Hormone Receptor Status and Survival in a Population-Based Cohort of Patients with Breast Carcinoma

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BACKGROUND. The objective of this study was to assess hormone receptor status as an independent predictor of survival in a population-based cohort of women with breast carcinoma who were followed for up to 11 years.

METHODS. Since 1990, the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program has collected data on hormone receptor status among patients with breast carcinoma. In a cohort of 205,736 women with breast carcinoma age ≥ 20 years at diagnosis who were entered into the SEER data base between 1990 and 2000, the authors analyzed the association of hormone receptor status with year of diagnosis, patient age, disease stage, tumor histology, tumor grade, race/ethnicity, and metropolitan/statewide residence areas. Kaplan–Meier survival curves were compared according to hormone receptor status, and Cox proportional-hazards regression models were used to assess the association of hormone receptor status with breast carcinoma-specific and all-cause mortality controlling for age, disease stage, tumor grade, tumor histology, race/ethnicity, and SEER region.

RESULTS. Women who had tumors that were positive for both estrogen and progesterone hormone receptors had significantly better survival than other women with breast carcinoma in the overall cohort, within each stage, and in the younger and older age groups, although the survival advantage was greater among women age ≤ 50 years than among older women. Hormone receptor status was associated with mortality even when patient age, disease stage, tumor grade, tumor histology, race/ethnicity, and metropolitan/statewide residence areas were taken into account.

CONCLUSIONS. Hormone receptor status was identified as an independent predictor of outcome in women with breast carcinoma. Data from clinical trials with long follow-up may shed light on whether and how the benefit of hormonal and other treatment varies with hormone receptor status. *Cancer* 2005;103:2241–51.
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Both observational studies and randomized trials have found that women with breast carcinoma who have tumors that test positive for estrogen receptor (ER) and/or progesterone receptor (PR) live longer than women who have tumors that test negative for both hormone receptors.[1–17] In large studies with more than a decade of follow-up, such as those from San Antonio and from the National Surgical Adjuvant Breast and Bowel Project,[8,18–20] the presence of hormone receptors has been associated with a 10% survival advantage. Some investigators have suggested that the early survival advantage of patients with ER-positive (ER+)/PR+ tumors disappears over

time and that the survival curves converge due to late recurrences in patients with ER+ tumors.[21,22] Hormone receptor status among patients with breast carcinoma also is associated with disease stage, race, age, and socioeconomic status.[23–28] Thus, although hormone receptor status is used in treatment decision-making, the benefits of having a hormone receptor-positive tumor may not be attributable entirely to treatment.

In 1990, the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program began collecting data on hormone receptor status in women with breast carcinoma.[29] The objective of the current study was to analyze the association of hormone receptor status with survival, taking other predictors of survival into account, in a very large cohort of patients listed in the SEER population-based registries with the diagnosis breast carcinoma between 1990 and 2000.

MATERIALS AND METHODS

Since 1973, the SEER cancer registries have collected data on cancer incidence, mortality, and survival from the cancer registries of states and metropolitan areas throughout the U.S. Representing approximately 14% of the population of the U.S.,[29] the SEER registries are located in the cities of Los Angeles, CA; San Jose, CA; Atlanta, GA; Detroit, MI; San Francisco-Oakland, CA; and Seattle, WA; and in the states of Connecticut, New Mexico, Hawaii, Utah, and Iowa. The Los Angeles and San Jose cancer registries joined SEER in 1992. The registries routinely collect data on patient demographics, primary tumor site, morphology, stage at diagnosis, first course of treatment, and vital status at follow-up.[29] The program's case ascertainment is reported as 98%, and a sample of cases is reabstracted each year to assess the quality of the data collected from the medical records.[29] Further details regarding the SEER Program have been reported elsewhere.[30]

Because the registries obtain their data on ER and PR status in breast carcinomas from laboratory reports in patient medical records,[29–27] reported receptor status depends on the criteria and quality of local laboratories in each SEER region. During the study period, most laboratories determined hormone receptor status by immunochemical assay.

Our study sample consisted of women registered in the SEER data base who were at least age 20 years at the time they were diagnosed with primary invasive breast carcinoma, diagnosed between January 1, 1990, and December 31, 2000, and who were followed through the latter date. The patients were categorized with respect to hormone receptor status as ER+/PR+, ER+/PR-negative (ER+/PR−), ER−/PR+, ER−/PR−, PR missing, ER missing, both missing, or ER and/or PR borderline. We included groups with missing information on a single hormone receptor and those with borderline hormone receptor data in our analysis of the distribution of covariates by hormone receptor status, but we excluded them from the survival analysis.

In our analysis of the association between age and hormone receptor status, we categorized age groups into decades as follows: ages 20–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, 70–79 years, and ≥ 80 years. In our survival analyses, we followed the common practice in studies of females with unknown menstrual history or status by using an age categorization of ≤ 50 years and > 50 years as a proxy for menopausal status.[31] Race/ethnicity was classified as non-Hispanic white, non-Hispanic black, Hispanic, and other (Native American, Filipino, Chinese, Korean, Vietnamese, and Indian/Pakistani). Stage at diagnosis was categorized using the American Joint Committee on Cancer TNM classification scheme as State I, Stage II, Stage III, Stage IV, or unstaged. We grouped patients histologically by labeling tumors with the *International Classification of Diseases for Oncology-Second Edition* (ICD-O-2) morphology codes 8500/3, 8503/0, 8521/3, and 8541/3 as *ductal*; labeling tumors with ICD-O-2 codes 8520/3 as *lobular*; labeling tumors with ICD-O-2 codes 8522/3 as *mixed*, and labeling tumors with all other ICD-O-2 breast carcinoma codes as *other*. Tumors were graded as Grade 1, Grade 2, Grade 3, Grade 4, or other/unknown. Patients in the data base also were categorized by year of diagnosis and SEER region, which included either metropolitan (urban/suburban) or statewide (including rural areas). We assessed the association of hormone receptor status with other demographic and clinical variables using contingency tables and chi-square tests.

For our survival analyses, we calculated survival from the date of diagnosis to the date of either death or last follow-up. We conducted the analyses using both all-cause and breast carcinoma-specific mortality. We estimated survival curves using the Kaplan–Meier method,[32] and we used the log-rank test[3y3] to assess the association of survival with hormone receptor status within the cohort as a whole and within stage strata.

To determine whether or not hormone receptor status was a significant predictor of death (breast carcinoma-specific and all-cause mortality) when age, race/ethnicity, disease stage, histology, tumor grade, and metropolitan/statewide residence were taken into account, we conducted multivariable analyses of survival using Cox[34] proportional hazards regression models. We used the SAS (version 8.0; SAS Institute,

Cary, NC) and R statistical software packages for these analyses.

RESULTS

Demographic and Clinical Characteristics

The cohort consisted of 205,736 women with histologically confirmed invasive breast carcinoma diagnosed from January 1, 1990 through December 31, 2000. Table 1 presents the distribution of patients with breast carcinoma by hormone receptor status, among those with hormone receptor status reported, and by year of diagnosis, age group at diagnosis, disease stage, histology, tumor grade, race/ethnicity, and metropolitan versus statewide residence.

Among 155,890 women who had their hormone receptor status reported in the data base, nearly two-thirds had ER+/PR+ tumors (63.5%). Approximately 20.0% of women had ER−/PR− tumors, 12.8% of women had had ER+/PR− tumors, and approximately 3.3% of women had ER−/PR+ tumors.

During the 11 years covered by the data set, the numbers of newly diagnosed patients for whom hormone status was reported more than doubled. The proportion with ER+/PR+ tumors increased from 63.6% to 65.5%, and the proportion with ER−/PR− tumors increased from 19.0% to 20.0%. The proportion of women with ER+/PR− tumors varied from a low of 11.7% to a high of 14.5 but showed no pattern of change over time, whereas the proportion of women with ER−/PR+ tumors declined from 4.5% to 1.7%.

In the cohort overall, hormone receptor status was associated significantly with age, and ER+ tumors were associated positively and ER− tumors were associated negatively with older age. Non-Hispanic white women were much more likely than non-Hispanic black women to have ER+/PR+ tumors and were much less likely to have ER− tumors. The proportion of hormone receptor-negative tumors increased with stage and grade, and ductal and other tumors were much less likely than lobular and mixed tumors to be ER+.

Survival

Figure 1 depicts Kaplan–Meier curves for breast carcinoma-specific and overall survival by hormone receptor status. The four hormone receptor status curves depicting breast carcinoma-specific survival begin to separate in the first year and remain separate throughout the follow-up period (Fig. 1A); ER+/PR+ tumors were associated with the best survival, followed by ER+/PR− tumors, ER−/PR+ tumors, and ER−/PR− tumors. In terms of all-cause mortality, patients with ER+/PR+ tumors also had a survival advantage compared with other patients until at least 10

TABLE 1
Percentage Distribution of Hormone Receptor Status by Demographic and Clinical Characteristics among Women with Breast Carcinoma who had Estrogen Receptor and Progesterone Receptor Status Reported in the Surveillance, Epidemiology, and End Results Program, 1990–2000

	Hormone receptor status (%)				
Characteristic	ER+/PR+	ER+/PR−	ER−/PR+	ER−/PR−	Total
No. of patients	99,042	19,887	5165	31,796	155,890
Percentage of patients	63.53	12.76	3.31	20.40	100.00
Yr of diagnosis[a]					
1990[b]	63.56	12.96	4.45	19.03	9850
1991[b]	62.58	14.46	3.79	19.71	9839
1992[c]	61.47	13.95	4.01	20.57	13,255
1993[c]	60.45	13.47	4.36	21.72	13,328
1994[c]	62.51	12.66	3.76	21.07	13,904
1995[c]	63.41	11.71	4.04	20.84	14,271
1996[c]	63.51	11.83	3.48	21.18	14,911
1997[c]	64.50	12.15	3.08	20.28	16,061
1998[c]	64.85	12.15	2.94	20.06	17,011
1999[c]	64.87	13.09	2.18	19.86	17,404
2000[c]	65.45	12.80	1.72	20.03	17,056
Age group at diagnosis[a]					
20–29 yrs	41.55	8.56	5.02	44.86	876
30–39 yrs	49.12	8.90	6.03	35.95	9605
40–49 yrs	60.50	8.07	5.34	26.09	29,576
50–59 yrs	61.48	12.41	3.52	22.59	34,355
60–69 yrs	66.02	14.21	2.47	17.30	34,236
70–79 yrs	68.54	15.32	2.00	14.15	31,615
≥ 80 yrs	68.31	16.62	1.78	13.29	15,627
Race/ethnicity[a]					
Non-Hispanic white	65.51	12.97	3.04	18.47	121,906
Non-Hispanic Black	48.03	12.45	4.53	34.99	11,884
Hispanic	57.50	12.07	4.07	26.36	9567
Other	63.58	11.45	4.24	20.73	12,533
AJCC stage at diagnosis[a]					
Stage I	68.82	12.65	2.95	15.57	68,283
Stage II	60.42	12.21	3.53	23.84	58,754
Stage III	51.63	13.90	3.95	30.52	10,548
Stage IV	52.75	16.38	4.19	26.68	5518
Unstaged	64.05	13.32	3.36	19.27	12,787
Histology[a]					
Ductal	62.77	12.20	3.37	21.66	115,858
Lobular	73.19	17.70	2.46	6.65	12,481
Mixed	76.06	13.66	2.61	7.67	9573
Other	55.06	12.44	3.94	28.56	17,978
Grade[a]					
Well differentiated	80.78	12.87	1.93	4.42	21,220
Moderately differentiated	73.88	12.97	2.56	10.60	54,011
Poorly differentiated	45.15	12.02	4.58	38.25	49,337
Undifferentiated/anaplastic	46.29	10.58	4.41	38.71	4,169
Other/unknown	65.52	13.92	3.42	17.13	27,153
Regions					
Metropolitan	64.04	12.49	3.42	20.05	52,989
Statewide	63.27	12.90	3.26	20.57	102,901

ER: estrogen receptor; +: positive; PR: progesterone receptor; −: negative; AJCC: American Joint Committee on Cancer.

[a] $P < 0.0001$.

[b] Based on nine regions.

[c] Based on 11 regions.

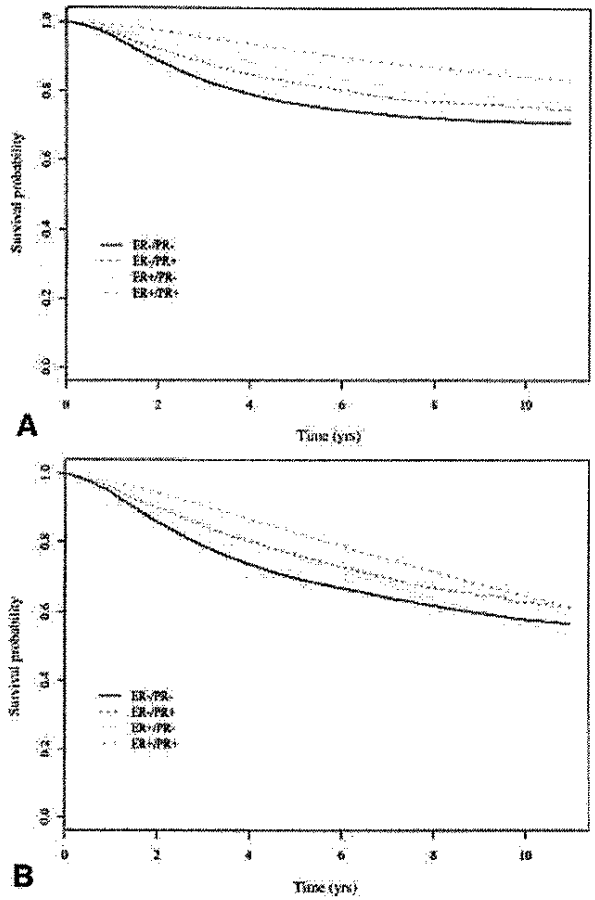

FIGURE 1. (A) Breast carcinoma-specific survival is illustrated according to estrogen receptor (ER) and progesterone receptor (PR) status. (B) Overall survival is illustrated according to ER/PR status. +: Positive; −: negative.

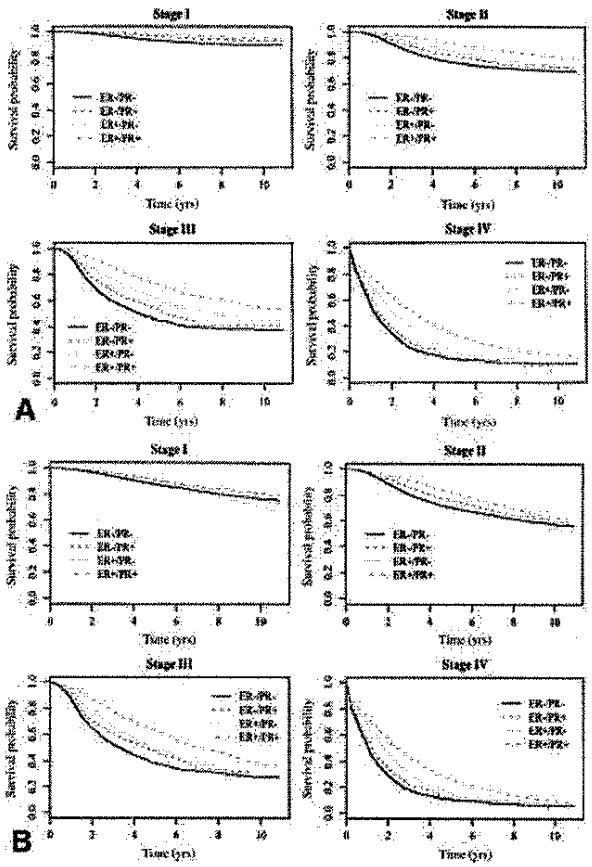

FIGURE 2. (A) Breast carcinoma-specific survival is illustrated according to estrogen receptor (ER) and progesterone receptor (PR) status and disease stage. (B) Overall survival is illustrated according to ER/PR status and disease stage. +: Positive; −: negative.

years after diagnosis. Beyond 10 years, the all-cause survival curve for patients with ER+/PR+ tumors converge with the curve for patients with ER−/PR+ tumors, crossing the ER+/PR− curve at about 5 years (Fig. 1B). The 2 PR− curves cross at about 9 years after diagnosis, because the downward slopes of the 2 ER− groups are steeper in the first 5 years than thereafter.

Figure 2 depicts survival by hormone receptor status and disease stage. Although, in terms of both breast carcinoma-specific mortality (Fig. 2A) and all-cause mortality (Fig. 2B), patients with ER+/PR+ tumors appear to have an advantage that increases with stage, that advantage is most apparent in the breast carcinoma-specific analysis. The stage-specific curves also highlight the high mortality of patients with ER− tumors in the first 5 years after diagnosis, especially in patients with late-stage disease.

Table 2 presents 5-year and 10-year Kaplan–Meier survival percentages with 95% confidence intervals (95% CIs) overall and by disease stage for the 4 hormone receptor status groups. The tables show that the survival benefits of ER+/PR+ status increase with stage of disease, although stage has a much greater impact than hormone receptor status on survival.

