Tissue Microarray for Routine Clinical Breast Biomarker Analysis

The British Columbia Cancer Agency 2008 Experience

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Abstract

Clinical use of tissue microarrays for immunohistochemical analysis of breast biomarkers, namely estrogen receptor, progesterone receptor, and HER2, was instituted in our laboratory in 2008. The method has proved reliable and cost-effective. We report the results of the initial year of testing with this method.

In 2007, a validation study undertaken in our laboratory to assess tissue microarray (TMA) as an alternative to whole-section immunohistochemical testing for breast biomarkers indicated a high level of concordance between the 2 methods. In 2008, based on these results, we initiated routine TMA testing of a breast biomarker panel consisting of estrogen receptor (ER), progesterone receptor (PR), and HER2 for suitable cases. The method and results for this initial year are reported.

Materials and Methods

Cases submitted for a breast biomarker panel had an H&E stain and 4 unstained slides prepared. Histology staff then assessed the suitability of each case for TMA. Core biopsy cases and cases with minimal tissue (small biopsy specimens) immediately underwent routine whole-section immunohistochemical analysis. Cases with apparent adequate tissue were sent to a pathologist who assessed the H&E-stained sections for TMA suitability using set guidelines. Cases with sufficient tumor to allow removal of three 0.6-mm cores and still leave diagnostic tissue were acceptable for TMA and had core locations marked.

Histology staff constructed a TMA weekly from accumulated suitable breast biopsy blocks. TMA sections were stained for H&E, ER, PR, and HER2. The reporting pathologist received the stained TMA sections, the original H&E-stained sections of the tumors for each case, the test requisitions, and pathology reports. The pathologist followed a set reporting protocol that included verification of case demographics and a morphologic cross-check,
comparing the morphologic features of the tumors in the original H&E-stained sections with the morphologic features of tumors in the TMA cores. Each antibody-stained array slide also contained a set of control cores that were examined.

Each array core stain was individually interpreted using the maximum stain intensity in the core on a scale of 0 to 3. For ER and PR, the highest stain intensity in any of 3 cores was reported. For ER, a negative stain (0) generated a “reflex” repeated ER stain on one of the previously cut unstained whole sections. This stain was interpreted before reporting the case. The reporting protocol established rules for reporting HER2 (Table 2). All equivocal HER2 cases had HER2 by fluorescence in situ hybridization (FISH) performed on a previously cut whole section.

Results

TMA Performance

In 2008, the laboratory constructed 48 TMAs containing 3 cores per case from a total of 526 breast cancer cases submitted from 18 hospitals. The average number of cases per TMA was 11 (range, 4-24).

Noninformative Cases

Of the 1,578 core sections in the arrays, 21 (1.3%) were missing, 5 (0.3%) were not interpretable, and 18 (1.1%) had no tumor.

For HER2, there were 32 cases (6.1%) with 1 core unsatisfactory, 7 cases (1.3%) with 2 cores unsatisfactory, and 3 cases (0.6%) with 3 cores unsatisfactory. This resulted in 10 cases (the cases with 2 or 3 unsatisfactory cores) that needed to be repeated on whole sections. Because 2+ cores automatically made a case equivocal, requiring FISH, repeated immunohistochemical staining on a whole section was not needed for some cases that had 1 or more unsatisfactory cores.

ER Results

There were 445 cases positive (84.6%) for ER and 81 cases negative (15.4%). All 81 negative cases were repeated on whole sections. In this repeated testing, 15 cases (19%) were positive (1 case was scored Allred 3; the remainder, having only rare, weakly positive nuclei, were scored as Allred 2).

