Annals of Diagnostic Pathology 19 (2015) 296–300

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

The role of immunohistochemistry in the evaluation of gynecologic pathology part 2: a comparative study between two academic institutes

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ARTICLE INFO

Keywords:
Gynecologic pathology
Immunohistochemistry
p16
p53
ki-67

ABSTRACT

Use of specific immunohistochemistry (IHC) marker, singly or in panels, differs and is influenced by practice setting, individual experience beside other factors. This is a part 2 study where we surveyed the application of IHC in gynecologic (gyn) pathology. Our specific aim in this part was to identify what specific stains are preferentially used. A retrospective chart review on all cases accessioned to the gyn pathology specialty sign out service during a 1-year period was performed at two academic pathology departments. Outside referral and consult as well as gyn cytology cases were excluded from the study. The most commonly ordered markers in diagnostic gyn pathology in descending order of frequency were as follows: P16, ki-67, p53, estrogen receptor (ER), progesterone receptor (PR), and CK7. P16 was used mainly in establishing the diagnosis/grading of squamous intraepithelial lesions (SIL) and differentiating serous from endometrioid carcinomas (ECs). P53 was used particularly in the diagnosis of serous carcinomas and establishing the diagnosis of differentiated vulvar intraepithelial neoplasia. Positive p16 was documented in all high-grade SIL, endocervical carcinomas, and serous carcinomas. In contrast, p16 was negative in all benign, low-grade SIL, and ECs. ER and PR were used in panels with p16, p53, vimentin, and carcinoembryonic antigen to assign tumors to specific site, in differentiating EC from serous carcinomas and in establishing the diagnosis of endocervical adenocarcinomas. Immunohistochemistry was used in 4.7% and 8.7% of gyn surgical path cases at two institutions. P16, ki-67, and p53 were the most commonly used markers especially in grading SIL. This study documents the most commonly used IHC biomarkers at two tertiary care academic centers for defining benchmarks for IHC use.

1. Introduction

Morphology is the cornerstone for diagnosis and characterization of different lesions. However, sometimes distinguishing benign from malignant process and knowing the cell of origin especially in poorly differentiated tumors are a challenge. Immunohistochemistry (IHC) has become an integral part in diagnostic pathology as an adjunct technique helping in resolving diagnostic dilemmas and used as predictive and prognostic markers beside others [1–7]. The use of IHC is widely varied in different practices and even among different pathologists in the same practice [8].

The use of panels rather than single IHC marker is advisable especially if used for diagnostic purposes [3]. It is necessary and required to perform adequate positive and negative controls, without which the results in most instances are invalid [3]. Familiarity with different clones and epitopes is important to avoid misinterpretation of the results [3]. It is advisable and required by credentialing bodies for every lab to establish its own titration and working conditions and participate in proficiency tests for quality assurance.

In the recent circumstances with budget restriction affecting many practices, proper use of IHC to reduce medical care costs without drastically affecting the efficiency of the health care is required [9]. Recently, we reviewed the use of IHC in diagnostic gynecologic (gyn) pathology at Vanderbilt University Medical Center (VU), where we documented 4.8% use rate in routine gyn surgical pathology cases with an average number of 2.4 stain per case where the IHC was used [10]. Additionally, we reported that the years of practice and time spent on gyn service significantly affected IHC use among pathologists, with less use for practitioners with more than 10 years of practice and more than 10 weeks/year of service. The specific aims of the current part 2 report were (1) to compare the use of IHC in diagnostic gyn pathology between 2 academic tertiary care centers, namely, VU and Ohio State University (OSU); (2) to identify what IHC stains are preferentially used in diagnostic gyn pathology; and (3) to document the value of the most commonly used IHC markers in diagnostic gyn pathology. Toward these aims, we retrospectively reviewed the IHC biomarkers used in diagnostic gyn pathology at the two academic centers, which are located at two different geographic locations.

2. Materials and methods

The laboratory information systems at VU and the OSU were queried for gyn surgical pathology cases that were evaluated between October

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http://dx.doi.org/10.1016/j.anndiagpath.2015.06.005
1092-9134/

2013 and September 2014. Cases in which IHC stains were used were identified and characterized in detail. In the current report, we documented the specific markers that were performed, the diagnosis, rationale for the most commonly used markers, and whether, in our judgment, any of the markers ultimately helped in the accurate categorization of the case. Referrals, outside consults, and gyn cytopathology cases were excluded from the study. Microsatellite instability for the mismatch repair genes is used as the reflex testing at VU, whereas IHC is the test of choice for the mismatch repair proteins at OSU for screening of Lynch syndrome in cases with endometrial carcinomas. Therefore, IHC performed for the mismatch repair proteins was excluded from the analysis of the current data.

3. Results

At OSU, IHC markers were used in 263 (8.7%) of 3028 cases, which is almost as twice as that at VU where IHC was used in 195 (4.7%) of 4168 cases, the ratio of which is almost maintained throughout the different organ systems (Table 1). Table 2 listed the commonly used IHC markers at the 2 institutions with 479 stains applied to 195 cases (2.5%) at VU compared to 446 stains applied to 263 cases (1.7%) at OSU.

