Annals of Diagnostic Pathology 18 (2014) 313–318

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

Original Contributions

The clinical use of a P63/cytokeratin7/18/cytokeratin5/14 antibody cocktail in diagnostic breast pathology

Emily S. Reisenbichler, MD [a,*,1], John R. Ross, MD [b,1], Omar Hameed, MD [a,c,d]

[a] Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, 1161 21st Ave South CC-3322 Medical Center North, Nashville, TN 37232-2561, USA
[b] Department of Pathology, University of Alabama at Birmingham, P210 West Pavilion 619 South 19th St Birmingham, AL 35233-7331, USA
[c] Department of Surgery, Vanderbilt University Medical Center, 1161 21st Ave South CC-3322 Medical Center North, Nashville, TN 37232-2561, USA
[d] Vanderbilt Ingram Cancer Center, Vanderbilt University Medical Center, 1161 21st Ave South CC-3322 Medical Center North, Nashville, TN 37232-2561, USA

ARTICLE INFO

Keywords:
Breast
Immunohistochemistry
Antibody Cocktail
Cytokeratin 7/18
Cytokeratin 5/14
p63

ABSTRACT

An antibody cocktail directed against p63, cytokeratin (CK)5/14, and CK7/18 is reported to be useful in distinguishing noninvasive from invasive breast lesions and for the characterization of intraductal epithelial proliferations. However, limited studies evaluate its use in clinical practice. A retrospective review of breast material at a university medical center identified cases that were immunostained with the above antibody cocktail. Additional p63 immunostaining alone was performed to further determine the utility of the antibody cocktail in the evaluation of invasion. Of 50 breast cases identified, the antibody cocktail was used to confirm or exclude invasion in 44 (88%). Twenty-two (50%) of these had easily identifiable p63/CK5/14-positive myoepithelial cells, whereas the remainder lacked such staining, confirming the diagnosis of invasive carcinoma. In 27 cases with available diagnostic material for additional p63 immunostaining, the cocktail better highlighted myoepithelial cells by staining nuclei and cytoplasm. Easier identification of invasion was also facilitated by CK7/18 expression in invasive foci, especially those composed of single cells. Ten cases were immunostained to help determine the nature of an intraductal proliferation. The cocktail demonstrated a mosaic staining pattern of both CK7/18- and CK5/14-positive epithelial cells in 3 (30%) cases consistent with usual hyperplasia; homogenous CK7/18 expression in the remaining cases supported the diagnosis of atypical ductal hyperplasia or carcinoma in situ. In summary, the p63/CK7/18/CK5/14 cocktail stain appears to be a useful tool in diagnostic breast pathology, in the evaluation of possible invasion, particularly in the setting of minute foci of invasion as well as in epithelial proliferations.

1. Introduction

Screening methods for the detection of breast lesions, including the routine use of mammograms and ultrasound as well as newer modalities such as magnetic resonance imaging (MRI), in specific patient populations, have allowed for the detection of smaller lesions [1]. Although identifying malignancies at an early stage may allow for more conservative surgical procedures and increase the potential for curative treatment, smaller lesions can be more challenging to diagnose. As a result, utilization of multiple immunohistochemical stains in combination (antibody cocktails) has become indispensable in the evaluation of smaller diagnostic tissue specimens, most notably prostate needle biopsy material [2] and, more recently, other tissue types, such as breast [3,4]. One such antibody cocktail is a marker for low molecular weight cytokeratins (CK7 and CK18), high molecular weight CKs (CK5 and CK14), and p63. This combination allows for the staining of both myoepithelial cells (CK5/14 and p63-positive; cytoplasmic and nuclear, respectively) as well as carcinoma cells (CK7/18 positive) on a single slide. Although several studies [3,4] have evaluated the use of this antibody cocktail in different series of breast lesions, there are limited studies aimed at assessing its diagnostic utility in actual clinical use. To specifically address this, we retrospectively reviewed all breast specimens evaluated at our institution with this antibody cocktail to determine what diagnostic dilemmas on hematoxylin and eosin (H&E) led to the use of this cocktail. In select cases, the utility of the antibody cocktail was compared with that of a single p63 immunostain.

