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Re-evaluating Adjuvant Breast Cancer Trials: Assessing Hormone Receptor Status by Immunohistochemical Versus Extraction Assays

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Background: **Tumor levels of steroid hormone receptors, a factor used to select adjuvant treatment for early-stage breast cancer, are currently determined with immunohistochemical assays. These assays have a discordance of 10%–30% with previously used extraction assays. We assessed the concordance and predictive value of hormone receptor status as determined by immunohistochemical and extraction assays on specimens from International Breast Cancer Study Group Trials VIII and IX. These trials predominantly used extraction assays and compared adjuvant chemoendocrine therapy with endocrine therapy alone among pre- and postmenopausal patients with lymph node–negative breast cancer. Trial conclusions were that combination therapy provided a benefit to pre- and postmenopausal patients with estrogen receptor (ER)–negative tumors but not to ER-positive postmenopausal patients. ER-positive premenopausal patients required further study.** ***Methods:*** **Tumor specimens from 571 premenopausal and 976 postmenopausal patients on which extraction assays had determined ER and progesterone receptor (PgR) levels before randomization from October 1, 1988, through October 1, 1999, were re-evaluated with an immunohistochemical assay in a central pathology laboratory. The endpoint was disease-free survival. Hazard ratios of recurrence or death for treatment comparisons were estimated with Cox proportional hazards regression models, and discriminatory ability was evaluated with the *c* index. All statistical tests were two-sided.** ***Results:*** **Concordance of hormone receptor status determined by both assays ranged from 74% (κ = 0.48) for PgR among postmenopausal patients to 88% (κ = 0.66) for ER in postmenopausal patients. Hazard ratio estimates were similar for the association between disease-free survival and ER status (among all patients) or PgR status (among postmenopausal patients) as determined by the two methods. However, among premenopausal patients treated with endocrine therapy alone, the discriminatory ability of PgR status as determined by immunohistochemical assay was statistically significantly better (*c* index = 0.60 versus 0.51; *P* = .003) than that determined by extraction assay, and so immunohistochemically determined PgR status could predict disease-free survival.** ***Conclusions:*** **Trial conclusions in which ER status (for all patients) or PgR status (for postmenopausal patients) was determined by immunohistochemical assay supported those determined by extraction assays. However, among premenopausal patients, trial conclusions drawn from PgR status differed—immunohistochemically determined PgR status could predict response to endocrine therapy, unlike that determined by the extraction assay. [J Natl Cancer Inst 2006;98:1571–81]**

The levels of estrogen receptor (ER) and progesterone receptor (PgR) in the primary tumor of a patient with early-stage invasive breast cancer are powerful predictors of that patient's response to adjuvant endocrine therapies and chemosensitivity of the primary tumor *(1–3)*. The 2005 International Expert Consensus on the Primary Therapy of Early Breast Cancer recognized that endocrine responsiveness of the primary tumor should be the first consideration for selecting adjuvant systemic therapies *(4)*.

Early studies establishing the predictive and prognostic value of steroid hormone receptors measured levels of ER and PgR in

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tumor cytosols with extraction assays *(5)*. Over the past 30 years, when the importance of evaluating steroid hormone receptor levels was being established and when the need to assess the value of adjuvant therapies according to receptor level in clinical trials was being recognized, the standard method of determining hormone receptors changed from extraction assays to immunohistochemical assays. This change was brought about by the development of immunohistochemical assays in general, followed by the wide commercial availability of ER antibodies and PgR antibodies in the 1990s. Immunohistochemical assays are less labor intensive and less expensive than extraction assays. They are also suitable for small tumors, allow morphologic correlation, and can be used on retrospectively collected specimens.

When hormone receptor status of tumor tissue determined by immunohistochemical assay has been compared with that determined by extraction assays *(6–10)*, discordance of 10%–30% has been found for both ER and PgR status. There has been remarkable consistency in this discordance across the literature, especially when one considers that previous studies were predominantly cohort studies, in which patient cohorts varied widely across and within studies (e.g., by disease stage and characteristics, treatments received, age, and menopausal status), and that assay methods varied widely (e.g., type of tissue and tissue preparation, type of extraction assays, antibodies used in the immunohistochemical assay, and scoring and cut points used to classify tumors as negative or positive) *(6–14)*.

Hormone receptor status determined by immunohistochemical assay has also been found consistently to have similar or superior predictive and prognostic value than that determined by extraction assays *(6,7,14–16)*. It is important to note, however, that many of these studies were limited by short follow-up and that the predictive and prognostic value was confounded by the inclusion in the cohort of women treated with a range of therapies *(11)*.

We therefore decided to use specimens from well-defined randomized clinical trial cohorts to investigate the extent to which the method of assessing hormone receptor status may affect trial conclusions. The clinical trials that we selected were two International Breast Cancer Study Group (IBCSG) randomized clinical trials of adjuvant therapy in pre- and perimenopausal patients (Trial VIII) and in postmenopausal patients (Trial IX) with lymph node–negative breast cancer. In such patients, the receipt of chemotherapy in addition to endocrine therapy is highly dependent on hormone receptor status. IBCSG Trial VIII concluded that premenopausal women with ER-negative (i.e., endocrine nonresponsive), lymph node–negative breast cancer should receive adjuvant chemotherapy, whereas for patients with ER-positive (i.e., endocrine responsive) disease, the combination of chemotherapy with ovarian function suppression or other endocrine agents and the use of endocrine therapy alone should be studied further *(17)*. IBCSG Trial IX concluded that postmenopausal patients with lymph node–negative breast cancer benefited substantially from adjuvant chemotherapy if their cancer was ER negative (i.e., endocrine nonresponsive); in contrast, if their cancer was ER positive (i.e., endocrine responsive), they obtained no benefit from the combination treatment compared with endocrine therapy alone *(18)*.

We investigated the concordance of hormone receptor status as determined by extraction assays originally in local pathology offices when patients were randomly assigned to treatment and as determined, more recently, by immunohistochemical assay in the IBCSG Central Pathology Laboratory. We examined the extent to which the assessment of treatment responsiveness in the two randomized trials differed when ER status was determined by immunohistochemical assay and by extraction assays. We further examined the assessment of treatment responsiveness according to PgR status, which was not considered in the primary trial analyses, because, in particular, we wanted to clarify whether PgR status as determined by either assay could predict treatment responsiveness. PgR requires a more technically difficult extraction assay than that for ER, and the PgR level in the primary tumor has been discounted by some as not useful in clinical practice *(19)*, although it has been endorsed by others as supplementing information on ER level *(20–22)*.

