Comparison of Anti–Estrogen Receptor Antibodies SP1, 6F11, and 1D5 in Breast Cancer

Lower 1D5 Sensitivity but Questionable Clinical Implications

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Abstract

We compared the anti–estrogen receptors (ER) SP1, 6F11, and 1D5 antibodies in breast carcinoma cases with different ranges of positive cells to evaluate whether this could generate different therapies for patients. We selected 66 cases of breast cancer, each of which was immunostained with the 3 antibodies. 1D5 was less sensitive than SP1 and 6F11, as seen in 26, 20, and 21 negative cases, respectively. Nine cases showed differences in endocrine-therapy indications, of which 8 1D5-negative cases showed low positivity for SP1 and/or 6F11. However, these cases were prevalently G3, progesterone receptor–negative or low-positive, with high Ki-67 and positive HER-2 findings, all biological features associated with endocrine resistance. Finally ER values obtained with these 3 antibodies had no implications for chemotherapy.

Estrogen receptors (ER) are powerful predictors of breast cancer response to endocrine therapy. In addition, chemotherapy is significantly more beneficial in patients with ER-negative than in ER-positive breast cancer.[1] Therefore it is of paramount importance to use reliable assays for determining ER status to ensure adequate therapy for patients. From the 1970 to the early 1990s, a ligand-binding assay (LBA) was used, which allowed ER levels to be quantitatively assessed. The availability of ER antibodies and the advent of heat-mediated antigen retrieval made it possible to assess ER with immunohistochemistry (IHC) on paraffin sections in a procedure that is less laborious and less expensive than LBA.[2] Compared with LBA, ER status determined with IHC showed a concordance rate of 70% to 90%; however, it has been found consistently to have similar or superior predictive and prognostic value.[3,4] ER status of breast cancer is currently assessed in routine diagnosis using IHC on formalin-fixed, paraffin-embedded tissue. Nevertheless, significant variability is seen in laboratories around the world[5-7] related to preanalytic variables, antigen retrieval techniques, use of controls, interpretation, and scoring practices. An important issue is the selection of the most reliable antibody. Because there is no gold standard, the American Society of Clinical Oncology (ASCO)–College of American Pathologists (CAP) guidelines recommend the use of antibodies employed in studies showing clinical benefit from endocrine therapy in ER-positive breast cancer patients. ER antibodies that have met these criteria are clones 1D5 (DAKO, Carpinteria, CA), 6F11 (Vector Laboratories, Burlingame, CA), SP1 (Lab Vision, Thermo Fisher Scientific, Kalamazoo, MI), and 1D5+ER.2.123 (DAKO).[3] It is important to note that studies comparing different antibodies consider discordant cases

showing ER staining values lower or higher than 1%, but they rarely specify how much these values deviate from this threshold.[8-15] For example, a tumor may show 0.5% positive cells with an antibody, becoming an ER-negative tumor, and 2% or 40% with other antibodies, becoming ER positive. However, these 2 different conditions may have important differences in clinical practice. For example, some authors indicate that tumors with ER expression of more than 50% do not show significant improvement with the addition of chemotherapy to endocrine-therapy.[16]

The aim of this study was to compare the clinically validated, most commonly used antibodies for the study of ER levels in breast carcinomas and to evaluate whether potential differences were significant for therapeutic decisions.

Materials and Methods

From January 2008 to December 2010, 362 consecutive infiltrating breast carcinomas were diagnosed at the Institute of Pathology of Sacro Cuore Hospital, Negrar, Italy. For study purposes, the tumors were subdivided into 4 categories based on the rate of ER expression assessed using the clone SP1 (Ventana Medical Systems, Tucson, AZ) and obtained from the pathology records: less than 1%, ER negative[3]; 1% to 9%, low ER positive; 10% to 50%, intermediate ER positive tumors[3,4]; and more than 50%,[16] highly ER positive. Patient age, tumor dimension, grade, vascular invasion, nodes status, and Ki-67 and HER-2 values were obtained from the histology reports as well. Tissue samples were handled according to the ASCO-CAP recommendations[3]: time from tissue acquisition to fixation was 20 to 30 minutes; samples were sliced at 5-mm intervals and placed in sufficient volume of 10% buffered formalin for 8 to 24 hours.

