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LETTER TO THE EDITOR

# Tissue Microarray (TMA) Versus Whole Section Immunohistochemistry in the Assessment of ER/PR and Her-2/neu Status in a Breast Cancer Series from Sudan

To the Editor:

Tissue microarray immunohistochemistry (TMA-IHC) allows cost reduction relative to standard IHC, which could be important for breast cancer (BC) pathology in developing countries (1–3). However, there are very few studies on the validity of the technique in low-resource settings (1–3). We tested TMA versus standard IHC for ER, PR, and Her-2/neu in a native Sudanese BC series ($n$ = 67) selected among the archival BC cases diagnosed between 2004 and 2005 at the Department of Histopathology and Cytopathology, Radiation & Isotope Centre (RICK), Khartoum, Sudan. Histologic classification, grading, staging, ethical approval, IHC quality control, dilutions and sources of antibodies, antigen retrieval methods, and scoring followed the previously described criteria (4). TMA blocks (18 tumors per block, 4 cores of 1.0 mm per tumor) were constructed using an automated TMA station (Galileo TMA CK3500, ISE, Milan, Italy). All sections were cut shortly prior to immunostaining.

The mean age of study subjects was 54.8 ± 13.6 years (range 25.0–80.0 years, 22/67 had missing age information). Most tumors were of large size (mean: 4.2 ± 2.0 cm; range 1.0–10.0 cm, 10/67 tumors had missing size data). The major histologic type was invasive ductal carcinoma (86.6%, 58/67), others were mucinous (5/67, 7.5%), invasive lobular (3/67, 4.5%), and medullary (1/67, 1.5%). Regarding tumor grade, 31.3% (21/67) were grade 2 and 68.7% (46/67) grade 3. Early stage tumors were 41/67 (61.2%; T1, 8/67, 11.9%; T2, 33/67, 49.3%), late stage tumors were 17/67 (25.4%; T3, 14/67, 20.9%; T4, 3/67, 4.5%), while 9/67 (13.4) were of unknown stage. Lymph node status of 47/67 cases (70.1%) was unknown, 17/67 (25.4%) cases were lymph node positive and 3/67 (4.5%) lymph node negative.

By TMA-IHC, the tumors that scored ER+, PR+, and Her-2/neu+ were 30/67 (44.8%), 38/67 (56.7%), and 8/65 (12.3%), respectively. The frequency of the cases with missing or noninformative cores (3/67, 4.5%) was within the reported range (5). Using whole section IHC, the positivity scores were higher: ER+, 44/67 (65.7%); PR+, 51/67 (76.1%); Her-2/neu+, 10/65 (15.4%). Thus, compared with whole section IHC, TMA-IHC had sensitivities of 77.8%, 82.8%, and 83.3% for ER, PR, and Her-2/neu, respectively. The negative predictive values were 64.7% for ER, 72.2% for PR, and 96.4% for Her-2/neu. Overall, the efficiency of TMA-IHC was 84.2% for ER, 88.1% for PR, and 97.0% for Her-2/neu. After excluding the three cases (4.5%) that lost all the 4 TMA cores, concordance rates were 81.15% (52/64), 84.4% (54/64), and 87.5% (56/64) for ER, PR, and Her-2/neu, respectively. The mean size of the tumors that scored ER+ by both TMA and whole section IHC was 3.7 ± 1.9 cm (range: 1.0–8.0 cm), whereas those with mismatched ER status were larger (mean size: 4.9 ± 3.1 cm, range: 1.0–10.0 cm). On the other hand, the tumors that scored PR+ by both approaches were in the size range of those with mismatched PR status (mean: 3.9 ± 2.0 cm–range: 1.5–7.0 cm, versus mean: 4.3 ± 2.1 cm–range: 1.0–10.0 cm). One of the only two tumors with mismatched Her-2/neu status measured 3.5 cm, whereas size was not recorded for the other case.

Of note, in five tumors weak ER+ staining by whole section IHC, with negative internal controls (normal breast ducts), suggested fixation problems. In this small subset, the mismatch between TMA-IHC and whole section IHC was substantial for all of the tested markers (ER+: 3/5, PR+: 2/5, Her-2/neu+: 2/5).

In conclusion, an acceptable negative predictive value of 96.4% was found for Her-2/neu, but for ER and PR the negative predictive values were low (ER: 64.7%, PR: 72.2%), reflecting false-negative results,

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that may reflect both preservation/fixation problems and intra-tumor heterogeneity (3, 4, 6), particularly in the case of ER status. The higher negative predictive value of Her-2/neu TMA-IHC might be related to the small number of Her-2/neu+ tumors in this study (only 10 cases). Overall, our results suggest that the application of TMA-IHC to the classic BC prognostic markers is problematic when dealing with large tumors in low-resource settings, where quality control in pathology processing may be less than optimal. Additional studies, with more cases, are needed for definitive conclusions.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest. IB is shareholder and present Scientific Director of ISE.

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## REFERENCES

1. Shebl AM, Zalata KR, Amin MM, El-Hawary AK. An inexpensive method of small paraffin tissue microarrays using mechanical pencil tips. *Diagn Pathol* 2011;6:117.

2. Sharma SK, Deka L, Gupta R, Gupta S, Singh DK, Singh S. Tissue microarray construction from gross specimens: development of a novel simple technique. *J Clin Pathol* 2010;63:782–5.

3. Al Kuraya K, Simon R, Sauter G. Tissue microarrays for high-throughput molecular pathology. *Ann Saudi Med* 2004;24:169–74.

4. Awadelkarim KD, Arizzi C, Elamin EO, *et al*. Pathological, clinical and prognostic characteristics of breast cancer in Central Sudan versus Northern Italy: implications for breast cancer in Africa. *Histopathology* 2008;52:445–56.

5. Thomson TA, Zhou C, Chu C, Knight B. Tissue microarray for routine analysis of breast biomarkers in the clinical laboratory. *Am J Clin Pathol* 2009;132:899–905.

6. Tolles J, Bai Y, Baquero M, Harris LN, Rimm DL, Molinaro AM. Optimal tumor sampling for immunostaining of biomarkers in breast carcinoma. *Breast Cancer Res* 2011;13:R51.