Patients and Methods

Trial Cohorts

The designs of IBCSG Trials VIII *(17)* and IX *(18)* have been described. Briefly, Trial VIII enrolled pre- and perimenopausal patients with lymph node–negative breast cancer. The trial evaluated whether sequential treatment with six 28-day courses of classical chemotherapy with cyclophosphamide, methotrexate, and fluorouracil (CMF) followed by 18 monthly subcutaneous implants of the gonadotropin-releasing hormone analog goserelin was statistically significantly associated with longer disease-free survival than six 28-day courses of classical CMF chemotherapy alone or 24 monthly implants of goserelin alone. From March 1, 1990, through October 1, 1999, a total of 1063 assessable patients were randomly assigned to treatment. Trial IX enrolled postmenopausal patients with lymph node–negative breast cancer and evaluated whether sequential treatment with three 28-day courses of classical CMF chemotherapy followed by tamoxifen for 57 months was associated with statistically significantly longer disease-free survival than tamoxifen alone for 60 months. From October 1, 1988, through August 1, 1999, a total of 1669 eligible and assessable patients were randomly assigned to treatment. Previously reported trial results were presented according to CONSORT guidelines and included participant flow diagrams *(17,18)*. Institutional review boards reviewed and approved the protocols, and informed consent was required according to the criteria established within the individual countries.

In both trials, patients with ER-positive, ER-negative, or ER-unknown tumors (ER-unknown status was allowed only if ER determination was not possible because of the lack of tumor material) were eligible until August 1, 1998. At that time, protocol amendments restricted enrollment to patients with ER-positive tumors because other trials had found that, for patients with ER-negative tumors, tamoxifen was not effective and ovarian ablation also might not be effective *(23,24)*. More than 94% of patients in these two trials were randomly assigned to treatment before release of the amendments. Randomization was stratified according to ER status and Trial IX was statistically powered for subset analyses according to ER status (the entire trial cohort had 80% power to detect a relative reduction in relapse risk of 33% for the ER-positive stratum and 50% for the ER-negative stratum).

Pathology Methods

Before randomization in both trials, concentrations of steroid hormone receptor in the primary tumors were determined by local pathologists with the use of standard assay methods *(25,26)*. At the trials' outset, ER status was determined by extraction assays, including a sucrose-gradient, dextran-coated charcoal assay or an enzyme-linked immunosorbent assay (ELISA); determination of ER status by immunohistochemical assay was allowed later in the study. A case report form collected data about all available quantitative and qualitative results from steroid hormone receptor assays. In Trials VIII and IX, 65% and 71% of patients, respectively, had their hormone receptor levels determined by an extraction assay. The assay method used was dextran-coated charcoal assay or ELISA in approximately 55% and 45% of these patients, respectively.

More than 80% of patients randomly assigned to treatment in Trials VIII and IX had archival tumor material available for immunohistochemical hormone receptor evaluation. Retrospective tissue collection was carried out in accordance with institutional guidelines and national laws. Immunohistochemical assays of the expression of ER and PgR in the primary tumors were carried out at the IBCSG Central Pathology Laboratory in Milan, Italy, in a blinded manner, as previously described *(27)*. Tumor specimens of 571 premenopausal and 976 postmenopausal patients on which extraction assays had determined ER and PgR levels before patients were randomly assigned to treatment were re-evaluated with an immunohistochemical assay in the IBCSG Central Pathology Laboratory.

To assess hormone receptor levels in archival formalin-fixed and paraffin-embedded tumor tissue specimens, sections were dewaxed, pretreated with 3% hydrogen peroxide for 5 minutes to block endogenous peroxidase activity, and then treated with 0.001 *M* EDTA (pH 8.0) at 99 °C for 20 minutes to retrieve antigenicity. Sections were then incubated with specific primary mouse monoclonal antibodies against human ER (clone 1D5, 1 : 100 dilution) or human PgR (clone 1A6, 1 : 800 dilution) (both obtained from Dako, Glostrup, Denmark) for 30 minutes at room temperature by use of an automatic immunostainer (Autostainer, Dako). Antibody–antigen complexes were subsequently visualized with a high-sensitivity detection kit (EnVision+ System-HRP; Dako) according to the manufacturer's instructions. Peroxidase activity was visualized as a brown–black product by use of 3,3′-diaminobenzidine and copper sulfate (Sigma Chemical Co, St Louis, MO) as the chromogen.

According to the recommendations for all IBCSG trials, we evaluated immunohistochemical results by the following protocol. 1) Tissue was checked for expected immunostaining of non-neoplastic breast tissue (intense nuclear staining of at least a minor percentage of luminal epithelial cells) and lack of immunoreactivity of myoepithelial, stromal, and inflammatory (if any) cells. 2) The staining pattern of the neoplastic component was evaluated at low or intermediate magnification (×100 to ×250) that took into account any biologically significant heterogeneity of staining in different parts of the invasive tumor. Staining of the intraductal (or in situ) component was not considered. 3) At higher magnification (×400), any definite nuclear localization of the immunostaining was assessed, without considering as specific any membrane or cytoplasmic staining. This nonspecific staining may appear on occasion (especially when apocrine or squamous metaplasia is present), and it does not interfere with the specific nuclear staining of the hormone-responsive neoplastic cells. 4) For tumors with homogeneous staining throughout, at least 10 high-power fields (containing a minimum of 2000 invasive tumor cells) were randomly selected, and the number of cells showing nuclear immunostaining (irrespective of the staining intensity) was counted and compared with the total number of neoplastic cells. The results were recorded as an overall percentage. For tumors with biologically significant staining heterogeneity, 10 high-power fields were selected to mirror the degree of staining heterogeneity. For example, if only 20% of the invasive tumor area showed diffuse nuclear staining and the remaining tumor had only occasional (or no) immunoreactive cells, then only two high-power fields were counted in the former area and eight high-power fields were counted in the latter area. In tissues with about 10% immunoreactive cells, additional fields were counted and/or another pathologist checked the results.

Statistical Considerations

Extraction assay values (expressed as femtomoles per milligram of cytosol protein) and immunohistochemistry values (expressed as the percentage of immunoreactive cells) were initially categorized as follows: 0 = none; 1–9 = low; 10 or more = high, with units corresponding to the assay used. The choice of cut points for the extraction assays reflected the standard practice: a value of 10 fmol/mg of cytosol protein or more was used to indicate a hormone receptor–positive status and a value of less than 10 fmol/mg of cytosol protein was used to indicate a hormone receptor–negative status. The choice of cut point for the immunohistochemical assay also reflected the frequent practice: 10% immunoreactive cells or more were used to indicate a hormone receptor–positive status and less than 10% immunoreactive cells were used to indicate a hormone receptor–negative status, as well as evidence that the presence of any immunoreactive cells appeared to indicate the endocrine responsiveness of the tumor *(11)*. Thus for extraction assays, our dichotomization was negative (<10 fmol/mg of cytosol protein) and positive (≥10 fmol/mg of cytosol protein). For immunohistochemical assays, our dichotomization was absent (0% stained cells) and present (>0% stained cells) or was negative (<10% stained cells) and positive (≥10% stained cells).

Concordance denoted the number or percentage of tumors with the same classification by the two assay methods. Agreement denoted the chance-corrected concordance as measured by a κ statistic, which was weighted (κ_w) for ordinal hormone receptor status variables and unweighted for dichotomous variables; 95% confidence intervals (CIs) were also calculated.

