The Clinical Significance of Human Papillomavirus Type in Women with Recurrent Cytological Atypia

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ABSTRACT: **Background:** The human papillomavirus (HPV) test has proven to be efficient in triaging women with abnormal Pap findings in women with low cytological atypia, but there is no data about the accuracy for large loop excision of transformation zone in cases of recurrent atypia.

Objectives: To assess the clinical correlation between results of HPV typing and conization histology in women who had recurrent abnormal Pap test results with no colposcopy findings.

Methods: Our retrospective cohort study included 138 women enrolled in the Maccabi Healthcare Services who had consecutive atypical Pap test results for 2 years in which no abnormal colposcopic findings were detected. These women had an HPV typing and then conization.

Results: Among the total study population (n=138), 71.7% had negative histology, 19.6% had ≤ cervical intraepithelial neoplasia grade 1 (≤ CIN1), and 8.7% had CIN2+. With regard to HPV typing, 34.8% were negative and 65.2% were positive. Of those testing positive, 34.4% were positive for HPV 16 or 18. Sensitivity, specificity, positive predictive value, and negative predictive values of HPV typing for women were 89.7%, 44.4%, 38.9%, and 91.7%, respectively, and for HPV 16 or 18: 71.4%, 67.7%, 32.3%, and 100.0%, respectively. After stratification by cytological grades, for women with high-grade cervical cytology, the sensitivity and negative predictive values of the HPV typing were higher than among low-grade cervical cytology, while specificity and positive predictive values were lower.

Conclusions: HPV typing is a useful tool for the management of patients with persistently abnormal Pap test results.

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The first time a woman has atypical cells of undetermined significance (ASCUS) cytology, she has three options:

- Colposcopy
- Pap test after 6 and 12 months and colposcopy if one test was found positive
- Human papillomavirus (HPV) test and colposcopy if high-risk strain is found

Women having low-grade squamous intraepithelial lesion (LGSIL) and abnormal cytology are referred for colposcopy [1,2].

There are currently no clear guidelines for women who have recurrent abnormal Pap test results with no colposcopy findings. In these cases, diagnostic large loop excision of transformation zone (LLETZ) is an option. Because the obstetric implications of the LLETZ procedure is required in defining a high-risk population among this group, the HPV test for high-risk types may be helpful.

HPV test has proved to be efficient for the triage for colposcopy in women with low cytological atypia [3], but there is no data about the accuracy of triage for LLETZ in cases of recurrent atypia. The aim of this study was to understand how to use HPV typing in the management of women who had recurrent abnormal Pap test results with no colposcopy findings.

PATIENTS AND METHODS

This retrospective cohort study was comprised of 138 women enrolled in the Maccabi Healthcare Services who had consecutive atypical Pap test results for 2 years with no colposcopy findings, including endocervical curettage (ECC), between 2009 and 2013.

HPV typing was performed at Maccabi facilities for two reasons: recurrent atypia in Pap test results with no abnormal colposcopy findings and a 6 month following conization with histological cervical intraepithelial neoplasia (CIN) margins [4].

Women with an atypical Pap test result were divided into two groups:

- High-grade cervical cytology: at least one high-grade result in one Pap test result within 2 years, including high-grade squamous intraepitheliel lesion (HGSIL), atypical squamous cells that cannot rule out HGSIL (ASCH), and atypical glandular cells (AGC)
- Low-grade cervical cytology: including LGSIL and ASCUS in a Pap test result within the 2 years.

The women enrolled in the study had an HPV typing and then conization performed at the same clinic in Tel Aviv under

the observation of the director of the Women's Health Center, Maccabi Healthcare Services. Conization was performed using LLETZ under local anesthesia. Conization results were divided into two groups: ≤ CIN1 for women with CIN1 or lower and CIN2+ for women with CIN2 or worse.

To perform the HPV DNA detection and typing, 5 µl of the DNA was extracted and tested for the presence of HPV. The f-HPV/Genomed kit (Genomed Diagnostics, Wollerau, Switzerland) was used according to the manufacturer's instructions. The kit uses 15 primers amplifying within E6 and E7 regions of the HPV genome. Extracted DNA was amplified using a multiplex polymerase chain reaction (PCR) with a set of 14 fluorescently labeled primers recognizing HPV 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 (high-risk HPV). A human short tandem repeat analysis was used as an internal control. This sequence is added to check DNA integrity and PCR inhibitors. Different labeling of primers allows amplicons to be generated during the same PCR reaction. The kit uses five dyes for detection technology. The presence of HPV was detected as fluorescent peaks in the electrophoretogram. The type was assessed by size and color of the peak, corresponding to one or more of the 15 types [5].

