of controls and calibrators to enhance precision and reproducibility. Normalization with five reference genes addresses differences in RNA quality stemming from preanalytic factors,[22,23] such as delay to fixation and duration of fixation.[6,24] Cronin et al[6,25] recently documented the operational performance of the standardized Onco*type* DX assay: linearity over a more than 2,000-fold RNA concentration range with an average accuracy of 0.3%, coefficients of variation for the assay process below 5.7%, and assay variability contributed by instruments, operators, reagents, and day-to-day variation limited to less than 0.5 expression units (standard deviation).

Although the overall concordance for ER status was high in this study, discordances that may be a result of biology (in some cases protein expression and mRNA expression may be truly different due to epigenomic factors) and/or to assay performance issues were observed. There was an imbalance in the distribution of the discordant cases between central IHC and central RT-PCR. IHC ER-negative cases that were RT-PCR positive were observed more commonly than IHC ER-positive cases that were RT-PCR negative. These discordant cases that were IHC ER negative and RT-PCR ER positive showed quantitative ER levels by RT-PCR that ranged from 6.5 to 10.5 expression units (16-fold range). In contrast, those cases that were IHC ER positive but RT-PCR negative all tended to cluster within 2 units (four-fold range) of the RT-PCR cutoff. The relatively low incidence of IHC ER-positive cases that are RT-PCR ER negative in this study is

Table 4. Concordance of Central RT-PCR by Onco*type* DX and Central and Local IHC for HR Status

Measure	Central IHC+ No.	Central IHC+ %	Central IHC– No.	Central IHC– %	Onco*type* DX Total	Local IHC+ No.	Local IHC+ %	Local IHC− No.	Local IHC− %	Oncotype DX Total
Central RT-PCR+	432	94	24	8	456	423	93	38	12	461
Central RT-PCR–	27	6	286	92	313	32	7	283	88	315
Total central IHC	459		310		769	455		321		776
Concordance, %			93					91		
95% CI			91%, 95%					89%, 93%		
Kappa, %			86					81		
95% CI			83%, 90%					77%, 86%		
Central IHC+						415	92	44	14	459
Central IHC−						34	8	276	86	310
Total local IHC						459		320		769
Concordance, %								90		
95% CI								88% to 92%		
Kappa, %								79		
95% CI								75% to 83%		

Abbreviations: RT-PCR, reverse-transcriptase polymerase chain reaction; IHC, immunohistochemistry; HR, hormone receptor.

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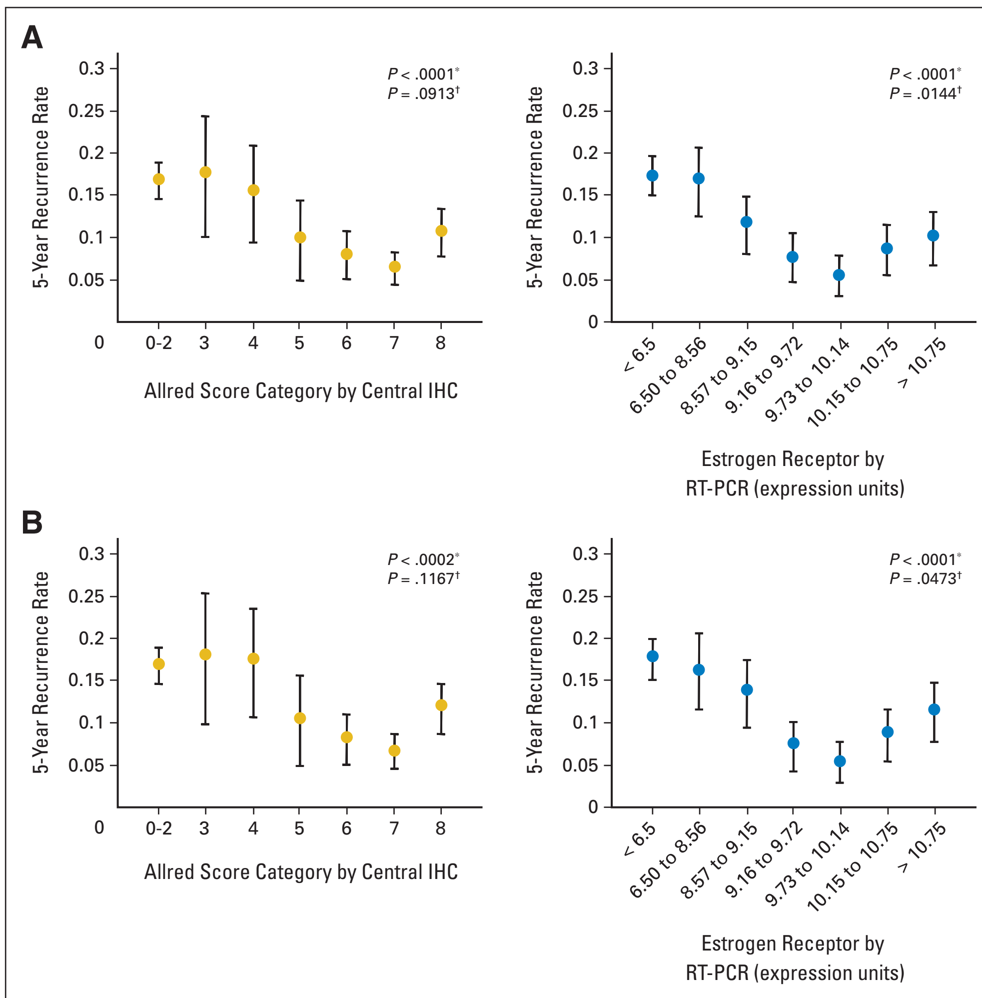