The primary outcome in both trials was disease-free survival, defined as the length of time from the date of randomization to any recurrent disease (including ipsilateral breast recurrence), the appearance of a second primary cancer (including contralateral breast cancer), or death, whichever occurred first. Hazard ratios (HRs) of recurrence or death (with corresponding 95% confidence intervals) and log-rank tests were reported for comparisons of disease-free survival between treatment arms according to hormone receptor status. Cox proportional hazards regression models were used to estimate hazard ratios and 95% confidence intervals. To check assumptions of proportionality, curves of the log of the cumulative hazard versus time were plotted and assessed visually to determine if the vertical shift

between the curves was constant over time and by testing for an interaction term of treatment arm with time in the models.

The discriminatory ability of hormone receptor status to predict disease-free survival was evaluated separately according to treatment by use of the *c* index *(28,29)*. In this study, the *c* index was interpreted as an estimate of the probability that, for two randomly chosen patients on the same treatment, the patient with a receptor-positive (or a receptor present) tumor will have longer disease-free survival than the patient with a receptor-negative (or a receptor absent) tumor. The *c* indices were compared between assay methods by use of a *U* statistic *(28,29)*. Disease-free survival among patients whose tumors were concordant or discordant by the two assay methods according to treatment were summarized by use of Kaplan–Meier plots. All statistical tests were two-sided, and *P* values of less than .05 were considered to be statistically significant.

All analyses were undertaken separately for the two trials. The data analysis used SAS version 9.1 (SAS Institute Inc, Cary, NC) and S-PLUS version 6.1 (Insightful Corp, Seattle, WA).

Results

Among the pre- and perimenopausal patients randomly assigned to treatment in Trial VIII and the postmenopausal patients randomly assigned to treatment in Trial IX, 571 (54%) of 1063 patients and 976 (58%) of 1669 patients, respectively, had hormone receptor levels measured both locally by an extraction assay and centrally by an immunohistochemical assay and were included in these analyses. The clinical characteristics of patients in this study and those of the trial cohorts were comparable, although, in this analysis cohort, there were slightly fewer patients (123 [8%] of 1547 total patients) with tumors that were 0–1 cm in diameter than there were in the overall trial cohorts. Median duration of follow-up was 8.2 and 9.4 years in the Trials VIII and IX analysis cohorts, respectively; follow-up was more than 1.5 years longer in these analysis cohorts than among patients who were not included in our study because patients in the analysis cohorts likely entered the trials earlier when only extraction assays were used to establish ER status at randomization.

The distributions of ER and PgR values are summarized in Table 1. Spearman's rank correlation coefficients for the quantitative values obtained by the two assay methods were .46 for ER and .61 for PgR among premenopausal patients and were .53 for ER and .61 for PgR among postmenopausal patients, indicating moderately strong positive associations between the quantitative assay values.

Concordance and Agreement

The classification of tumors by hormone receptor status is presented in Table 2. The concordance and agreement of hormone receptor status as determined by extraction and immunohistochemical assays are presented in Table 3. For the different hormone receptor status variables in both pre- and postmenopausal patients, few tumors were classified into the low category by immunohistochemical assay (1%–9% of cells stained), and few tumors were classified as none (0 fmol/mg of cytosol protein) by extraction assays. Thus, the concordance of the three categories was in the range of 55%–74% (κ_W, indicating agreement, ranged from 0.33 to 0.47).

In contrast, concordance and agreement were better for extraction assay results dichotomized as negative or positive compared with immunohistochemical assay results dichotomized as absent or present or as negative or positive; concordance ranged from 74% to 88% (κ ranged from 0.48 to 0.66). For ER status, concordance and agreement were observed to be higher among postmenopausal patients (88%; κ = 0.66) than among premenopausal patients (81%; κ = 0.53). However, concordance and agreement for PgR status were lower among postmenopausal patients (76%; κ = 0.49) than among premenopausal patients (80%; κ = 0.51), and concordance for ER status and PgR status was similar among premenopausal patients (Fig. 1).

ER status from extraction assays indicated that 179 (32%) of 562 tumors in premenopausal patients were ER negative and 231 (24%) of 959 tumors in postmenopausal patients were ER negative. When the ER status of these ER-negative tumors was determined by immunohistochemical assay, 76 (42%) of the 179 ER-negative tumors in premenopausal patients were ER present and 72 (31%) of the 231 ER-negative tumors in postmenopausal patients were ER present. Of 383 and 728 tumors in premenopausal and postmenopausal patients, respectively, as determined by extraction assays to be ER positive, 30 (8%) and 44 (6%) were ER absent by immunohistochemical assay. Thus, approximately one-third of the 25%–30% of patients who would not have been eligible for the trials after ER-negative tumors were deemed ineligible would have been considered as appropriate candidates for the trials according to immunohistochemical assay results, and approximately 10% of patients who were eligible according to extraction assay results would not have been considered as appropriate trial candidates using immunohistochemical assays.

PgR results from extraction assays indicated that 162 (29%) of the 557 tumors in premenopausal patients were PgR negative and that 337 (37%) of the 919 tumors in postmenopausal patients were PgR negative. Among the 162 PgR-negative

Table 1. Distributions of ER and PgR by extraction assays and by immunohistochemical (IHC) assay in premenopausal (Trial VIII) and postmenopausal (Trial IX) patients with lymph node–negative breast cancer*

		Extraction assays, fmol/mg of cytosol protein		IHC, % of stained cells		
Receptor	No. of patients	Median (IQR)	Range	Median (IQR)	Range	Spearman's rank correlation coefficient
Premenopausal (Trial VIII)						
ER	562	19 (7–52)	0–600	71 (2–90)	0–100	.46
PgR	557	39 (8–145)	0–7027	60 (0–90)	0–100	.61
Postmenopausal (Trial IX)						
ER	959	50 (10–188)	0–1815	90 (21–95)	0–100	.53
PgR	919	19 (4–100)	0–2564	15 (0–75)	0–100	.61

*ER = estrogen receptor; PgR = progesterone receptor; IQR = interquartile range.

Table 2. Classification of tumors by extraction assays (fmol/mg of cytosol protein) and by immunohistochemical (IHC) assay (% of stained cells) in premenopausal (Trial VIII) and postmenopausal (Trial IX) patients with lymph node–negative breast cancer*