Table 3 presents the results of the multivariable analysis of the association of hormone receptor status with breast carcinoma-specific and all-cause mortality, controlling for disease stage, patient age, histology, tumor grade, race/ethnicity, and SEER region, for patients who had their hormone receptor status reported. All of the variables in both models, especially disease stage, were associated with mortality; patients who had Stage IV disease had a hazard rate of 52.73 (95% CI, 49.67–55.98) for breast carcinoma-specific mortality and a hazard rate of 16.74 (95% CI, 16.08–17.43) for all-cause mortality, compared with patients who had Stage I disease. However, controlling for disease stage and for the other factors shown, patients who had ER− tumors appeared to have twice the breast carcinoma-specific mortality rate of patients

TABLE 2
Five-Year and 10-Year Breast Carcinoma-Specific Survival and Overall Survival Percentages and 95% Confidence Intervals by Hormone Status and Disease Stage

	Survival rate (95% confidence interval)			
Disease stage	ER+/PR+	ER+/PR−	ER−/PR+	ER−/PR−
Disease-specific survival				
All stages				
5 yrs	91.6 (91.4–91.8)	85.8 (85.2–86.4)	82.4 (81.2–83.6)	76.2 (75.6–76.8)
10 yrs	84.2 (83.7–84.7)	77.4 (76.3–78.5)	75.5 (73.7–77.3)	70.0 (70.1–71.7)
Stage I				
5 yrs	98.1 (97.9–98.3)	97.2 (96.7–97.6)	96.2 (95.2–97.2)	93.3 (92.7–93.9)
10 yrs	94.9 (94.4–95.4)	93.1 (91.9–94.3)	93.3 (91.6–95.1)	90.2 (89.3–91.1)
Stage II				
5 yrs	90.8 (90.4–91.2)	84.7 (83.7–85.8)	81.9 (79.9–83.8)	76.1 (75.3–77.0)
10 yrs	80.5 (79.7–81.4)	73.5 (71.5–75.5)	72.9 (10.0–75.9)	69.6 (68.3–70.9)
Stage III				
5 yrs	72.3 (70.8–73.8)	60.5 (57.3–63.8)	52.1 (46.7–58.2)	44.6 (42.5–46.9)
10 yrs	53.5 (50.5–56.8)	43.8 (38.5–49.8)	40.4 (34.2–47.6)	37.3 (34.6–40.2)
Stage IV				
5 yrs	33.3 (31.1–35.7)	19.7 (16.3–23.8)	14.3 (9.3–22.0)	14.5 (12.2–17.1)
10 yrs	16.6 (13.8–20.0)	8.13 (4.66–1.42)	4.42 (0.928–21.0)	10.5 (8.17–13.6)
Unstaged				
5 yrs	90.9 (90.1–91.7)	86.2 (84.2–88.3)	81.8 (77.9–86.0)	75.3 (73.4–77.4)
10 yrs	83.0 (81.1–84.9)	76.4 (72.3–80.7)	71.9 (64.9–79.5)	68.5 (65.6–71.5)
Overall survival				
All stages				
5 yrs	82.8 (82.5–83.1)	75.7 (74.9–76.5)	76.1 (74.7–77.5)	69.4 (68.8–70.1)
10 yrs	64.7 (64.0–65.4)	56.4 (54.9–58.0)	62.7 (60.3–65.1)	57.9 (56.9–59.0)
Stage I				
5 yrs	90.9 (90.6–91.3)	88.6 (87.7–89.5)	91.2 (89.7–92.8)	87.7 (86.9–8.6)
10 yrs	75.8 (74.8–76.8)	70.3 (68.0–72.8)	81.0 (77.7–84.5)	77.6 (76.0–79.2)
Stage II				
5 yrs	82.2 (81.7–82.8)	75.0 (73.7–76.3)	76.6 (74.4–78.8)	70.1 (69.1–71.1)
10 yrs	62.8 (61.7–63.9)	54.5 (52.1–57.1)	61.0 (57.2–65.0)	57.4 (55.7–59.1)
Stage III				
5 yrs	63.4 (61.7–65.0)	50.6 (47.3–54.0)	46.2 (40.8–52.4)	38.6 (36.5–40.8)
10 yrs	38.3 (35.3–41.6)	25.6 (20.3–32.2)	28.3 (20.7–38.7)	28.3 (25.5–31.4)
Stage IV				
5 yrs	26.5 (24.4–28.7)	15.2 (12.2–18.8)	11.5 (7.3–18.3)	10.54 (8.7–12.9)
10 yrs	9.6 (7.5–12.4)	6.1 (3.4–10.8)	3.2 (1.0–15.7)	5.8 (4.0–8.4)
Unstaged				
5 yrs	73.9 (72.7–75.1)	68.9 (66.1–71.8)	70.1 (65.1–75.5)	63.7 (61.4–66.1)
10 yrs	51.8 (49.4–54.3)	48.9 (44.7–53.6)	54.3 (46.8–63.1)	48.6 (44.9–52.7)

ER: estrogen receptor; +: positive; PR: progesterone receptor; −: negative.

who had ER+/PR+ tumors; the effect of hormone receptor status on all-cause mortality was weaker but remained statistically significant. The breast carcinoma-specific mortality rate in non-Hispanic black patients was nearly 50% higher than the rate in non-Hispanic white patients; the rate in Hispanic patients was only slightly higher, but statistically significantly, and the rate in other patients was lower. Patients who had carcinoma of lobular or mixed histology had slightly lower mortality rates compared with other patients. All-cause mortality among women age > 50 years was almost twice as high as that among younger women, but breast carcinoma-specific mortality was only slightly higher.

Table 4 presents the results of a separate, multivariable analysis of hormone receptor status with breast carcinoma-specific and all-cause mortality in patients with Stage I disease. In this group, hormone receptor status generally was a weaker predictor of all-cause mortality, but ER−/PR− tumors were associated with a nearly threefold increase in breast carcinoma-specific mortality. Older age was not associated with breast carcinoma-specific mortality but was associated with a threefold increase in all-cause mortal-

TABLE 3
Hazard Ratios for Breast Carcinoma-Specific and All-Cause Mortality Associated with Hormone Receptor Status and Other Demographic and Clinical Factors[a]

	Mortality (%)			
	Breast carcinoma-specific		All-cause (%)	
Factor	HR	95% CI	HR	95% CI
Hormone receptor status				
ER+/PR+	1.00	Reference	1.00	Reference
ER+/PR−	1.46	1.39–1.53	1.25	1.21–1.29
ER−/PR+	1.82	1.70–1.96	1.36	1.27–1.44
ER−/PR−	2.10	2.03–2.18	1.51	1.47–1.56
Age at diagnosis				
≤ 50 yrs	1.00	Reference	1.00	Reference
> 50 yrs	1.24	1.19–1.28	2.01	1.95–2.07
Stage at diagnosis				
Stage I	1.00	Reference	1.00	Reference
Stage II	4.04	3.83–4.26	2.04	1.97–2.10
Stage III	12.52	11.79–13.29	5.04	4.85–5.23
Stage IV	45.54	42.87–48.37	15.44	14.82–16.08
Unstaged	4.16	3.87–4.46	2.76	2.66–2.88
Histology				
Other	1.00	Reference	1.00	Reference
Ductal	1.02	0.98–1.07	0.99	0.96–1.03
Lobular	0.77	0.72–0.83	0.83	0.79–0.87
Mixed	0.92	0.84–1.00	0.83	0.78–0.88
Disease stage				
Stage I	1.00	Reference	1.00	Reference
Stage II	2.22	2.00–2.47	1.24	1.17–1.30
Stage III	3.54	3.19–3.94	1.67	1.59–1.76
Stage IV	3.63	3.20–4.11	1.65	1.53–1.78
Unstaged	2.73	2.46–3.04	1.38	1.31–1.45
Race/ethnicity				
Non-Hispanic white	1.00	Reference	1.00	Reference
Non-Hispanic black	1.47	1.39–1.54	1.37	1.32–1.42
Hispanic	1.07	1.00–1.14	0.97	0.93–1.02
Other	0.85	0.80–0.91	0.73	0.70–0.77
Region				
Metropolitan	1.0	Reference	1.0	Reference
Statewide	1.07	1.04–1.11	1.07	1.04–1.10

HR: hazard ratio; 95% CI: 95% confidence interval; ER: estrogen receptor; +: positive; PR: progesterone receptor; −: negative.
[a] The hazard ratios and 95% confidence intervals shown represent estimates that were derived from a multivariable model controlling for all of the other variables shown.

ity. Breast carcinoma-specific mortality was 50% higher, but all-cause mortality was only 40% higher, among black non-Hispanic women than among white non-Hispanic women with Stage I disease. Differences between Hispanic and non-Hispanic white women were not statistically significant, but breast carcinoma-specific mortality was higher, and all-cause mortality was lower, among Hispanic women. Women of other ethnicity had lower mortality rates than white women with Stage I disease.

TABLE 4
Hazard Ratios for Breast Carcinoma-Specific and All-Cause Mortality Associated with Hormone Receptor Status and Other Demographic and Clinical Factors among Women with Stage I Breast Carcinoma[a]

	Mortality (%)			
	Breast carcinoma-specific		All cause	
Characteristic	HR	95% CI	HR	95% CI
Hormone receptor status				
ER+/PR+	1.00	Reference	1.00	Reference
ER+/PR−	1.53	1.32–1.77	1.17	1.10–1.25
ER−/PR+	1.66	1.31–2.12	1.00	0.87–1.15
ER−/PR−	2.89	2.59–3.22	1.17	1.09–1.25
Age at diagnosis				
≤ 50 yrs	1.00	Reference	1.00	Reference
> 50 yrs	1.09	0.98–1.22	3.10	2.85–3.35
Grade				
Grade 1	1.00	Reference	1.00	Reference
Grade 2	2.00	1.61–2.47	1.13	1.05–1.22
Grade 3	3.70	2.99–4.58	1.42	1.31–1.54
Grade 4	3.43	2.48–4.74	1.19	1.00–1.43
Other/unknown	2.40	1.93–2.99	1.24	1.15–1.35
Histology				
Other	1.00	Reference	1.00	Reference
Ductal	1.38	1.17–1.62	1.05	0.98–1.14
Lobular	0.97	0.74–1.27	0.94	0.84–1.06
Mixed	1.10	0.82–1.47	0.85	0.74–0.97
Race/ethnicity				
Non-Hispanic white	1.00	Reference	1.00	Reference
Non-Hispanic black	1.45	1.22–1.72	1.37	1.24–1.50
Hispanic	1.15	0.93–1.42	0.93	0.82–1.05
Other	0.66	0.53–0.83	0.57	0.51–0.65
Region				
Metropolitan	1.00	Reference	1.00	Reference
Statewide	1.02	0.92–1.13	1.07	1.02–1.12

HR: hazard ratio; 95% CI: 95% confidence interval; ER: estrogen receptor; +: positive; PR: progesterone receptor; −: negative.
[a] The hazard ratios and 95% confidence intervals shown represent estimates that were derived from a multivariable model controlling for all of the other variables shown.

Table 5 presents the results of a separate analysis among women who were diagnosed at or before age 35 years. In this age group, the associations of breast carcinoma-specific and all-cause mortality with the variables in the model were almost identical. The association of hormone receptor status with mortality was weaker among these young women compared with the overall cohort. Like in the overall cohort, disease stage was the most important predictor of mortality. Patients with ER−/PR+ tumors had higher breast carcinoma-specific mortality than patients in the other hormone receptor status categories, but they had the same all-cause mortality as the patients with ER−/PR− tumors. Patients with tumors graded > Grade I had approximately 3 times the risk of mortality as patients with Grade I tumors. Patients who

TABLE 5
Hazard Ratios and for Breast Carcinoma-Specific and All-Cause Mortality Associated with Hormone Receptor Status and Other Demographic and Clinical Factors among Women Age ≤ 35 Years[a]

	Mortality rate (%)			
	Breast carcinoma-specific		All cause	
Characteristic	HR	95% CI	HR	95% CI
Hormone receptor status				
ER+/PR+	1.00	Reference	1.00	Reference
ER+/PR−	1.13	0.88–1.46	1.17	0.93–1.47
ER−/PR+	1.50	1.15–1.94	1.36	1.05–1.75
ER−/PR−	1.39	1.18–1.63	1.34	1.15–1.55
Stage at diagnosis				
Stage I	1.00	Reference	1.00	Reference
Stage II	3.63	2.74–4.79	3.00	2.35–3.84
Stage III	10.21	7.54–13.83	8.29	6.33–11.87
Stage IV	24.92	17.89–34.71	20.47	15.19–27.59
Unstaged	4.18	2.94–5.93	3.59	2.62–4.91
Histology				
Other	1.00	Reference	1.00	Reference
Ductal	1.31	1.07–1.60	1.24	1.03–1.50
Lobular	1.32	1.07–1.60	1.28	0.75–2.21
Mixed	1.50	1.00–2.27	1.43	0.97–2.11
Grade				
Well differentiated	1.00	Reference	1.00	Reference
Moderately differentiated	2.69	1.19–6.11	2.61	1.22–5.57
Poorly differentiated	3.54	1.58–7.96	3.59	1.70–7.61
Undifferentiated/anaplastic	3.64	1.55–8.54	3.61	1.63–7.98
Other/unknown	3.09	1.36–7.02	3.09	1.44–6.60
Race/ethnicity				
Non-Hispanic white	1.00	Reference	1.00	Reference
Non-Hispanic black	1.51	1.26–1.83	1.60	1.35–1.91
Hispanic	1.24	1.01–1.52	1.21	0.99–1.47
Other	0.97	0.75–1.23	0.98	0.78–1.23
Region				
Metropolitan	1.00	Reference	1.00	Reference
Statewide	1.089	0.94–1.27	1.08	0.93–1.24

HR: hazard ratio; 95%CI: 95% confidence interval; ER: estrogen receptor; +: positive; PR: progesterone receptor; −: negative.
[a] The hazard ratios and 95% confidence intervals shown represent estimates that were derived from a multivariable model controlling for all of the other variables shown.

had tumors with ductal histology had at least a 25% higher mortality rate than patients who had tumors with other histology. The hazard ratios associated with nonwhite race/ethnicity were higher among these young women compared with the cohort overall.

Table 6 shows the distribution of missing or borderline data on hormone receptor status. Nearly 25% of the cohort lacked or had borderline results for either or both hormone receptors. The proportion of patients with breast carcinoma who had hormone receptor status data present increased year by year, from 68.7% in 1990 to 80.5% in 1999, and the proportion with borderline results declined from 2.7% in 1990 to 0.6% in 2000. Women who were diagnosed before age 30 years or after age 79 years, non-Hispanic black women, Hispanic women, and women with advanced or unstaged disease were less likely than others to have hormone receptor status reported.

Table 7 presents the distribution of hormone receptor status by year of diagnosis for non-Hispanic white women. It indicates that ER+/PR+ tumors were more prevalent in this subgroup than in the sample as a whole at baseline and that, although prevalence increased both in the sample as a whole and in white women, the increase was greater in white women.

DISCUSSION

For more than 2 decades, hormone receptor status has played a role in treatment decisions for patients with newly diagnosed breast carcinoma and for patients with recurrent disease; and increasingly, hormone receptor assays have come into wider use.[27] The increase we observed from 1990 to 2000 in the proportion of SEER patients with breast carcinoma who had records that included hormone receptor assays shows the timeliness of SEER's decision to collect these data. Overall, in our cohort of > 205,000 women who were diagnosed with invasive breast carcinoma, > 155,000 women had data on both PR status and ER status.

In this large cohort, patients with ER+/PR+ tumors, especially those with advanced disease, had better survival compared with other patients. Although the Kaplan–Meier analyses showed that, among patients with Stage I disease and among others who survived > 10 years after diagnosis, having a tumor with ER−/PR+ status may be even more advantageous, proportional hazards analysis taking other factors into account did not support that conclusion for the cohort overall. Among women with breast carcinoma diagnosed in Stage I (Table 4), patients with ER−/PR+ tumors did not differ from patients with ER+/PR+ tumors in terms of all-cause mortality, but they had significantly worse breast carcinoma-specific mortality. Patients who were diagnosed at age ≤ 35 years with ER+/PR− tumors did not differ in terms of mortality from other young patients with ER+/PR+ tumors, but patients with ER− tumors fared worse.