HER2 Results

There were 342 (65.4%) of 523 reportable cases (3 cases had 3 unsatisfactory cores) that were immunohistochemically

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Pathologist Guidelines for Marking Core Biopsy Specimens</th>
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<tr>
<td>1. If ability to select 3 good tissue microarray (TMA) cores is in doubt, do not use TMA. Send for whole-section (WS) analysis. Mark the H&amp;E-stained slide as “WS,” and note the reason, eg, not sufficient quantity (NSQ), complex (mixed ductal carcinoma in situ [DCIS] invasive; more than one tumor phenotype.</td>
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<tr>
<td>2. Mark foci with invasive tumor only, taking care not to sample DCIS.</td>
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<tr>
<td>3. An adequate tumor block allows removal of 3 TMA cores (each 0.6 mm in diameter) and leaves residual diagnostic tissue. Usually 1 low-power field of tumor (~8 mm diameter) is sufficient.</td>
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<tr>
<td>4. Mark the TMA core locations with a “dot;” usually place 2 dots near the tumor edge and 1 in the tumor center.</td>
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| Image 1 | Breast cancer section of about 8 mm² (1 low-power field). A, Section marked with dots to indicate location for technologist to remove cores for tissue microarray (TMA) (H&E, ×100). B, Section appearance after three 0.6-mm TMA cores have been removed from the paraffin block. Adequate residual diagnostic tissue is present (H&E, ×100). |
negative. HER2 FISH data on 73 of the immunohistochemically negative cases were available from an unrelated internal study: 1 case was amplified, 1 was equivocal/amplified (HER2/CEP17 ratio, 2.0-2.2), and 1 was equivocal/not amplified (HER2/CEP17 ratio, 1.8 to <2.0) Table 4.

There were 126 (24.1%) of 523 cases that were immunohistochemically equivocal. All had HER2 FISH analysis on a whole section: 106 (84.1%) were not amplified, 9 (7.1%) were amplified, 8 (6.3%) were equivocal/amplified, and 3 (2.4%) were equivocal/not amplified.

Of 522 cases, there were 55 (10.5%) that were immunohistochemically positive. Of 13 with FISH data available, 12 were amplified and 1 was equivocal/amplified.

Immunohistochemical analysis of HER2 on TMA compared with HER2 studies by FISH on whole sections Table 5 revealed 98.8% concordance for nonamplified vs amplified if defined as the recommended HER2/CEP17 ratio of less than 1.8 vs greater than 2.22 and a 97.7% concordance if defined as an HER2/CEP17 ratio of less than 2.0 vs greater than 2.0 Table 6.

Estimated Costs

TMA Costs

In our laboratory in 2008 Canadian dollars, the cost to build, analyze, and report an 18-case TMA was $255. The 48 TMAs therefore cost $12,240 (48 × $255). The repeated whole-section stains for ER (81 × ~$30 = $2,430) and HER2 (9 × $30 = $270) brought the total cost to almost $15,000.

Immunohistochemical Whole Section Costs

In our laboratory in 2008, the cost of a breast cancer biomarker panel of 3 antibodies tested on whole sections

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**Image 2** Stained tissue microarray (TMA) sections. **A**, Section of 0.6-mm cores from 15 cases, 3 cores per case. Slide orientation is provided by limiting the first row to 3 cases and inserting cores of muscle tissue at the end of the first row and below the first column (H&E, ×20). **B**, Estrogen receptor stain using SP1 antibody on a Ventana Benchmark stainer (×20). **C**, HER2 stain using 4B5 antibody on a Ventana Benchmark stainer (×20). The image is truncated. Control sections and slide labels are not shown.
using a Ventana staining system (Ventana, Tucson, AZ) was $95. The 526 cases that were tested by TMA would have cost almost $50,000 (526 × $95 = $49,970) had they been analyzed using whole sections. Therefore, the estimated saving using TMA for analysis was about $35,000 ($50,000 – $15,000 = $35,000).