Demographic data of the patients (age, birth country, religion, and nationality) were collected from the Maccabi Healthcare Services database and the Israeli Ministry of Interior.

The study was approved by the institutional review board at Maccabi Health Services.

STATISTICAL ANALYSIS

Descriptive analysis was performed. Validity measures (sensitivity, specificity, positive predictive value, and negative predictive value) were used for detecting CIN2+ for the whole population and for stratified data according to cytological grade, using histologic conization results as the gold standard. Statistical Analysis System (SAS) software release 9.1.3 (SAS Institute Inc., USA).

RESULTS

The demographic characteristics of the study population are presented in Table 1. The mean age and standard deviation of the study population was 38.7 ± 9.3 years. Of the women, 96.3% were Jewish and 76.8% had been born in Israel.

In the total study population (n=138), 71.7% had negative conization histology, 19.6% had ≤ CIN1, and 8.7% had CIN2+ [Table 2]. With regard to HPV typing, 34.8% were HPV negative and 65.2% were HPV positive. Of the HPV positive group, 34.4% were positive for HPV 16 or 18 [Table 2],

For women having high-grade cervical cytology (n=26), 19.2% were HPV negative and 80.8% were HPV positive. Of those who were positive for HPV, 42.9% were positive for HPV 16 or 18 [Table 2], 76.9% had no dysplasia in the conization histology, 11.5% had ≤ CIN1, and 11.5% had CIN2+. In the HPV negative group no one had positive conization histology [Table 2].

For women having low-grade cervical cytology (n=112), 38.4% were HPV negative and 61.6% were HPV positive. Of those who were positive for HPV, 31.9% were positive to HPV 16 or 18 [Table 2]. No dysplasia was found for 70.5% in the conization histology, 21.4% ≤CIN1, and 8.0% CIN2+ [Table 2].

Table 3 describes sensitivity, specificity, positive predictive value, and negative predictive value of HPV typing for women found to be positive for HPV, and especially for women found to be positive for HPV 16 or 18. Validity measures are described for the total population, for those with high-grade cervical cytology and for those with low-grade cervical cytology in association with high-grade cervical cytology in colposcopy (CIN2+).

Table 1. Demographic characteristics of the study population

		HPV positive		HPV negative (n=48)	Total (N=138)
		Total (N=90)	High risk HPV (n=31)		
Age, years (mean ± standard deviation)		39.0 ± 10.0	37.3 ± 7.7	38.2 ± 8.1	38.7 ± 9.3
Age group, years, n (%)	20–34	42 (46.7)	13 (41.9)	19 (39.6)	61 (44.2)
	35–49	34 (37.8)	17 (54.9)	25 (52.1)	59 (42.8)
	50–64	13 (14.4)	1 (3.2)	4 (8.3)	17 (12.3)
	65+	1 (1.1)	0	0	1 (0.7)
Religion, n (%)	Jewish	81 (94.2)	24 (85.7)	48 (100.0)	129 (96.3)
	Other	5 (5.8)	4 (14.3)	0	5 (3.7)
Birth country, n (%)	Israel	68 (75.6)	22 (71.0)	38 (79.2)	106 (76.8)
	Other	22 (24.4)	9 (29.0)	10 (20.8)	32 (23.2)

Table 2. HPV typing in correlation with conization histology

		CIN2+	≤ CIN1 + HPV	Negative
Total population (n=138)				
HPV positive	HPV 16,18 (n=31)	6	4	21
	HPV others	5	20	34
	Total (N=90)	11	24	55
HPV negative (N=48)		1	3	44
High-grade (n=26)				
HPV positive	HPV 16,18 (N=9)	1	0	8
	HPV others	2	3	7
	Total (N=21)	3	3	15
HPV negative (n=5)		0	0	5
Low-grade (n=112)				
HPV positive	HPV 16,18 (n=22)	5	4	13
	HPV others	3	17	27
	Total (n=69)	8	21	40
HPV negative (n=43)		1	3	39

Table 3. Sensitivity, specificity, positive predictive value, and negative predictive value of HPV typing for women found to be positive for HPV, and especially for women found to be positive for HPV 16 or 18. Validity measure are described for the total population, for those with high-grade cervical cytology and for those with low-grade cervical cytology in association with high-grade cervical cytology in colposcopy (CIN2+). Sensitivity, specificity, positive predictive value, and negative predictive value are expressed as percentages

	Total population (n=138)		High grade (n=26)		Low grade (n=112)	
	Total	HPV 16, 18	Total	HPV 16, 18	Total	HPV 16, 18
Sensitivity	89.7	71.4	100.0	100.0	87.9	69.2
Specificity	44.4	67.7	25.0	38.5	49.4	75.0
Positive predictive value	38.9	32.3	28.6	11.1	42.0	40.9
Negative predictive value	91.7	100.0	100.0	100.0	90.7	90.7

DISCUSSION

We aimed to determine whether HPV typing can improve the ability to identify women with CIN2+ in those with consecutive cytological atypia.