Hilsenbeck et al.[21] have suggested that, because the hormone receptor status curves converge or cross over time, the data may violate the assumptions of proportional hazards regression analysis. Model diagnostics indicate that the ER/PR status data indeed may violate those assumptions. However, it has been argued that even "statistically significant" violations of proportional hazards generally should not affect the interpretation of data derived from such models, especially estimates based on large samples.[35] Indeed, residual plots in this data set indicate only slight variation in the parameters relative to the magnitude of

TABLE 6
Percentage Distribution of Missing or Borderline Hormone Receptor Status Information by Demographic and Clinical Characteristics among Women with Breast Carcinoma in the Surveillance, Epidemiology, and End Results Program, 1990–2000

	Hormone receptor status (%)					
Characteristic	Present	PR missing	ER missing	ER/PR missing	ER/PR borderline	Total
No. of patients	155,890	5036	227	41,771	2812	205,736
%	75.8	2.45	0.11	20.30	1.37	100.00
Yr of diagnosis[a]						
1990[b]	68.7	2.3	0.2	26.1	12.7	12,902
1991[b]	73.7	2.0	0.2	21.3	12.8	13,349
1992[c]	71.7	2.2	0.1	23.7	12.3	18,499
1993[c]	72.7	2.0	0.1	23.3	11.9	18,344
1994[c]	74.2	2.2	0.1	22.0	11.5	18,722
1995[c]	74.0	2.4	0.1	22.5	11.1	19,304
1996[c]	75.8	3.2	0.1	20.1	10.9	19,680
1997[c]	77.9	2.8	0.1	18.3	10.9	20,626
1998[c]	80.0	2.2	0.1	17.1	10.8	21,311
1999[c]	80.5	2.3	0.1	16.4	10.7	21,619
2000[c]	79.8	2.9	0.1	16.5	10.6	21,380
Age group at diagnosis[a]						
20–29 yrs	72.7	1.4	0.3	24.1	11.6	1,06
30–39 yrs	76.5	1.8	0.2	19.5	11.9	12,551
40–49 yrs	76.8	2.3	0.1	19.1	11.7	38,501
50–59 yrs	77.1	2.7	0.1	18.8	11.3	44,550
60–69 yrs	76.0	2.7	0.1	20.0	11.3	45,054
70–79 yrs	75.3	2.5	0.1	21.0	11.2	41,982
≥ 80 yrs	71.5	2.1	0.1	25.3	11.1	21,892
Race/ethnicity[a]						
Non-Hispanic white	76.9	2.4	0.1	19.1	11.4	158,386
Non-Hispanic black	68.9	2.6	0.1	27.1	11.4	17,264
Hispanic	68.9	2.9	0.1	27.0	11.1	13,877
Other	77.4	2.1	0.1	19.4	11.1	16,209
AJCC stage at diagnosis[a]						
Stage I	77.9	2.8	0.1	18.0	11.2	87,684
Stage II	80.8	2.3	0.1	15.3	11.6	72,820
Stage III	76.9	1.7	0.1	19.7	11.6	13,715
Stage IV	62.6	2.1	0.1	34.0	11.1	8,814
Unstaged	56.4	2.1	0.1	40.4	10.1	22,703
Histology[a]						
Ductal	77.6	2.3	0.1	18.7	11.4	149,368
Lobular	76.7	3.2	0.1	19.0	11.1	16,299
Mixed	78.2	3.7	0.0	16.7	11.3	12,240
Other	64.6	2.4	0.1	31.5	11.4	27,829
Grade[a]						
Well differentiated	77.41	3.65	0.12	17.90	10.92	27,411
Moderately differentiated	80.04	2.62	0.09	16.18	11.07	67,484
Poorly differentiated	78.94	1.81	0.13	17.46	11.66	62,503
Undifferentiated/anaplastic	79.61	2.31	0.06	15.72	12.31	5237
Other/unknown	63.00	2.35	0.11	32.97	11.56	43,101
Regions[a]						
Metropolitan	74.7	2.7	0.1	21.4	11.0	137,635
Statewide	77.8	2.0	0.1	18.1	12.1	68,101

ER: estrogen receptor; +: positive; PR: progesterone receptor; −: negative; AJCC: American Joint Committee on Cancer.
[a] $P < 0.0001$.
[b] Based on nine regions.
[c] Based on 11 regions.

TABLE 7
Estrogen Receptor/Progesterone Receptor Status by Year of Diagnosis for Non-Hispanic White Women

Yr of diagnosis[a]	Hormone receptor status (%)				
	ER+/PR+	ER+/PR−	ER−/PR+	ER−/PR−	Total
No. of patients	79,864	15,817	3707	22,518	121,906
%	65.51	12.97	3.04	18.47	100.00
1990[b]	65.65	13.09	4.10	17.15	7334
1991[b]	64.52	14.41	3.40	17.67	8210
1992[c]	63.74	14.05	3.69	18.52	10,428
1993[c]	62.27	13.51	4.09	20.13	10,557
1994[c]	64.36	13.01	3.37	19.27	10,916
1995[c]	65.30	12.00	3.72	18.98	11,138
1996[c]	65.31	12.02	3.24	19.44	11,558
1997[c]	66.55	12.47	2.81	18.16	12,482
1998[c]	66.60	12.66	2.56	18.18	13,072
1999[c]	66.96	13.21	1.99	17.83	13,290
2000[c]	67.90	12.92	1.58	17.61	12,921

ER: estrogen receptor; +: positive; PR: progesterone receptor; −: negative.
[a] $P < 0.0001$.
[b] Based on nine regions.
[c] Based on 11 regions.

the estimates, at least in the first 7 or 8 years. Longer follow-up may have led to underestimates rather than to exaggeration of the effects of hormone receptor status. Moreover, in the breast carcinoma-specific analyses, the curves did not converge. Hence, although the Cox model cannot be interpreted uncritically, in these circumstances, it remains a valuable tool for analyzing predictors of mortality in a multivariate setting.

We found that hormone receptor status was associated with age, race/ethnicity, disease stage, histology, tumor grade, and SEER region. We also found that, when we controlled for age, race/ethnicity, disease stage, histology, tumor grade, and residence, hormone receptor status remained an independent predictor of both breast disease-specific mortality and all-cause mortality, although it was weaker than disease stage at diagnosis or tumor grade.

The absence of hormone receptor data also was associated with year of diagnosis, age, disease stage, histology, and tumor grade. By 2000, nearly 85% of SEER records included hormone receptor status, and the proportion of borderline readings was well below 1%, suggesting changes in both practice and interpretation. Very young or very old women, non-Hispanic black or Hispanic women, and women with advanced or unstaged disease or ungraded tumors were less likely than other women to have their hormone receptor status reported.

In another large cohort study, the recurrence rate among ER− patients was higher compared with ER+ patients in the first 3 years after diagnosis, but it slowed later on.[21] The SEER data suggest a similar pattern.[29] Others have reported that, in ER+ tumors, higher PR levels are associated with a better response to treatment, reduction in recurrence, and longer survival.[15] Although < 5% of tumors are ER−/PR+, these tumors respond to hormone therapy,[22] and PR status is predictive of response to hormone manipulation. It has been suggested that PR may be a better indicator of endocrine responsiveness than ER alone.[36] We found that both receptors are important.

Hormone receptor assays have changed in the past decade. Ligand binding initially was used to assess hormone receptor status, but immunohistochemical assays are now used more commonly, because they are easier to perform, safer, less expensive, and equivalent in their ability to predict response to hormone therapy. Although the results of these two methods are correlated highly, few clinical studies have demonstrated the predictive abilities of immunohistochemistry specifically for both receptors.[37]

SEER does not collect information on the type of assay used or on the cut-off levels used for positive assays, and the criteria used for the hormone receptor assays in our cohort may have varied considerably.[38] Moreover, some tumors with only slight ER activity nonetheless respond to hormone therapy. However, misclassification in the SEER data base would bias the observed association of hormone receptor status with survival toward the null.

SEER also does not include data on chemotherapy or hormone therapy in its public-use data files. Such data may have helped to account for the association of survival with hormone receptor status and for the relative weakness of that association in young women, whose treatment is less likely to be dependent on hormone receptor status compared with the treatment of older women.[39]

Li et al.[40] observed an increase in the incidence of ER+ tumors during the 1990s. In that period, breast cancer incidence increased, but disease stage at diagnosis moved downward, probably as a result of the increasing utilization of mammography. Hormone receptor status, as indicated in Table 1, is associated with disease stage. The increase in ER+ tumors, therefore, may be a byproduct of the increase in Stage I carcinomas. It also may reflect the widespread use of hormone-replacement therapy, especially among non-Hispanic white women (see Table 7), during the same period.[41–43] However, SEER does not collect data on patients' past use of hormone-replacement therapy.

Tumors with ER−/PR− status tend to have higher proliferation rates, more cells in S-phase, and less likelihood of response to hormone therapy than other

tumors.[22] Li et al. suggested that both biologic and environmental factors may contribute to these associations.[27] In our SEER cohort, patients who were non-Hispanic black or Hispanic were less likely than non-Hispanic white patients or other patients (predominantly East and South Asian) to have tumors with ER+/PR+ status and were more likely to have ER−/PR− tumors or missing data on hormone receptor status. They also had higher breast carcinoma-specific and all-cause mortality rates. Patients of other ethnicity had slightly lower mortality rates, especially in the all-cause analyses, but not in the analysis of very young patients. Because of selection factors associated with immigration patterns, these patients may have been higher in socioeconomic status and, thus, may have differed in both etiologic exposures and access to treatment from other nonwhite women in the cohort.

In a comparison of the SEER registry population with the general population, Nattinger et al. found that SEER sampling largely is representative of the nation as a whole. Although SEER under-represents residents of rural areas, the difference between the SEER sample and the U.S. population rarely exceeds 5%.[44]

In the current study, we analyzed outcomes associated with the ER and PR status of breast carcinomas diagnosed in the years 1990–2000. Although many reports based on clinical trials have presented data on these markers, relatively few have presented > 5 years of follow-up on a large cohort. In this study, utilizing the latest SEER public-use data set, we analyzed mortality in relation to these markers, controlling for age, race/ethnicity, disease stage, histology, tumor grade, and SEER region, in a population-based cohort of > 155,000 women with up to 11 years of follow-up.

Hormone receptor status was a significant predictor of both breast carcinoma-specific and all-cause mortality. Although it had a greater impact on the former than the latter, not all deaths due to breast carcinoma or its treatment were attributable on this manner, especially among older women and among women who were diagnosed with early-stage disease. Therefore, we considered both outcomes important.

In addition, given the role of hormone receptor status in treatment decisions, we considered the presence of hormone receptor status reports a marker for quality of care. Nearly 25% of patients in the cohort lacked data on hormone receptor status, and minorities, the elderly, and individuals with advanced or unstaged disease were over represented in this group (Table 6). The SEER data set enabled us to assess these patterns.

To our knowledge to date, the SEER registries have not collected information on such variables as exogenous hormone use, family history, and body mass index, or on other biomarkers, such as HER-2/*neu* expression. Several other receptor variants and isoforms with different functions, as well as other biomarkers, have shown some potential value as predictive or prognostic factors in breast carcinoma but have not yet been included in the SEER data base. Of course, SEER can collect only those data that routinely appear in patient charts.

Use of the SEER data base in our analysis revealed that the survival benefits of hormone receptor status persist for up to 11 years. The SEER data, as the program extends its geographic coverage and lengthens its follow-up, will be an increasingly valuable resource for hypothesis generation. However, without collecting accurate data on medical therapy, currently available information from the SEER data base cannot tell us whether the survival benefit associated with ER+/PR+ status is due to hormone therapy or to inherently lower aggressiveness on the part of ER+/PR+ tumors. Longer follow-up of patients enrolled in randomized clinical trials will be needed to understand whether and how chemotherapy, newer hormone antagonists, and/or biomodulators may interact with hormone receptor status to improve the long-term survival of patients with breast carcinoma.

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Since the early 1980s, radiation oncologists have examined central issues of glioblastoma treatment in a number of randomized studies, such as total dose, fractionation, and target volume studies), and have thus contributed greatly to evidence-based medicine. So did Roa et al,[1] who have to be congratulated on their work.

In this context, we were astonished that in an accompanying editorial[4] written by a radiation oncologist, resection of glioblastomas in elderly patients was favored vigorously. This recommendation was based on a recently published Finnish trial.[5] The latter represents the only prospective neurosurgical glioma trial to date, and included 23 patients. Nineteen of them had a WHO criteria stage IV tumor. Shaw concluded that except for patients older than 50 years, with a Karnofsky performance status of less than 70, all patients should undergo resection. In our opinion, this view is not sufficiently substantiated by prospective data. Certainly "it is not reasonable to discuss the value of resection in patients who are deemed ineligible for anything but a biopsy, nor is it appropriate to recommend biopsy for a respectable symptomatic tumor".[6]

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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Time for Reappraisal of Progesterone-Receptor Testing in Breast Cancer Management

To THE EDITOR: Banerjee et al[1] published a report in the July 1, 2004, issue of the *Journal of Clinical Oncology* demonstrating the prognostic value of progesterone receptor (PR) status in a series of 1,055 patients with stage I-III breast cancer. Using recursive partitioning, a nonparametric statistical technique, they were able to determine four distinct prognostic groups defined by the number of positive nodes, tumor size, PR status, differentiation, race, and marital status. In the May 1, 2004, issue of the *Journal*, a letter to the editor by Olivotto et al[2] advocated the interruption of PR testing in breast cancer patients on the basis of a study of 192 estrogen-receptor (ER)–negative breast cancer patients among which 191 were also PR-negative. PR status was determined by an immunohistochemical technique that used clone 1A6. In the opinion of Olivotto et al,[2] routine PR testing is not warranted for its prognostic value, and may only serve to identify ER-negative patients who may respond to hormonal therapy. Given their results the authors were convinced that PR status determination did not give any additional information to ER determination. Following these publications, we would like to contribute to the debate on the utility of testing PR status for prognostication in early breast cancer. In 1996, we published a report concerning 942 patients with T1-3 breast cancer who had been surgically treated between 1980 and 1986 with a median follow-up period of 117.9 months.[3] The purpose of this study was to validate the immunohistochemical detection of PR (IHC-PR) by comparing it with a standard dextran-coated charcoal (DCC) method, and to assess its prognostic significance in early breast cancer. Mean patient age in the series was 56 years, and there were 398 node-negative (42%) and 544 node-positive (58%) patients. IHC-PR tumor status was determined using the PgR-ICA Abbott monoclonal antibody. Five hundred and fifty tumors (58.4%) were IHC-PR positive. Concordance between the IHC and the DCC methods in the series was 83.2%. In the node-negative group of patients, IHC-PR status was the only independent prognostic factor for overall survival (OS; odds ratio [OR] = 3; 95% CI, 1.8 to 5.3; $P < .0001$) in a multivariate analysis, using a Cox proportional hazards model, including patients' age, menopausal status, tumor size, Scarff-Bloom-Richardson grade, and ER status. Furthermore, in the node-negative group of patients, IHC-PR status was an independent prognostic factor for disease-free survival (OR = 1.8; 95% CI, 1.2 to 2.8; $P = .008$) and metastasis-free survival (OR = 1.8; 95% CI, 1.1 to 3; $P = .02$), alongside with tumor size and Scarff-Bloom-Richardson grade. The five-year OS in the whole group (node-negative and node-positive patients) was 74% and 85% for patients with less than 10% and 10% to 49% IHC-PR–positive tumor cells ($P = .0002$), respectively, and 85% and 93% for patients with 10% to 49% and ≥ 50% IHC-PR–positive tumor cells ($P = .008$), respectively. Subsequent to this publication, we performed the survival analysis again, using Dako PgR636, a different antibody specific for PR, in the same group of 398 node-negative patients with a longer median follow-up

(170 months), and by adding peritumoral vascular emboli to the previous Cox proportional hazard model. PR status determined by PgR636 was again an independent prognostic factor for OS (relative risk [RR] = 2; 95% CI, 1.4 to 2.9); $P = .0002$), metastasis-free survival (RR = 1.9; 95% CI, 1.2 to 3; $P = .001$) and disease-free survival (RR = 1.5; 95% CI, 1.1 to 2.2; $P = .02$) alongside with peritumoral vascular emboli.

In our studies, PR status, as determined by accurate immunohistochemical methods, is a strong prognostic factor, and survival is correlated to the proportion of PR-positive cells in breast tumors. In contrast, ER status is of lesser prognostic significance. PR testing should be performed on patients with breast cancer, and the results should be used for correct determination of their prognosis and management.

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Authors' Disclosures of Potential Conflicts of Interest

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IN REPLY: We appreciate the letter by MacGrogan et al contributing to the debate on the utility of testing progesterone receptor (PR) status in patients with early breast cancer. Whereas Olivotto et al[1] argue that routine PR testing is not warranted because it has little use in guiding therapy decisions, our data are not consistent with that opinion. PR is a protein in which synthesis is positively regulated by estrogen receptor (ER), and the presence of PR may therefore indicate a more functionally intact ER pathway. It is therefore not surprising that PR status has been found useful by others as a predictive as well as a prognostic factor, and that combining ER and PR allows more accurate prediction of clinical outcome.[2] In our series of 1,055 patients with stage I-III breast cancer, PR status was a strong prognostic factor in patients with ≥ four positive lymph nodes. In fact, PR status was a stronger prognostic factor than ER status in this subgroup of patients. Patients with PR-positive tumors had a significantly better prognosis than those with PR-negative tumors (5-year recurrence free survival rate, 55% *v* 27%, respectively). Previous inconsistencies in clinical results may have been in part due to the difficulty in accurately measuring PR. In any case, it remains to be seen if the superiority of PR over ER will hold up when aromatase inhibitors are used as adjuvant therapy instead of tamoxifen.

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Optimizing End Points and Outcomes in Cancer-Associated Wasting

TO THE EDITOR: We congratulate Jatoi et al[1] in completing a large, well-powered, phase III pharmaconutritional study in cancer-associated wasting (also referred to as cancer cachexia). This study highlights several points that deserve further discussion.

Although incompletely understood, cancer-associated wasting is common and debilitating. To date, efforts to mitigate it have met with limited success. Cancer-associated wasting is likely to be a multifactoral process. To date, this has not been reflected in the design and reporting of clinical studies. To better understand cancer-associated wasting and the effects of intervention, a consensus research definition incorporating contemporary knowledge is needed. This should attempt to characterize patients by, for example, the severity of wasting, the presence or absence of systemic inflammation, and predominant symptoms or symptom clusters. These parameters may reflect underlying differences in pathophysiology and influence the response to intervention. Such consensus in trial design should make it easier to compare and analyze wasting studies.