IHC

The most informative block from each tumor that should include normal breast tissue as internal control was selected for the IHC reactions using different anti-ER antibodies. Reactions with SP1 (Ventana) were repeated. The protocol used for each single antibody is reported in **Table 1**.

The presence of some ER-positive nuclei with heterogeneous staining pattern of luminal cells and the absence of immunoreactivity of myoepithelial, endothelial, and inflammatory cells served as positive and negative controls, respectively, for all reactions. For assessment of ER expression using the different antibodies, at least 10 high-power fields were randomly selected for each tumor. The percentage of positive tumor cells was recorded. Every case was assessed by 2 different pathologists (G.B. and L.B.) and, in case of significant discordance, revaluation at the multiheaded microscope was performed by 3 pathologists (G.B., L.B., and G.Z.). To evaluate whether the results obtained with each single antibody could modify the therapeutic approach, a dedicated breast oncologist (M.V.) reviewed the clinicopathologic reports of all cases and correlated them with the results obtained with each single antibody, proposing the more appropriate therapy derived from the IHC results.

Statistical Analysis

Data were recorded using Microsoft Excel software (Microsoft, Redmond, WA) and transferred to Stata/IC for Windows version 11.1 software (StataCorp, College Station, TX). The Wilcoxon matched-pair signed-rank test was used to compare the ER results found with different antibodies. A P value of less than .05 was considered significant.

Results

Based on the results of clone SP1 (Ventana) and using a bimodal classification, 51 cases (14%) were negative (<1% ER+) and 311 (86%) were positive (>1% ER+). The positive cases were subdivided as follows: 12 cases with low ER expression (1%-9% ER+); 14 cases showed intermediate ER positivity (10%-50% ER+); and finally 285 cases were highly positive with more than 50% ER+ tumor cells **Figure 1**. For the clinicopathologic correlation, all cases with low and intermediate ER expression were considered, and the first 20 cases were selected among the negative and highly expressing tumors, for a total of 66 cases **Table 2**.

Table 1
Protocol Used for Each Single Antibody

Antibody	Company	Clone	Dilution	Immunostainer	Antigen Retrieval	Incubation	Detection
Confirm ER Rmab	VMS	SP1	PD	BenchMarch XT, VMS	CC1 pH 8,2 x 30′	37°C x 20′	UltraView Universal Dab Detection
ER Mmab	DAKO	1D5	1:100	Bond MaX, Leica	ER2 pH 9.0 x 30′	RT x 15′	Bond Polymer Refine Detection
ER Mmab	Leica	6F11	1:80	Bond MaX, Leica	ER2 pH 9.0 x 30′	RT x 15′	Bond Polymer Refine Detection

ER Rmab, estrogen receptor rabbit monoclonal antibody; ER Mmab, estrogen receptor mouse monoclonal antibody; DAKO, Dako, Carpinteria, CA; Leica, Leica Biosystems, Newcastle, England; VMS, Ventana Medical Systems, Tucson, AZ

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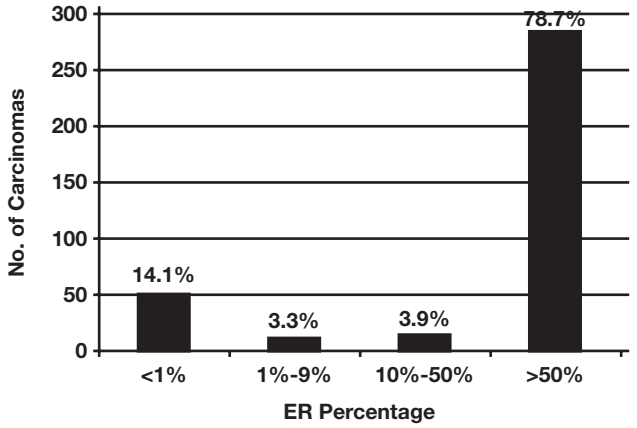

Figure 1 Estrogen receptor (ER) distribution of 362 breast carcinomas.

