Annals of Diagnostic Pathology 17 (2013) 526–530

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

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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ARTICLE INFO

Keywords:
Immunohistochemistry
Quality improvement
Tissue microarray
Web-based conferences

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.

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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1092-9134
http://dx.doi.org/10.1016/j.anndiagpath.2013.07.003

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 +

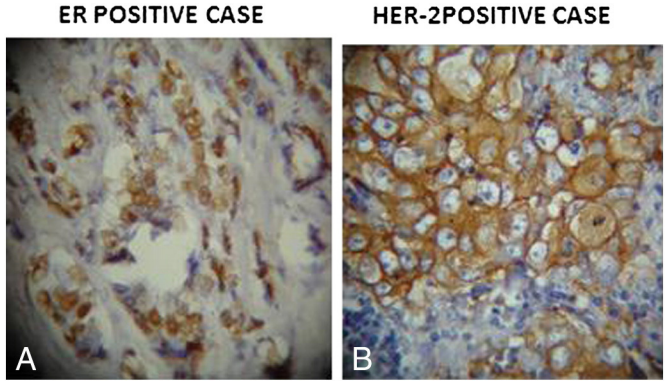

Fig. 1. Photomicrographs of immunostained breast cancer whole sections from Ibadan: ER-positive case (A) and HER2-positive case (B).