2. Materials and methods

After obtaining the institutional review board approval for the study, a retrospective search of the surgical pathology files at the University of Alabama at Birmingham was used to identify breast tissue specimens immunostained with a p63/CK7/18/CK5/14 pre-diluted antibody cocktail ("ADH-5"; Biocare Medical, Concord, CA) over a 15-month period. The clinical, radiologic, and pathologic

* Corresponding author. Vanderbilt University Medical Center, MCN C-3321, 1161 21st Ave S, Nashville, TN 37232-2561. Tel.: +1 615 343 4307; fax: +1 615 343 7023.
E-mail address: Emily.s.reisenbichler@Vanderbilt.Edu (E.S. Reisenbichler).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.anndiagpath.2014.08.007
1092-9134/

findings in these cases were reviewed to determine the indication(s) for immunohistochemistry (IHC), quality of staining, and interpretation of findings.

As noted above, the prediluted cocktail included antibodies directed against CK 5 (clone XM26), CK 14 (clone LL002), p63 (clone BC4A4), CK 7 (clone BC1), and CK18 (clone E431-1). An automated immunostainer (Thermo Scientific–Labvision 720; Lab Vision Corporation, Fremont, CA) and manufacturer (Biocare)-provided protocols and reagents were used as follows: after deparaffinization, heat-induced antigen retrieval, and blocking of endogenous peroxidase, sections were incubated for 45 minutes with the antibody cocktail. The secondary reagent, DS KIT-2 (Biocare), was then applied for 30 minutes. The reaction was visualized by incubation with DAB (Biocare) for 5 minutes followed by incubation with Vulcan Fast Red (Biocare) for 10 minutes. Slides were then counterstained with hematoxylin, dehydrated (air dry and xylene), and coverslipped. Normal breast ducts and lobules were used as positive control. Additional p63 immunostaining (clone 4A4, 1:200 dilution; Biogenex, San Ramon, CA) was performed on all cases with available tissue.

Positive staining for CK5/14 was identified as brown cytoplasmic staining within intraductal/luminal epithelium and myoepithelial cells. Positive staining for CK7/18 was identified as red cytoplasmic staining within intraductal/luminal epithelial cells. Positive staining for p63 was defined as brown nuclear staining in myoepithelial cells.

3. Results

The antibody cocktail was applied on a total of 50 breast cases, representing 2.3% of total institutional breast cases evaluated during the search period. The cases included 24 (48%) needle core biopsies and 26 (52%) excision specimens. Most, 44 (88%), cases were immunostained to evaluate foci of possible invasive carcinoma. These included 3 (6%) cases, where the immunostained sections were also used to better define the nature of an associated intraluminal epithelial proliferation. Another 6 (12%) cases were solely immunostained to further assess the nature of an intraductal epithelial proliferation.

Of the 44 foci suspicious for invasion, easily identifiable p63/CK5/14-positive myoepithelial cells were observed in 22 (50%) foci excluding such a diagnosis (Figs. 1-3). Of these, most consisted of circumscribed nests of atypical proliferative epithelium demonstrating diffuse CK7/18 expression, consistent with diagnoses of ductal carcinoma in situ (DCIS; Figs. 1 and 2) or lobular carcinoma in situ. Papillary architecture was noted within 2 circumscribed lesions demonstrating the presence of peripheral myoepithelial cells but absence of myoepithelial cells lining fibrovascular cores. Mimics of invasive carcinoma, including atypical apocrine adenosis (Fig. 3A and D) and sclerosing lesions involved by DCIS (Fig. 3B and E) or usual ductal hyperplasia, demonstrated p63/CK5/14-positive myoepithelial cells surrounding irregularly shaped epithelial proliferations.