This study, like others, places the outcome emphasis on absolute weight gain. This may not be appropriate. First, the

Journal of Clinical Oncology June 1, 2005

cells.[5] Therefore, we believe that the antitumor activity of gefitinib is due, at least in part, to its ability to affect tumor cell proliferation and survival. In this respect, cancer cells' sensitivity and resistance to this agent is clearly related to the presence of molecular alterations that have been shown to render tumor cell growth dependent or independent on EGFR signaling.[5-8]

In conclusion, several different mechanisms of action are likely be involved in the antitumor activity of anti-EGFR agents, including effects on nontumor cell types. This observation makes even more difficult the identification of markers to predict the probability of cancer patients to respond to gefitinib.

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Progesterone Receptor Testing: Not the Right Time to Be Buried

To the Editor: Olivotto et al state that progesterone receptor (PgR) testing should be discontinued,[1] but we disagree with their conclusions for several reasons. It is well known that PgR is an estrogen receptor (ER) –regulated protein and that its expression indicates a functional ER pathway. In the 70% to 80% of breast cancer cases that are ER-positive, we think that PgR testing has some utility. While its prognostic role is not clearly defined, it does provide predictive information. ER-positive and PgR-negative tumors are, in fact, less responsive to endocrine therapy (particularly tamoxifen) than ER-positive and PgR-positive tumors in the metastatic setting.[2,3] Other authors have reported that the presence of both receptors is a marker of a greater probability of benefit from adjuvant tamoxifen than ER alone.[4,5] Consequently, PgR negativity can influence the therapeutic decision to offer adjuvant chemotherapy in addition to adjuvant endocrine therapy in selected patients. The observation in the Oxford Overview that the reduction of recurrence for patients with ER-positive/PgR-negative tumors after adjuvant tamoxifen is similar to that obtained in patients with ER-positive/PgR-positive tumors[6] could be due to technical difficulties in measuring PgR in some of the earlier trials included in the meta-analysis.[7] Moreover, recent preliminary data from the Arimidex or Tamoxifen Alone or in Combination (ATAC) trial, presented by Dowsett at the 2003 San Antonio Breast Cancer Symposium, show no difference in disease-free survival between tamoxifen and anastrozole in the subgroup of patients with ER- and PgR-positive tumors, while anastrozole was found to be significantly superior to tamoxifen in the subgroup of ER-positive PgR-negative patients.[8] While these are retrospective data that need to be confirmed, they are provocative. Furthermore, it is now known that human PgR proteins exist in two isoforms, PgR-A and PgR-B, which seem to have different functions as shown by in vitro and in vivo data,[9,10] even if they are transcribed from the same gene under the control of separate promoters.[11] The two isoforms were measured by immunoblotting of tumor lysates from node-positive patients treated with tamoxifen. A high ratio between the two isoforms (PgR-A/PgR-B) was found to identify a subgroup of patients with ER and PgR positive tumors resistant to tamoxifen in both univariate and multivariate analysis.[12] If confirmed, these data offer a new opportunity to better select patients who are good candidates for tamoxifen. For all the above reasons, it does not seem to be the right time to bury PgR testing, but instead, to start refining its purpose.

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It Is Not Time to Stop Progesterone Receptor Testing in Breast Cancer

To the Editor: We have read the provocative letter to the Editor by Olivotto et al,[1] in which the authors suggest that progesterone receptor testing in breast cancer management should be stopped. Their recommendation is based on their estrogen receptor (ER) and progesterone receptor (PR) immunohistochemistry results, which show that only one of 942 patients had an ER−/PR+ tumor (0.1%).

Prompted by their observations, we have analyzed the combined ER and PR values in a series of 1,228 consecutive patients from the Hospital 12 de Octubre in Madrid, Spain, treated during the period from 1992 to 1998. In this series, follow-up is available, which allows a true predictive evaluation of the hormone receptor status. Stage distribution was the following: stage I, 268 (21.9%); stage II, 693 (56.5%); stage III, 145 (11.8%); and stage IV, 120 (9.8%). Hormone receptors were determined using monoclonal antibody-based commercial immunoassay (Abbott Laboratories, Abbott Park, IL). Both receptors were known in 1,153 cases. Median follow-up in the series was 5.8 years. In the nonmetastatic patients, the proportion of cases treated with adjuvant tamoxifen was 69%, and this was more frequent in ER+ and/or PR+ than in ER− and PR− cases (84% ER+/PR+, 75% ER−/PR+, 83% ER+/PR−, and 31% ER−/PR−). During the follow-up, 306 patients have died, and 255 nonmetastatic patients have relapsed. Our hormone receptor subgroup results contrast markedly with those reported by Olivotto et al; in our series, we have found that the number of ER−/PR+ patients is not insignificant (7%, 82 cases). The number of patients ER+/PR+ was 534 (46%); ER+/PR− was 215 (19%); and ER−/PR− was 322 (28%).

Although the techniques of immunohistochemistry and immunoassay for determining ER and PR have equivalent

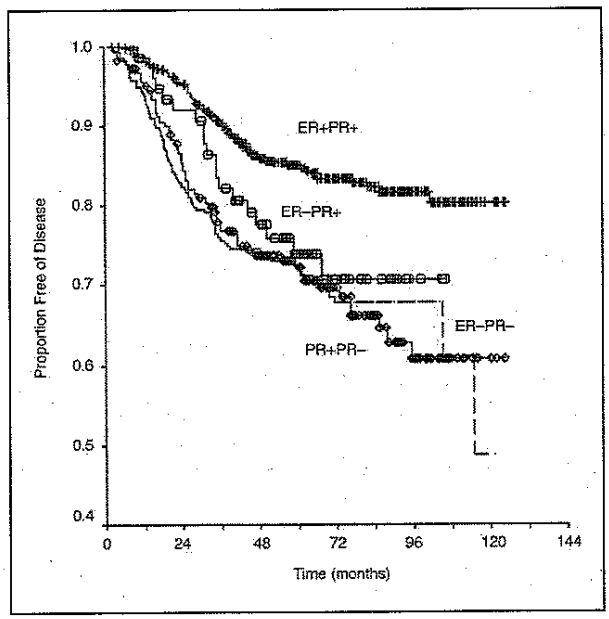

Fig 1. Disease-free survival curves by hormone receptor subgroups (N = 1,039). ER, estrogen receptor; PR, progesterone receptor.

Tissue microarrays: a new approach for quality control in immunohistochemistry

J Packeisen, H Buerger, R Krech and W Boecker

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TECHNICAL REPORT

Tissue microarrays: a new approach for quality control in immunohistochemistry

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Aims: To improve the interpretation of immunohistochemistry (IHC) staining results the use of a tissue microarray technique was established in a routine setting.
Methods: A tissue microarray was constructed by harvesting 600 µm tissue cores from paraffin wax embedded samples available in a routine pathology department. The punches originating from non-tumorous tissue were placed on host paraffin wax blocks. The microarray contained 12 different tissue samples, with a wide antigen profile and a dimension of 3.5 × 3 mm. One section of the multitissue array was placed as an "internal" positive control on each slide of the patient tissue to undergo identical immunohistochemical procedures.
Results: Using the tissue microarray technique as a tool for internal quality control, the interpretation of immunohistochemical staining of more than 20 different antigens in routine IHC was improved. The tissue microarray did not influence the staining results in conventional IHC or in different automated IHC settings.
Conclusion: The regular use of an institution adapted tissue microarray would be useful for internal positive control in IHC to enable different laboratory demands. Furthermore, this technique improves the evaluation of staining results in IHC.

Immunohistochemical methods are routinely used in surgical pathology. For confidence in the immunohistochemistry (IHC) result it is necessary to perform valid quality controls.[1–5] An internal positive control in IHC is essential to ensure that the immunostaining is working properly. A separate slide containing tissue known to be immunoreactive with the test antibody (external control) is a widely used but costly method. Furthermore, it does not completely guarantee that IHC has worked properly for the patient tissue. In many institutes, different positive control tissues for each test case are already in use, but these are often associated with logistical difficulties. The use of multitissue blocks in IHC has been described previously.[6] The tissue microarray technique was invented by Kononen *et al* in 1998[7] and is a promising tool in modern pathology, with almost an infinite number of applications.[8] We established a tissue microarray, which serves as a positive control microarray, as a new application for the tissue microarray technique. Because of the small size (3.5 × 3 mm) of the microarray, the staining of the test tissue was not affected and there was a clear demarkation of control and test tissue.

METHOD

We took core needle biopsies with a diameter of 0.6 mm from donor paraffin wax embedded tissue blocks of 12 different tissues (table 1), obtained from our routine histological workload, using a dedicated tissue array instrument (Beecher Instruments, New Jersey, USA). These tissue cores were arrayed into "host" paraffin wax blocks of 15 × 15 mm, creating similar arrays of 4 × 3 dots (fig 1) in the different blocks. To combine donor cores with the recipient block, the paraffin wax was reheated for five minutes at 80°C. At least 110 to 150 sections of 5 µm were cut and mounted on to adhesive coated slides and stored in a dry environment until use. A paraffin wax sectioning aid system (as described previously) was not used.[7] Two different automated staining systems from Dako (Autostainer and TechMate; Glostrup, Denmark) were used for immunohistochemical staining, in addition to manual procedures. Table 1 lists the antibodies used for staining.

RESULTS

The microarray positive control tissue array blocks were used over a period of six months for 1000 test cases. There was an overall loss of control dots of < 1.5% while processing. A loss of staining after storage of multitissue sections (up to three to four weeks) was not seen. In general, the positive control dots stained brightly (fig 1), and non-specific staining patterns could easily be excluded. Antibody and antigen retrieval problems resulted in the failure of staining in individual slides in about 1.4% of the test cases. In most of these cases, the control tissue also showed a negative staining reaction, which prompted a repetition of IHC. The time taken for the preparation of the control slides was low, even when it was divided into array building and cutting. It took about five minutes to construct the 12 dot array, and 20–30 minutes for the cutting and mounting of 150 control slides.

DISCUSSION

The use of an internal positive control is the most reassuring method for quality control in IHC, with multitissue controls being the most effective. Nevertheless, the preparation of multitissue blocks (so called "sausage technique") is time consuming and complicated in a routine setting. However, the microarray technique described here for building multitissue controls was easier and less time consuming because the control tissues for the multitissue blocks could be harvested from pre-existing blocks of paraffin wax embedded tissue. The consumption of "donor" tissue was low—0.6 mm tissue cores were sufficient so that the availability of rare tissues, particularly tumours with overexpression of tumour specific markers (for example, c-erbB-2 in breast cancer), was better than for conventional techniques. In addition, the amount of time needed for the preparation of a microarray control block was lower than described previously.[9 10] However, at the moment the costs for the array instrument cannot be neglected, but we solved this problem by a multi-institute cooperation. In the near future, the wider use of these arrays should lead to the commercial availability of costume designed test blocks, which would circumvent this limitation. Other equipment or special tools that are not available in a routine histopathology

Table 1 Control tissues used for immunohistochemistry

Positive staining tissue	Antigen	Clone
Myometrium	Progesterone	1°6
Myometrium/breast cancer	Oestrogen	1D5
Tonsil lymphoid tissue	B cell (CD20)	L26
Tonsil lymphoid tissue	T cell (CD3)	Polyclonal
Tonsil lymphoid tissue	Lymphocyte common antigen (LCA)	2B11/PD7/26
Colon carcinoma	Carcinoembryonic antigen (CEA)	11-7
Prostate	Cytokeratin/high molecular	34βE12
Colon carcinoma/prostate	Cytokeratin	MNF-116
Myometrium	Smooth muscle actin	HHF 35
Prostate	Prostate specific antigen	Polyclonal
Ovarian carcinoma	Ca 125	M11
Placenta	Plap	8a9
Brain tissue	S-100	Polyclonal
Myometrium	Desmin	D33
Thyroid	Thyroglobulin	Polyclonal
Breast cancer	p53 protein	Do-7
Placenta	Choriogonadotrophin	Polyclonal
Myometrium	Vimentin	V9
Mast cells in various tissue	c-kit (CD117)	Polyclonal
Tonsil germinal center	Ki-67	Polyclonal
Breast cancer	c-erbb2	Polyclonal
Thyroid	Thyroid transcription factor 1 (TTF-1)	8g7g3/1
Blood vessels/placenta	Factor VIII related antigen	Polyclonal
Melanoma	Hmb 45	

All antibodies were obtained from Dako.

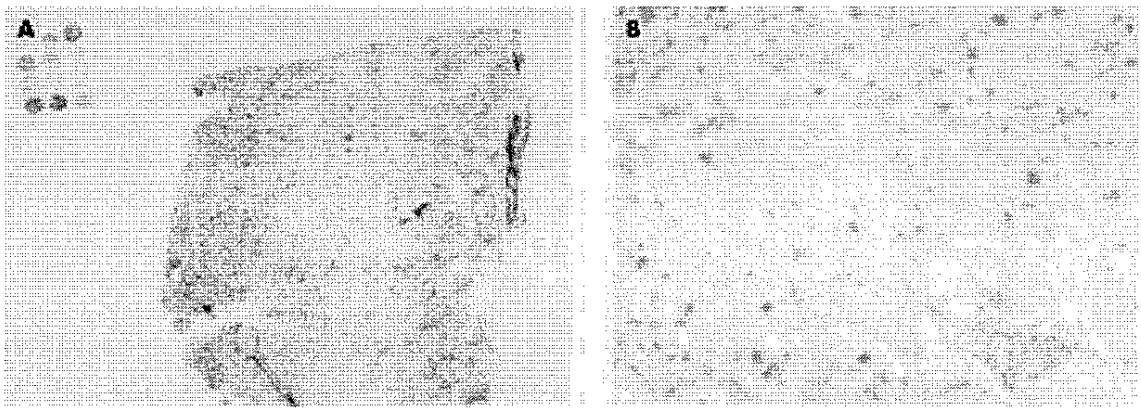

Figure 1 (A) Multitissue control array mounted at the end of the slide near to a tumour sample. (B) Magnification of the tumour stained negative for thyroid transcription factor 1 (TTF-1) with a missing staining reaction of the internal positive control. The positive staining result of the thyroid array element showed that the immunostaining had worked properly (inset right corner).

laboratory are not required. In addition, the amount of microarray control tissue on individual slides is low, so that the amount of case tissue samples needed is not affected. It is also worth mentioning that the volume of antibody required is not increased. The control array did not affect the staining of the case tissue and there was always a clear demarkation between control tissue and the patient sample.

> "The amount of time needed for the preparation of a microarray control block was lower than described previously"

Because this control array can be modified it could easily be adjusted to meet the individual needs of different laboratories. It is possible that arrays with dots of 5 × 5 (25 different tissues) could be devised, enabling the determination of a very wide antigen spectrum. The space required would be only 4.5 × 4.5 mm. This technique could also be applied to other staining procedures, such as fluorescent staining methods and brightfield in situ hybridisation.[11]

Aging of the tissue on the pre-prepared slides did not seem to influence the IHC results. Any possible aging, with consecutive loss of the immunoreactivity, would result in a false negative staining pattern of the internal control tissue, which would lead to repeated testing for that particular antigen. The heterogeneity of the donor tissues with regard to the different antigens might be seen as a disadvantage of this technique. Nevertheless, because the core biopsies function as "positive internal controls", negative staining of the tissue microarray for a specific antigen in a specific dot would lead to it being withdrawn from further use.

We found that the control microarray could also be used with different automated IHC staining systems, and could be useful for monitoring the efficiency of the staining procedure by comparing the immunohistochemical staining intensity in different batches.

In conclusion, the internal multitissue control in IHC is a new application for the tissue microarray technique. We suggest that quality control in IHC would benefit from the use of multitissue microarray controls.

Take home messages

- The tissue microarray technique is a useful new approach to internal multitissue control in immunohistochemistry (ICH)
- The quality control of IHC would benefit from the use of multitissue microarray controls

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Audit and internal quality control in immunohistochemistry

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Short reports

Audit and internal quality control in immunohistochemistry

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Abstract

***Aims*—Although positive and negative controls are performed and checked in surgical pathology cases undergoing immunohistochemistry, internal quality control procedures for immunohistochemistry are not well described. This study, comprising a retrospective audit, aims to describe a method of internal quality control for immunohistochemistry. A scoring system that allows comparison between cases is described.**

***Methods*—Two positive tissue controls for each month over a three year period (1996–1998) of the 10 antibodies used most frequently were evaluated. All test cases undergoing immunohistochemistry in the months of April in this three year period were also studied. When the test case was completely negative for a given antibody, the corresponding positive tissue control from that day was examined. A marking system was devised whereby each immunohistochemical slide was assessed out of a possible score of 8 to take account of staining intensity, uniformity, specificity, background, and counterstaining. Using this scoring system, cases were classified as showing optimal (7–8), borderline (5–6), or unacceptable (0–4) staining.**

***Results*—Most positive tissue controls showed either optimal or borderline staining with the exception of neurone specific enolase (NSE), where most slides were unacceptable or borderline as a result of a combination of low intensity, poor specificity, and excessive background staining. All test cases showed either optimal or borderline staining with the exception of a single case stained for NSE, which was unacceptable.**

***Conclusions*—This retrospective audit shows that immunohistochemically stained slides can be assessed using this scoring system. With most antibodies, acceptable staining was achieved in most cases. However, there were problems with staining for NSE, which needs to be reviewed. Laboratories should use a system such as this to evaluate which antibodies regularly result in poor staining so that they can be excluded from panels. Routine evaluation of immunohistochemical staining should become part of everyday internal quality control procedures.**

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Keywords: immunohistochemistry; audit; internal quality control

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In recent years, increasing attention has focused on pathology laboratories with regard to many aspects of the quality of routine work. Internal quality control procedures should be in place in all laboratories whereby a variety of criteria, including the standard of staining, are checked routinely on a daily basis. These procedures, as well as being part of internal quality control, are assessed by bodies such as Clinical Pathology Accreditation (CPA), UK. Histopathology laboratories should also routinely audit part of their own work and this is carried out in many institutions. For example, laboratories may audit a proportion of randomly selected biopsies. During this audit, many factors pertaining to the biopsy might be evaluated including accuracy of clerical details, turnaround time, quality of staining, and pathological content and accuracy.[1–3] To date, there has been little focus on the quality of immunohistochemical staining and, apart from the routine performing and checking of positive and negative controls, there are few recommendations for internal quality control of immunohistochemistry. The aim of our study was to perform a retrospective audit to assess the quality of immunohistochemical staining in our institution. To this end, we devised a scoring system that allows comparison of immunohistochemical staining between cases and antibodies over a period of time.