SP1 antibody reassessment showed no significant differences compared with the original reports. The distribution of ER staining values, obtained with the 3 antibodies, is shown in **Table 3**. Using a threshold of less than 1%, of these 66 tumors, 20 were negative with SP1, 21 with 6F11, and 26 with 1D5. The average percentage of stained cells was 36.6%, 33.6%, and 31.2% for SP1, 6F11, and 1D5, respectively. The Wilcoxon test showed that the percentage of cells stained was significantly greater with SP1 than 6F11 ($P = .002$) and 1D5 ($P < .0001$) and with 6F11 than 1D5 ($P = .0005$). The same results were obtained using semiquantitated data according to the Allred score (data not shown).

In 15 cases, the different percentage of ER-positive nuclei obtained with the 3 antibodies led to a shift in the IHC range **Table 4**. In 9 of these cases (cases 1-9, Table 4), the different ER value could influence the therapeutic management,

Table 2
Clinical and Pathologic Features of 66 Breast Carcinomas

Feature	Finding
Age, y	
Median	53
Range	32-93
Size, cm	
Mean	17
Range	0.5-8
Histologic type	
Ductal	57
Lobular	5
Others	4
Grading	
G1	10
G2	20
G3	36
Vascular invasions	
Absent	51
Present	15
Lymph node metastases	
Absent	39
Present	16
Unknown	11
PR status	
<1%	26
≥1%	40
HER2 status	
Positive	18
Negative	48

ER, estrogen receptor; PR, progesterone receptor.

Table 3
ER Values Obtained With the 3 Antibodies

	<1%	1%-9%	10%-50%	>50%
SP1	20	12	14	20
6F11	21	10	14	21
1D5	26	9	12	19

ER, estrogen receptor.

Table 4
Cases With ER Category Shift With the 3 Antibodies

								SP1			6F11			1D5		
Case No.	Age	Size, mm	Grading	ALM	PR	Ki-67	HER2	Value, %	CT	ET	Value, %	CT	ET	Value, %	CT	ET
1	80	40	3	4	0	40	+	0	No	No	2	No	Yes	5	No	Yes
2	71	30	3	0	15	60	–	0	Yes	No	5	Yes	Yes	0	Yes	No
3	41	50	2	0	2	30	–	2	Yes	Yes	0	Yes	No	0	Yes	No
4	57	12	3	0	0	30	+	5	Yes	Yes	5	Yes	Yes	0	Yes	No
5	80	20	3	0	0	30	–	5	No	Yes	0	No	No	0	No	No
6	38	7	3	0	0	60	–	5	Yes	Yes	5	Yes	Yes	0	Yes	No
7	54	18	3	0	1	35	+	8	Yes	Yes	2	Yes	Yes	0	Yes	No
8	81	80	3	2	0	30	+	8	No	Yes	0	No	No	0	No	No
9	42	20	3	14	5	70	–	8	Yes	Yes	8	Yes	Yes	0	Yes	No
10	47	NA	3	NA	30	25	+	8	Yes	Yes	20	Yes	Yes	8	Yes	Yes
11	43	10	2	0	40	15	–	8	Yes	Yes	15	Yes	Yes	8	Yes	Yes
12	74	35	2	0	0	15	–	20	Yes	Yes	15	Yes	Yes	5	Yes	Yes
13	48	25	2	NA	2	5	–	20	Yes	Yes	8	Yes	Yes	5	Yes	Yes
14	42	15	3	10	5	25	+	40	Yes	Yes	15	Yes	Yes	5	Yes	Yes
15	45	25	2	2	8	15	–	60	Yes	Yes	60	Yes	Yes	40	Yes	Yes

ALM, axillary lymph node metastases; CT, chemotherapy; ET, endocrine therapy; NA, not available; –, negative; +, positive.

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with differences seen in indications for endocrine therapy but not chemotherapy. In particular, 2 SP1-negative were 6F11- and/or 1D5-positive cases; 3 6F11-negative were SP1- and/or 1D5-positive cases; 8 1D5-negative were SP1- and/or 6F11-positive cases. All cases that would receive a therapeutic change were in the category of tumors with ER staining values between 0% and 9% **Image 1**.