			IHC			
			Absent	Present		
Trial	Receptor	Extraction assay	None (0)	Low (1–9)	High (≥10)	Total
Premenopausal (Trial VIII)	ER	Negative				
		None (0)	21 (3.7)	3 (0.5)	8 (1.4)	32 (5.7)
		Low (1–9)	82 (14.6)	6 (1.1)	59 (10.5)	147 (26.2)
		Positive				
		High (≥10)	30 (5.3)	6 (1.1)	347 (61.7)	383 (68.1)
		Total	133 (23.7)	15 (2.7)	414 (73.7)	562 (100)
	PgR	Negative				
		None (0)	24 (4.3)	2 (0.4)	10 (1.8)	36 (6.5)
		Low (1–9)	80 (14.4)	12 (2.2)	34 (6.1)	126 (22.6)
		Positive				
		High (≥10)	53 (9.5)	20 (3.6)	322 (57.8)	395 (70.9)
		Total	157 (28.2)	34 (6.1)	366 (65.7)	557 (100)
Postmenopausal (Trial IX)	ER	Negative				
		None (0)	26 (2.7)	1 (0.1)	10 (1.0)	37 (3.9)
		Low (1–9)	133 (13.9)	8 (0.8)	53 (5.5)	194 (20.2)
		Positive				
		High (≥10)	44 (4.6)	9 (0.9)	675 (70.4)	728 (75.9)
		Total	203 (21.2)	18 (1.9)	738 (77.0)	959 (100)
	PgR	Negative				
		None (0)	55 (6.0)	9 (1.0)	16 (1.7)	80 (8.7)
		Low (1–9)	169 (18.4)	27 (2.9)	61 (6.6)	257 (28.0)
		Positive				
		High (≥10)	105 (11.4)	53 (5.8)	424 (46.1)	582 (63.3)
		Total	329 (35.8)	89 (9.7)	501 (54.5)	919 (100)

*ER = estrogen receptor; PgR = progesterone receptor.

tumors in premenopausal patients, 58 (36%) were PgR present by immunohistochemical assay, and among the 337 PgR-negative tumors in postmenopausal patients, 113 (34%) were PgR present by immunohistochemical assay. Among the 395 and 582 PgR-positive tumors by extraction assays in premenopausal and postmenopausal patients, respectively, 53 (13%) and 105 (18%) were PgR absent by immunohistochemical assay.

Among the few tumors staining ER low by immunohistochemical assay, six (40%) of the 15 such tumors in premenopausal patients and nine (50%) of the 18 such tumors in postmenopausal patients were ER positive by the extraction assay. Among tumors staining PgR low by immunohistochemical assay, 20 (59%) of the 34 such tumors in premenopausal patients and 53 (60%) of the 89 such tumors in postmenopausal patients were PgR positive by the extraction assay. This finding suggests that it was reasonable to consider tumors classified as hormone receptor low by immunohistochemical assay as also endocrine responsive. Thus, in subsequent analyses, we focused on immunohistochemical assay values that were dichotomized as absent versus present and

Table 3. Concordance and agreement of hormone receptor status between the extraction assay and the immunohistochemical (IHC) assay and corresponding κ statistic (95% CI) by categorization*

	Extraction assay versus IHC†		
Receptor	None/low/high versus none/low/high	Negative/positive versus negative/positive	Negative/positive versus absent/present
Premenopausal (Trial VIII)			
ER status (n = 562)			
No. concordant (%)	374 (67)	459 (82)	456 (81)
κ (95% CI)	0.41 (0.35 to 0.47)	0.56 (0.48 to 0.63)	0.53 (0.46 to 0.61)
PgR status (n = 557)			
No. concordant (%)	358 (64)	440 (79)	446 (80)
κ (95% CI)	0.37 (0.31 to 0.43)	0.52 (0.44 to 0.59)	0.51 (0.43 to 0.59)
Postmenopausal (Trial IX)			
ER status (n = 959)			
No. concordant (%)	709 (74)	843 (88)	843 (88)
κ (95% CI)	0.47 (0.42 to 0.51)	0.66 (0.61 to 0.72)	0.66 (0.60 to 0.71)
PgR status (n = 919)			
No. concordant (%)	506 (55)	684 (74)	701 (76)
κ (95% CI)	0.33 (0.29 to 0.38)	0.48 (0.42 to 0.53)	0.49 (0.43 to 0.55)

*ER = estrogen receptor; PgR = progesterone receptor; CI = confidence interval. κ statistics were weighted for ordinal hormone receptor status and unweighted for dichotomous hormone receptor status.

†None, low, and high are 0, <10, and ≥10, respectively, with appropriate units of measure; negative versus positive are <10 versus ≥10; and absent versus present are 0 versus >0. For the extraction assay, units are fmol/mg of cytosol protein. For IHC, units are percentage of stained cells.

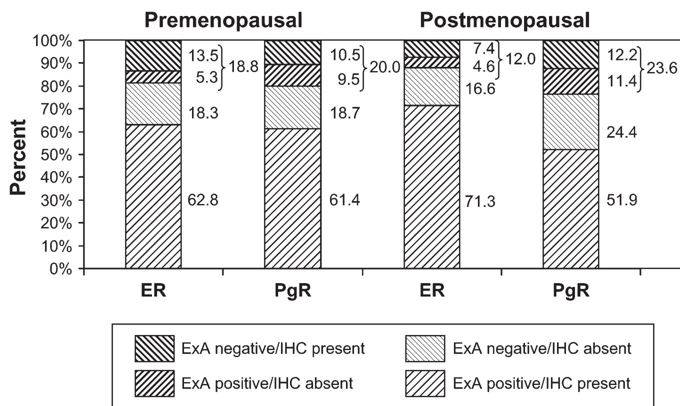

Fig. 1. Concordance of hormone receptor status as determined by extraction assays (ExA) and immunohistochemical (IHC) assays, for premenopausal and perimenopausal (Trial VIII) and postmenopausal (Trial IX) patients. For ExA, the cut-point values for negative versus positive tumors were <10 or ≥10 fmol/mg of cytosol protein. For IHC, the cut-point values for absent versus present tumors were 0% or >0% immunoreactive cells. The discordance, shown at the top of the bars, ranged from 12.0% to 23.6%. ER = estrogen receptor; PgR = progesterone receptor.

extraction assay values that were dichotomized standardly as negative versus positive at a cut point of 10 fmol/mg of cytosol protein.

Trial Results

The forest plot in Fig. 2 summarizes evaluations of outcomes for Trials VIII and IX that are based on the patients included in this analysis. For each trial, disease-free survival was compared between treatment arms according to hormone receptor status, which was determined by extraction assays (negative or positive) and by an immunohistochemical assay (absent or present). Hazard ratios of recurrence or death and corresponding 95% confidence intervals are presented.

