Review

ACTA CYTOLOGICA

Acta Cytologica 2016;60:534–539
DOI: 10.1159/000446271

Received: April 13, 2016
Accepted after revision: April 19, 2016
Published online: June 2, 2016

Review of Cytology Practice at Thomas Jefferson University Hospital before and after High-Risk Human Papillomavirus Testing

Marluce Bibbo[a] Zi-Xuan Wang[b] Krister Jones[a] Charalambos Solomides[a]
Rossitza Draganova-Tacheva[a] Robert Stapp[c]

[a]Cytopathology Laboratory, [b]Molecular Biology Laboratory and [c]Pathology Informatics, Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, Pa., USA

Key Words
ThinPrep test · Papanicolaou test · Human papillomavirus · Reflex test · Cotesting · Cervical intraepithelial neoplasia

Abstract
Objective: We performed a retrospective review of Papanicolaou (Pap) testing to assess whether the cytology practice in our institution was affected by the introduction of high-risk (HR) human papillomavirus (HPV) assays over time. ***Study Design:*** Cytology, HPV and histopathology records were retrieved from our laboratory information system from 2003 to 2015. Records for Digene Hybrid Capture 2®, Hologic Cervista® and Roche Cobas® HPV assays were obtained. A 3-month follow-up for HPV detected cases was performed, and results were correlated with cytology and biopsies. A 1-year follow-up of HPV 16/18 and other HR HPV detected cases was also performed. ***Results:*** From 2008 to 2015, a noticeable decrease in Pap testing volume occurred, from 11,792 to 4,664, while the percentage of HPV testing increased from 19 to 59%. Similar HPV detection rates and follow-up results for both reflex and cotesting were observed in the 3 HPV assays. ***Conclusions:*** The decrease in Pap testing was due to the lengthening of the test interval when cotesting results were negative. Practitioners adhering to guidelines accounts for increased molecular testing volume. A trend towards higher-grade cervical intraepithelial neoplasia in the follow-up of detected HPV 16/18 was noted. So far there has been no demand for HPV as a stand-alone test.

Introduction

Over time, cervical cancer screening has evolved from a single glass slide smear to a test involving liquid-based processing and molecular HPV testing in the residual material.

Since 1988 the Bethesda System for reporting cervical/vaginal cytology introduced the atypical squamous cells of undetermined significance (ASCUS) category under epithelial squamous cell abnormality [1]. It was noted, however, by the editors of the 1994 monograph [2] that cervical/vaginal cytopathology includes an element of subjective interpretation and consequently the application of criteria should be viewed within this context. Nu-

KARGER

E-Mail karger@karger.com
www.karger.com/acy

0001–5547/16/0606–0534$39.50/0

Correspondence to: Dr. Marluce Bibbo
Department of Pathology, Anatomy and Cell Biology
Thomas Jefferson University Hospital, Main Building, Suite 260, 132 South 10th Street
Philadelphia, PA 19107 (USA)
E-Mail Marluce.Bibbo@Jefferson.edu

merous clinicians were unhappy with the growing number of ASCUS interpretations issued by cytology laboratories. In 2001 the America Society for Colposcopy and Cervical Pathology (ASCCP) issued guidelines for human papillomavirus (HPV) testing [3], and reflex HPV testing was recommended for cases interpreted as ASCUS in patients >20 years of age. When high-risk (HR) HPV was positive, the patient was referred to colposcopy and, when negative, returned to routine screening.

The use of HPV cotesting was approved as a cervical cancer screening test in the USA in 2003 and endorsed by the American Cancer Society (ACS) [4]. It was approved in combination with Papanicolaou (Pap) testing, for women 30 years or older every 3–5 years, but it was not approved as a stand-alone test.

Updated consensus guidelines from the ACS, ASCCP and American Society for Clinical Pathology were issued in 2012 [5]. In 2014 Roche Cobas® became the first HPV test approved by the FDA to replace the Pap test for primary screening of cervical cancer [6].

Assays for HPV DNA testing in our practice have included Digene Hybrid Capture 2 (HC 2)® approved by the FDA in 1999, Hologic Cervista® approved in 2009 for detection of HR HPV, and Roche Cobas® approved in 2011 for detection of HR HPV and genotyping HPV types 16 and 18.

The purpose of this retrospective review on Pap testing was to assess whether the cytology practice in our institution was affected by the introduction of HR HPV assays over time.