Fig 2. Five-year recurrence rates by estrogen receptor (ER) expression. (A) All patients and (B) excluding patients who were not known to have received hormonal therapy. IHC, immunohistochemistry; RT-PCR, reverse-transcriptase polymerase chain reaction. (A) (*) Calculated using a Cox proportional hazards model including all ER values (n = 769 for IHC and n = 776 for RT-PCR); (†) Calculated using a Cox proportional hazards model including only positive ER values (n = 409 for IHC and n = 459 for RT-PCR); (B) (*) Calculated using a Cox proportional hazards model including all ER values (n = 723 for IHC and n=714 for RT-PCR); (†) Calculated using a Cox proportional hazards model including only positive ER values (n = 363 for IHC and n = 397 for RT-PCR).

similar to the 2% discordance rate between IHC ER-positive and RT-PCR ER-negative cases seen in over 20,050 reported cases from the GHI Clinical Laboratory.[26]

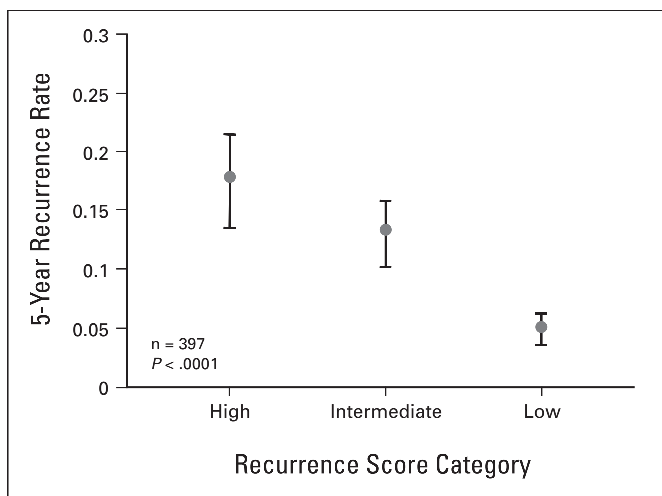

Fig 3. Five-year recurrence rates by recurrence score. Shown here are results for estrogen receptor–positive patients excluding those who were not known to receive hormonal therapy. Similar results were observed in the analysis, which included all estrogen receptor–positive patients.

In contrast to ER, the discordant PR cases were evenly distributed. The PR discordances are not explained by differences in PRA and PRB expression as the RT-PCR PR primer/probe set identifies both species. Finally, when either ER-positive and/or PR-positive cases were assessed as HR positive, the overall concordance was also high (≥ 90%); nevertheless, between 8% (central IHC) and 12% (local IHC) of samples classified as HR negative by IHC were HR positive by RT-PCR.

