Tissue Microarray Technique in Evaluation of Proliferative Activity in Invasive Ductal Breast Cancer

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Abstract. Aim: Investigation of the relationship between expression of Ki-67 antigen and minichromosome maintenance 2 (MCM-2) protein by using an immunohistochemical study on whole sections (WS) and sections of invasive ductal breast cancer (IDC) obtained in the tissue microarray (TMA) technique. Materials and Methods: Material consisted of 51 archival paraffin blocks of IDC. Tissue microarrays were composed of 0.6 mm core punches. Reactions were performed using specific antibodies (anti-Ki-67 and anti-MCM-2). Intensity of the marker expression was evaluated using computer-assisted image analysis. For statistical purposes, three different tests were used. Results: Spearman rank correlation revealed a strong positive correlation between expression of tested markers: Ki-67 (TMA) vs. Ki-67 (WS) (r=0.91, p<0.05) and MCM-2 (TMA) vs. MCM-2 (WS) (r=0.87, p<0.05). Mann Whitney U-test showed no significant differences between the two markers in both analysed techniques. Moreover, the Bland–Altman plot demonstrated a low level of bias between the analysed methods. Conclusion: This study shows that TMA could have a great potential in evaluation of proliferative activity in IDC and that MCM-2 protein might be a specific and sensitive marker of cell proliferation.

Tissue microarray (TMA) technology was described by Wan et al. in 1987 (1). They published a modification of an idea of H. Battifora in 1986 of a so–called “multitumor (sausage) tissue block” (2). In 1998, J. Kononen and collaborators developed a device that was able to rapidly and reproducibly produce TMAs (3).

Key Words: Tissue microarray, TMA, breast cancer, Ki-67, MCM-2.
single cell division (20, 21). In view of the presented facts, expression of MCM family proteins, including MCM-2, may be the most frequently applicable marker for clinical and pathological purposes in the future, especially for tracking rapidly dividing cells (22-28).

Most studies assay biomarkers on TMAs using immunohistochemical (IHC) techniques, but to our knowledge we are the first to test for reproducibility and accuracy of proliferation marker assessment on TMAs as compared to traditional assessment in whole tissue sections.

Materials and Methods

Patient samples. The studies were performed on 51 archival paraffin blocks containing fragments of invasive ductal breast cancer (IDC), sampled in 2000 during procedures of mammectomy in the Lower Silesian Oncology Centre in Wroclaw. All the patients were female and the grade of malignancy (G) was G1 in 10 cases, G2 in 30 cases and G3 in 11 cases.

TMA construction and tissue samples. Tissue samples were fixed in 10% buffered formalin. Subsequently they were dehydrated and embedded in paraffin blocks. For the construction of the TMA blocks, a new section was made from paraffin donor block and stained with hematoxylin-eosin (HE). Two additional sections were cut for performing IHC on whole tissue sections. The HE sections were examined by two independent pathologists (CK, BP) under light microscopy (BX-42; Olympus, Tokyo, Japan) and areas of interest were circled using a permanent marker. From the corresponding paraffin blocks, three 0.6 mm core punches were taken for each case using a Manual Tissue Arrayer I (Beecher Instruments Inc, Sun Prairie, Wisconsin, USA) and transferred into the recipient paraffin block.

IHC. Immunohistochemical reactions were performed on paraffin sections (4 μm) mounted on Superfrost Plus slides (Menzel Gläser, Braunschweig, Germany) cut from whole tissue and TMA paraffin blocks. Deparaffinization and antigen retrieval were performed in Target Retrieval Solution, pH 6 (97°C, 20 min) and PT Link Rinse Station. The sections were then washed in Tris-buffered saline (TBS) and incubated with primary antibodies: anti-Ki-67 (MIB-1, 1:100; Dako Cytomation, Glostrup, Denmark) and anti-MCM-2 (CRCT2.1, 1:50; Novocastra Laboratories, Newcastle, UK) in a Link48 Autostainer (room temperature, 20 min). EnVision FLEX was used for the visualization of the antigens, in accordance with the manufacturer’s instructions. All slides were counterstained with Mayer’s haematoxylin. All the antibodies, reagents and equipment except for the anti-MCM2 antibody were obtained from Dako Cytomation.

Evaluation of the intensity of IHC reaction. For the evaluation of Ki-67 and MCM-2 in each paraffin section and TMA core three fields with the highest number of tumour cells yielding a positive reaction were selected (hot spots). The percentage of positive cells in each hot spot was evaluated under ×400 magnification, scoring the brown-labeled cell nuclei of cancer cells (BX-42 light microscope equipped with Cell√D software for computer-assisted image analysis; Tokyo, Japan). The general result for every sample was an average of the three hot spot percentages. The intensity of the IHC reactions in coded preparations were independently evaluated by two pathologists (CK, BP). Moreover in doubtful cases, a re-evaluation with a double-headed microscope was performed until a consensus was achieved.

Statistical analysis. The results were subjected to statistical analysis using Statistica 7.1 PL and Prism 5.0 (Statsoft, Krakow, Poland and GraphPad, La Jolla, CA, US, respectively). The correlation between the expression of both markers and grade of malignancy was examined using Spearman’s rank correlation test. Mann–Whitney U-test and Bland-Altman test were used to compare the results from whole tissue sections and TMAs (29, 30). In all analyses, results were considered to be statistically significant when p<0.05.