**Discussion**

This article reports the initial year of operational experience with TMA in our clinical laboratory following a reported validation study of the method. The British Columbia Cancer Agency central reference laboratory performs, on average, about 200 breast biomarker tests per month on tissue blocks

![Image](image1.png)

**Table 2**

Pathologist Tissue Microarray (TMA) Scoring Protocol

1. Verify patient and specimen identification with the original requisition and pathology report.
2. Compare morphologic features of the tumor in the H&E-stained whole section slides with the tumor morphologic features in the TMA cores of the cases as a check for block sorting error.
3. Interpret each core for the maximum intensity of any invasive tumor cells.
4. Report estrogen receptor (ER) and progesterone receptor (PR) as positive or negative, and give the maximum intensity (0-3).
   - An Allred score of 2 (any stained nucleus) or greater is considered positive. (Revised to Allred score of 3; see text.)
5. IF ER is negative, repeat ER testing on a whole section before issuing a report.
6. Report HER2 as negative (no stain or 1+), positive (3 cores 3+), or equivocal (any 2+ or 3+ in <3 cores).
7. If the HER2 result is equivocal, order HER2 by fluorescence in situ hybridization (FISH) on a whole section. Add a comment to the report: "HER2 by FISH has been requested and will be reported separately."

* The reporting pathologist receives the TMA sections, the original dotted H&E-stained sections, a copy of the pathology report, and the original test requisition for each case.
from cases from our institution and from community hospitals in British Columbia.

Correct identification of patient tumor in the TMA block is critical. To ensure this, the clerical, technical, and pathology staff involved is limited to personnel trained to follow a specified operating procedure. When the array is reported, the pathologist is given the H&E-stained sections that were marked for core removal to compare the tumor morphologic features with the morphologic features of the H&E-stained tumor in the TMA cores (Images 1A and 2A). The test requisition and a copy of the original pathology report are provided for each case in the array. In our laboratory, a clerk enters the data from each core into a data sheet, allowing the pathologist to devote full attention to the interpretation of the array without the distraction of recording. The clerk is also trained to ensure that the pathologist “follows the rules” and indicates on the data sheet that quality assurance activities have been done. The clerk generates a report sheet that contains patient demographics, specimen numbers and source, the results for each core in the array, and a note if additional tests are required (repeated ER or FISH). A copy of this report sheet is given to the reporting pathologist, the transcriptionist typing the final report, and the histology laboratory as a cross-check that final whole-section analyses on selected cases are ordered. To date, no errors have occurred with the protocol outlined.

Sampling error is a potential issue. The validation study revealed very high concordance with results from whole section and TMA. Many studies have noted high concordance between limited samples and larger samples of tumor.3,4 Nevertheless, potential tumor heterogeneity must be considered by the pathologist in all phases of sampling from amount of tumor submitted for microscopic examination, which blocks are chosen for biomarker analysis, and morphologic variability in the sections. Our protocol tries to minimize the risk of tumor heterogeneity by careful morphologic assessment of the original H&E-stained sections. Cases defined as “complex” are specifically excluded from the TMA. These include cases with phenotypic heterogeneity and those that have a close mix of invasive and in situ carcinoma. The use of 3 cores ensures reasonable sampling of the tissue in the block.

Interpretation of the cores for maximal stain intensity is generally simple and quick. In the validation study,1 it was found that the maximal intensity seen in the TMA correlated best with the result obtained using the matched whole section. The temptation to average the intensity score must be avoided, particularly for HER2. Nonspecific marginal staining of cores (“edge effect”) is not seen in our laboratory (Image 3). Although the cause for edge effect is not well understood, this observation may be informative about the cause. We can conceive of 2 explanations. Edge effect may relate to the influence of fixation

### Table 3
ER Results for Tissue Microarrays 1 Through 48 (526 Cases)*

<table>
<thead>
<tr>
<th>ER Result</th>
<th>Total</th>
<th>Whole Section Negative</th>
<th>Whole Section Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive†</td>
<td>445 (84.6)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Negative</td>
<td>81 (15.4)</td>
<td>66 (81)</td>
<td>15 (19)</td>
</tr>
</tbody>
</table>

ER, estrogen receptor.
* Data are given as number (percentage).
† Allred score of 2 or greater.