We have shown high sensitivity and low specificity of HPV typing in both low-grade cervical cytology and high-grade cervical cytology women. These findings correspond with those observed in an evaluation performed using the Cobas HPV assay [6], which were compared to the results of colposcopy (tissue biopsy). In the evaluation made by the researchers, a clinical endpoint of CIN2+ was considered positive, the sensitivity was 91.4%, and specificity was 31.2% [6]. Our results may be associated to the high prevalence of HPV positivity found in our study population (65.2%), based on women with recurrent cytological atypia. Veijalainen and colleagues [7] showed a similar proportion among a repeat LGSIL group, where 68.6% were positive for high-risk HPV.

The high sensitivity of HPV typing found in our study indicates the ability of the test to correctly identify those presenting as CIN2+ (low false negative rates) and suggests high contribution in applying HPV typing to avoid unnecessary procedures. The low specificity of HPV typing found in our study indicates the low ability of the test to correctly identify those not having CIN2+ and high false positive rates, implying an increase in both the burden on the healthcare system (high cost) and the anxiety and worry induced in those women, both unnecessary. Li et al. [8] showed that HPV typing demonstrates high sensitivity (77.4%) and low specificity (48.5%) in detecting CIN2+.

We found high negative predictive value in all patients (low-grade and high-grade), implying that women with a negative HPV typing result most probably do not have CIN2+, thus unnecessary referral for conization procedures, intervention with potential complications, and obstetric implications may be avoided [9].

We have shown that the presence of HPV 16 or HPV 18 did not increase the sensitivity of HPV typing to identify CIN2+ but increased the specificity. These findings were also reported at the Mayo Laboratories [6] in their evaluation of the Cobas HPV assay using HPV 16 or 18 with the same clinical endpoint (CIN2+) where lower sensitivity of 51.9% and higher specificity of 86.6% were reported.

The high-sensitivity and low-specificity values among women with high-grade cervical cytology emphasize that HPV typing is redundant among this group and conization should be performed immediately. Veijalainen and co-authors [7] showed that among LGSIL patients, the sensitivity and specificity of high-risk HPV testing using the Hybrid Capture 2 or Abbott Realtime instruments (94.1% and 35.6%, respectively) were almost similar to the sensitivity and specificity found in our study (91.7% and 44.4%, respectively). The authors concluded that a high prevalence of HPV infection combined with a low specificity limit the use of HPV typing in the case of LGSIL [7].

The low positive predictive value found indicates that although women were positive in HPV typing, it does not necessarily mean they have CIN2+. This fact raises doubts about the effectiveness of the test among these patients.

HPV typing has already proven to be useful for the optimization of triaging strategies among women with low-grade cervical cytology [7,10,11]. The ALTS study [11] suggested that HPV DNA testing, under certain circumstances, could be used in a triage scheme for women with low-grade cervical cytology to determine if colposcopy is needed.

HPV typing may reduce unnecessary conizations using management strategies in low-risk patients [12,13]. In an evaluation to find the most efficient and cost-effective management strategy for women in the United States diagnosed as having ASCUS, HPV typing (and cytology) provided the same, or greater, life expectancy benefits and was more cost-effective than other management strategies [12]. Nakamura and colleagues [14] reported that women with LGSIL cytology who test negative for at least eight of the highest risk types of HPV (16, 18, 31, 33, 35, 45, 52, and 58) may not need immediate colposcopy and biopsy, thus reducing by 40% the number of expected referrals. Persson and collaborators [15] reported a low risk of developing high-grade cervical disease among HPV negative patients, highlighting the high contribution of HPV test in helping inform clinical management.

To the best of our knowledge, this study was the first in Israel to included patients with persistent cytological atypia for whom management of their disease is unclear. Our results may help gynecologists with this important issue. The internal validity of the study is high since conizations were performed in a single clinic.