In Trial VIII, we observed remarkably similar treatment comparison results between the two assay methods among subgroups of premenopausal patients defined by ER status, i.e., the estimated hazard ratios and 95% confidence intervals comparing treatments among the ER-negative subgroup defined by extraction assays are virtually identical to the those among ER-absent

Disease-free survival hazard ratios

Patient Group	Events/Pts	Hazard Ratio (95% CI)	Log-rank p-value
Estrogen Receptor (ER)			
Premenopausal (Trial VIII) CMF:Goserelin			
ExA ER negative	35/121	0.6 (0.3 , 1.17)	0.135
ExA ER positive	66/248	0.87 (0.54 , 1.42)	0.587
IHC ER absent	32/89	0.61 (0.3 , 1.26)	0.181
IHC ER present	69/280	0.86 (0.53 , 1.37)	0.518
Premenopausal (Trial VIII) CMF→Goserelin:Goserelin			
ExA ER negative	32/117	0.49 (0.23 , 1.01)	0.054
ExA ER positive	68/255	0.85 (0.53 , 1.37)	0.516
IHC ER absent	31/90	0.48 (0.23 , 1.01)	0.053
IHC ER present	69/282	0.85 (0.53 , 1.36)	0.495
Postmenopausal (Trial IX) CMF→Tamoxifen:Tamoxifen			
ExA ER negative	72/231	0.49 (0.3 , 0.8)	0.004
ExA ER positive	199/728	0.96 (0.73 , 1.27)	0.785
IHC ER absent	65/203	0.46 (0.27 , 0.77)	0.003
IHC ER present	206/756	0.95 (0.73 , 1.25)	0.739
Progesterone Receptor (PgR)			
Premenopausal (Trial VIII) CMF:Goserelin			
ExA PgR negative	33/112	0.93 (0.47 , 1.84)	0.83
ExA PgR positive	63/252	0.71 (0.43 , 1.17)	0.175
IHC PgR absent	38/104	0.62 (0.32 , 1.19)	0.152
IHC PgR present	58/260	0.92 (0.55 , 1.54)	0.752
Premenopausal (Trial VIII) CMF→Goserelin:Goserelin			
ExA PgR negative	26/102	0.61 (0.28 , 1.35)	0.227
ExA PgR positive	70/265	0.79 (0.49 , 1.26)	0.326
IHC PgR absent	36/105	0.46 (0.23 , 0.9)	0.025
IHC PgR present	60/262	0.99 (0.6 , 1.65)	0.985
Postmenopausal (Trial IX) CMF→Tamoxifen:Tamoxifen			
ExA PgR negative	106/337	0.7 (0.47 , 1.03)	0.067
ExA PgR positive	157/582	0.86 (0.63 , 1.18)	0.349
IHC PgR absent	100/329	0.76 (0.51 , 1.13)	0.174
IHC PgR present	163/590	0.81 (0.59 , 1.1)	0.177

95% CI (CT±ET : ET)

0.25 0.5 1.0 2.0 4.0

Favors CT±ET Favors ET

Fig. 2. Disease-free survival. Single-agent endocrine therapy was compared with chemotherapy, with or without endocrine therapy for hormone receptor status, as determined by extraction assays (ExA) and by immunohistochemical (IHC) assays for premenopausal (Trial VIII) and postmenopausal (Trial IX) patients included in this study. Hazard ratios of recurrence or death are presented. For ExA, the cut-point values for negative versus positive tumors were <10 or ≥10 fmol/mg of cytosol protein. For IHC, the cut-point values for absent versus present tumors were 0% or >0% immunoreactive cells. Results were remarkably consistent, with the exception of comparisons within the premenopausal progesterone receptor (PgR) subgroups, which are enclosed in a **dashed box**. *P* values were calculated from two-sided log-rank tests. Pts = patients; CI = confidence interval; CT = chemotherapy; ET = endocrine therapy; CMF = cyclophosphamide, methotrexate, and fluorouracil chemotherapy.

Table 4. Evaluation of the discriminatory ability (*c* index) of hormone receptor status variables determined by extraction assays and by immunohistochemical (IHC) assay separately by treatment*

Receptor	Extraction assay, negative versus positive†	IHC, absent versus present†	*P* value‡
Premenopausal (Trial VIII)			
Goserelin (n = 182)			
ER	0.54	0.56	.87
PgR	0.51	0.60	.003
CMF (n = 193)			
ER	0.49	0.53	.32
PgR	0.53	0.55	.83
CMF → goserelin (n = 196)			
ER	0.48	0.50	.49
PgR	0.48	0.50	.87
Postmenopausal (Trial IX)			
Tamoxifen (n = 491)			
ER	0.56	0.58	.37
PgR	0.55	0.56	.23
CMF → tamoxifen (n = 485)			
ER	0.49	0.50	.47
PgR	0.52	0.54	.83

*ER = estrogen receptor; PgR = progesterone receptor; CMF = cyclophosphamide, methotrexate, and fluorouracil chemotherapy. The *c* index estimates the probability that, of two randomly chosen patients receiving the same treatment, the patient with a receptor-positive tumor will have longer disease-free survival than the patient with a receptor-negative tumor (or for IHC, receptor-present versus receptor-absent tumor). Values of *c* index that are near to 0.5 indicate that receptor status is no different than chance in determining which patient will have longer disease-free survival, and values that are near to 0 or 1 indicate that receptor status virtually always determine which patient has longer disease-free survival.

†Extraction assays negative versus positive are <10 versus ≥10 fmol/mg of cytosol protein, respectively; IHC assays absent versus present are 0 versus >0% of immunoreactive cells, respectively.

‡The *P* value that compared the *c* index of extraction assay result of negative or positive with that of IHC result of absent or present is from a two-sided *U* statistic.

subgroup defined by immunohistochemical assay (e.g., for the comparison CMF versus goserelin: ER-negative HR = 0.60, 95% CI = 0.30 to 1.17; ER-absent HR = 0.61, 95% CI = 0.30 to 1.26). In contrast, we observed different results among subgroups defined by PgR status that were determined by the two assay methods, especially in comparisons that included goserelin. In the PgR-absent subgroup as defined by immunohistochemical assay, the decreased hazard of recurrence or death in the CMF-alone arm or the CMF–goserelin arm, as compared with that in the goserelin-alone arm, was more striking (for the comparison CMF versus goserelin, HR = 0.62, 95% CI = 0.32 to 1.19; for the comparison CMF–goserelin versus goserelin, HR = 0.46, 95% CI = 0.23 to 0.90); in the PgR-present subgroup defined by immunohistochemical assay, the ratios of hazards of recurrence or death were not statistically different from unity (for the comparison of CMF versus goserelin, HR = 0.92, 95% CI = 0.55 to 1.54; for the comparison CMF–goserelin versus goserelin, HR = 0.99, 95% CI = 0.60 to 1.65).

These observations were confirmed in an assessment of how well ER status and PgR status predicted disease-free survival within a given treatment arm (Table 4) and in a comparison of hormone receptor status as determined by extraction assays and by immunohistochemical assay. Among patients treated with goserelin alone, ER status as determined by extraction assays and that determined by immunohistochemical assay had similar discriminatory ability (*c* index = 0.54 and 0.56, respectively; *P* = .87). However, the discriminatory ability of PgR status as determined by immunohistochemical assay was statistically significantly better than that determined by extraction assays (*c* index = 0.60 and 0.51, respectively; *P* = .003), with PgR status determined by extraction assays having little ability to predict disease-free survival among goserelin-treated patients. To illustrate these results, disease-free survival among patients randomly assigned to the goserelin-alone arm was summarized (Fig. 3) for ER status and PgR status as classified by the two assay methods. Among the 15 patients whose PgR status was PgR present by the immunohistochemical assay and PgR negative by the extraction assay (Fig. 3, B), only one patient experienced a recurrence or death; whereas, among the 15 patients whose PgR status was PgR absent by the immunohistochemical assay and PgR positive by the extraction assay, eight patients experienced recurrence or death (Fig. 3, B).