Although immunohistochemistry detects protein expression while RT-PCR measures mRNA transcription levels, numerous groups have shown a high degree of concordance between the two, particularly with regard to ER assessment.[6,10,11,27,28] Concordance assessment of HR status examines protein and gene expression as dichotomous variables, as either positive or negative; however, equally important is whether HR biology is dichotomous or continuous.[5,29] The LBA reported ER protein on a continuous scale with a large dynamic range. For IHC the Allred system was developed for semiquantitative ER assessment using a specific protocol and antibody and was superior to LBA.[3] The Allred system semiquantifies both the percentage of positive cells and the intensity of staining: either 10% weakly staining nuclei or 1% intermediately staining nuclei represent the cut point for HR positivity. AS has been optimized for use with other protocols and antibodies, most recently in the validation of the FDA-approved ER/PR pharmDx Kit. For the semiquantitation of PR,

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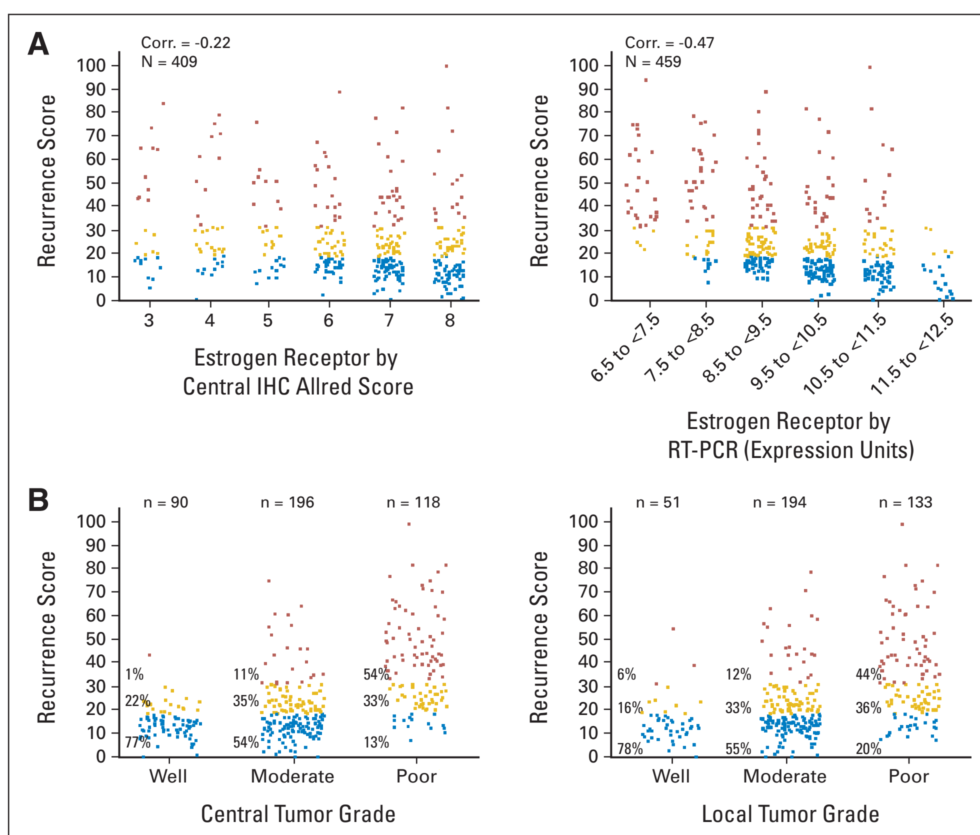

Fig 4. Relationship between estrogen receptor (ER), tumor grade, and recurrence score. (A) ER expression and recurrence score in ER-positive patients. (B) Tumor grade and recurrence score in ER-positive patients. IHC, immunohistochemistry; mod, moderate; corr, Spearman rank correlations.

Mohsin et al[30] demonstrated the utility of the Allred system with similar success. However, implementation of the AS system in different laboratories may give results that differ from the specific methodology employed in the original Allred studies. As shown, the Onco*type* DX RT-PCR measures of ER and PR exhibit a continuous distribution of expression over a 3,000-fold and 1,000-fold range, respectively, for ER and PR. In contrast, using the ER clone 1D5 and PR clone 636 AS tended to be bimodal.[27]

