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Use of Web-based training for quality improvement between a field immunohistochemistry laboratory in Nigeria and its United States–based partner institution

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ABSTRACT

The importance of hormone receptor status in assigning treatment and the potential use of human epidermal growth factor receptor 2 (HER2)–targeted therapy have made it beneficial for laboratories to improve detection techniques. Because interlaboratory variability in immunohistochemistry (IHC) tests may also affect studies of breast cancer subtypes in different countries, we undertook a Web-based quality improvement training and a comparative study of accuracy of immunohistochemical tests of breast cancer biomarkers between a well-established laboratory in the United States (University of Chicago) and a field laboratory in Ibadan, Nigeria. Two hundred and thirty-two breast tumor blocks were evaluated for estrogen receptors (ERs), progesterone receptors (PRs), and HER2 status at both laboratories using tissue microarray technique. Initially, concordance analysis revealed κ scores of 0.42 (moderate agreement) for ER, 0.41 (moderate agreement) for PR, and 0.39 (fair agreement) for HER2 between the 2 laboratories. Antigen retrieval techniques and scoring methods were identified as important reasons for discrepancy. Web-based conferences using Web conferencing tools such as Skype and WebEx were then held periodically to discuss IHC staining protocols and standard scoring systems and to resolve discrepant cases. After quality assurance and training, the agreement improved to 0.64 (substantial agreement) for ER, 0.60 (moderate agreement) for PR, and 0.75 (substantial agreement) for HER2. We found Web-based conferences and digital microscopy useful and cost-effective tools for quality assurance of IHC, consultation, and collaboration between distant laboratories. Quality improvement exercises in testing of tumor biomarkers will reduce misclassification in epidemiologic studies of breast cancer subtypes and provide much needed capacity building in resource-poor countries.

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1. Introduction

Breast cancer is the most prevalent cancer of women worldwide [1,2]. Although breast cancer survival has improved over the past decades in some developed countries [3], significant differences in breast tumor stage, treatment options, and mortality rates still exist in the world with regard to race and ethnicity [2,4,5]. Despite the rapid expansion of novel diagnostics designed to personalize breast cancer care [6], there remain several significant unmet needs for improving the accuracy and reproducibility of tests that are already in common daily clinical practice [7].

Over the past decades, breast tumor markers such as estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) have become very useful in predicting prognosis and determining therapy options for patients with breast cancer. This has been particularly helpful in identifying those patients who would benefit from antiestrogen therapy and also those who would likely respond to HER2-based therapy. The effective use of

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these markers, therefore, would naturally depend on the accurate, reliable, and reproducible determination of their presence and levels in individual breast tumors.

Immunohistochemistry (IHC) has so far been used for ER, PR, and HER2 determination. However, despite long-term use worldwide, there still exists significant intralaboratory and interlaboratory variability of IHC results. Some identified reasons for interlaboratory discrepancies include the following: type of antibody clone used, differences in antigen retrieval techniques, formalin fixation time, and different scoring/reporting systems. As a result, an ad hoc consensus conference, consisting of directors from a broad range of IHC laboratories, was convened in 2006, which put together recommendations aimed at standardizing IHC laboratory practices [8,9]. The factors considered at the conference included preanalytic factors, which focus on fixation techniques; analytic factors, which talk about antigen retrieval procedure; and postanalytic factors, which discuss the scoring and reporting systems. Some of the specific recommendations made at this conference include the exclusive use of formalin fixatives, the need for adequate fixation time, careful selection of antibody clone, and the use of an efficient scoring system.

In Nigeria, IHC determination of breast tumor markers has just recently been introduced. Only a handful of laboratories offer these tests currently, and it is important to set up quality measures to assure accurate performance of IHC analysis of breast tissues to guide treatment decisions. The aim of this study therefore is to examine the reproducibility of test results obtained from a field laboratory in Nigeria in comparison with data obtained from a well-established laboratory at the University of Chicago, Illinois, using tissue microarray (TMA) technology. We also assessed the feasibility of Web-based conferences and digital microscopy in ensuring quality assurance.