3.1. P16, P53, ki-67, and WT1

P16 is a surrogate marker of the presence of high-risk human papilloma virus [11] and a protein that is frequently expressed in many high-grade neoplasms as a function of cell cycle dysregulation. P16 stain is nuclear and cytoplasmic and was evaluated as positive (moderate or strong diffuse staining in more than 10% of cells) (Figure E) or negative (mild, faint staining, or moderate or strong mosaic staining in less than 10% of cells) [12,13] (Figure B). P53 is a tumor suppressor protein containing transcriptional activation and DNA binding that responds to diverse cellular stresses to regulate other genes, thereby inducing cell cycle arrest, apoptosis, senescence, and DNA repair [14,15].

P53 immunoreactivity was interpreted as normal (wild type) in which the cells show heterogeneous weak p53 staining (Figure C), completely negative p53, in which no staining was found in any of the cells (which is surrogate marker for nonsense mutation of the *TP53* gene) and positive (excessive) p53 in which more than 85% of the cells of interest showed homogeneous moderate or strong immunopositivity [16,17] (Figure F).

The ki-67 antibody *MIB-1* clone is a well-known proliferation marker widely used in diagnostic surgical pathology. ki-67 stain was scored as low (≤10%), intermediate (>10% and ≤20%), and high (>20%) [18]. For the purpose of the analysis in the current report, ki-67− includes all cases reported as low (≤10%) positivity, while ki-67+ cases includes cases with intermediate (>10% and ≤20%) and high (>20%) positivity.

The most commonly used IHC stain at the 2 institutions was p16 (125 cases and 120 at VU and OSU, respectively) (Table 2). P16 was used with and without ki-67 mainly in establishing the diagnosis/grading squamous intraepithelial lesions (SIL) in the lower genital tract and differentiating endometrioid from serous carcinomas (Figure B and E).

Table 1
Comparison between VU and OSU in IHC use in diagnostic gyn pathology according to specific organ site.

	VU[a]	OSU
Vulva	22/236 (9.3%)	27/165 (16.4%)
Vagina	13/142 (9.2%)	23/118 (19.5%)
Cervix	92/1557 (5.9%)	57/673 (8.5%)
Uterus	51/1690 (3.0%)[b]	110/1359 (8.1%)
Fallopian tube	1/311 (0.3%)	3/425 (0.7%)
Ovary	16/232 (6.9%)	43/288 (14.9%)
Total	195/4168 (4.7%)	263/3028 (8.7%)

[a] See ref. [10].
[b] Eight cases stained for the mismatch repair proteins were excluded in the current report.

Table 2
Immunohistochemistry markers used at VU and OSU in diagnostic gyn pathology (cases where IHC was used/total number of cases = 203/4216 and 263/3028 at VU and OSU, respectively).

IHC marker	No. of stain	
	VU	OSU
p16	125	120
ki-67	69	45
p53	59	59
ER	35	34
Vimentin	10	32
PR	23	23
CD10	10	23
CK7	14	16
Inhibin	3	16
Desmin	7	14
WT1	6	12
CK20	8	12
ck5/6	6	11
CEA	7	10
SMA	4	10
Pax-8	10	0
Others	83	9
Total	479	446

Strong diffuse p16 stain positivity was documented in all cases of high-grade SIL, and negative in all cases of low grade SIL and reactive/benign lesions (Table 3). P16 was strongly and diffusely positive in all endocervical adenocarcinomas, serous carcinomas (Figure E), cervical squamous cell carcinomas and carcinosarcomas in contrast to negative (mosaic) staining in endometrioid carcinomas (ECs) (Table 4) (Figure B).

P53 was the second and third most frequently used IHC stain at OSU and VU, respectively (n = 59 at each institution) (Table 2). P53 was used in conjunction with p16 and/or ki-67 particularly in the diagnosis of serous carcinomas (p16+, p53+) (Table 4, Figure F) and establishing the subtle differentiated vulvar intraepithelial neoplasia (VIN) lesions (Table 5). P53 showed strong nuclear staining in 9 of 11 and 10 of 10 serous carcinomas at VU and OSU, respectively, and 1 of 1 clear cell carcinoma. P53 was negative (wild type) in all benign/reactive, endometrioid (Figure C) and miscellaneous carcinomas (Table 4). P53 staining was positive in all differentiated VIN (Table 5).

3.2. Estrogen and progesterone receptors

The IHC staining for these markers was used jointly in 23 cases at the 2 institutions, whereas 12 and 11 cases were stained with estrogen receptors (ER) alone at VU and OSU, respectively. The ER and progesterone receptors (PR) were requested as predictive markers. These 2 markers, mainly ER, were used in panels with other markers, for example, p16, p53, vimentin, and carcinoembryonic antigen (CEA), to assign tumors to specific site in the female genital tract. Table 6 summarizes the use of ER, in conjunction with p16, in the setting of differentiating endometrioid adenocarcinomas from serous carcinomas and in establishing the diagnosis of endocervical adenocarcinomas.

3.3. Melanocytic and spindle cell lesion markers

The use of markers of spindle cell lesions was not uncommon. Melanocytic markers, including S100 protein, HMB-45, and Melan-A were used in 9 and 5 cases at VU and OSU, respectively. The markers were used mostly in vulvar specimens to evaluate melanocytic lesions and to rule out malignant melanoma, a lesion which not uncommonly arises in this anatomic site. With further regard to smooth muscle markers, Desmin was the most commonly used (14 and 7 cases at VU and OSU, respectively).