Results

The majority of the examined tumours manifested nuclear expression of Ki-67 antigen and MCM-2 protein (Figure 1). Statistical analysis of the relationship between expression of the same marker on whole sections (WS) and TMA demonstrated a strong positive correlation for Ki-67 antigen: Ki-67 (TMA) vs. Ki-67 (WS) (r=0.91, p<0.05) and for MCM-2 protein: MCM-2 (TMA) vs. MCM-2 (WS) (r=0.87, p<0.05). Statistical analysis also revealed positive, significant correlations of both analysed antigens in whole tissue sections, as well as in TMAs, with the grade of malignancy. These results are summarized in Table I. Mann Whitney U-test showed no significant differences between the percentage of positive cells in whole tissue sections and TMAs regarding Ki-67 and MCM-2 expression (Figure 2A and B). Similar results were noted for both antigens regarding the grade of malignancy, when scores for each antigen were analysed within the same grade (Figure 2C and D). Bland–Altman test revealed a low level of bias between analysed tissue specimens for both Ki-67 antigen and MCM-2. Standard deviation of bias for Ki-67 (WS) vs. Ki-67 (TMA) was 9.52%, whereas for MCM-2 (WS) vs. MCM-2 (TMA) it was 11.67%.

Table I. Correlation between investigated proliferation markers (Ki-67 and minichromosome maintenance 2 (MCM-2) protein) assessed in whole tissue sections (WS) and tissue microarrays (TMA).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Ki-67 (WS)</th>
<th>Ki-67 (TMA)</th>
<th>MCM-2 (WS)</th>
<th>MCM-2 (TMA)</th>
<th>Tumour grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki-67 (WS)</td>
<td>X</td>
<td>0.91</td>
<td>0.60</td>
<td>0.59</td>
<td>0.51</td>
</tr>
<tr>
<td>Ki-67 (TMA)</td>
<td>X</td>
<td>0.65</td>
<td>0.65</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>MCM-2 (WS)</td>
<td>X</td>
<td>0.87</td>
<td>0.38</td>
<td>0.38</td>
<td>0.38</td>
</tr>
<tr>
<td>MCM-2 (TMA)</td>
<td>X</td>
<td>0.44</td>
<td>0.44</td>
<td>0.44</td>
<td>0.44</td>
</tr>
<tr>
<td>Tumour grade</td>
<td>X</td>
<td></td>
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**Discussion**

Development of hardware for TMA preparation, is essential, since the interest in this diagnostic and research tool has increased and has recently become very popular. TMAs in cancer research give the opportunity to analyse the frequency of a molecular alteration in different tumour types, evaluate prognostic markers, test potential diagnostic markers and optimize antibody staining conditions. Research teams continue to investigate the advantages, disadvantages and possibilities of using this method. Camp et al. reviewed the literature describing the decade of development of TMA (31).

Depending on the spectrum of scientific research, different tissues are examined using TMA. Most research in this field was conducted to compare the expression of specific markers on whole sections and TMA. In our study, we analysed the expression of Ki-67 antigen and MCM-2 protein in a group of IDC cases. We examined the correlation between these proliferation markers in whole tissue sections and in TMAs. Spearman correlation showed a strong positive correlation between these proteins in whole tissue sections and as well as in TMAs. Additionally, the positive correlations of both Ki-67 and MCM-2 expression with the grade of malignancy were observed. In our previous studies, we have shown that Ki-67 strongly correlates with MCM-2 expression in breast cancer on classical whole tissue sections (28). This study confirms our previous observations.

An important aspect of the TMA is also the diameter of the punch. A well-known fact is that pathologists frequently feel more confident of the results if larger core size is used. There is no evidence in the literature showing that large cores are better nor worse than small cores for assessment of TMAs. Anagnostou et al. conducted experiments with different sizes of cores and found that TMAs with 0.6 mm cores are as representative as those with any common larger sized core (34). Karlsson et al. conducted comparative studies of 0.6 and 1 mm cores on breast, lung and endometrial cancer tissue and found that both core sizes were equally informative regarding Ki-67 expression (35).

Studies were also conducted on TMA testing specimens obtained from regular needle biopsy. In their work, Vogel and Büllmann suggested that biopsy material may be used for TMA construction without proper equipment in order to reduce costs and could be considered as an optimal solution in pathological practice (36). Moreover, Munirah et al. stated that TMA may provide easy access to similar samples examined by the

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**Figure 1.** Expression of Ki-67 antigen (A) and minichromosome maintenance 2 (MCM-2) protein (B) in TMA cores of invasive ductal breast cancer.
pathologist and therefore improving the repeatability of the results (37). Furthermore, comparison of TMA with whole tissue sections may be proposed as an internal laboratory control for improving the credibility of this method.

In conclusion, through our study we have shown that TMA is a reliable technique for examining a large set of tumours. We validated this technique in breast cancer specimens by comparing the IHC staining results obtained for proliferation markers Ki-67 and MCM-2 in TMA with those from classical whole tissue sections. As no significant differences were observed between the two methods, we suggest that TMA may be useful for a wide spectrum of histological examination of various tissue preparations and IHC markers.

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References


34 Anagnostou VK, Lowery FJ, Syrigos KN, Cagle PT and Rimm DL: Quantitative evaluation of protein expression as a function of tissue microarray core diameter: is a large (1.5 mm) core better than a small (0.6 mm) core? Arch Pathol Lab Med 134: 613-619, 2010.

