Validation of EP1 Antibody Clone for Estrogen Receptor Immunohistochemistry for Breast Cancer

CAROLINE DIORIO1,2,4, DANIELA FURRER1,4, ANNICK MICHAUD1, SOPHIE LABERGE2,3,5, ION POPA2,3,5, SIMON JACOB2,3,5, LOUISE PROVENCHER1,2,6 and JEAN-CHARLES HOGUE2

1Oncology Unit, Quebec University Hospital Research Center – Laval University, Quebec City, QC, Canada; 2Deschênes-Fabia Breast Diseases Center, and 3Pathology Department, Saint-Sacrement Hospital, Quebec University Hospital Research Center – Laval University, Quebec City, QC, Canada; Departments of 4Preventive and Social Medicine, 5Molecular Biology, Medical Biochemistry, and Pathology and 6Surgery, Laval University, Quebec City, QC, Canada

Abstract. Background: SP1 Rabbit monoclonal antibody to estrogen receptor (ER) has long been the standard for determination of ER status in breast cancer but has been replaced by the rabbit EP1 clone. Aim: To validate the EP1 antibody clone for use in determination of breast cancer ER status in a large clinical population against the previous standard SP1. Materials and Methods: ER immunohistochemistry was assessed in 523 consecutive cases from a clinical setting using tissue microarrays. Results: The kappa statistic showed that the agreement of ER status between SP1 and EP1 was considered to be almost perfect (kappa=0.97, 95% confidence interval=0.94-1.00). Sensitivity was 99.3%, specificity was 98.6% and overall agreement was 99.2%. Conclusion: The EP1 antibody was herein validated regarding its use in breast cancer with almost perfect agreement with the previously used standard SP1 antibody.

Determination of estrogen receptor (ER) status is crucial for breast cancer treatment (1, 2). The first evidence for ER assessment as a prognostic marker was reported in 1973 (3). About 82% of invasive breast cancer cases are ER-positive (1) and binding of estrogens to ER activates specific estrogen-responsive elements that promote cancer growth (2). ER-positive tumors are responsive to endocrine therapy such as tamoxifen and aromatase inhibitors (4-10).

Recent literature has shown that rabbit monoclonal antibodies (such as SP1) are superior to mouse antibodies (such as 1D5 and 6F11) (11-14). One of the most widely used antibodies for clinical determination of ER status in breast cancer is the SP1 rabbit clone (15). A previous study by our group validated the new EP1 clone according to the validation process for a new antibody in ER status determination in breast cancer (16). A previous study suggested that EP1 could result in a better and easier interpretation of the ER status (17).

Therefore, the aim of the present short communication was to report the validation of the EP1 clone in a large population of patients with breast cancer with the SP1 clone as the reference.

Materials and Methods

Case selection. All cases used in the present validation were obtained from the St. Sacrement Hospital’s Pathology Service, which is a reference center for breast cancer in the Province of Quebec, Canada.

A total of 523 prospective consecutive cases were included. This study was approved by the Ethical Committee of the Quebec University Hospital - Laval University (#DR-002-1420). All women provided written informed consent.

Tissue microarray (TMA) construction and immunohistochemistry. TMAs were constructed as previously described (18). Breast cancer cell lines (MCF-7, MDA-231, and SKBR-3) were included in duplicate on each array block and served as positive (MCF-7) and negative (MDA-231 and SKBR-3) controls, respectively. One 4-μm-thick section from each TMA block was stained with hematoxylin and eosin for reference histology. On average, approximately three cores per patient were available for immunohistochemistry. The FLEX20 protocol was used for EP1 staining, and the FLEX30 protocol was used for SP1 staining (Dako, Burlington, ON, Canada), as previously described (16).

All slides were initially blindly assessed by one pathologist. Results were considered positive when 10-100% of the cells were stained; weakly positive when 1-9% of the cells were stained; and negative when fewer than 1% of the cells were stained (19, 20).

Correspondence to: Caroline Diorio, Oncology Unit, Quebec University Hospital Research Center – Laval University, 1050 chemin Ste-Foy, Quebec City, QC, G1S 4L8, Canada. Tel: +1 4186827511 Ext. 84726, e-mail: caroline.diorio@crchudequebec.ulaval.ca

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Discordant results between clones were blindly assessed by a second pathologist. Discordant results between the first two pathologists were assessed by a third pathologist and a consensus was reached between the three when necessary.