2. Materials and methods

2.1. Study settings

This study was conducted at both the University of Chicago and the Institute for Advanced Medical Research and Training (IAMRAT) at Ibadan. It involved online IHC training sessions. The study was approved by institutional review boards of the coordinating institutions in Ibadan and the University of Chicago [10]. After an initial training (stage 1), the first performance evaluation (stage 2) was conducted followed by a review of process and then a session of online training and discussion (stage 3) and a second evaluation of performance (stage 4). Statistical analysis was performed with StataSE software, version 10 (STATA, Cary, North Carolina). The κ statistic served to test inter-tester agreement in IHC staining and scoring. Overall inter-tester agreement was obtained based on κ coefficient calculated using a 4×4 weighting matrix.

3. Results

3.1. Initial training of personnel

A Nigerian pathologist (O.F.I.) obtained training at the Department of Pathology of the University of Chicago. The training focused on IHC testing techniques, antigen retrieval techniques, and handling red flags in IHC testing. The tests focused on ER, PR, and other prognostically relevant biomarkers for breast cancer. After this training program, he went back to the University of Ibadan to set up an IHC laboratory within the IAMRAT and also train technical personnel to man the laboratory. The necessary equipments needed were shipped down to Nigeria from the United States, and these included antibodies, ice racks, weighing equipment, and others. The training session in Nigeria was an intensive course lasting 12 weeks. Training sessions included seminars, use of academic literatures, and involved practical demonstrations giving opportunity for hands-on experience. The training was extended to a laboratory technician at IAMRAT and a board-certified pathologists from the University College Hospital, Ibadan, Nigeria. After the training was completed, information was sent to physicians and pathologists in hospitals around the country through letters and e-mails informing them about the commencement of IHC services in the laboratory. Immunohistochemistry analysis for ER, PR, and HER2 of breast cancer tissues was provided at no cost to referring physicians.

3.2. IHC testing in Ibadan

Samples of breast cancer cases from various teaching hospitals in Nigeria were referred to the IAMRAT Laboratory at the University of Ibadan for IHC analysis. Pathologists from the various referring institutions were requested to send 2 formalin-fixed tumor tissue blocks and fill in a laboratory consultation form detailing patients biodata, clinical and pathologic information, and diagnosis. Four-micron-thick sections were prepared using a Rotary microtome machine from each of 235 formalin-fixed, paraffin-embedded (FFPE) tissue blocks received in the IAMRAT Laboratory representing 165 patients in all. These were subjected to immunohistochemical analysis for ER, PR, and HER2 status (Fig. 1). The stained slides were then scored by the pathologist based in Ibadan (O.A.O.) using the Dako ER/PR pharmDx Interpretation Manual (Carpinteria, CA).

3.3. Construction of TMAs in Chicago

Tissue microarrays were successfully constructed in Chicago from 232 tumor samples of the total 235 FFPE blocks received from Nigeria. Cores were precisely arrayed into a new recipient paraffin block using the automated tissue microarrayer ATA-27 (Beecher Instruments, Sun Prairie, Wisconsin), with the method described by Kononen et al [11]. Three tissue blocks were excluded because of small biopsy sample or low tissue quality.

3.4. Immunohistochemistry testing in Chicago

Paraffin specimens were cut into 4-μm sections and mounted on positively charged slides. The sections were deparaffinized and rehydrated in xylene and then decreasing grades of alcohol respectively and were then washed in Tris-buffered saline. Immunohistochemical assays were performed manually in the Ibadan laboratory using standardized conditions with antibodies, antigen unmasking, and scoring systems, as detailed in Table 1. Slides were incubated in 0.03% hydrogen peroxide for 5 minutes to block endogenous peroxidase activity, followed by incubation for 20 minutes in a protein-blocking solution (Protein Block Serum-free solution; DAKO) to reduce nonspecific background. Envision +

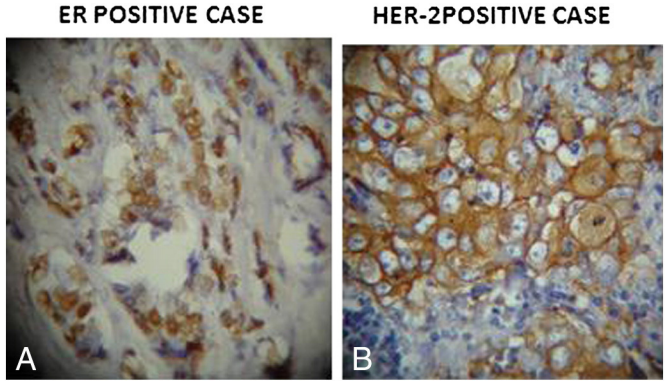

Fig. 1. Photomicrographs of immunostained breast cancer whole sections from Ibadan: ER-positive case (A) and HER2-positive case (B).