Table 1 Details of the 10 most commonly used antibodies

Antigen/antibody	*Clone*	*Supplier*	*Dilution*	*Pretreatment*
CD45	2B11+ PD7/26	Dako	1/50	Microwave
CD20	L26	Dako	1/50	Microwave
CD3	Pab	Dako	1/50	Microwave
CAM5.2	CAM5.2	Becton Dickinson	1/10	Trypsin
AE1/3	AE1/3	Dako	1/50	Trypsin
CEA	11-7	Dako	1/50	Trypsin
NSE	Pab	Incstar	1/2	None
Chromogranin A	Dak-A3	Dako	1/50	None
S100	Pab	Signet	1/20	Trypsin
Desmin	D33	Dako	1:100	None

Pab, polyclonal antibody.

Table 2 Scoring system used in our study

Staining criteria	*Score and criteria for scoring*			
Staining intensity	0 (no staining)	1 (weak staining)	2 (moderate staining)	3 (strong staining)
Uniformity of staining	0 (not uniform throughout)	1 (uniform throughout)	NA	NA
Specificity of staining	0 (non-specific staining present)	1 (only specific staining present)	NA	NA
Absence of background staining	0 (excessive background staining that interferes with interpretation)	1 (background staining present but does not interfere with interpretation)	2 (no background staining present)	NA
Counterstaining	0 (inadequate)	1 (adequate)	NA	NA

NA, not applicable.

Table 3 Scores of positive tissue controls in immunohistochemistry 1996–8

Antigen/antibody	*Unacceptable (%) Score 0–4*	*Borderline (%) Score 5–6*	*Optimal (%) Score 7–8*
CD45 (n = 72)	1.4	33.3	65.3
CD20 (n = 70)	0	10	90
CD3 (n = 69)	1.5	17.4	81.1
CAM5.2 (n = 71)	2.8	36.6	60.6
AE1/3 (n = 69)	7.3	30.4	62.3
*CEA (n = 71)	7.0	23.9	69.1
NSE (n = 58)	36.1	60.4	3.5
*Chromogranin A (n = 67)	10.5	26.8	62.7
S100 (n = 72)	0	25.0	75.0
Desmin (n = 64)	1.6	12.5	85.9

*Includes two slides where inappropriate tissue was used as control (scored as 0).

Materials and methods

SPECIMENS

Cases were retrieved from the files of the department of pathology, Royal Group of Hospitals Trust, Belfast. Two positive tissue controls (where available) from each month over a three year period (1996–8) for the CAM5.2 and AE1/3 antibodies and antibodies directed against CD45, CD20, CD3, carcinoembryonic antigen (CEA), neurone specific enolase (NSE), chromogranin A, S100, and desmin were retrieved from file (table 1). These ten antibodies were chosen because they were the most commonly used antibodies during the study period. All routine test cases undergoing immunohistochemistry within the months of April were also retrieved. Where a test case was negative with an antibody and where there was no internal positive control, the positive tissue control for that antibody performed on that day was used. Negative controls, where the primary antibody was replaced with buffer (Tris buffered saline), for all cases were also reviewed .

IMMUNOHISTOCHEMICAL STAINING

All slides were stained manually using a standard methodology of peroxidase streptavidin–biotin (Duet StABC; Dako, Ely, Cambridgeshire, UK) with diaminobenzidine as the chromogen. Counterstaining was with Harris's haematoxylin. All antibody incubations were conducted at room temperature for 30 minutes. Some cases, as well as undergoing manual staining, were also stained using an automated immunostainer (Ventana NEXES; Ventana, Strasbourg, France), which was being evaluated in our department during part of the study period. Automated protocols followed the manufacturer's recommended procedures with antibody incubations at 37°C for 30 minutes using the Ventana detection and counterstain systems. Pretreatment by microwaving was conducted using a Matsui domestic oven delivering 850 W for 20 minutes in 0.01 M citrate buffer (pH 6.0). Trypsin digestion (ICN, Aurora, Ohio, USA) was performed using a 0.1% solution in 0.1% calcium chloride at 37°C (pH 7.8) for 10 minutes. Protein digestion on the Ventana NEXES was performed at 37°C using the manufacturer's digestion kit.

SLIDE ASSESSMENT

Each slide was assessed out of a possible score of 8. Parameters measured (table 2) were staining intensity (0, 1, 2, 3), uniformity (0, 1), specificity (0, 1), absence of background staining (0, 1, 2), and counterstaining (0, 1). A score of 0–4 was considered to be unacceptable, 5–6 borderline, and 7–8 optimal. In cases where the intensity of staining was 0 (negative), the staining was considered to be unacceptable and all other parameters were also considered to be 0. If the degree of background staining was judged to interfere with interpretation (a score of 0), the stain was also considered unacceptable and given a score of 0. In those cases where both manual and automated immunostaining were performed, the final numerical scores were compared. The two authors assessed each slide over a double headed microscope.

Results

There was no staining of negative controls. Table 3 shows the numbers of positive controls examined and the proportions of these showing unacceptable, borderline, and optimal staining. Four slides (two staining for CEA and two for chromogranin A) were completely negative as a result of the selection of an inappropriate positive control. These were scored as 0. Most positive tissue controls showed optimal staining and in most cases staining was either borderline or optimal. The exception was staining for NSE where there were consistent problems: staining was typically weak and lacking in specificity, with excessive background. Using the same control material, chromogranin A staining was superior with only 10% of cases showing unacceptable staining.

There were 44, 42, and 46 test cases for review in 1996, 1997, and 1998, respectively. Of these, six of 44, five of 42, and six of 46, respectively, were not on file at the time of review. Within the test cases, 55 different antibodies were used, ranging in frequency from 1 to 39 requests. All test cases audited (including those that were negative and where the positive tissue control for that day was used), showed either borderline or optimal staining except for a single case of staining for NSE, which was unacceptable owing to non-specific staining and excessive background staining. Table 4 shows the percentage scores for each of the criteria for those slides stained manually. As can be seen, over 90% of cases gained maximum marks for staining intensity, uniformity, specifi-

Table 4 Scores in percentages of all test slides reviewed in our study, including positive tissue controls where the test case slide was negative

	Scores for each year											
	0			1			2			3		
Staining criteria	*1996*	*1997*	*1998*	*1996*	*1997*	*1998*	*1996*	*1997*	*1998*	*1996*	*1997*	*1998*
Staining intensity	0.0	0.0	0.0	0.8	1.1	0.0	0.0	8.9	8.3	99.2	90.0	91.7
Uniformity of staining	5.0	4.4	0.0	95.0	95.6	100.0	NA	NA	NA	NA	NA	NA
Specificity of staining	0.8	6.7	2.8	99.2	93.3	97.2	NA	NA	NA	NA	NA	NA
Absence of background staining	0.3	1.1	0.0	36.1	17.8	38.9	63.6	81.1	61.1	NA	NA	NA
Counterstaining	0.0	1.1	0.0	100.0	98.9	100.0	NA	NA	NA	NA	NA	NA

Total slides reviewed by year (manual methodology only): 1996, 119; 1997, 90; 1998, 72.
NA, not applicable.

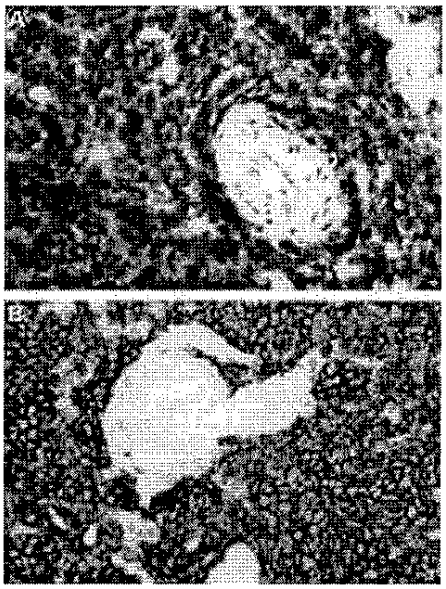

Figure 1 Comparison of staining for CD20 between manual (A) and automated (B) methods. Automated immunostaining resulted in more intense, crisp membrane staining.

city, and adequacy of counterstaining. Background staining was more of a problem, with only 61–81% of cases achieving the maximum score.

Twenty five different antibodies were used for automated immunostaining in our study. Overall, automated staining resulted in higher scores than manual staining. The overall mean score for manually stained slides was 7.6, whereas the mean score for automated stained slides was 7.9. Slides stained on the Ventana NEXES generally showed more intense staining of serial sections than those stained manually. In addition, background staining with the Ventana NEXES was eliminated without affecting the intensity of staining (fig 1). One exception to this was bcl2 staining, which required an amplification protocol supplied by the manufacturer. There was generally no difference in the uniformity or specificity of staining or the adequacy of counterstaining between the manual and automated methods.

Discussion

The aim of our study was to evaluate the standard of immunohistochemical staining in our department, which comprises a busy teaching hospital. External quality assurance programmes such as that managed by UK National External Quality Assurance (UKNEQAS) for Immunocytochemistry (London) and a laboratory's own internal quality control systems are two means of assessing performance in immunohistochemistry. The UKNEQAS organisation holds regular regional workshops and updates participants through official publications. Internal quality control systems, however, have been more difficult to formalise and, although positive and negative control material are checked on a daily basis, more formal assessment is probably not carried out in most laboratories. Organisations such as the National Committee for Clinical Laboratory Standards (NCCLS) (USA) have published guidelines on best practice, and recent publications have shown that there is an interest in setting out goals and objectives for quality control procedures in immunohistochemistry.[4 5] Other groups have attempted to identify good practice and have made recommendations regarding quality standards in immunohistochemistry.[6] These quality issues are likely to assume increasing importance with the advent of clinical governance. Data from UKNEQAS for Immunocytochemistry show that increasing numbers of laboratories sometimes struggle to maintain standards.[7]

The internal quality control procedures carried out in our laboratory, namely positive tissue controls containing the antigen under test and a negative control section from each test block, appear to meet the minimum required criteria of those that are reasonably expected to be conducted by a routine diagnostic immunohistochemistry laboratory. Although it is common practice to review at the end of each day both sets of controls along with the test material, we have conducted a three year review of a proportion of the positive control material of the most commonly used antibodies in our laboratory, together with a proportion of test cases. Our study assesses only the standard of immunohistochemistry and makes no attempt to determine whether reporting pathologists have used an appropriate or adequate panel of antibodies, or whether they have interpreted the results correctly.

The scoring system we devised was an attempt to assess the elements looked for when examining an immunohistochemical slide. We did not consider the problem of interobserver and intra-observer variation of this scoring system but, rather, the two authors examined the slides together using a double headed micro-

scope. The intensity of staining was judged on a four point scale from negative (0) to intense (3). The specificity and uniformity of staining and the adequacy of counterstaining were scored as either 0 or 1. The degree of background staining was judged on a reversed three point scale from 0 (background stain interferes with interpretation) to 2 (no background). Other scoring systems such as that used in the UKNEQAS for Immunocytochemistry scheme score each slide out of a total of 20, this being a composite score from four independent assessors each scoring out of 5. In this scoring scheme, however, criteria vary and are dependent on the antibody under examination.[8] Using our system, we found that evaluating different antibodies on a common scale was possible, allowing for comparison between antibodies over a period of time.

The review of positive tissue controls showed that with most antibodies there was optimal or borderline staining, with only a small proportion showing unacceptable staining. Most test cases also showed optimal or borderline staining. The exception to this was staining for NSE, where only a small proportion of control cases showed optimal staining and over one third were unacceptable. The main problems were low intensity of staining, non-specific staining, and excessive background. Staining for NSE has a reputation for poor specificity and this was confirmed in the study, suggesting that NSE positivity is not conclusive evidence of neuroendocrine differentiation. Many pathologists still use antibodies to NSE as part of a panel to confirm neuroendocrine differentiation and we suggest that alternative antibodies such as those to chromogranin A and PGP 9.5 might be more suitable for this purpose. However, it might be that other laboratories may achieve better results with NSE staining using the same or a different antibody. Other laboratories may find consistent problems with other antibodies and might wish to exclude these from their immunohistochemical panels.

Automated immunohistochemistry using the Ventana NEXES system marginally improved the overall scoring, usually by producing a very clean background without loss of intensity. The exception to this was bcl2 staining, which required an amplification protocol provided by the manufacturer. This shows that each antibody must be evaluated individually when introducing automated immunohistochemical staining into a laboratory. Although the overall mean score for automated immunostaining (7.9) was only marginally greater than that for manual staining (7.6), there was a trend towards greater intensity with a cleaner background. The cost of such automated systems and the effect automation may have on staffing levels are beyond the scope of this paper.

The number of cases not retrievable from the files is perhaps excessive, but is indicative of the diverse system of reporting and the ongoing research interests of a large teaching hospital. Implementing a system of review, such as we are suggesting, and reporting the incidence of missing slides to pathologists and laboratory staff may result in an improved awareness of the need to return slides for filing and to file the slides correctly.

In summary, this retrospective audit describes a method for improving the daily internal quality control of immunohistochemical staining. Laboratories might wish to carry out similar procedures on a regular basis to ensure that their immunohistochemistry is of a high standard. In this way, antibodies that consistently result in substandard staining can be identified. Steps can be taken to correct this, either by using different protocols or by excluding these antibodies from routine use. We would recommend that laboratories devise a system such as ours to assess their standard of immunohistochemical staining. This assessment should be performed regularly as part of internal quality control.

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MEMORIAL U. PRINT JOURNAL

Influence of menstrual cycle, parity and oral contraceptive use on steroid hormone receptors in normal breast.

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Steroid receptor was assessed immunohistochemically in 158 samples of normal breast for variation through the menstrual cycle. Patterns and intensity of reaction were used in a semi-quantitative scoring system to examine the influence of cycle phase, cycle type, parity and age. The changes in oestrogen receptor for natural cycle and oral contraceptive (OC) cycles indicated down-regulation by progestins. Progesterone receptor did not vary significantly in natural cycles, but increased steadily through OC cycles. This study provides strong evidence that both oestrogen and progesterone influence breast epithelium, but dissimilarities from the endometrium are apparent. The interval since pregnancy had a significant negative effect on frequency and score of oestrogen receptor and score of progesterone receptor. Multivariate analysis established the phase of cycle and OC use as independent significant influences on oestrogen receptor. The interval since pregnancy was an independent significant factor for both oestrogen and progesterone receptor presence.

PIP: Presence, distribution, and quantity of estrogen and progesterone receptors (ER, PR) were determined by immunohistochemical techniques in 158 breast tissue samples, and results scored and analyzed for age, cycle phase, and oral contraceptive use. Frozén specimens fixed by standard histologic methods were analyzed with the ER-ICA kit using rat monoclonal antibody for ER (Abbott), or the mouse monoclonal antibody against rabbit uterine PR. One section from each case was scored, counting all terminal duct lobular units (TDLU), and accounting for staining intensity, percentage of positive TDLUs, and staining pattern. Most of the sections showed mixed positive and negative areas, sometimes a sporadic pattern, and less often a ring pattern. 38% head positive ER, and 72% were positive for PR. ER scores ranged from 0-513 (median 0), and PR scores from 0-600 (median 186). ER appeared in 47% of cycling women, significantly more often on

days 1-13, while there were more low and moderate scores but fewer negatives on days 14-28. 70% of cycling women and 70% of pill users had PR. 26% of the oral contraceptive users were positive for ER, with scores ranging from 9-417, significantly lower than those seen in the natural cycle. There were no significant variations in ER throughout the cycle; PR scores were significantly higher on days 14-28 of the oral contraceptive cycle. There were no effects of age, breast age, or parity on ER or PR. Among parous women, however, ER and PR were detected much less frequently in women naturally cycling and 5 years postpartum. In multivariate analysis, controlling for cycle phase, oral contraceptives significantly lowered frequency of staining, and time postpartum also lowered ER and PR staining significantly. In the discussion it was noted that the decline in ER in the 2nd half of the cycle in breast parallels that in endometrium, but PR rise in breast is in contrast to falling in endometrium in the later half of the natural or pill cycle. These data show a blunted response in numbers of steroid receptors after pregnancy, as has been reported in other indicators of breast proliferation.