Discussion

In this study, we showed that the ER expression obtained using SP1, 6F11, and 1D5 antibodies in breast carcinomas could modify the therapeutic approach for patients with breast cancer. Using the SP1 antibody in routine diagnoses, we could divide our case series into positive and negative cases using a bimodal distribution with a 1% cutoff as suggested in the literature.[17-19] However, studies on ER status using LBA[20] or polymerase chain reaction[21] revealed a broad range of ER values among ER-positive breast cancer. The amount of ER demonstrated with the IHC assay may be as much a function of preanalytic factors and assay sensitivity.[17] It may be possible that the dichotomization of a continuous variable can result in loss of clinical information.[22] Because the ER bimodal distribution results in a shift of cases with low or intermediate positive ER to higher levels,[17] the likely consequence is a reduction of chemotherapy indications.

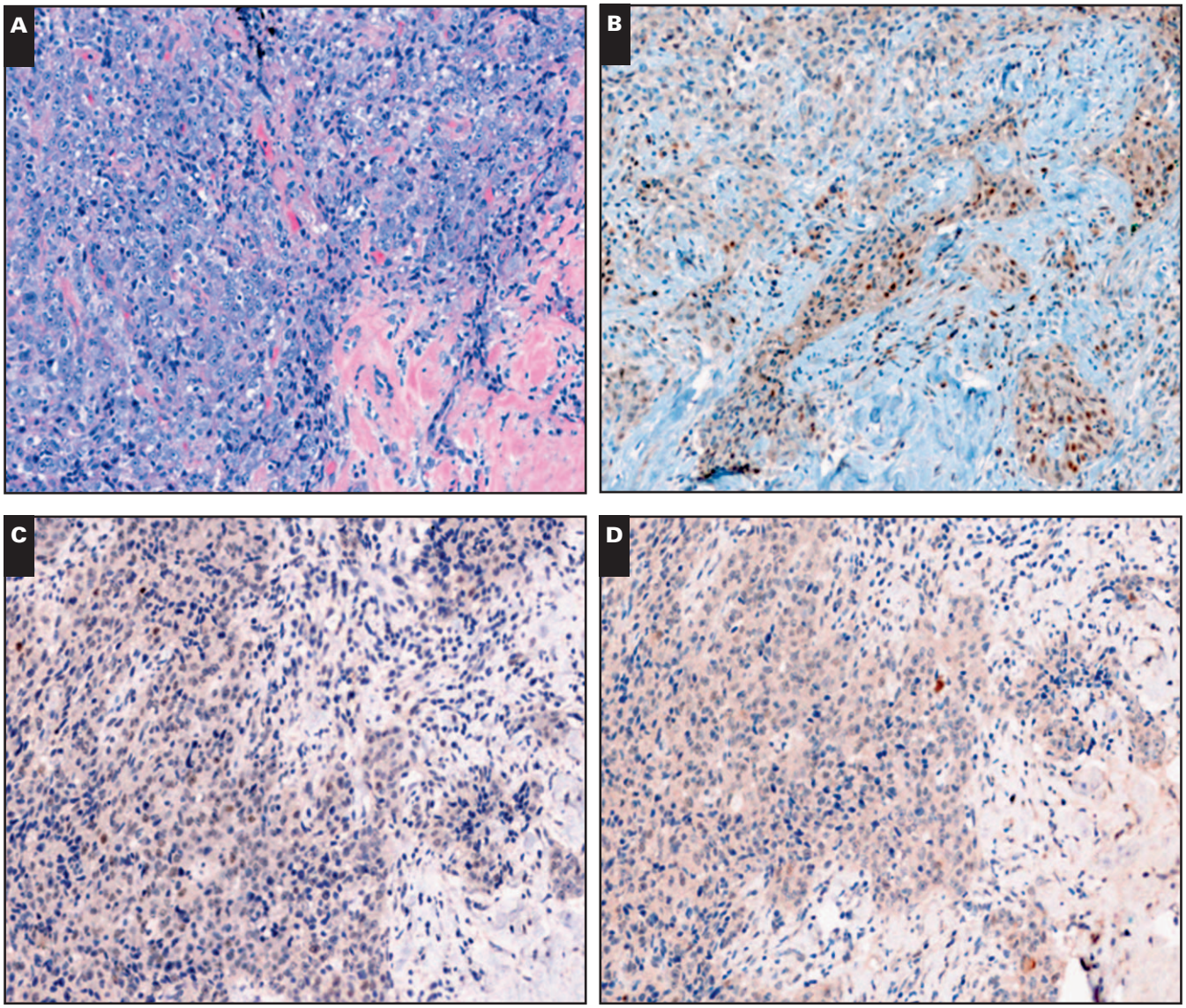

Image 1 Example of a ductal carcinoma (**A**, H&E) with different immunostaining expression for SP1 (8%; **B**), 6F11 (2%; **C**), and 1D5 (negative; **D**).