Table 1
Antibodies and conditions used for immunohistochemical analyses [12–14]

Antibody	Clone	Dilution	Source	Pretreatment	Scoring
ER	1D5	1:50	Dako	Microwave 30 min, citrate buffer (pH 6.0)	Nuclear; 0, ≤10%; 1, 11%-30%; 2, 30%-70%; 3, ≥70% (13)
PR	636	1:50	Dako	Microwave 30 min, citrate buffer (pH 6.0)	Nuclear; 0, ≤10%; 1, 11%-30%; 2, 30%-70%; 3, ≥70% (13)
HER2	HercepTest	Ready to use	Dako	Microwave 15 min, Epitope retrieval solution (HercepTest catalog no. K5207)	Membranous; 0, 1+, 2+, 3+ (17)
Vimentin	V9	1:50	Dako	None	Staining to monitor the quality of tissue fixation in archival tumors (12)

reagents (DAKO) were used as a detection system. Slides were then treated for 5 minutes with 3-3′-diaminobenzidine chromogen, counterstained with hematoxylin, and coverslipped. Appropriate negative controls for the immunostaining were prepared by omitting the primary antibody step.

3.5. First evaluation of immunostainings

The results of immunostainings for ER and PR were scored semiquantitatively, independently by the 2 study pathologists (A.I.K., O.A.O.) at the 2 study centers using 4-point scale of Reiner et al [12] based on the intensity and percentage of IHC reaction. Regarding staining with vimentin, V9 served as a control to monitor the quality of tissue fixation in archival tumors [13].

3.6. Initial concordance analysis

An initial concordance analysis was performed comparing IHC results from the Nigerian laboratory, which used whole sections, with corresponding IHC results from the Chicago laboratory derived from TMAs. The IHC test results for ER and PR from Nigeria were initially scored into 5 categories based on percentage and intensity of IHC reaction (Dako ER/PR pharmDx Interpretation Manual) [15], whereas in Chicago, the results were scored using the 4-point scale of Reiner et al [12]. For the purpose of comparison, we used the conversion table proposed by Shousha [16]. Assessment of HER2 status was performed according to recommendations of the American Society of Clinical Oncology and College of American Pathologists [17]. For this concordance analysis, we had 155 comparable ER results, 164 comparable PR results, and 154 comparable HER2 results. The κ for ER results was 0.42, which indicates a moderate agreement. The PR results had a similar concordance with a κ of 0.41. The HER2 test results had a κ of 0.38, which demonstrates a fair agreement.

3.7. Process review and Web-based training

In this stage, 23 digital microscopic images of randomly selected cases were taken by the histotechnologist in Ibadan from the study histologic slides with appropriate positive and negative controls for ER, PR, and HER2 markers using a digital camera, which was mounted on a microscope. The images were then transmitted in JPEG format over the Internet as e-mail attachments to the pathologist in Chicago. The images were reviewed by the pathologists in Chicago for scoring, image quality, and staining characteristics using free graphic software Picasa from Google and ImageScope, Aperio Technologies (Vista, CA). The images were then jointly reviewed by both pathologists during the Web conferences. The purpose of this stage was to assess the quality of digital images and staining techniques in Nigeria and further train the attending pathologist at the Ibadan field IHC laboratory on techniques of assessment of staining quality and scoring.

Tissue microarray slides created at the University of Chicago were also scanned using Automated Cellular Imaging System (Fig. 2). Digital images of discordant cases were then jointly reviewed online by pathologists using freely downloadable viewing software Image Scope Viewer (Aperio Technologies) and WebEx, a virtual meeting manager that enabled us to share desktops, presentations, and images in real time. Web-based conferences were held biweekly, during which we discussed IHC staining protocols, standardized scoring systems, and resolved discrepant cases.