### Table 4
HER2 Results for 212 Cases With Both Immunohistochemical and Fluorescence In Situ Hybridization† Testing†

<table>
<thead>
<tr>
<th>HER2 Immunohistochemical Results</th>
<th>No. of Cases</th>
<th>Amplified</th>
<th>Not Amplified</th>
<th>Equivocal/Amplified</th>
<th>Equivocal/Not Amplified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>73</td>
<td>1 (1)</td>
<td>70 (96)</td>
<td>1 (11)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Equivocal</td>
<td>126</td>
<td>9 (7.1)</td>
<td>106 (84.1)</td>
<td>8 (6.3)</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>12 (92)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* HER2/CEP17 ratio: amplified, >2.2; not amplified, <1.8; equivocal/amplified 2.0-2.2; equivocal/not amplified, 1.8 to <2.0.
† Data are given as number (percentage) unless otherwise indicated.

### Table 5
HER2 Immunohistochemical Analysis on Tissue Microarray vs FISH Results on Whole Sections

<table>
<thead>
<tr>
<th>HER2 by Immunohistochemical Analysis</th>
<th>HER2/CEP17 Ratio &lt;1.8 vs &gt;2.2</th>
<th>HER2/CEP17 Ratio &lt;2.0 vs &gt;2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amplified</td>
<td>Not Amplified</td>
</tr>
<tr>
<td>Positive</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>70</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>70</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization.
of tissue being more intense at the edge of the specimen and is, therefore, not seen in cores removed from the tumor deep within the paraffin blocks. Second, edge effect possibly relates to drying of the reagents at the periphery of the section during the staining process, possibly associated with surface tension in the fluid medium. It is conceivable that there is less drying associated with smaller pieces of tissue in the section.

TMA performance was better than expected from our validation study, with fewer noninformative (missing or no tumor) cores. By comparing the validation study with the present study, we found there were fewer missing cores (1.7% vs 1.3%) and fewer with no tumor (7.1% vs 1.1%) in the present study. Comparison of HER2 analysis on the validation study vs the present study again revealed fewer noninformative cores in the present study (16% vs 6.1%). This was also true for cases with 2 or more noninformative cores in the present study (8.1% vs 1.3%). The improved TMA performance probably reflects greater experience by pathologists choosing core sites and improved accuracy of technical staff in removing cores.

The results of HER2 immunohistochemical testing on TMA were highly concordant with those of HER2 testing by FISH on whole sections. There was a 98.8% concordance for amplified vs nonamplified with the current recommended criteria of an HER2/CEP17 ratio of less than 1.8 and more than 2.2.2

In the period of this study, the laboratory had a very low threshold for positive interpretation of ER and PR stains, and any nuclear staining was considered a positive result. For this reason, routine repeated immunohistochemical analysis on whole sections for TMA ER-negative cases was performed: 15 (19%) of 81 TMA ER-negative cases were found to have nuclear staining for ER on a whole section. The majority of TMA ER false-negative tests were scored as Allred 2 (weak stain, <1%) on repeated whole-section stain.5 Recently, following guidelines recommended by the College of American Pathologists,5 we have changed our threshold for positivity from a score of Allred 2 to Allred 3 because this is regarded as a more clinically relevant positive threshold. False-negative cases with this higher threshold would likely be less common, although given the importance of avoiding false-negatives, repeated testing of TMA ER-negative cases will continue.

A drawback to implementing TMA is increased turnaround time. In our laboratory, results can be delayed up to 9 days. Probably in many laboratories this would not be acceptable, but to date, this has not been a problem for our clinicians. If a case is “urgent,” it is immediately processed as a routine whole section and reported in less than 48 hours.

Cost control is a problem for all laboratories. Because savings by TMA are directly related to test volume, low-volume laboratories would likely not find this method useful.

Breast needle core biopsies represent a substantial portion of biomarker cases. We have successfully constructed TMAs from breast needle core biopsy specimens. However, we decided not to include them in our TMA cases because we found that only a small proportion of needle core biopsy samples have sufficient tumor to allow removal of 3 TMA cores and still leave diagnostic tumor tissue in the original needle cores. Analyzing these few suitable needle core biopsy specimens by TMA was not considered cost-effective.

Our initial year of experience has confirmed that, with careful application, the TMA method of analysis is cost-effective and accurate for routine breast biomarker analysis in the clinical laboratory.

References