Materials and Methods

With institutional review board approval, we retrospectively reviewed cytology, HPV and histopathology records from our laboratory information system from 2003 to 2015. These records were obtained in collaboration with the pathology informatics director (R.S.).

No records for 2003, 2004, and 2005 HR HPV assays were found in our practice. Records for HC 2® HPV testing were obtained from 2006 to 2008, for Cervista® from 2009 to 2011 and for the Cobas® from 2012 to 2015.

The HPV assays were performed in the residual material of ThinPrep specimens by the molecular pathology laboratory.

A 3-month (January to March) follow-up of cases with detected HR HPV was performed for 2008 HC 2®, for 2011 Cervista®, and for 2013 Cobas®. The results of the 3 HR HPV assays were correlated with Pap cytology results. In addition, HR HPV positivity of various cytologic interpretations in Pap specimens and follow-up biopsies were obtained.

A 1-year follow-up of ASCUS cases with HPV 16/18 and other HR HPV detected by Roche Cobas® was also obtained for 2014.

Table 1. Testing volume and percentage of HR HPV tests

Year	Pap, n	Thin-Prep, n	HPV		Reflex		Cotest	
			n	%	n	%	n	%
2003	18,271	17,180	0	0	–	–	–	–
2004	14,858	14,389	0	0	–	–	–	–
2005	13,893	13,667	0	0	–	–	–	–
2006	13,518	13,294	12	0	12	100	0	0
2007	11,687	11,388	218	0	123	56	95	44
2008	12,158	11,792	2,239	19	441	20	1,798	80
2009	10,557	10,166	2,534	25	425	17	2,109	83
2010	9,583	9,235	2,920	31	650	22	2,270	78
2011	8,611	8,352	2,676	32	607	22	2,069	77
2012	6,870	6,708	2,841	42	448	15	2,393	84
2013	5,991	5,814	2,788	48	334	12	2,454	88
2014	5,234	4,999	2,862	57	344	12	2,518	88
2015	4,923	4,664	2,746	59	267	10	2,479	90

Total Pap tests, ThinPreps and HR HPV testing including reflex and cotesting for the years 2003–2015.

Results

Table 1 displays the volume of Pap tests (conventional and ThinPrep) and HR HPV assays performed from 2003 to 2015. No HPV tests are listed for 2003, 2004, and 2005, and a small number of tests are listed for 2006 and 2007. This table also shows the number and percent of reflex HPV and cotesting from 2006 to 2015. From 2008 to 2015, a noticeable decrease in Pap testing occurred from 11,792 to 4,664, while the percentage of HPV testing increased from 19 to 59%. The percentage of reflex HPV tests remained stable from 2008 to 2011 and showed a decrease from 2012 to 2015. The percentage of cotesting during the same time period remained relatively stable.

Testing volumes of all Pap tests, ThinPrep, and HR HPV from 2003 to 2015 appear in figure 1a. A progressive decrease in Pap test volume and an increase in HPV testing are seen over time. Figure 1b shows the volume of HPV reflex and cotesting for the years 2006–2015. A higher volume of cotesting compared to reflex testing is evident.

Table 2 shows a 3-month window of HR HPV testing at Thomas Jefferson University Hospital by 3 different assays: HC 2® in 2008, Cervista® in 2011 and Cobas® in 2013. The percentage of molecular tests increased from 18% in 2008 to 33% in 2011 and to 47% in 2013. Similar HPV detection rates in both reflex (32, 34, 27%) and cotesting (12, 10, 10%) in the 3 HR HPV assays were observed.