The relationship between recurrence risk and ER expression by central IHC and central RT-PCR was explored. Five-year recurrence rate estimates were obtained for all patients, and separately for the ER-positive patients who received chemohormonal therapy. As expected, ER by both central IHC and central RT-PCR were significantly associated with relapse when all patients were included in the analysis ($P < .0001$ for both). When the ER-positive subgroup was analyzed, ER expression by central IHC AS was marginally associated with recurrence ($P = .091$), while ER expression by central RT-PCR was significantly associated with recurrence ($P = .014$). The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14 Onco*type* DX study in ER-positive patients treated with placebo or tamoxifen showed that quantitative ER was the best single predictor of tamoxifen benefit (interaction $P = .0007$).[27] Possibly in this study, where all ER-positive patients received combined chemohormonal therapy, ER is less associated with recurrence risk because chemotherapy benefit was lower in patients with higher ER.[31] In contrast, in this subgroup of ER-positive patients, the RS, which incorporates other biologic pathways beyond ER, was a highly significant predictor of recurrence ($P < .0001$).

In summary, this study demonstrated a high level of concordance for ER, PR, and HR between local and central IHC laboratories and central RT-PCR using the Onco*type* DX assay. Quantitative RT-PCR using the Onco*type* DX assay is an alternative method for determining hormone receptor status.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

Conception and design: Sunil S. Badve, Robert P. Gray, Barrett H. Childs, Steve C. Rowley, Steven Shak, George W. Sledge, Lori J. Goldstein, Joseph A. Sparano
Financial support: Barrett H. Childs, Steve C. Rowley

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Administrative support: Robert P. Gray, George W. Sledge
Provision of study materials or patients: Robert P. Gray, Edith D. Perez, Lawrence J. Shulman, Silvana Martino, Nancy E. Davidson, George W. Sledge, Lori J. Goldstein
Collection and assembly of data: Frederick L. Baehner, Robert P. Gray, Steven Shak, Joseph A. Sparano
Data analysis and interpretation: Sunil S. Badve, Frederick L. Baehner, Robert P. Gray, Barrett H. Childs, Tara Maddala, Mei-Lan Liu, Steve C. Rowley, Steven Shak, Lori J. Goldstein, Joseph A. Sparano
Manuscript writing: Sunil S. Badve, Frederick L. Baehner, Robert P. Gray, Barrett H. Childs, Tara Maddala, Mei-Lan Liu, Steve C. Rowley, Steven Shak, Nancy E. Davidson, George W. Sledge, Joseph A. Sparano
Final approval of manuscript: Sunil S. Badve, Frederick L. Baehner, Robert P. Gray, Barrett H. Childs, Tara Maddala, Mei-Lan Liu, Steve C. Rowley, Steven Shak, Edith D. Perez, Lawrence J. Shulman, Silvana Martino, Nancy E. Davidson, George W. Sledge, Lori J. Goldstein, Joseph A. Sparano

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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Glossary Terms

ER (estrogen receptor): Belonging to the class of nuclear receptors, estrogen receptors are ligand-activated nuclear proteins present in many breast cancer cells that are important in the progression of hormone-dependent cancers. After binding, the receptor-ligand complex activates gene transcription. There are two types of estrogen receptors (á and â). ERá is one of the most important proteins controlling breast cancer function. ERâ is present in much lower levels in breast cancer and its function is uncertain. Estrogen-receptor status guides therapeutic decisions in breast cancer.

RT-PCR (reverse-transcriptase polymerase chain reaction): PCR is a method that allows logarithmic amplification of short DNA sequences within a longer, double-stranded DNA molecule. Gene expression can be measured after extraction of total RNA and preparation of cDNA by a reverse-transcription step. Thus, RT-PCR enables the detection of PCR products on a real-time basis, making it a sensitive technique for quantitating changes in gene expression.

Immunohistochemistry: The application of antigen-antibody interactions to histochemical techniques. Typically, a tissue section is mounted on a slide and is incubated with antibodies (polyclonal or monoclonal) specific to the antigen (primary reaction). The antigen-antibody signal is then amplified using a second antibody conjugated to a complex of peroxidase-antiperoxidase (PAP), avidin-biotin-peroxidase (ABC) or avidin-biotin alkaline phosphatase. In the presence of substrate and chromogen, the enzyme forms a colored deposit at the sites of antibody-antigen binding. Immunofluorescence is an alternate approach to visualize antigens. In this technique, the primary antigen-antibody signal is amplified using a second antibody conjugated to a fluorochrome. On UV light absorption, the fluorochrome emits its own light at a longer wavelength (fluorescence), thus allowing localization of antibody-antigen complexes.

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