**Analysis of results.** Results were analyzed according to the method recommended by Fitzgibbons et al. (19) and by Hammond et al. [American Society of Clinical oncology (ASCO)/College of American Pathologists (CAP)] (20) for the validation of hormonal receptor status assays in breast cancer. Kappa statistics were used to compare EP1 and SP1. All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NY, USA).

**Results**

In this study, 523 consecutive breast cancer cases were assayed for ER status by immunohistochemistry using the SP1 and EP1 clones.

The kappa statistic showed that the agreement of the results of the ER status determined using SP1 and EP1 was considered to be almost perfect (kappa=0.97, 95% confidence interval=0.94-1.00) (Table I). Among the 76 samples which tested as ER-negative using EP1, 73 (96.1%) were also considered negative using SP1, and three were considered positive. Among the seven samples tested as weakly ER-positive using EP1, six were weakly positive using SP1 and one was positive. Finally, among the 440 samples tested as ER-positive using EP1, 429 (97.5%) were positive using SP1, 10 were weakly positive, and 1 was negative. Therefore, sensitivity was 99.3%, specificity was 98.6% and overall agreement was 99.2%.

**Discussion**

The aim of the present study was to validate the EP1 antibody clone against the previous standard SP1 for use in determination of ER status in breast cancer in a large clinical population. EP1 clone was assessed in 523 consecutive cases treated in a breast cancer unit. The kappa statistic showed a good agreement rate of the results of ER status between SP1 and EP1. Positive agreement was 99.3%, negative agreement was 98.6% and overall agreement was 99.2%. These agreement rates are above the recommended thresholds for ER testing validation of ≥90% for positive results and ≥95% for negative results (19).

The determination of the ER status is crucial to the management of breast cancer (1, 2). Changing the antibody used in a specific immunohistochemistry assay is considered a major change to the assay and requires a validation process. A validation process specific to ER was proposed by Fitzgibbons et al. in 2010 (19) and ensures that two different antibodies (with different sensitivity and specificity) will provide results that are similar (19, 20). It has been reported that many laboratories do not validate changes in immunohistochemical methods (21). Our laboratory is a reference center for breast cancer in the Province of Quebec, and is one of the centers with the largest case volume in Canada. We previously published our validation process for EP1 with SP1 as the reference standard (16) and the validation process recommended by ASCO/CAP (19, 20). The previous study showed a good concordance between EP1 and SP1 (16), which is confirmed in the present study using unselected consecutive samples encountered in our day-to-day practice.

In conclusion, the EP1 antibody has been validated for use in breast cancer with an agreement with the previously used antibodies against ER (cocktail of 1D5 and ER-2-123) (17). Furthermore, EP1 is a rabbit antibody, which has been shown to be better for ER immunohistochemistry compared to mouse antibodies (such as 1D5 and 6F11) (11-14).

This study might be limited by the small number of cases with weakly positive staining for ER. However, this was a study performed in consecutive unselected patients and no effort was made to select these patients. Nevertheless, our previous validation process showed a positive agreement of 53.8% (7/13 cases) among weakly positive cases compared to 60.0% (6/10 cases) in the present study.

In conclusion, the EP1 antibody has been validated for use in breast cancer with an agreement with the previously used SP1 antibody that is almost perfect.

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**Table I. Agreement between immunohistochemistry for the estrogen receptor status by EP1 (FLEX20) and SP1 (FLEX30) for a cohort of 523 consecutive patients with breast cancer.**

<table>
<thead>
<tr>
<th>EP1</th>
<th>SP1, n</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Weakly positive</td>
</tr>
<tr>
<td>Negative</td>
<td>73</td>
<td>0</td>
</tr>
<tr>
<td>Weakly positive</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Total, n (%)</td>
<td>74 (14.2)</td>
<td>16 (3.1)</td>
</tr>
<tr>
<td>Kappa (95% CI)</td>
<td>0.97 (0.94-1.00)</td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>99.3%</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>98.6%</td>
<td></td>
</tr>
<tr>
<td>Overall agreement</td>
<td>99.2%</td>
<td></td>
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</tbody>
</table>

95% CI: 95% Confidence interval.
Conflicts of Interests

All Authors declare that they have no conflict of interests.

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References


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