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The staining comparison of the 3 antibodies showed that 1D5 was less sensitive than SP1 and 6F11. In addition, the percentage of stained cells was significantly greater with SP1 than 6F11/1D5 and with 6F11 than 1D5. Studies have compared different anti-ER antibodies in breast carcinoma. Vassallo et al[15] (20 cases), Arihiro et al[8] (89 cases), and Kaplan et al[11] (592 cases) showed no significant differences in sensitivity between 1D5 and 6F11. In particular, Kaplan et al[11] showed a 97.5% concordance rate, even though 6F11 stained a significantly higher percentage of cells and more intensely. Cheang et al[10] demonstrated that SP1 is superior to 1D5 for identifying patients with good prognosis among those with ER-positive breast carcinomas. Although results of this study are in line with the results of Treaba et al,[14] the study has been criticized because it was conducted on tissue microarray specimens from frozen material left over after LBA; this artifact may differentially affect the epitope targeted by 1D5 rather than SP1. This study also had a larger-than-expected proportion of LBA-positive/IHC-negative tumors.[23] A subsequent study comparing SP1 and 1D5 immunostaining results in 508 formalin-fixed and paraffin-embedded breast cancers showed a 99.6% concordance rate, with only 2 discordant SP1-positive/1D5-negative cases. These 2 cases included poorly differentiated invasive carcinoma showing very rare and weakly positive nuclei with 1D5 (<1%) and 5% and 8% positive nuclei with SP1, respectively.[9] Few studies comparing 6F11 and SP1 showed no significant sensitivity differences between the 2 antibodies.[12,13] However, one of these studies showed that SP1 stained a significantly higher percentage of cells and more intensely.[13] Finally the United Kingdom National External Quality Assessment Scheme found that laboratories using 6F11 and SP1 had more satisfactory performances than those using 1D5.[24]

All the aforementioned studies analyzed the performances of anti-ER antibodies on nonselected cases, and none evaluated the effect on therapeutic management based on different staining values. For this reason, we focused our attention on selected cases with different ranges of ER positivity, using thresholds related to therapeutic indications. In 9 of our cases (Table 4, cases 1-9) the indication for endocrine therapy was influenced by the antibodies used for IHC staining because the tumor was considered negative with one (<1% ER+) and positive with another (1% to 9% ER+) antibody. These cases were prevalently G3, progesterone receptor–negative or low-positive, with high Ki-67 and positive HER-2. All these biological features are associated with endocrine-resistance[25-27] and suggest chemotherapy as a useful option.

The threshold used to define ER positivity is debatable. Although the ASCO-CAP panel recommended considering endocrine therapy in patients whose breast tumor shows at least 1% ER+ cells, they recognize that these recommendations will result in a slight increase in the application of endocrine therapy in some cases. They also recognize that it is reasonable for oncologists to discuss the pros and cons of endocrine therapy with patients whose tumor contains low levels of ER on IHC (1% to 10% weakly positive cells) and to make an informed decision based on the balance.[3] In a recent retrospective study on 1,257 patients with breast cancer who had ER staining of less than 10%, the authors concluded that the prognosis for tumors with low ER expression (1%-10%), especially ER staining of 1% to 5%, does not differ significantly from tumors with undetectable ER levels (<1%).[28]

In conclusion, this study demonstrated higher sensitivity of anti-ER SP1 and 6F11 clones compared with 1D5 clone. However, cases negative with anti-ER 1D5 showed low positivity (1% to 9%) with SP1 and 6F11 and were also related to other biological features associated with endocrine resistance. The ER values obtained with these 3 antibodies had no implications for chemotherapy.

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