Among postmenopausal patients in Trial IX, similar estimates of hazard ratios were obtained when the two assay methods were used to determine hormone receptor status, and the same conclusions would be reached—i.e., adjuvant CMF in addition to tamoxifen increased disease-free survival compared with tamoxifen alone among patients with ER-negative tumors but not among patients with ER-positive tumors (Fig. 2). A similar pattern was found for PgR status, although results that were based on PgR status appeared more discrepant. Among patients on the tamoxifen-alone arm, ER status and PgR status as determined by extraction assay or by an immunohistochemical assay could predict response to tamoxifen, with *c* index values ranging from 0.55 to 0.58, but values were not statistically significantly different between assay methods (for ER status, *P* = .37; for PgR status, *P* = .23; Table 4). As an illustration, disease-free survival among patients in the tamoxifen-alone arm was summarized according to ER status and PgR status as classified by both assay methods (Fig. 3). Disease-free survival appeared slightly, although not statistically significantly, better for patients whose ER status was ER present by an immunohistochemical assay and ER negative by the extraction assay than for patients whose ER status was ER absent by an immunohistochemical assay and ER positive by the extraction assay (Fig. 3, C).

Discussion

In this re-evaluation of data from two IBCSG randomized clinical trials of adjuvant therapy in premenopausal (Trial VIII) and postmenopausal (Trial IX) patients with lymph node–negative breast cancer, conclusions that were based on ER status determined by immunohistochemical assay performed at a central laboratory confirmed those that were based on ER status determined by extraction assays performed at local pathology laboratories (i.e., premenopausal and postmenopausal women with ER-negative, lymph node–negative breast cancer benefited [in terms of better disease-free survival] from the addition of adjuvant chemotherapy to endocrine therapy, whereas if their cancer was ER positive, postmenopausal women obtained no benefit from the combination treatment compared with endocrine therapy alone and for premenopausal women the use of combination treatment or endocrine therapy alone required further study). When disease-free survival was evaluated according to PgR status of the primary tumor, which was not examined in the primary trial analyses, PgR status that was determined by an

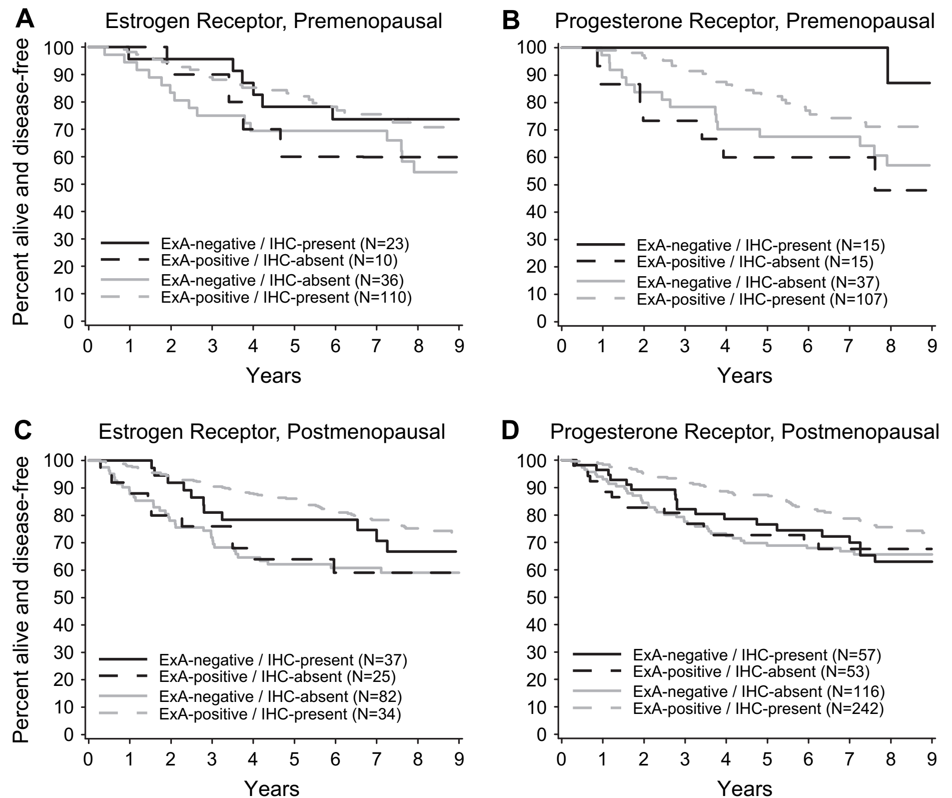

Fig. 3. Kaplan–Meier estimates of disease-free survival according to concordance of hormone receptor status determined by extraction assay (ExA) and by immunohistochemical (IHC) assay. **Black lines** indicate discordant assay results; **shaded lines** indicate concordant assay results. **A**) Estrogen receptor (ER) status among premenopausal patients on the goserelin-only arm of Trial VIII. The numbers of patients at risk at times 0, 5, and 8 years were 23, 17, and 10 for those with ExA-negative and IHC-present tumors; 10, 6, and 5 for those with ExA-positive and IHC-absent tumors; 36, 25, and 13 for those with ExA-negative and IHC-absent tumors; and 110, 74, and 39 for those with ExA-positive and IHC-present tumors. Estimates of 5-year disease-free survival were 78% (95% confidence interval [CI] = 55% to 90%), 60% (95% CI = 25% to 83%), 69% (95% CI = 52% to 82%), and 82% (95% CI = 73% to 88%), respectively. **B**) Progesterone receptor (PgR) status among premenopausal patients on the goserelin-only arm of Trial VIII. The numbers of patients at risk at times 0, 5, and 8 years were 15, 15, and 7 for those with ExA-negative and IHC-present tumors; 15, 9, and 3 for those with ExA-positive and IHC-absent tumors; 37, 25, and 15 for those with ExA-negative and IHC-absent tumors; and 107, 70, and 40 for those with ExA-positive and IHC-present tumors. Estimates of 5-year disease-free survival were 100% (95% CI = 78% to 100%), 60% (95% CI = 32% to 80%), 68% (95% CI = 50% to 80%), and 82% (95% CI = 73% to 88%), respectively. **C**) ER status among postmenopausal patients on the tamoxifen-only arm of Trial IX. The numbers of patients at risk at times 0, 5, and 8 years were 37, 24, and 17 for those with ExA-negative and IHC-present tumors; 25, 13, and 11 for those with ExA-positive and IHC-absent tumors; 82, 49, and 31 for those with ExA-negative and IHC-absent tumors; and 340, 255, and 162 for those with ExA-positive and IHC-present tumors. Estimates of 5-year disease-free survival were 78% (95% CI = 61% to 89%), 64% (95% CI = 42% to 79%), 62% (95% CI = 51% to 72%), and 86% (95% CI, 82% to 89%), respectively. **D**) PgR status among postmenopausal patients on the tamoxifen-only arm of Trial IX. The numbers of patients at risk at times 0, 5, and 8 years were 57, 36, and 24 for those with ExA-negative and IHC-present tumors; 53, 30, and 22 for those with ExA-positive and IHC-absent tumors; 116, 78, and 50 for those with ExA-negative and IHC-absent tumors; and 242, 184, and 114 for those with ExA-positive and IHC-present tumors. Estimates of 5-year disease-free survival were 77% (95% CI = 63% to 86%), 73% (95% CI = 58% to 83%), 69% (95% CI = 60% to 76%), and 87% (95% CI = 82% to 91%), respectively.

immunohistochemical assay could predict response to endocrine therapy better than that determined by extraction assays, particularly among pre- and perimenopausal patients. These results provide confidence in the use of steroid hormone receptor status determined by immunohistochemical assay to select adjuvant treatment, even though the clinical trials on which these evidence-based recommendations were established had determined hormone receptor status by locally performed extraction assays, which are expected to have a discordance of 10%–30% with respect to immunohistochemical assay.