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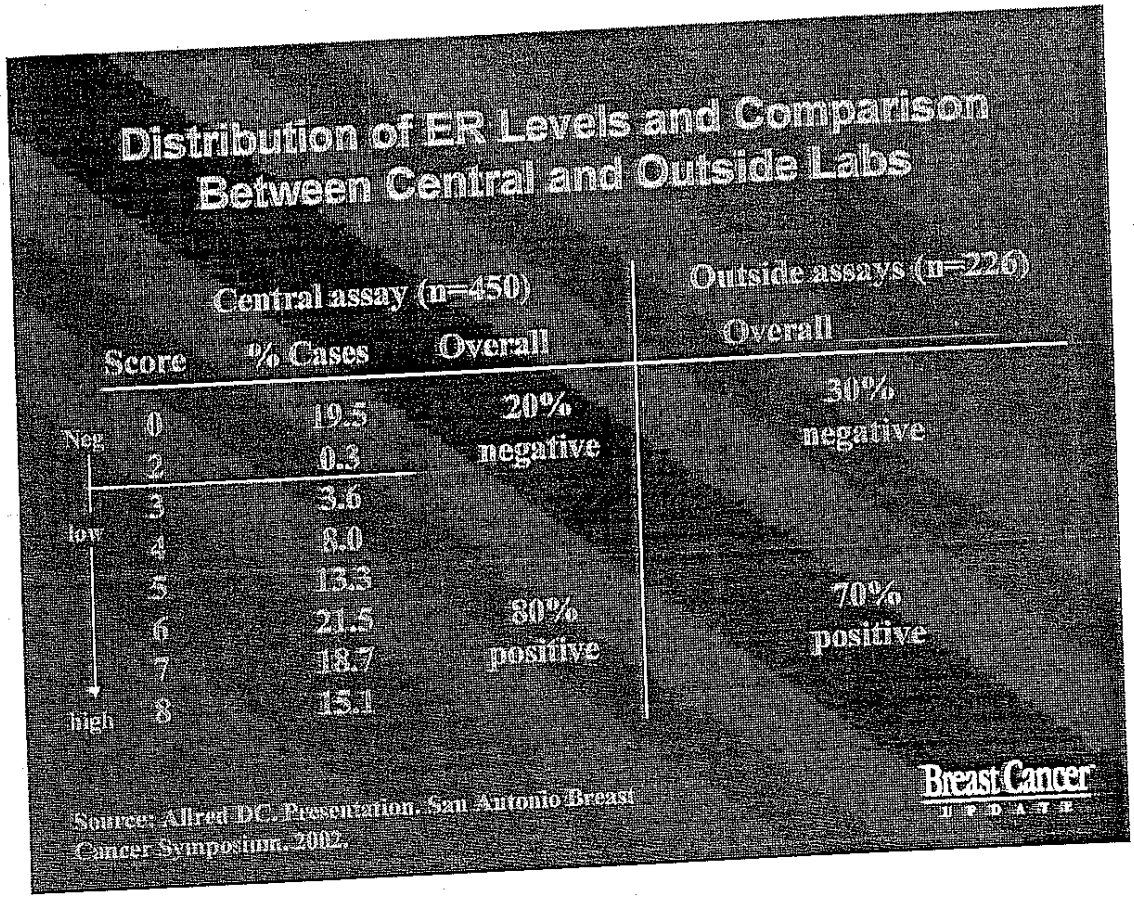
Distribution of ER Levels and Comparison Between Central and Outside Labs
Central assay (n=450)
Score
% Cases
Overall
Outside assays (n=226)
Overall
Neg
low
high
0
2
3
4
5
6
7
8
19.5
0.3
3.6
8.0
13.3
21.5
18.7
15.1
20% negative
80% positive
30% negative
70% positive
Source: Allred DC. Presentation. San Antonio Breast Cancer Symposium. 2002.
Breast Cancer UPDATE

BCU 7 | 2004

Editor's Note

Team in need of a coach

Every medical oncology fellow quickly learns about interdisciplinary cancer care, but thank God for the American College of Surgeons' mandate for tumor boards, because without them, we might be strangers. Personally, I don't like to think about any surgeon, radiation oncologist or medical oncologist not regularly attending one of these valuable meetings. However, the truth is that we really don't report to anyone, and our collaboration is pretty much voluntary.

This issue of our audio series attempts to demonstrate how critical it is that interdisciplinary team members talk to each other. We begin with the local control guys, and Pat Borgen and Frank Vicini comment on a plethora of surgical and radiation therapy research issues that profoundly affect systemic management decisions.

For example, Dr Vicini is the principal investigator of a critical NSABP-RTOG randomized clinical trial evaluating partial breast irradiation (PBI). This historic collaboration between two premier collaborative clinical trial groups will provide much-needed answers about PBI, albeit many years from now. In the interim, the pace at which this accelerated and patient-friendly treatment strategy permeates into the nonprotocol management algorithm utilized in the community treatment setting is anyone's guess.

While we wait for definitive research results, patients should seek input from every team member regarding the advisability of PBI and which technique is preferable. Pat Borgen cautions us that local control may have much more of an impact on long-term survival than previously recognized, and one might imagine that PBI could either have a deleterious effect (if it results in suboptimal local tumor control) or could be a more effective modality (because treatment can be implemented prior to chemotherapy).

With an increasing number of patients receiving taxane-based adjuvant regimens that can take up to six months to complete, earlier radiation therapy could have a potential antitumor advantage.

From a quality of life perspective, avoiding six weeks of daily treks for radiation therapy is appealing, particularly after the physical and emotional trauma of adjuvant chemotherapy. However, patients will surely want to know what their medical oncologist has to say on this issue before they opt for an unproven treatment modality.

Input from Craig Allred, the pathologist for the interdisciplinary team collaborating on this issue of *Breast Cancer Update*, is unfortunately very disheartening. I have nothing personal against pathologists or Craig, who is a really nice man, but if Adam Brufsky's interview provides ample documentation that contemporary systemic therapy of breast cancer is essentially target-driven, then Craig's comments leave us wondering if we have the ability to measure the most critical targets every oncologist must consider — ER, PR and HER2 status. (My apologies to Phillip Roth for that very long sentence.)

I keep expecting some rebel breast cancer patient advocacy group to stage a massive protest at the NCI to demand that pathologists provide impeccable ER, PR and HER2 assays. At the present time, however, women are going to continue to relapse unnecessarily or receive suboptimal palliative care because we can't get their pathology right. Even if recent history tells us that our usually capable nation is not totally effective in military intelligence gathering, we should be able to at least gather accurate

information for the war on cancer.

Maybe we need more than ACOS-mandated tumor boards. Maybe we need someone to rally and guide the entire team — including nurses, pharmacists, radiologists, psychologists, social workers and others — and take a deep breath, and really figure out how to work together better so patients can receive the very best care we have.

— Neil Love, MD
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Select publications

Gene expression profiling studies have reconfirmed the previously realized biologic importance of ER in breast cancer. Perou et al[59] published the results of their breast cancer gene expression analysis in 2000 and found that expression profile patterns largely separated tumors into ER positive and ER negative categories. These findings have been confirmed by others using different sampling methods and expression profiling techniques.[60–62] Results from gene microarray studies have further categorized breast cancers into several major subtypes based on their patterns of gene expression, including the ER positive luminal subtype and the ER negative basal subtype.[59,60,63] The existence of these breast cancer phenotypes have been verified by immunohistochemical studies of protein expression.[64,65]

ER has complex relationships with other biomolecules relevant in breast cancer. The majority of cancers express ER and HER2 in an inverse manner, and a subset of tumors (approximately 10%) express both.[66–69] Although individual luminal cells of the normal breast rarely co-express ER and the proliferation marker Ki-67, a substantial proportion of breast cancer cells show this coexpression.[70] The interactions of ER with growth factors and signal transduction molecules appear to be important in the development of resistance to endocrine therapy.[71]

Although ER often retains its functionality during endocrine therapy, evidence suggests that adaptive signal transduction pathways stimulate tumor progression independent of ER-ligand interactions.[72] Currently, clinical ER testing assesses for the presence or absence of detectable ER protein regardless of its functional state.

ER TESTING

Interlaboratory Variability

Multiple reports addressing interlaboratory variability for ER testing have been published in the past several years, mostly from European institutions.[23,25,73–76] The most notable of these studies were conducted by Rhodes and colleagues under the auspices of the United Kingdom's national external quality assessment scheme for immunocytochemistry (NEQAS-ICC).[25,75,76] The NEQAS-ICC is presently comprised of 200 participating laboratories from 26 countries in Europe and Asia. For its first published comparative study, the NEQAS-ICC investigators circulated to participating laboratories unstained composite tumor sections known to possess low, medium, or high ER levels.[25] Only 37% of the participating laboratories were able to obtain a positive result for the presence of ER in tumors with low ER levels using the traditional 10% staining cutoff, but 66% reported a positive result if a 1% cutoff was used.[25]

The high rates of interlaboratory variability found through the NEQAS-ICC quality assessment scheme prompted further investigation into the causative factors of such variability. In a second study, tumors fixed and processed by the NEQAS-ICC centralized laboratory were assayed by the participants, and the results were compared with those obtained using tumors fixed and processed by the participating laboratories themselves.[76] Overall testing results were found to be equivalent for the two sets of tumors, validating the scheme's quality assurance mechanism (ie, distribution of unstained composite tumor sections). Moreover, their findings strongly suggested that preanalytical variables (tissue handling, fixation, and processing) do not greatly affect ER testing results using IHC.

In a later NEQAS-ICC report, the length of time for heat antigen retrieval was identified as the most important variable for improving ER testing standardization.[75] Additionally, using an elegant statistical analysis of their ER testing results over 2 years, NEQAS-ICC ranked their participants as "high assay sensitivity" or "low assay sensitivity" laboratories. NEQAS-ICC high assay sensitivity laboratories had a mean rate of positive ER testing for all patients of 77% (compared with 72% for low sensitivity laboratories).[21] Obviously, ER testing results for an individual laboratory will depend to some extent on the characteristics of the patient population studied, especially patient age and the clinical setting in which the testing is performed (eg, primary cancers versus recurrences or metastases). Nevertheless, interlaboratory comparisons of testing results such as those provided in the NEQAS-ICC studies could assist in identifying specific laboratories that could benefit from technical improvements in their ER testing methodologies.

Additional interlaboratory comparisons of ER testing performed in Austria and Sweden addressed staining technique and scoring reproducibility, respectively.[73,74] Although variation was demonstrated in both of these studies, the authors concluded that improvements in testing could be made through automation and training. A German study demonstrated poor reproducibility of ER testing using tissue microarrays with ER detection failure rates similar to those reported by the NEQAS-ICC.[77]

Layfield et al[23] published results demonstrating a disagreement rate of 26% among three laboratories in the United States independently testing 35 breast cancers for ER using IHC. That study was a follow-up to an earlier laboratory survey (in the form of questionnaires) that also demonstrated poor standardization for ER testing.[27] The more recent of the two studies is the only published interlaboratory comparison of ER testing in the United States in which unstained slides were circulated.[23]

ER testing findings for intraductal carcinoma from NSABP Protocol B-24 have recently been presented by Allred et al.[22] The predictive value of a positive ER status for response to tamoxifen therapy was demonstrated by these data. Additionally, it was observed that cases analyzed by participating institutions using non-standardized methods were more frequently ER negative compared with those tested by a centralized IHC laboratory (where a clinically validated and standardized testing method was used). The findings of Layfield[23] and by NSABP B-24[22] indicate that significant interlaboratory variability for ER testing does occur in the United States.

Currently, there are legitimate concerns worldwide that ER immunohistochemical testing methodologies are insufficiently standardized and that clinically significant false negative rates exist.[24,78] The interlaboratory comparisons of Rhodes et al[25] and Layfield et al[27] have convincingly revealed interlaboratory variability in ER testing methodologies and results. A concerted effort by laboratories to adopt reproducible and clinically validated testing standards for ER

IHC will be necessary to properly address this problem. If successfully implemented, standardization of ER testing could serve as a paradigm for the multitude of predictive markers that will likely be assayed by IHC in the future.

Technical Considerations

Standardization of ER detection methods (ie, specimen selection, processing, scoring, and quality measures) is of paramount importance for the accurate analysis of ER status and appropriate patient management. IHC is a commonly used and widely commercialized technique that already has achieved a marked level of standardization. As a complex multistep laboratory procedure, IHC requires highly trained personnel for its proper performance. Indeed, seemingly minor differences in testing procedures may lead to marked variability of results. An additional level of complexity is encountered when evaluating markers requiring quantitation, such as ER or HER2 for breast cancer. Multiple parameters, such as those listed in Table 1, should be considered when performing IHC to detect ER.[79,80] In the subsequent paragraphs we review these variables and discuss their importance.

When to Test

ER testing is indicated for all primary invasive breast carcinomas because of its proven prognostic and predictive value.[9,13,81,82] Many centers are now also performing ER testing in cases of ductal carcinoma in-situ (Fig. 3), a trend based primarily on the recently presented findings from NSABP Protocol B-24.[22] The true utility of ER testing for ductal carcinoma in-situ, however, remains controversial, and further studies are pending.

TABLE 1. Variables for ER Detection by Immunohistochemistry

Preanalytical variables
Timing of testing
Specimen type
Fixative type
Fixation time
Processing method
Analytical variables
Automated versus manual procedure
Antibody and titer
Antigen retrieval time
Blocking procedure
Detection kit used
Staining method
Interpretive variables
Manual scoring versus image analysis
Scoring systems
Scoring cutoffs
Quality assurance and control
Types of controls
Internal
External
Quantitative
Quality assurance procedures
External quality assessment programs

ER testing may also be indicated in the settings of recurrent and/or metastatic breast cancer (when a change of ER status would affect treatment decisions) because of potential alterations of the ER status of tumors over time.[83–86] It has been demonstrated that the ER status in approximately one third of breast cancers reverses during disease progression, both from positive to negative and from negative to positive.[85,86] These ER status conversions typically require several years to occur, but conversion from ER positivity to ER negativity has been documented in less than one year.[86] An ER status change to ER positive from ER negative may be beneficial to patients undergoing hormonal treatment.[83] Conversely, conversion to ER negative from ER positive can be associated with aggressive, therapy-resistant disease.[84] The ER status of the recurrent and metastatic disease should be considered as the current ER status of a given patient.

Types of Specimens

ER analysis by IHC is traditionally performed on formalin-fixed, paraffin-embedded histologic tumor sections chosen during diagnostic review of the hematoxylin and eosin-stained slides. Typically, tumors are sectioned from excisional or mastectomy specimens as part of the routine pathologic evaluation, and the amount of tumor available for analysis can vary widely based on the stage of disease. Analysis of ER in smaller-sized, paraffin-embedded specimens (such as needle biopsies) and air-dried or alcohol fixed direct smears can also be performed.[87]

Measurement of ER in large gauge needle core biopsies has been validated against results from excisional specimens in several studies.[88–90] Many centers, including ours, routinely assess breast tumor markers on needle core biopsy specimens (Fig. 4).[91] Intratumoral heterogeneity for ER expression can be biologic or artifactual in nature, and reduced staining is most often observed in the center of the tumor compared with periphery.[5,92] This heterogeneity does not substantially affect ER results obtained using needle core biopsy specimens. If the ER results measured on needle core biopsy are questioned (usually due to small tumor volume), repeat testing of the excision specimen is warranted.

The analysis of cytologic specimens for ER using immunocytochemistry (Fig. 5) has recently been reviewed by one of the authors (NS).[93] Prognostic and predictive markers of breast cancer, including ER, can be reliably assessed on cytologic material by IHC. Comparative studies have demonstrated concordance rates ranging from 80 to 90% for ER analysis of cytologic versus histologic specimens.[94–96] Clinically, ER analysis of cytologic specimens is important for patients receiving neoadjuvant chemotherapy and only when core needle biopsy is not available. In that setting, when response to therapy is dramatic, pretreatment cytologic smears of primary or meta-static disease may represent the only material available for ER analysis.

Tissue Handling, Fixation and Processing

Methods used for tissue handling, fixation, and processing can affect ER analysis by IHC. Gross examination of specimens and tissue submission techniques vary between institutions, but overall they are relatively standardized. It is

Biotech Histochem. 1992 Mar;67(2):110-7.

Quality assurance and standardization in immunohistochemistry. A proposal for the annual meeting of the Biological Stain Commission, June, 1991.

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Quality assurance, quality control, proficiency testing, reagent documentation and validation are standard parts of everyday practice in clinical laboratories throughout the United States. Immunohistochemical stains employ reagents and principles in common with immunoenzyme methods utilized in the clinical laboratory. However, immunohistochemistry has not routinely been subjected to similar standardization and quality assurance procedures that manufacturers and pathologists alike have applied to essentially the same techniques in the clinical laboratory environment. The current proposal was invited by the Biological Stain Commission with the charge of incorporating the findings of previous workshops on quality control in immunohistochemistry into a practical design for implementation. The status of quality assurance, quality control and standardization in immunohistochemistry is reviewed and a phased strategy for implementation is proposed.

Biotech Histochem. 1991;66(4):194-9.

The taming of immunohistochemistry: the new era of quality control.

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The most critical factor for interpreting the results of immunohistochemistry is verification of antibody sensitivity and specificity. While some manufacturers supply material data sheets with this information, many do not. This paper describes a well-defined quality assurance program for testing immune reagents. This program can be used to provide commercial suppliers of antisera with analyses of their products destined for government licensure applications. This paper illustrates the protocol and explains the testing philosophy developed over the last eight years.