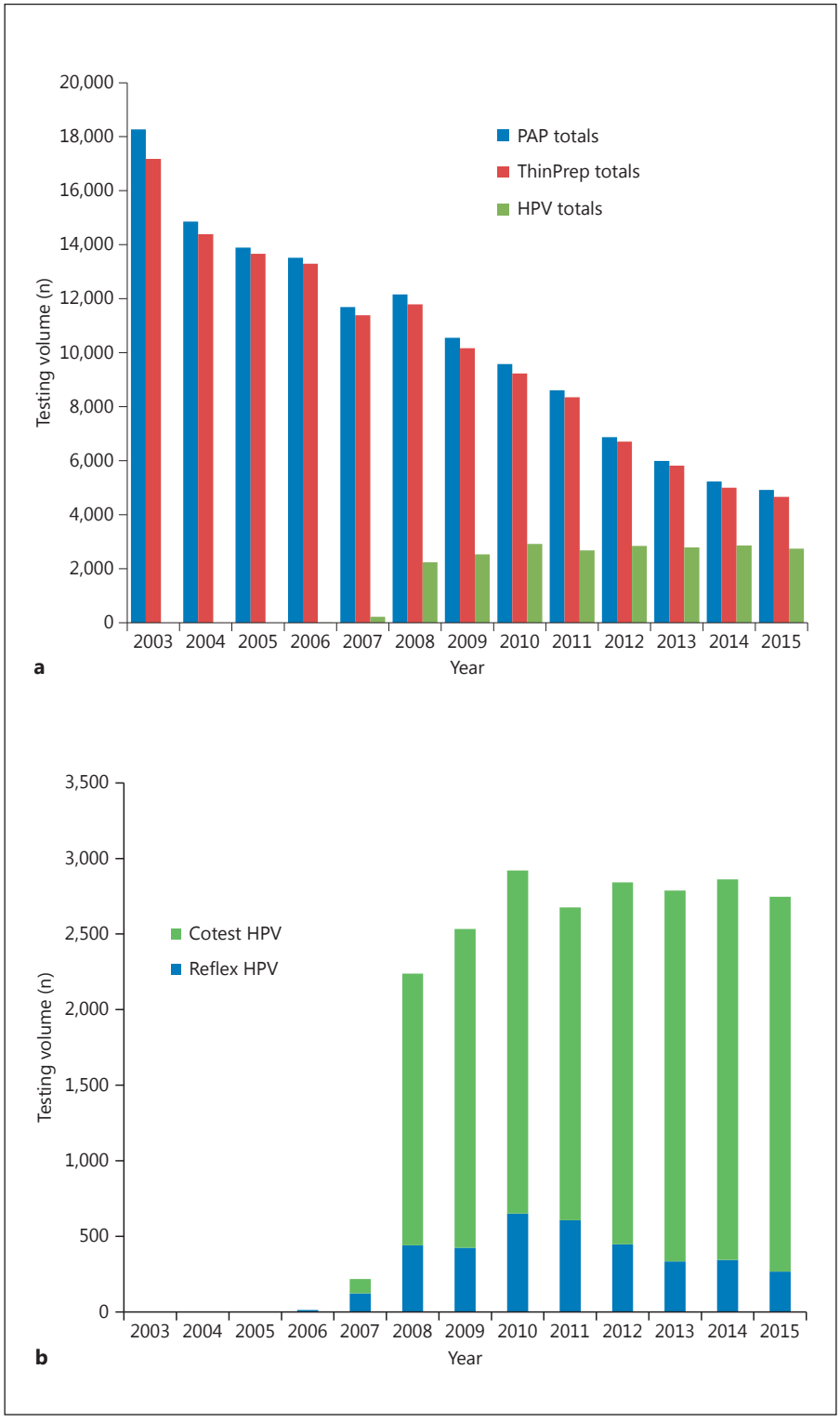

Fig. 1. a Testing volume of all Pap tests, from ThinPrep and HR HPV testing for the years 2003–2015; see table 1 for details. **b** HPV reflex and cotesting for the years 2003–2015.

Acta Cytologica 2016;60:534–539
DOI: 10.1159/000446271

Table 2. Three-month analysis of HPV testing from 3 assays used at Thomas Jefferson University Hospital

	Digene HC 2®	Hologic Cervista®	Roche Cobas®
Date range	January to March 2008	January to March 2011	January to March 2013
ThinPrep tests, n	2,757	2,236	1,547
Total molecular tests	514/2,757 (18%)	735/2,236 (33%)	732/1,547 (47%)
Reflex HPV	94/514 (18%)	207/735 (28%)	110/732 (15%)
HPV detected	30/94 (32%)	71/207 (34%)	30/110 (27%)
Cotesting	420/514 (82%)	528/735 (72%)	622/732 (85%)
HPV detected	50/420 (12%)	51/528 (9.6%)	64/622 (10%)