We investigated data from two randomized clinical trial populations of patients with lymph node–negative, early-stage invasive breast cancer. The trial populations were pre- and perimenopausal patients and postmenopausal patients who underwent similar well-defined treatment strategies to evaluate the combination of chemoendocrine therapy compared with endocrine therapy alone. Only two other studies *(14,30)* used randomized clinical trial populations who received adjuvant therapy, but the cohorts of patients with lymph node–positive disease were smaller in both studies than those in Trials VIII and IX. In the IBCSG trial populations of patients with lymph node–negative breast cancer, immunohistochemical assay–determined hormone receptor status and extraction assay–determined status had rates of concordance and agreement that were consistent with those previously reported *(6–14)*, and we confirmed that immunohistochemical assay–determined hormone receptor status was a valid discriminator of endocrine responsiveness and chemosensitivity.

In particular, among pre- and perimenopausal patients in Trial VIII, the benefit of increased disease-free survival associated with CMF followed by goserelin, compared with goserelin alone, was most clearly evident among PgR-absent tumors, as determined by immunohistochemical assay. In contrast, among postmenopausal patients in Trial IX, ER status demonstrated the benefit of increased disease-free survival associated with adjuvant CMF among patients with ER-negative (or ER-absent) tumors, regardless of assay method. Colleoni et al. *(31)* also found that PgR status was more predictive of response to perioperative chemotherapy than ER status among premenopausal patients with lymph node–negative disease, whereas ER status was more predictive of response than PgR status among postmenopausal patients.

When we compared the results of locally assessed extraction assays with those of centrally assessed immunohistochemical assay in these trial populations of patients with lymph node–negative disease, the immunohistochemical assay appeared to increase the fraction of ER-present and PgR-present tumors in both pre- and perimenopausal patients and in postmenopausal patients, most likely because of the overall higher sensitivity of the immunohistochemical assay. The fraction of tumors that scored positive by use of extraction assays and absent by use of an immunohistochemical assay was much higher for PgR levels than

for ER levels in both trials, suggesting that extraction assays were more likely to yield false-positive results for PgR status than for ER status. This result appeared to be especially true among pre- and perimenopausal patients because, when extraction assays were used, the fraction of patients with PgR-positive tumors was greater than the fraction of patients with ER-positive tumors, a result that is at variance with the current knowledge on the coordinate expression of the two receptors *(32)*. The false-positive results for PgR status as determined by extraction assays may explain its inability to predict disease-free survival among pre- and perimenopausal patients treated with endocrine therapy alone and its inability to demonstrate disease-free survival benefit of chemoendocrine therapy compared with endocrine therapy alone in PgR-negative tumors. These abilities emerged clearly when we reassessed PgR status among pre- and perimenopausal patients by immunohistochemical assay.

The main advantages of immunohistochemical assays compared with extraction assays are that immunohistochemical assays are easier and quicker to perform and may be performed on very small tumors or tumor specimens, such as those obtained by fine-needle aspiration biopsy or core biopsy examinations. Immunohistochemical assays allow precise determination of hormone receptor status at the individual cell level, by taking into account only the neoplastic cells of the invasive component of the tumor, and thereby accommodate the heterogeneity of hormone receptor expression in tumors, which cannot be evaluated by extraction assays. Extraction assays, in contrast, generally provide an overall score for the entire cell population of the tumor tissue, including non-neoplastic cells and in situ components of the tumor, and thus may lead to false-positive or false-negative findings, depending on the relative proportions of truly invasive tumor cells and other cell types in the tumor tissue. Extraction assays also quantitatively evaluate the hormone receptor content of the sample, which may be more difficult to achieve by immunohistochemical assay. In fact, the quantitative or semiquantitative assessment of the percentage of immunoreactive cells may not be truly representative of the quantitative differences in hormone receptor content of different tumors. Both intensity of staining and number of immunostained cells in different tumor samples may be affected by preanalytical (e.g., type and length of fixation) and analytical (e.g., type and concentration of the immunohistochemical reagents or the immunostaining protocol) variables and by interpretative bias. As a consequence, intra- and interlaboratory reproducibility of the results of immunohistochemical assays for hormone receptor levels may reduce the predictive power of hormone receptor status and thus affect the choice of the most appropriate therapeutic intervention. It is essential that the reproducibility of immunohistochemical assay results be substantially improved by active participation of all involved laboratories in quality assurance and quality control programs and that standardized procedures, such as those used by IBCSG and also used in this study, be adopted to satisfy the continual need for accurate evaluation of receptor status. The standardized approach to evaluation and immunostaining used by IBCSG minimized interlaboratory technical variations and underscores the value of tissue collection and central review of hormone receptors as part of randomized clinical trials evaluating adjuvant systemic therapies for breast cancer.

When interpreting the results of this study, certain limitations should be taken into account. First, we were unable to determine whether a cut point of 1% or more or of 10% or more stained cells in immunohistochemical assays was more concordant with tumors classified as ER positive (≥10 fmol/mg of cytosol protein) by extraction assays because too few tumors were classified into the ER low category by immunohistochemical assay (1%–9% of cells stained). Second, the small numbers of tumors with discordant classification prevented direct comparisons of disease-free survival between patients whose tumors were hormone receptor absent by immunohistochemical assay and positive by extraction assay with patients whose tumors were hormone receptor present by immunohistochemical assay and negative by extraction assay. Finally, about 20% of patients randomly assigned to treatment in Trials VIII and IX did not have archival tumor material available for hormone receptor evaluation, though the clinical characteristics of patients included in this study and those of the overall trial cohorts were comparable, with the exception that there were slightly fewer patients with tumors that were 1 cm or less in diameter in this study.

Despite the 10%–30% discordance in steroid hormone receptor status determined by immunohistochemical assay versus extraction assays, on average in this population of lymph node–negative invasive breast cancers, the presence or absence of ER determined by an immunohistochemical assay confirmed randomized clinical trial results that were based on ER status determined by extraction assays. PgR status determined by immunohistochemical assay improved the ability of PgR to predict response to endocrine therapy, particularly among pre- and perimenopausal patients.

Appendix

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References

(1) Clark GM. Prognostic and predictive factors. In: Harris J, Lippman ME, Morrow M, et al., editors. Diseases of the breast. Philadelphia (PA): Lippincott-Raven; 1996. p. 461–84.