Of the remaining 22 foci that were suspicious for invasion, 21 showed no p63/CK5/14-positive myoepithelial staining yet showed diffuse, strong CK7/18 expression, confirming the diagnosis of invasive carcinoma. The invasive carcinoma in these situations often appeared as circumscribed nests of tumor cells resembling solid DCIS (Fig. 1) or cribriform DCIS (Fig. 2). The angulated tubules of invasive tubular carcinoma (Fig. 3C and F) were distinguished from the distorted glands of sclerosing lesions by the absence of myoepithelial cells. Invasive carcinoma was present as single cells in 3 cases (Fig. 4). The remaining case, also composed of solid nests of tumor cells, demonstrated diffuse p63/CK5/14 expression without any CK7/18 staining (Fig. 1F), consistent with a basal-type invasive mammary carcinoma.

The antibody cocktail was used to determine the nature of a proliferative epithelial lesion in 10 foci, 3 of which showed possible invasion as well. Most cases showed foci of epithelium with homogenous CK7/18 expression, supporting the diagnoses of atypical/carcinomatous proliferations. Lesions demonstrating only CK7/18 expression included cases of invasive ductal carcinoma with both nested (Fig. 1E) and tubular

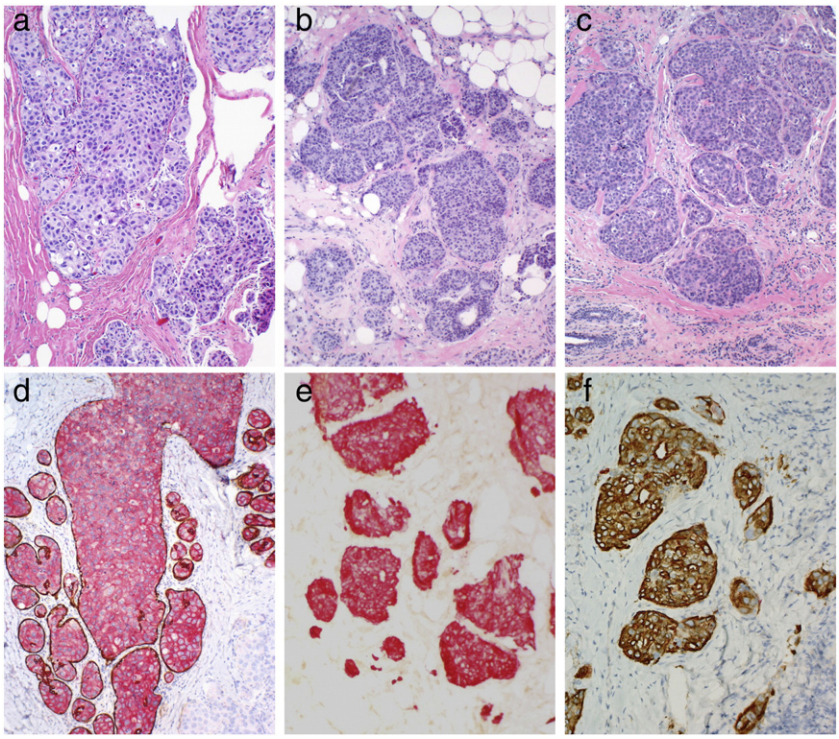

Fig. 1. Comparison of p63/CK7/18/CK5/14 antibody cocktail staining in solid DCIS and clustered solid nests of invasive carcinoma that mimic solid DCIS. Ductal carcinoma in situ (a) showing diffuse CK 7/18 expression with surrounding p63 nuclear staining of myoepithelial cells (d). Invasive carcinoma (b) showing homogenous CK 7/18 expression with absence of p63 staining (e). Invasive carcinoma (c) showing homogenous CK 5/14 expression with absence of p63 staining (f) consistent with basal-type invasive mammary carcinoma.