Publication Types:
- Review
- Review, Tutorial

Reliability of immunohistochemical demonstration of oestrogen receptors in routine practice: interlaboratory variance in the sensitivity of detection and evaluation of scoring systems

A Rhodes, B Jasani, D M Barnes, L G Bobrow, K D Miller

Abstract

***Aims*—To investigate interlaboratory variance in the immunohistochemical (IHC) detection of oestrogen receptors so as to determine the rate of false negatives, which could adversely influence the decision to give adjuvant tamoxifen treatment.**

***Methods*—To ensure that similar results are obtained by different institutions, 200 laboratories from 26 countries have joined the UK national external quality assessment scheme for immunocytochemistry (NEQAS-ICC). Histological sections from breast cancers having low, medium, and high levels of oestrogen receptor expression were sent to each of the laboratories for immunohistochemical staining. The results obtained were evaluated for the sensitivity of detection, first by estimating threshold values of 1% and 10% of stained tumour cells, and second by the Quick score method, by a panel of four assessors judging individual sections independently on a single blind basis. The results were also evaluated using participants' own threshold values.**

***Results*—Over 80% of laboratories were able to demonstrate oestrogen receptor positivity on the medium and high expressing tumours, but only 37% of laboratories scored adequately on the low expressing tumour. Approximately one third of laboratories failed to register any positive staining in this tumour, while one third showed only minimal positivity.**

***Conclusions*—There is considerable interlaboratory variability, especially in relation to the detection of breast cancers with low oestrogen receptor positivity, with a false negative rate of between 30% and 60%. This variability appears to be caused by minor differences in methodology that may be rectified by fine adjustment of overall technique.**

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Keywords: immunohistochemistry; oestrogen receptors; interlaboratory variation

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The importance of establishing the oestrogen receptor status of tumours for the treatment of women with breast cancer has recently been emphasised.[1] The authors concluded that the fundamental question to be asked when predicting the likely outcome for a particular woman receiving adjuvant tamoxifen treatment is not whether she is young or old, with or without nodal involvement, or receiving chemotherapy—but whether or not her tumour is completely oestrogen receptor negative. Oestrogen receptor status is now often established by an immunohistochemical (IHC) test employing monoclonal antibodies.[2–4] This assay has been shown to be at least as sensitive as the biochemical ligand binding assay[5 6] and has the advantages of being applicable to small tumours and Tru-Cut biopsy samples, and of allowing only tumour cells to be assessed for oestrogen receptor status. The IHC assay can be conducted inexpensively[7 8] on routinely processed tissue sections, with no need for specialised equipment. Consequently in many countries IHC analysis has become the chosen technique for establishing oestrogen receptor status in a routine pathology setting.[9 10]

In view of the increasing use of the oestrogen receptor IHC assay, it is vital that good quality assurance procedures are in place to assess the quality of the assays carried out by different laboratories.[10] The United Kingdom national external quality assessment scheme for immunocytochemistry[11] (UK NEQAS-ICC) currently assesses the quality of many immunohistochemical techniques carried out in the majority of UK clinical laboratories and in various laboratories based outside the United Kingdom. Since April 1994 the scheme has provided an external quality assessment (EQA) programme for the demonstration of oestrogen and progesterone receptors on routinely processed breast tumours.

In this paper we report on the degree of variability between 200 laboratories in demonstrating oestrogen receptors by immunohistochemistry on the same cases. The main aim of the study was to establish the proportion of laboratories able to demonstrate oestrogen receptors reliably in a weakly positive tumour, as there is a danger that these tumours could be erroneously reported as negative if the IHC assay is not of adequately high sensitivity.

Methods

Laboratories participating in the UK NEQAS-ICC programme for steroid hormone receptors (table 1) were sent two unstained slides containing histological tissue sections of formalin fixed and paraffin processed breast tumours showing different levels of receptor expression. Included in the composite tumour block, comprising three different oestrogen receptor positive infiltrating ductal carcinomas

Table 1 Countries with laboratories participating in the UK NEQAS-ICC programme for steroid hormone receptors

Country	No of Labs
Australia	6
Austria	1
Belgium	3
Canada	1
Denmark	3
Finland	1
France	25
Germany	4
Greece	1
Hong Kong	1
Hungary	1
Ireland	14
Malaysia	1
Malta	1
New Zealand	1
Norway	1
Portugal	1
Saudi Arabia	1
Singapore	2
South Africa	1
Slovenia	1
Sultanate of Oman	1
Spain	1
Sweden	9
Switzerland	9
United Kingdom	138
USA	2

Table 2 Evaluation of the staining achieved by the organising centre and participants who are known to have validated their immunohistochemical assay by published clinical studies

	Low expressor (X)			Medium expressor (Y)			High expressor (Z)			
Lab‡	Quick score	% nuclei positive	Result*	Quick score	% nuclei positive	Result*	Quick score	% nuclei positive	Result*	Cut off used*
a	3	>10	+	3	>10	+	6	>10	+	20%
b	2	>10	+	5	>10	+	6	>10	+	H score†
c	2	>10	−	7	>10	+	6	>10	+	15%
d	0	0	−	3	>10	+	6	>10	+	10%
e	2	>10	+	5	>10	+	6	>10	+	2%
f	2	>1	+	5	>10	+	6	>10	+	5%
g¶	3	>10	+	5	>10	+	6	>10	+	10%

*The result using participants' own threshold value.
†H score – cut off value of 50.
‡In order to preserve anonymity, the laboratories have been coded by letters.
¶Results of the initial testing conducted by the UK NEQAS-ICC organising laboratory.

(X, Y, and Z), was some normal glandular breast tissue which acted as an internal control. In order to ensure that all sections contained a similar proportion of oestrogen receptor positive cells, every 100th section was immunostained for oestrogen receptors by the organising laboratory. Each participant was asked to demonstrate oestrogen receptors and to return the best stained slide, along with their own in-house control slide and a completed questionnaire giving methodological details (including details of the threshold value used by the laboratory), to the UK NEQAS-ICC coordinating centre for assessment. An expert panel of four, comprising pathologists (BJ, LB) and biomedical and clinical scientists (AR, DB), examined the slides and assessed the quality of the IHC assay performed by each laboratory.

METHODS OF EVALUATION

For the purposes of the present study, the "Quick" score method of assessment[12 13] was used to assess the range of immunostaining performed by the participating laboratories. With this method the intensity of the immunohistochemical reaction as viewed under the light microscope was recorded as follows: 0, negative (no staining of any nuclei even at high magnification); 1, weak (only visible at high magnification); 2, moderate (readily visible at low magnification); 3, strong (strikingly positive even at low power magnification). The proportion of tumour nuclei showing positive staining was also recorded as: 0 (none); 1 (approximately 1–25%); 2 (26–50%); 3 (51–75%); or 4 (76–100%). The score for intensity was added to the score for proportion, giving the Quick score, with a range of 0–7 for each individual tumour.

The proportion of cells stained in each tumour in the composite block was also recorded as either 0, ≥ 1% but < 10%, or ≥ 10%. The absence or presence of staining of the nuclei of non-neoplastic ducts in adjacent tissue was also recorded. This served as an internal control. Slides which failed to show any staining in the normal internal control or which showed excessive non-specific immunostaining in the stromal component were deemed unsatisfactory and were excluded from statistical analysis.

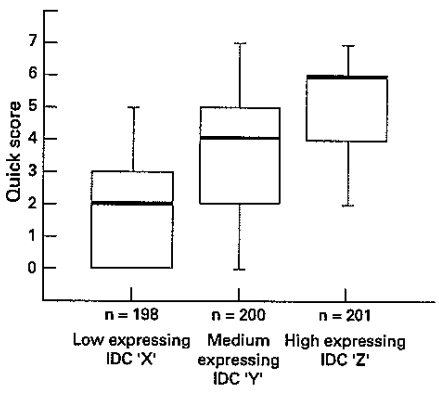

Figure 1 Distribution of the results of the "Quick" score evaluation conducted on the three infiltrating ductal carcinomas (IDC), X, Y, Z, used at assessment. The bold line represents the median score, the bottom and top of the boxes, the 1st and 3rd quartiles, respectively, and the range bars, the lowest and highest scores, respectively. The slightly different numbers for the three tumours reflect loss of tissue from the microscope slides; n, number of participating laboratories.

OESTROGEN RECEPTOR STATUS OF THE REFERENCE TUMOURS X, Y, AND Z

From the UK NEQAS participants, six were identified as having published clinical studies relating oestrogen receptor positivity to tamoxifen treatment. These studies are not referred to in this paper as this would identify the laboratories concerned and in so doing transgress the UK NEQAS code of practice which confers anonymity to all participants.[14] The assessment results from these laboratories and the initial testing performed by the organising centre were used to establish the oestrogen receptor status of the tumours X, Y, and Z, and are recorded in table 2. Additional confirmation of the oestrogen receptor positive status was provided in the form of the results of previous biochemical assays conducted on these cases.

STATISTICAL ANALYSIS

Median values were established for the Quick scores achieved by participating laboratories on the infiltrating ductal carcinomas (IDC) labelled X, Y, and Z. Spearman's rank coefficient was used to test for correlation between the level of sensitivity achieved on the three different tumours and differences in the proportion of laboratories showing oestrogen receptor positivity at various threshold values was tested by means of the χ^2 test. Kendall's coefficient of

Table 3 Correlation of the Quick scores achieved by 190 laboratories on the infiltrating ductal carcinoma (IDC) with low oestrogen receptor expression with the scores achieved by the same laboratories on the IDC with medium oestrogen receptor expression

Quick scores, low expressor (X)	Quick scores, medium expressor (Y)							
	0.00	2.00	3.00	4.00	5.00	6.00	7.00	Total
0.00	19	18	4	12	5	2	1	61
2.00	2	20	9	21	16	3	3	74
3.00		3	3	6	14	3	8	37
4.00			1	3	6	1	4	15
5.00						2	1	3
6.00								
7.00								
Total	21	41	17	42	41	11	17	190

The Quick scores for the 4% of laboratories where staining was recorded as "uninterpretable" have been removed from the analysis.
Spearman correlation = 0.557, standard error = 0.053; significance = $p < 0.0001$.

Table 4 Correlation of the Quick scores achieved by 190 laboratories on the infiltrating ductal carcinoma (IDC) with low oestrogen receptor expression with the scores achieved by the same laboratories on the IDC with high oestrogen receptor expression

Quick scores, low expressor (X)	Quick scores, high expressor (Z)							
	0.00	2.00	3.00	4.00	5.00	6.00	7.00	Total
0.00		16	5	17	7	14	2	61
2.00	1	1	2	13	19	33	5	74
3.00				3	5	25	4	37
4.00					1	9	5	15
5.00						2	1	3
6.00								
7.00								
Total	1	17	7	33	32	83	17	190

The Quick scores for the 4% of laboratories where staining was recorded as "uninterpretable" have been removed from the analysis.
Spearman correlation = 0.528, standard error = 0.055; significance = $p<0.0001$.

Table 5 Correlation of the Quick scores achieved by 190 laboratories on the infiltrating ductal carcinoma (IDC) with high oestrogen receptor expression with the scores achieved by the same laboratories on the IDC with medium oestrogen receptor expression

Quick scores, medium expressor (Y)	Quick scores, high expressor (Z)							
	0.00	2.00	3.00	4.00	5.00	6.00	7.00	Total
0.00	1	12	3	4		1		21
2.00		4	3	14	9	11		41
3.00		1	1	3	4	8		17
4.00				8	10	23	1	42
5.00				4	7	24	6	41
6.00					1	6	4	11
7.00					1	10	6	17
Total	1	17	7	33	32	83	17	190

The Quick scores for the 4% of laboratories where staining was recorded as "uninterpretable" have been removed from the analysis.
Spearman correlation = 0.661, standard error = 0.044; significance = $p<0.0001$.

concordance (Kendall's W) was used to determine the level of agreement between assessors.

Results

When the staining results were analysed by the Quick score (fig 1) the median scores were 2 for tumour X (low oestrogen receptor expressor), 4 for tumour Y (medium oestrogen receptor expressor), and 6 for tumour Z (high oestrogen receptor expressor).

Spearman's rank coefficient showed a highly significant positive correlation between the level of sensitivity achieved by individual laboratories on the tumours of differing oestrogen receptor expression (tables 3–6).

When only the proportion of nuclei stained in the tumours was evaluated, 99.0% of participants demonstrated 10% or more of the nuclei of the high expressor, while 99.5% demonstrated 1% or more. For the medium expressor, 84.5% demonstrated 10% or more of nuclei, while 88.0% demonstrated 1% or more. For the low expressor, 37.3% demonstrated 10% or more of tumour nuclei, with 66.3% demonstrating 1% or more (fig 2). When the threshold values used by participants to designate a tumour as either oestrogen receptor positive or oestrogen receptor negative were used, the proportion of assays which would have recorded the high, medium, and low expressing tumours as oestrogen receptor positive fell to 98.0%, 80.0%, and 32.8%, respectively (for all evaluations, $p < 0.0001$, two tailed). Approximately one third of participants failed to demonstrate any tumour nuclei at all in the low expressor (fig 3).

Kendall's coefficient of concordance revealed a significant level of concordance between assessors in the evaluation of slides (Kendall's $W = 0.014$, $p = 0.040$).

Discussion

With immunocytochemistry for oestrogen receptors, it is a commonly observed phenomenon that the first sign of a fall in sensitivity of the IHC technique is a diminution in staining intensity, and this is followed by a reduction in the proportion of tumour nuclei demonstrated. For this reason, three methods of evaluation were used to assess one or both of these criteria.

The Quick score method was included on the basis that it was a previously validated system for evaluating oestrogen receptor status of each of the tumours,[12 13] in conjunction with a simple but clinically validated 10% oestrogen receptor positive threshold.[15–19] This threshold is commonly used by many laboratories to differentiate between breast tumours which are likely to respond to tamoxifen treatment and those which are not (table 7). We also included the recently recommended 1% threshold value, considered to be clinically relevant by some

Table 6 Comparison of the Quick scores achieved on the infiltrating ductal carcinoma (IDC) with medium oestrogen receptor expression with the proportion of nuclei stained in the IDC with low oestrogen receptor expression

% Nuclei stained, low expressor (X)	Quick scores, medium expressor (Y)							
	0.00	2.00	3.00	4.00 (median)	5.00	6.00	7.00	Total
No nuclei stained	18	17	3	12	5	2	1	58
Some nuclei stained but less than 10%	2	16	7	20	11		2	58
10% or greater		7	7	10	25	9	14	72
Total	20	40	17	42	41	11	17	188

Spearman correlation = 0.539, standard error = 0.057; $p < 0.0001$.

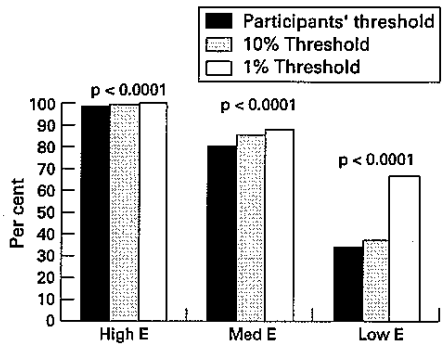

Figure 2 The proportion of laboratories from which immunohistochemistry reliably demonstrated the intraductal carcinomas X, Y, Z as being oestrogen receptor positive. χ^2 values were as follows:

High oestrogen receptor expressing tumour:
Proportion demonstrating oestrogen receptor positivity using own threshold value: 98.0% (n = 176), χ^2=167.201.*
Proportion demonstrating 10% or more nuclei: 99.0% (n = 198), χ^2 = 192.080.
Proportion demonstrating 1% or more nuclei: 99.5% (n = 199), χ^2 = 196.020.

Medium oestrogen receptor expressing tumour:
Proportion demonstrating oestrogen receptor positivity using own threshold value: 80.0% (n = 178), χ^2 = 65.528.*
Proportion demonstrating 10% or more nuclei: 84.5% (n = 143), χ^2 = 95.220.
Proportion demonstrating 1% or more nuclei: 88.0% (n = 176), χ^2 = 115.520.

Low oestrogen receptor expressing tumour:
Proportion demonstrating oestrogen receptor positivity using own threshold value: 32.8% (n = 58), χ^2 = 21.023.Proportion demonstrating 10% or more nuclei: 37.3.0% (n = 73), χ^2 = 12.755.*
Proportion demonstrating 1% or more nuclei: 66.3% (n = 130), χ^2 = 20.898.

**Where no threshold value was given it was assumed that, regardless of the value used, (a) no nuclear staining will always represent an oestrogen receptor negative status; (b) staining awarded a Quick score of >4 will always represent an oestrogen receptor positive status. p Values refer to all three threshold values and are two tailed.*

workers.[6 8 21] Positive IHC assays using this cut off value has been associated with a large improvement in disease-free survival in patients receiving adjuvant tamoxifen (~30% at five years), with nearly one tenth of all oestrogen receptor positive patients investigated having only 1–10% of oestrogen receptor positive nuclei in their tumours.[21] Lastly, the oestrogen receptor status of the tumours was evaluated using the threshold values employed in the participants' own laboratories.

The overall analysis showed that while the majority of laboratories had little difficulty in demonstrating the tumours with high oestrogen receptor expression, a significant proportion (62.7%, p < 0.0001) failed to demonstrate 10% or more of the nuclei of the low expressor (fig 2). Interestingly there was a three way split in these results, with approximately one third of the assays staining no nuclei at all, one third staining some nuclei but less than 10%, and one third staining 10% or more (fig 3). Clearly with such wide interlaboratory variation in the assay sensitivity, a 10% threshold value used in one laboratory is unlikely to be applicable in another. The same would apply to the Quick score, with relatively large interquartile ranges of 0–3 for the low expressing carcinoma and 2–5 for the medium expressing carcinoma (fig 1). This interlaboratory variance is not caused by inconsistencies at the time of evaluation, as the level of agreement between individual assessors was good, as it was in a previous study,[13] but instead it was caused by variations in the sensitivity of the IHC method. Consequently the oestrogen receptor status (positive or negative) of these tumours and the predicted response to adjuvant tamoxifen treatment are considerably influenced by which laboratory has performed the assay.