Table 3. Follow-up of HPV detected cases

HPV	Digene HC 2®	Hologic Cervista®	Roche Cobas®
Reflex from ASCUS			
Reflex total cases	94	207	110
HPV positive cases	30	71	30
Follow-up			
CIN 1	9 (30%)	25 (35%)	9 (30%)
CIN 2	4 (13%)	3 (4%)	4 (13%)
Negative CIN	5 (17%)	10 (14%)	5 (17%)
Negative Pap	7 (23%)	25 (35%)	7 (23%)
No follow-up	5 (17%)	8 (12%)	5 (17%)
Cotest			
Cotest total cases	420	528	622
HPV positive cases	50	51	64
Initial diagnosis			
Negative	35	33	47
Follow-up			
CIN 1	4 (11%)	1 (3%)	5 (11%)
Negative CIN	3 (9%)	4 (12%)	10 (21%)
Negative Pap	15 (43%)	12 (36%)	16 (34%)
No follow-up	13 (37%)	16 (49%)	16 (34%)
LSIL	13	11	13
Follow-up			
CIN1	7 (54%)	7 (64%)	7 (54%)
Negative Pap	6 (46%)	4 (36%)	6 (46%)
HSIL	2	7	4
Follow-up			
CIN 1	2 (100%)		
CIN 2		7 (100%)	4 (100%)

CIN 1/2 = Cervical intraepithelial neoplasia; LSIL = low-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion.

Table 4. ASCUS reflex HPV test by Roche Cobas®, 1-year follow-up

	Cases, n	Percent
ASCUS reflex HPV by Roche Cobas®		
Total ASCUS cases	344	100
Total detected	138	40
Total not detected	206	60
HR 16/18 positive	29	8
HR other positive	109	32
Follow-up biopsy for positive HPV 16/18		
Total biopsies	20/29	69
CIN 1	5	25
CIN 2	2	10
CIN 3	4	20
Negative CIN	9	45
Follow-up biopsy for positive HR HPV		
Total biopsies	55/109	50
CIN 1	21	38
CIN 2	2	4
CIN 3	3	5
Negative CIN	29	53

LSIL = Low-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion.

Table 3 shows the follow-up of HR HPV detected cases in both reflex and cotesting categories for the same 3-month window of HR HPV testing using HC 2®, Cervista® and Cobas®. For ASCUS cases where biopsy results were available, cervical intraepithelial neoplasia (CIN) 1 was frequently diagnosed, followed by CIN 2 and negative for CIN. Forty-three percent of ASCUS HPV cases detected by HC 2® (13/30 = 43%) and Cobas® (13/30 = 43%) showed CIN 1 or 2 on a follow-up biopsy

and 39% (28/71 = 39%) by Cervista®. Some cases were followed by repeat Pap tests, and some cases had no follow-up, as shown in table 3. Fewer cases of CIN 1 (4/35 = 11%, 1/33 = 3%, 5/47 = 11%) were found in the follow-up of negative cases with HPV detected by the 3 cotesting assays. Most cases diagnosed as low- and high-grade squamous intraepithelial lesions were confirmed by CIN 1 and CIN 2/3, respectively, in the histologic follow-up. No cases of invasive carcinoma were found.

Table 4 demonstrates the total number of HPV reflex tests of ASCUS by Roche Cobas® in 2014. One hundred thirty-eight cases out of 344 (40%) had HPV detected: 29 HPV 16/18 and 109 other HR HPV. The follow-up of these cases is also shown in table 4. A higher percentage of CIN 2 and CIN 3 was found in the follow-up biopsies of HPV 16/18 detected cases. No statistical analysis was performed, as the total case number is small.

To date, the molecular laboratory has no record of Cobas® HR HPV testing alone.

Discussion

In the last decade it has become clear that infection with HR HPV is required for the development of most cervical cancers and high-grade precursor lesions. Recent emphasis on molecular testing seeks to identify infection with HPV strains considered a high risk for carcinogenesis.

The clinical use of HPV testing started as additional screening for patients with ASCUS cervical cytology results in order to determine the need for colposcopy [3]. In our hospital practice, HR HPV reflex tests, automatically ordered by the cytology laboratory for ASCUS results, were performed from 2006 (when covered by insurance) and continue through the present.

In women 30 years and older, the HR HPV assay can be used in combination with cervical cytology to adjunctively screen for the presence or absence of HR HPV types. This information, together with the physician's assessment of the cytology history, other risk factors, and professional guidelines [5], may be used to guide patient management. Table 1 shows that HPV cotesting at Thomas Jefferson University Hospital started in 2007 and progressively increased from 44 to 90% by 2015. At the same time, the decrease in Pap testing is striking from 11,792 in 2008 when the percentage of HPV testing was 19%, to 4,664 in 2015 with 59% utilizing HPV testing. It is known that a negative HR HPV test represents a low risk of developing disease over 5 years and safely allows lengthening of the test interval. This rationale is reasonable to explain our observed decrease in Pap testing. Also noticeable is the percentage decrease in HPV reflex testing and increase in HPV cotesting. Most likely this was the result of increased adherence by practitioners to cotesting guidelines [5].