Interlaboratory Web-based conferences were performed using user-friendly Web-based tools such as Skype and WebEx. Skype is a software that enables one to make Internet calls free between users who have Internet access. WebEx is an online conferencing tool that enables conferencing from different physical location. It permits desktop sharing and thus permits off-site presentations and facilitates effective participation among the participants. The conferences were held from 2 locations, one at IAMRAT, Ibadan, Nigeria, and another at the University of Chicago in Illinois.

Some of the important reasons for the discrepancies identified during the Web conference training sessions include differences in staining protocols, antigen retrieval procedures, and scoring methods. After the Web-based discussion sessions, joint evaluation of digital slide images revealed almost complete agreement between both pathologists. In addition to reviewing images, technical issues relating to preanalytical, analytical, and postanalytical concept of IHC staining and testing were discussed, such as follows:

- Duration of formalin fixation of tissue
- Type of buffer used—commercial citrate vs locally prepared
- Choice of antibody clone used
- Antigen retrieval procedure—how long did they apply primary antibody
- Type of diluent used
- Dilution ratio
- Scoring systems—percentage vs intensity of staining

3.8. Second evaluation of immunostainings

After the Web-based training, unstained TMA slides of all the patients were then sent back to the laboratory at IAMRAT, Ibadan, where they were restained and evaluated immunohistochemically for ER, PR, and HER2, taking into cognizance all experiences acquired during the Web-based training. Results were e-mailed to the pathologist in Chicago for second concordance analysis. The concordance level was then assessed again for evidence of improved staining quality and scoring accuracy to assess the effectiveness of the online training. In the final evaluation after the Web conferences, the concordance between TMA slides in Chicago and TMA slides restained in Nigeria was shown to have improved with a κ of 0.64 (substantial agreement) for ER results, 0.60 (moderate agreement) for PR, and 0.75 (substantial agreement) for HER2 results (see Table 2).

4. Discussion

Our study incorporated both the use of digitized images taken with a digital camera by the technologists at a Nigerian laboratory and whole-slide imaging using Image Scope Aperio Technology in the University of Chicago laboratory for quality assurance of IHC tests. These digital images along with the Web-based conferences proved to be useful and cost-effective tools for long-distance training and pathology consultations, especially in developing countries with limited access to information, skilled specialists, and resources. This