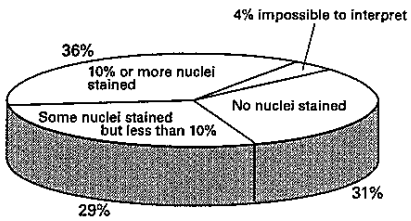

Figure 3 The proportions of 200 laboratories from which immunohistochemistry demonstrated either no nuclei, some nuclei but less than 10%, and 10% of more, in the "low" oestrogen receptor expressing infiltrating ductal carcinoma.

The choice of threshold value could compensate for the slightly differing levels of IHC sensitivity observed between laboratories. It has been recommended that threshold values should always be gauged against clinical outcome.[13] Consequently laboratories with different assay sensitivities could theoretically obtain the same result on the same tumour, as long as individual threshold values have been carefully adjusted to clinical outcome (assuming a similar proportion of patients respond to adjuvant tamoxifen treatment in different populations). In order to make allowance for this, the oestrogen receptor status of the tumours used in the present study was also established, using the participants' own threshold values. The fact that the interlaboratory variance persisted and if anything increased when the laboratories' chosen threshold values were used (fig 2) indicates that these would not compensate entirely for the differences in sensitivity observed between laboratories.

The positive oestrogen receptor status of the three tumours used in this study, as determined by the organising centre, is ratified by the results of the biochemical analyses. Furthermore the results of all six of the expert laboratories known to use clinically validated oestrogen receptor assays indicated that the high and medium expressing tumours were oestrogen receptor positive, and four of the six agreed that the low expressing tumour was positive, using either their own threshold value or a 10% cut off. Yet further support for the view that all the tumours were oestrogen receptor positive was obtained indirectly from the significant correlation between the Quick scores achieved on the medium expressing tumour and the proportion of nuclei stained on the low expressing tumour (table 6). Approximately 70% of laboratories who achieved higher than the median Quick score of 4 on the medium expressing tumour demonstrated ≥ 10% of nuclei in the low expressing tumour. In contrast only 18% of those scoring less than 4 on the medium expresser demonstrated ≥ 10% of nuclei in the

Table 7 The methods of evaluation for oestrogen receptors used by UK NEQAS-ICC participants

Threshold value	*Frequency (No of labs)*	*%*
10% or more of tumour nuclei demonstrated[15–19]	106	50.0
Histo (H) score[20 22 23]	17	8.1
20% or 25% and more of tumour nuclei demonstrated	13	6.1
5% or more of tumour nuclei demonstrated	10	4.7
Quick score[12 13 22]	6	2.8
1% or more of tumour nuclei demonstrated[6 8 21]	3	1.4
Category score[13 22]	2	0.9
50% or more of tumour nuclei demonstrated	2	0.9
Values known but each account for less than 0.9% of total	8	3.8
Unknown (information not provided by participant)	45	21.2
Total	212	100

low expresser. Consequently a Quick score of less than the median value on a relatively high oestrogen receptor expressing tumour correlates with < 10% of nuclei staining on the low expresser, while a Quick score greater than the median correlates with ≥ 10% of nuclei staining on the low expresser.

The significant positive correlation between the level of sensitivity achieved by the same laboratories on the different tumours (tables 3–6) indicates that less than optimum sensitivity on relatively high expressing tumours equates to poor and sometimes inadequate demonstration of very low expressers. This is because in the low expressing tumours the amount of oestrogen receptor present is much closer to the designated threshold value, and a slight fall in sensitivity can result in the number of nuclei demonstrated being below this value.

Interestingly, of all the threshold values investigated, the recently recommended 1% threshold value[6 8 21] would result in a significant number of laboratories recording all three categories of tumour used in the present study, including the low expressing intraductal carcinoma, as oestrogen receptor positive (fig 2). The reason for this is that the 1% threshold alone would make sufficient allowance for the observed interlaboratory variation in IHC sensitivity. However, it must be emphasised that a 1% threshold could result in detection of a higher proportion of oestrogen receptor positive unresponsive tumours from laboratories using a more sensitive method of detection. Hence, as emphasised by Barnes *et al*, a reasonable balance must be achieved between sensitivity and specificity in order to more accurately predict the proportion of patients likely to benefit from hormone treatment.[10 13]

Once improvement in interlaboratory consistency in carrying out the IHC assay has been achieved, it will be possible to address two outstanding questions: first the "accuracy" of the assay, and second the choice of cut off point. In the past, when the cytosol assay was used, there was always a small number of oestrogen receptor "negative" cases that responded to endocrine treatment. It is not clear whether these were genuinely negative or whether there was insufficient tumour in the sample used to prepare the cytosol. The advantage of IHC is that the presence of tumour can be confirmed by eye. Conversely there are also unresponsive oestrogen receptor positive cases. This may happen because the tumour burden is so great that treatment is ineffective or it could reflect the presence of oestrogen receptor in normal epithelial cells; again negative staining of tumour cells can now be checked visually.

The question of the cut off values remains a topic of much discussion. These may well differ according to whether the assay is to provide prognostic or predictive information. Much experience has been gained from the treatment of metastatic disease but less is available from the adjuvant setting. The increased use and improvements in quality of IHC will enable critical examination of relations between different cut off points and response. This in turn will lead to a consensus as to the "correct" values and make comparisons between studies easier.

CONCLUSIONS

In this study, we have investigated the ability of laboratories participating in the United Kingdom NEQAS-ICC for hormonal receptors to demonstrate positive staining in mammary carcinomas shown by experienced laboratories to have an oestrogen receptor positive status. The difficulties experienced by some laboratories in achieving this goal are highlighted and have since been communicated to the participants, with special emphasis on the false negative results. The reasons for the underachievement by some laboratories may lie in variations in the sensitivity of the overall staining technique. The sensitivity of the IHC assay is determined by several variables, which include the quality and concentration of the primary antibody used, the power of the antigen retrieval, and the secondary detection systems and quality of the fixation of the tissue. A superficial comparison of these variables among the assay systems used by different laboratories has failed to reveal any that are predominantly responsible for the differences observed. However, quality assurance is a continual process and the ongoing cycle of assessment runs, currently in progress for the oestrogen receptor IHC assay, may show that a combination of these factors is responsible for the observed interlaboratory variance. Better optimisation of such factors is needed to ensure that the results produced in one laboratory are comparable with those produced in another. This in turn may allow the chosen set of prognostic/therapeutic threshold values for selecting treatment for both primary and metastatic breast cancers to be safely applicable in the majority of laboratories offering the specialist oestrogen receptor IHC assay service.

We thank Elizabeth Anderson, Andre Balaton, Rudolf Baumann, and Rastko Golouh for providing us with invaluable assistance, and all the participants of UK NEQAS-ICC without whom this study would not have been possible.

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1: Breast Cancer. 2004;11(3):267-75. Related Articles, Links

Immunohistochemical assessment for estrogen receptor and progesterone receptor status in breast cancer: analysis for a cut-off point as the predictor for endocrine therapy.

Ogawa Y, Moriya T, Kato Y, Oguma M, Ikeda K, Takashima T, Nakata B, Ishikawa T, Hirakawa K.

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BACKGROUND: An immunohistochemical (IHC) method is commonly used for determining estrogen receptor (ER) and progesterone receptor (PR) status in breast cancer. However, the proper cut-off points of IHC have not been established. Cut-off points for ER and PR status as predictive factors for endocrine therapy are needed. METHODS: A total of 249 cases of female breast cancer were enrolled. ER and PR status by IHC were analyzed using the proportion of stained cells and staining intensity by Allred's score. RESULTS: Proportion score (PS) and intensity score (IS) were related to enzyme immunoassay (EIA) titers, for both in ER and PR ($p < 0.0001$, all). PS correlated with IS in both ER and PR ($R = 0.47$ and 0.41, respectively). ER status by IHC was related to tumor size and lymph node status, while PR was related to tumor size and menopausal status. In 152 patients who received endocrine therapy with a median follow-up term of 38 months, differences in disease-free survival were most significant using a cut-off point of PS 3 which indicated more than 10 % of cells stained positively for both ER and PR ($p = 0.0007$ and 0.0087, respectively). In addition, combination analysis of ER and PR using this cut-off point revealed a notable prognostic difference. CONCLUSION: A 10 % staining proportion may be an acceptable cut-off point for both ER and PR status by IHC, in terms of predicting response to endocrine therapy in breast cancer.

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1: Breast Cancer. 2003;10(2):97-104. Related Articles, Links

Significance of immunohistochemical assessment of steroid hormone receptor status for breast cancer patients.

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The assessment of steroid hormone receptors in resected breast cancer tissues is essential to decide whether endocrine therapy is indicated and to select the best treatment for each patient on the basis of receptor status. Both enzyme immunoassay (EIA) and immunohistochemistry (IHC) have been generally used as methods for examination of estrogen receptor (ER) and progesterone receptor (PgR). In some patients, receptor status cannot be examined for various reasons. A questionnaire survey in Japan clarified that ER status is not examined in approximately 40% of patients receiving breast conserving surgery. To eliminate "receptor unknown" cases, IHC examination on paraffin-embedded tissue is useful to assess the in situ receptor status. The concordance rate of ER and PgR status between EIA and IHC is very high and a study of 88 cases revealed a 97.7% concordance for ER and 92.0% for PgR at a cutoff point of 10%. The cutoff point of IHC is controversial and some studies demonstrated that patients showing 1% ER positive cancer cells would benefit from endocrine therapy. On the other hand, immunohistochemical expression of receptors is heterogeneous and some patients with ER negative invasive tumors have ER positive intraductal components. A study of 65 breast cancers demonstrated that ER positive intraductal components were detected in 3.1% cases of ER negative invasive lesions. According to these results and the recommendation of the St. Gallen International Conference, IHC is thought to be more useful than EIA in the assessment of steroid hormone receptor status for breast cancer patients.

Publication Types:
- Review
- Review, Tutorial

1: Anal Quant Cytol Histol. 2003 Apr;25(2):90-6.

Analysis of the reliability of manual and automated immunohistochemical staining procedures. A pilot study.

Biesterfeld S, Kraus HL, Reineke T, Muys L, Mihalcea AM, Rudlowski C.

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OBJECTIVE: To study the variation in the number of stained cells and staining intensity comparing 2 immunostainers and manual staining for estrogen receptor (ER) expression in breast carcinoma. STUDY DESIGN: In 5 cases, 15 consecutive paraffin sections were investigated after simultaneous immunohistochemical ER staining. The slides were evaluated using a CM-2 TV image analysis system (Hund, Wetzlar, Germany). One viewing field, identified around a histologic structure present on all 15 sections, was analyzed. The percentage of immunoreactive cells (PP), mean grey values of the immunopositive (GVpos.) and immunonegative nuclei (GVneg.), and immunohistochemical staining intensity (SI, defined as GVneg.-GVpos.) were calculated. RESULTS: The mean PP values were higher for immunostainers A (70.2%) and B (53.8%) than for manual staining (40.8%). The results were significantly different comparing the 2 immunostainers ($P = .0143$) or immunostainer A and manual staining ($P < .0001$). Also, the mean SI values were higher for immunostainers A (24.5 +/- 2.8% [CV]) and B (18.5 +/- 31.1%) than for manual staining (10.8 +/- 33.8%). These differences revealed statistical significance comparing the immunostainers with manual staining ($.0001 < P = .0048$). CONCLUSION: Our results underline the higher staining quality using immunostainers in comparison with manual staining.

Publication Types:
- Evaluation Studies

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☐ 1: Am J Clin Pathol. 1996 Sep;106(3):332-8.

MEMORIAL U. PRINT JOURNAL

Automated immunohistochemical assay for estrogen receptor status in breast cancer using monoclonal antibody CC4-5 on the Ventana ES.

Nichols GE, Frierson HF Jr, Boyd JC, Hanigan MH.

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Determination of breast cancer estrogen receptor (ER) status as a predictor of tumor response to adjuvant endocrine therapy remains a mainstay of breast cancer management. Recent second generation anti-ER antibodies and new epitope retrieval methods have produced paraffin-based immunohistochemical results that correlate closely with the dextran-coated charcoal (DCC) assay and appear to represent a superior method of ER assay. The authors determined the ER status of 103 invasive breast cancers by paraffin-based, automated immunohistochemistry on the Ventana ES using a new monoclonal antibody, CC4-5, and compared the results to those of parallel DCC biochemical analysis and manual immunohistochemical analysis using anti-ER monoclonal antibody ER1D5. The specificity of the CC4-5 antibody for ER protein was confirmed by Western blot analysis. Sixty of 103 cases were positive for ER by CC4-5 automated immunohistochemistry. With a ligand binding assay threshold value of 20 fmol/mg protein, there were 50 positive cases by biochemical assay. The biochemical results corresponded to an 88% rate of agreement with automated CC4-5 staining. Analysis of discordant cases revealed that the majority of CC4-5 immunopositive only cases (8 of 11) were strongly positive, stroma rich tumors, suggesting that corresponding biochemical measurements were diluted by non representative stromal tissue. There was only one immunonegative, biochemically positive case (27 fmol/mg protein). Semiquantitation of CC4-5 staining using percent positive tumor cells or weighted average staining intensity (HSCORE) showed moderate to good correlation with quantitative DCC results ($r = 0.64$ and 0.62, $P < .0001$). ER1D5 was not suitable for use on the Ventana ES, most likely due to temperature constraints of the instrument. By manual ER1D5 staining, 40 of 79 examined cases were positive corresponding to a 99% rate

of agreement with automated CC4-5 staining. Semiquantitation of ER1D5 staining by percent positive tumor cells and weighted average staining intensity (HSCORE) showed excellent correlation with semiquantitation of automated CC4-5 results ($r = 0.90$ and 0.88, $P < .0001$). Automated immunohistochemistry using the Ventana ES and monoclonal antibody CC4-5 is a reliable method for determining breast cancer ER status. As with other immunohistochemical methods, direct correlation with morphology precludes errors due to tissue sampling, allowing for accurate analysis of stroma-rich or partially necrotic tumors and small neoplasms that otherwise would yield insufficient tumor tissue for biochemical analysis.

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1: Am J Clin Pathol. 2005 Jan;123(1):21-7.

Comment in:

- Am J Clin Pathol. 2005 Jan;123(1):9-12.

Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers.

Nadji M, **Gomez-Fernandez C**, **Ganjei-Azar P**, **Morales AR**.

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Paraffin sections or fine-needle aspiration smears from 5,993 cases of invasive mammary carcinomas were assessed immunohistochemically for estrogen receptor (ER; 1D5) and progesterone receptor (PR; 636) expression. Staining pattern and intensity were correlated with histologic subtypes and nuclear grades of tumors. Positive nuclear staining for ER and PR was observed in 75% and 55% of invasive carcinomas, respectively. In 92% of ER+ cases, diffuse and uniform staining of most tumor cells was observed. In the remaining 8%, a focal ER reaction was seen, usually because of inadequate fixation. In 21% of PR+ tumors, the reaction was heterogeneous or focal but unrelated to fixation. There were no ER-, PR+ tumors. All pure tubular, colloid, and infiltrating lobular carcinomas were ER+. All medullary, apocrine, and metaplastic and most high-nuclear-grade carcinomas were ER-. With monoclonal antibody 1D5 and antigen retrieval, immunohistochemical reaction for ER in breast cancer usually is an all-or-none phenomenon; therefore, quantitation of results is unnecessary. Despite antigen retrieval, inadequate fixation can cause false-negative results; evaluation of internal positive control samples is imperative. ER positivity and negativity are predictable in certain histologic types and nuclear grades of breast cancer. The reaction for PR can be heterogeneous or focal.

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1: Am J Clin Pathol. 2005 Jan;123(1):9-12. Related Articles, Links

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- Am J Clin Pathol. 2005 Jan;123(1):16-20.
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Beneath the surface of the mud, part II: the dichotomization of continuous biologicvariables by maximizing immunohistochemical method sensitivity.

Swanson PE, Schmidt RA.

Publication Types:
- Comment
- Editorial

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1: Am J Clin Pathol. 2005 Jan;123(1):16-20. Related Articles, Links

Comment in:
- Am J Clin Pathol. 2005 Jan;123(1):9-12.

Bimodal frequency distribution of estrogen receptor immunohistochemical staining results in breast cancer: an analysis of 825 cases.

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Immunohistochemical analysis is used routinely to determine the estrogen receptor (ER) status of breast cancers in paraffin sections. However, lack of standardization has raised concerns that weakly ER+ tumors often are classified erroneously as ER-. To determine the frequency of weakly ER+ tumors, we reviewed ER immunostains of 825 breast cancers. For each case, we estimated the proportion of ER+ tumor cells and also determined an Allred score (which results in scores of 0 or 2 through 8, based on staining intensity and proportion of positive cells). In 817 cases (99.0%), tumor cells showed complete absence of staining or staining in 70% or more of the cells. Similarly, 818 cases (99.2%) exhibited Allred scores of 0 or of 7 or 8. Thus, with the immunohistochemical method used in our laboratory, ER staining is essentially bimodal. The overwhelming majority of breast cancers are either completely ER- or unambiguously ER+, and cases with weak ER immunostaining are rare.

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Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers.

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Department of Pathology, University of Miami-Jackson Memorial Hospital, FL 33136, USA.

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