(2) ASCO Tumor Marker Expert Panel. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. J Clin Oncol 1996; 14:2843–77.

(3) Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thurlimann B, Senn H-J. Meeting highlights: updated international expert consensus on the primary therapy of early breast cancer. J Clin Oncol 2003;21:3357–65.

(4) Goldhirsch A, Glick JH, Gelber RD, Coates AS, Thurlimann B, Senn H-J. Meeting highlights: international expert consensus on the primary therapy of early breast cancer 2005. Ann Oncol 2005;16:1569–83.

(5) Barnes DM, Millis RR, Beex LVAM, Thorpe SM, Leake RE. Increased use of immunohistochemistry for oestrogen receptor measurement in mammary carcinoma: the need for quality assurance. Eur J Cancer 1998;34: 1677–82.

(6) Pertschuk LP, Kim DS, Nayer K, Feldman JG, Eisenberg KB, Carter AC, et al. Immunocytochemical estrogen and progestin receptor assays in breast cancer with monoclonal antibodies. Cancer 1990;66:1663–70.

(7) Beck T, Weikel W, Brumm C, Wilkens C, Pollow K, Knapstein PG. Immunohistochemical detection of hormone receptors in breast carcinomas (ER-ICA, PgR-ICA): prognostic usefulness and comparison with the biochemical radioactive-ligand-binding assay (DCC). Gynecol Oncol 1994;53: 220–7.

(8) Stierer M, Rosen H, Weber R, Hanak H, Auerbach L, Spona J, et al. Comparison of immunohistochemical and biochemical measurement of steroid receptors in primary breast cancer: evaluation of discordant findings. Breast Cancer Res Treat 1998;50:125–34.
(9) Zafrani B, Aubriot M-H, Mouret E, de Cremoux P, de Rycke Y, Nicolas A, et al. High sensitivity and specificity of immunohistochemistry for the detection of hormone receptors in breast carcinoma: comparison with biochemical determination in a prospective study of 793 cases. Histopathology 2000;37:536–45.
(10) Chebil G, Bendahl P-O, Idvall I, Ferno M. Comparison of immunohistochemical and biochemical assay of steroid receptors in primary breast cancer. Acta Oncol 2003;42:719–25.
(11) Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 1999;17:1474–81.
(12) Gordts SL, Neven P, Van Hooff I, Tonglet R, Laurent C, Marechal S, et al. The immunocytochemical versus cytosol measurement of the oestrogen receptor in invasive breast cancer tissue. Eur J Cancer 2000;36:S13–23.
(13) Mohsin SK, Weiss H, Havighurst T, Clark GM, Berardo M, Roanh LD, et al. Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. Mod Pathol 2004;17:1545–54.
(14) Fisher ER, Anderson S, Dean S, Dabbs D, Fisher B, Siderits R, et al. Solving the dilemma of the immunohistochemical and other methods used for scoring estrogen receptor and progesterone receptor in patients with invasive breast carcinoma. Cancer 2005;103:164–73.
(15) Esteban JM, Kandalaft PL, Mehta P, Odom-Maryon TL, Bacus S, Battifora H. Improvement of the quantification of estrogen and progesterone receptors in paraffin-embedded tumors by image analysis. Am J Clin Pathol 1993;99:32–8.
(16) Molino A, Micciolo R, Turazza M, Bonetti F, Piubello Q, Corgnati A, et al. Prognostic significance of estrogen receptors in 405 primary breast cancers: a comparison of immunohistochemical and biochemical methods. Breast Cancer Res Treat 1997;45:241–9.
(17) International Breast Cancer Study Group. Adjuvant chemotherapy followed by goserelin versus either modality alone for premenopausal lymph node-negative breast cancer: a randomized trial. J Natl Cancer Inst 2003;95: 1833–46.
(18) International Breast Cancer Study Group. Endocrine responsiveness and tailoring adjuvant therapy for postmenopausal lymph node-negative breast cancer: a randomized trial. J Natl Cancer Inst 2002;94:1054–65.
(19) Olivotto IA, Truong PT, Speers CH, Bernstein V, Allan SJ, Kelly SJ, et al. Time to stop progesterone receptor testing in breast cancer management [letter]. J Clin Oncol 2004;22:1769–70.
(20) Fuqua SAW, Cui Y, Lee AV, Osborne CK, Horwitz KB. Insights into the role of progesterone receptors in breast cancer [letter]. J Clin Oncol 2005;23: 931–2.
(21) MacGrogan G, de Mascarel I, Sierankowski G, Mauriac L, Debled M, Durand M, et al. Time for reappraisal of progesterone-receptor testing in breast cancer management [letter]. J Clin Oncol 2005;23:2870–1.
(22) Regan MM, Gelber RD. Predicting response to systemic treatments: learning from the past to plan for the future. Breast 2005;14:582–93.
(23) Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomized trials. Lancet 1998;351: 1451–67.
(24) Early Breast Cancer Trialists' Collaborative Group. Ovarian ablation in early breast cancer: overview of the randomized trials. Lancet 1996;348: 1189–96.
(25) Davis BW, Zava DT, Locker GW, Goldhirsch A, Harmann WH. Receptor heterogeneity of human breast cancer as measured by multiple intratumoral assays of estrogen and progesterone receptor. Eur J Cancer Clin Oncol 1984;20:375–82.
(26) Berger U, Wilson P, McClelland RA, Davidson J, Coombes RC. Correlation of immunocytochemically demonstrated estrogen receptor distribution and histopathologic features in primary breast cancer. Hum Pathol 1987;18:1263–7.
(27) Manzotti M, Dell'Orto P, Maisonneuve P, Fornaro M, Languino LR, Viale G. Down-regulation of beta(1C) integrin in breast carcinomas correlates with high proliferative fraction, high histological grade, and larger size. Am J Pathol 2000;156:169–74.
(28) Harrell FE. Regression modeling strategies: with applications to linear models, logistic regression, and survival analysis. New York (NY): Springer-Verlag; 2001.
(29) Alzola CF, Harrell FE. An introduction to S and the Hmisc and Design libraries, 2001. Available at: http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/RS. [Last accessed: September 26, 2006.]
(30) Alberts SR, Ingle JN, Roche PR, Cha SS, Wold LE, Farr GH Jr, et al. Comparison of estrogen receptor determinations by a biochemical ligand-binding assay and immunohistochemical staining with monoclonal antibody ER1D5 in females with lymph node positive breast carcinoma entered on two prospective clinical trials. Cancer 1996;78:764–72.
(31) Colleoni M, Gelber S, Coates AS, Castiglione-Gertsch M, Gelber RD, Price K, et al. Influence of endocrine-related factors on response to perioperative chemotherapy for patients with node-negative breast cancer. J Clin Oncol 2001;19:4141–9.
(32) Nadji M, Gomez-Fernandez C, Ganjei-Azar P, Morales AR. Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers. Am J Clin Pathol 2005;123:21–7.

Notes

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