Of interest is the similar HR HPV detection rate in both reflex (32, 34, 27%) and cotests (12, 10, 10%) for the 3 HPV assays, as shown in table 2.

The follow-up biopsies for ASCUS cases, when available, revealed CIN 1 or 2 in 43% of cases detected by HC 2® and Cobas® and 39% by Cervista®. Several biopsies showed absence of CIN, and quite a few cases were followed only by negative Pap tests. For cotesting, 3–11% of negative Pap tests showed CIN 1 in the follow-up biopsy. The majority of cases had only negative Pap tests for follow-up. Most low-grade squamous intraepithelial lesion cases were confirmed by cervical biopsy and all high-grade squamous intraepithelial lesion cases were confirmed by histology as shown in table 3. It is not surprising that no cases of cervical carcinoma were found, since our female population is at low risk for cervical cancer.

Table 4 illustrates the number of HPV reflex tests with the Cobas® platform in 2014, separating the HR HPV 16/18 (29 = 8%) from other HR HPV types (109 = 32%). Follow-up shows 69% of the HPV 16/18 cases had subsequent biopsies, in comparison to 50% for other HR HPV cases. A trend towards higher CIN 2/3 for HPV 16/18 was noted.

The lack of demand for HR HPV stand-alone testing in our institution is of interest. Major changes to screening guidelines in the last decade include initiation of screening at the age of 21 years, conservative management of young women with abnormal cytology, extended screening intervals for women aged ≥30 years and cessation of screening in low-risk women over the age of 65 years [5]. HR HPV is a prerequisite for the development of almost all types of cervical cancer, therefore HR HPV has become an integral part of new screening strategies. With the FDA approval of the first HPV test for primary cervical cancer screening of women ≥25 years [6], clinicians in the USA now have 3 different first-line screening options that they may offer to patients: the Pap test, cotesting with Pap and HPV, and HPV testing as a stand-alone test [7]. The choice of the cervical screening method may vary for a variety of reasons including patient and provider preference along with geographic, socioeconomic, and practice settings.

Conclusions

It is evident in our practice that ThinPrep Pap testing decreased from 11,792 to 4,664 (60%) over 8 years, while the percentage of HPV molecular testing (reflex plus cotesting) increased from 19 to 59%. The decrease in Pap testing is most likely due to an increased interval between tests when cotesting results were negative. The HPV detection rates assessed during a 3-month interval showed that the 3 HR HPV assays used, namely HC 2®, Cervista®, and Cobas®, detected similar rates of HR HPV. A trend was noted towards higher-grade CIN in the follow-up of HPV 16/18 detected cases. So far, the choices of the cervical screening method in our practice include the Pap test, HR HPV reflex test and cotesting with no demand for the HPV stand-alone test.

Disclosure Statement

None of the authors have any relevant financial disclosures to report.

References

1 The 1988 Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses. Developed and Approved at the National Cancer Institute Workshop, Bethesda, Maryland, USA, December 12–13, 1988. Acta Cytol 1989;33:567–574.

2 Kurman RJ, Solomon D: The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses. Berlin, Springer, 1994.

3 Wright TC, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ: ASCCP-Sponsored Consensus Conference. 2001 consensus guidelines for the management of women with cytological abnormalities. JAMA 2002;287:2120–2129.

4 Massad LS: Guidelines for cervical cancer screening from the American Cancer Society. J Low Genit Tract Dis 2003;2:87–88.

5 Saslow D, Solomon D, Lawson HW, et al: American Cancer Society, American Society for Colposcopy and Cervical Pathology and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. Am J Clin Pathol 2012;137:516- 542.

6 Huh WK, Ault KA, Chelmow D, et al: Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Gynecol Oncol 2015;136:178–182.

7 Nayar R, Goulart RA, Tisconia-Wasserman PG, Davis D: Primary HPV screening for cervical cancer in the United States – US Food and Drug Administration approval, clinical trials and where we stand today. Cancer Cytopathol 2014;122:720–729.