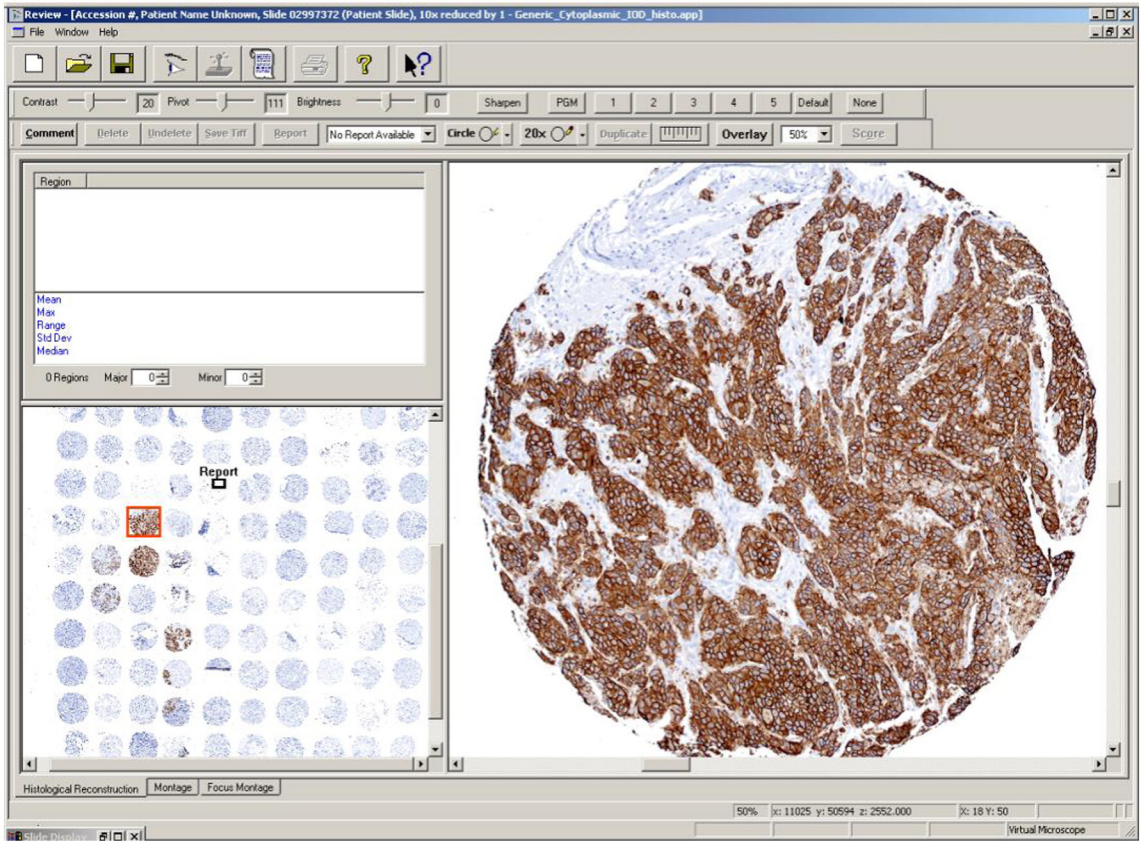

Fig. 2. View of the Automated Cellular Imaging System shows breast cancer TMA cores immunostained with HER2/neu.

was demonstrated by the ability of the pathologist to jointly review images and thus resolve discrepant cases and was further confirmed by an overall improvement in the concordance results during the second evaluation, which compared results from TMAs in Chicago with results of TMA slides restained and scored in Nigeria.

Similar benefits have been reported in other studies using Web-based tools for pathology consultation. A pilot project between the Italian hospital in Cairo, Egypt, and Civico hospital in Palermo reported benefits in costs reduction and ability to exchange knowledge of high scientific value, ultimately leading to better medical services through the use of static and dynamic techniques of telepathology [18]. This study, like ours, demonstrated the usefulness of simple Web-based conferencing tools such as Skype and MSN for analyzing digital images in off-site pathology consultations [19]. Another study conducted in the Mohs surgical laboratory compared the pathologic diagnosis between video images and conventional light microscopy [20]. This study used off-the-shelf consumer products like a digital video camera mounted on a microscope to transmit images from the Mohs surgery suite to a consultant viewing station off-site where the images were reviewed via iChat AV on a computer by a dermatopathologist. The Mohs laboratory group reported complete agreement between the iChat AV dynamic telepathology diagnosis and conventional light microscopy diagnosis for frozen section slides and 95% agreement for FFPE slides. These results further confirmed the potentials we found in the use of virtual images for pathologic consultation. However, in addition, we were able to demonstrate the use of these online tools in training local pathologists with demonstrable improvement in antigen retrieval, staining, and scoring techniques. In more advanced centers, high-speed, automated whole-slide imaging systems are being used to generate digital images of sufficient quality for pathologists to make reliable diagnostic decisions [21].

The major challenges encountered included poor quality of some the images from Nigeria and the relatively unstable Internet connection in Nigeria. Some of the images taken at Ibadan contained artifacts, and some had shadows cast on them possibly because of poor lighting and inadequate focus. Achieving excellent image quality is essential for virtual microscopy. Images should generally be at a sufficiently high resolution, well focused over the entire slide, accurately represent the colors exhibited on the glass specimen, reflect the representative area of tumor, and be free of compression and other image artifacts. The resolution of the images was, however, generally adequate for evaluation by our pathologists. The high level of agreement between both pathologists while reviewing the digital images online revealed the potentials of the use of virtual images for pathologic consultation and diagnosis. The findings thus support a need for enhanced medical photography and telepathology as a cost-effective means of determination of interlaboratory variability of IHC testing and Web-based quality improvement training and validation studies.

For our initial evaluation of concordance, we compared IHC scores reviewed from whole slides in Nigeria with the scores reviewed from TMAs in Chicago. We realize that the use of different technologies by both laboratories could be partly responsible for the poor results in the initial concordance analysis, but joint review of the images also revealed other reasons for discrepancies. These issues were discussed, and both pathologists agreed on ideal protocols and recommendations were made to the Nigerian laboratory. The improvement in results of the final concordance analysis is evidence of the success and usefulness of the Web conferences. For this analysis, we compared the results from Chicago with results from TMA slides sent from Chicago but restained and scored by the pathologists in Nigeria.

Furthermore, the significance of this study can also be viewed based on the therapeutic implications to the patients. Of 121 individual patients with breast cancer who had evaluable ER and/or

Table 2
Initial and final concordance analysis comparing results from Nigeria with those from TMAs in Chicago

		ER results from Nigeria				Total	κ Value
		0	1	2	3		
ER results from Chicago	0	109	0	2	0	111	
		133	**5**	**0**	**0**	**138**	
	1	3	1	2	0	6	
		5	**1**	**1**	**0**	**7**	
	2	6	0	3	0	9	
		3	**0**	**4**	**0**	**7**	
	3	1	1	1	0	3	
		0	**0**	**1**	**2**	**3**	
Total		119	2	8	0	129	0.42
		141	**6**	**6**	**2**	**155**	**0.64**

		PR results from Nigeria				Total	κ Value
		0	1	2	3		
PR results from Chicago	0	111	4	3	0	118	
		121	**8**	**3**	**0**	**132**	
	1	9	3	4	0	16	
		5	**6**	**5**	**1**	**17**	
	2	6	1	3	2	12	
		2	**2**	**6**	**2**	**12**	
	3	0	2	0	0	2	
		1	**0**	**0**	**2**	**3**	
Total		126	10	10	2	148	0.41
		129	**16**	**14**	**5**	**164**	**0.60**

		HER2 results from Nigeria				Total	κ Value
		0	1	2	3		
HER2 results from Chicago	0	68	14	11	5	98	
		113	**5**	**0**	**0**	**118**	
	1	1	2	10	1	14	
		6	**4**	**2**	**3**	**15**	
	2	1	1	1	2	5	
		1	**5**	**1**	**0**	**7**	
	3	2	1	1	7	11	
		1	**0**	**0**	**13**	**14**	
Total		72	18	23	15	128	0.39
		121	**14**	**3**	**16**	**154**	**0.75**

Values for final concordance comparing results from restained TMA slides in Nigeria with results from TMAs in Chicago are in bold font.

PR results from both laboratories, 7 of them were tested ER positive in Chicago but were not found to be positive after the initial testing in Nigeria based on a cutoff of 10% for both ER and PR positivities. Likewise, there were 10 individual cases that were detected as PR positive in Chicago but not found to be PR positive after the first testing in Nigeria. Therefore, based on the American Cancer Society guidelines that assign hormone treatment based on ER and/or PR positivity, 11 patients would have missed tamoxifen treatment based on the first evaluation of hormone receptors in Nigeria [22]. However, 6 of these 11 patients were later determined to be ER and/or PR positive after the final evaluation in Nigeria. Likewise, 10 of 124 individual patients were determined to be HER2 positive by the Chicago laboratory but not by the initial evaluation in the Nigeria laboratory. Five of them, however, were later determined to be HER2 positive after the final evaluation in Nigeria. These results further demonstrate the effect of our Web-based training in resolving at least 50% of discordant cases who would otherwise have missed vital, proven adjuvant breast cancer therapy [22,23].

Consistent with previous studies [24,25], we also showed that TMA use is an efficient and cost-effective tool for quality assurance and for assessing interlaboratory variability of IHC testing. Tissue microarrays allow researchers to validate new biomarkers or to discover and dissect molecular pathways, simultaneously, in hundreds of samples and was therefore very adequate for our study [26]. This has significant cost reduction benefits considering the high costs of antibodies, which would have otherwise been used to process each slide individually. Tissue microarray technology is, however, not yet being used conventionally in Nigerian laboratories possibly because of the initial high setup costs.

In summary, we have demonstrated the effectiveness of using simple and easily accessible Web-based tools for pathology consultation in different countries. We also showed that Skype and WebEx can be very useful tools for online conferencing and image sharing. In the near future, we hope to perform further concordance studies under similar standard conditions using prospective tissue samples received at the laboratory in Ibadan. These would further confirm the effectiveness of our training sessions. Accurate determination of breast cancer tumor markers has significant prognostic and therapeutic implications, so we recommend that validation studies such as these be performed in emerging laboratories and that virtual microscopy be used more to promote better collaboration between pathologists in distant laboratories.

References

[1] IARC. World Cancer Report, 2008. Boyle, P. and Levin, B.E. (eds), IARC Press, Lyon. 2008.
[2] Olofin I, Olopade OI. Eliminating global disparities in breast cancer care through clinical research. Am Soc Clin Oncol Ed Book 2009:681–4.
[3] Boyle P. Breast cancer control: signs of progress, but more work required. Breast 2005;14(6):429–38.
[4] Kurebayashi J, Moriya T, Ishida T, et al. The prevalence of intrinsic subtypes and prognosis in breast cancer patients of different races. Breast 2007;16(Suppl. 2):S72–7.
[5] Blackman DJ, Masi CM. Racial and ethnic disparities in breast cancer mortality: are we doing enough to address the root causes? J Clin Oncol 2006;24(14):2170–8.
[6] Olopade OI, Grushko TA, Nanda R, et al. Advances in breast cancer: pathways to personalized medicine. Clin Cancer Res 2008;14(24):7988–99.
[7] Ross JS, Symmans WF, Pusztai L, et al. Standardizing slide-based assays in breast cancer: hormone receptors, HER2, and sentinel lymph nodes. Clin Cancer Res 2007;13(10):2831–5.
[8] Goldstein N, Hewitt S, Taylor C, et al. Recommendations for improved standardization of immunohistochemistry. Appl Immunohistochem Mol Morphol 2007;15(2):124–33.
[9] Gown AM. Current issues in ER and HER2 testing by IHC in breast cancer. Mod Pathol 2008;21(Suppl 2):S8–S15.
[10] Huo D, Ikpatt F, Khramtsov A, et al. Population differences in breast cancer: survey in indigenous African women reveals over-representation of triple-negative breast cancer. J Clin Oncol 2009;27:4515–21.
[11] Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998;4:844–7.
[12] Reiner A, Neumeister B, Spona J. Immunocytochemical localization of estrogen and progesterone receptor and prognosis in human primary breast cancer. Cancer Res 1990;50(21):7057–61.
[13] Battifora H. Assessment of antigen damage in immunohistochemistry. The vimentin internal control. Am J Clin Pathol 1991;96(5):669–71.
[14] Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007;25(1):118–45.
[15] Interpretation Manual. Dako Inc.; http://www.dako.com/dist/index/knowledgecenter/kc_publications/kc_publications_interpret/er_pr_pharmdx__.htm. last accessed 6/12/2013
[16] Shousha S. Oestrogen receptor status of breast carcinoma: Allred/H score conversion table. Histopathology 2008;53(3):346–7.
[17] Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med 2007;131(1):18–43.
[18] Ayad E, Sicurello F. Telepathology in emerging countries pilot project between Italy and Egypt. Diagn Pathol 2008;3(Suppl 1):S2.
[19] Klock C, Gomes R de P. Web conferencing systems: Skype and MSN in telepathology. Diagn Pathol 2008;3(Suppl 1):S13.
[20] Mckenna JK, Florell SR. Cost-effective dynamic telepathology in the Mohs surgery laboratory utilizing iChat AV videoconferencing software. Dermatol Surg 2007;33(1):62–8.
[21] Gilbertson J, Ho J, Anthony L, et al. Primary histologic diagnosis using automated whole slide imaging: a validation study. BMC Clin Pathol 2006;6:4.
[22] Osborne CK. Tamoxifen in the treatment of breast cancer. N Engl J Med 1998;339:1609–18.
[23] Hortobagyi GN. Trastuzumab in the treatment of breast cancer. N Engl J Med 2005;353:1734–6.
[24] Hsu F, Nielsen T, Alkushi A. Tissue microarrays are an effective quality assurance tool for diagnostic immunohistochemistry. Mod Pathol 2002;15(12):1374–80.
[25] Milanes-Yearsley M, Hammond ME, Pajak TF, et al. Tissue micro-array: a cost and time-effective method for correlative studies by regional and national cancer study groups. Mod Pathol 2002;15(12):1366–73.
[26] Seligson D. The tissue micro-array as a translational research tool for biomarker profiling and validation. Biomarkers 2005;10(Suppl. 1):S77–82.