LIMITATIONS

This retrospective study comprised a low number of patients. All cases were tested at a single clinic, thus the external valid-

ity is questionable. Data regarding other risk factors, including smoking, were not accessible.

Based on our results, we recommend that women with repeat Pap smear results that indicate HGSIL should be referred for conization. In women with repeat Pap smear results indicating ASCUS or LGSIL with no colposcopic correlation, typing may be useful. The high negative predictive value of the HPV typing justifies conservative follow-up of women with negative results to avoid unnecessary conizations.

CONCLUSIONS

HPV typing is a very useful tool in the management of patients with persistent abnormal Pap test results, especially when HPV 16 or 18 present. Our study population was homogeneous and the results should be verified in different populations, for example in women with high incidence of HPV infections.

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References

1. Massad LS, Einstein MH, Huh WK, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. *J Low Genit Tract Dis* 2013; 17: S1-27.
2. Schejter E, Siegler E, Vaknin T, et al. Managing patients with abnormal cytology (Pap test) from the cervix - Position Paper Number 113. Available at: https://cdn.mednet.co.il/2015/04/%D7%A2%D7%93%D7%9B%D7%95%D7%9F-%D7%A0%D7%99%D7%99%D7%A8-%D7%A2%D7%9E%D7%93%D7%94-113-%D7%A0%D7%99%D7%94%D7%95%D7%9C-%D7%9E%D7%A9%D7%98%D7%97-%D7%A4%D7%90%D7%A4-%D7%9C%D7%90-%D7%AA%D7%A7%D7%99%D7%9F.pdf [Hebrew].
3. Andersson S, Dillner L, Elfgren K, Mints M, Persson M, Rylander E. A comparison of the human papillomavirus test and Papanicolaou smear as a second screening method for women with minor cytological abnormalities. *Acta Obstet Gynecol Scand* 2005; 84: 996-1000.
4. Zielinski GD, Bais AG, Helmerhorst TJ, et al. HPV testing and monitoring of women after treatment of CIN3: review of the literature and meta-analysis. *Obstet Gynecol Surv* 2004; 59: 543-53.
5. Genomed. F-HPV typing TM Multiplex Fluorescent PCR Kit for Human Papilloma Virus (HPV) Genotyping. User's Manual. 2009. Available at: http://www.f-hpv.com/files/f-hpv%2021%202%202007-1.pdf
6. Mayo Foundation for Medical Education and Research. Test ID: HPV Human Papillomavirus (HPV) DNA Detection with Genotyping, High-Risk Types by PCR, ThinPrep. Available at: http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/62598. [Accessed September 12, 2016].
7. Veijalainen O, Tuomisaari S, Luukkaala T, Mäenpää J. High risk HPV testing in the triage of repeat ASC-US and LSIL. *Acta Obstet Gynecol Scand* 2015; 94: 931-6.
8. Li K, Yin R. Repeat cytology and human papillomavirus screening strategies in detecting preinvasive cervical lesions. *Medicine (Baltimore)* 2015; 94: e435.
9. Jin G, LanLan Z, Li C, Dan Z. Pregnancy outcome following loop electrosurgical excision procedure (LEEP) a systematic review and meta-analysis. *Arch Gynecol Obstet* 2014; 289: 85-99.
10. Söderlund-Strand A, Eklund C, Kemetli L, et al. Genotyping of human papillomavirus in triaging of low-grade cervical cytology. *Am J Obstet Gynecol* 2011; 205: 145.e1-6.
11. Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group. *J Natl Cancer Inst* 2000; 92: 397-402.
12. Kim JJ, Wright TC, Goldie SJ. Cost-effectiveness of alternative triage strategies for atypical squamous cells of undetermined significance. *JAMA* 2002; 287: 2382-90.
13. Mandelblatt JS, Lawrence WF, Womack SM, et al. Benefits and costs of using HPV testing to screen for cervical cancer. *JAMA* 2002; 287: 2372-81.
14. Nakamura Y, Matsumoto K, Satoh T, et al. HPV genotyping for triage of women with abnormal cervical cancer screening results: a multicenter prospective study. *Int J Clin Oncol* 2015; 20: 974-81.
15. Persson M, Elfström KM, Olsson SE, Dillner J, Andersson S. Minor Cytological Abnormalities and up to 7-Year Risk for Subsequent High-Grade Lesions by HPV Type. *PLoS One* 2015; 10: e0127444.

Capsule

Belimumab for the treatment of early diffuse systemic sclerosis

Gordon and colleagues assessed the safety and efficacy of treatment with belimumab in patients with early diffuse cutaneous systemic sclerosis (dcSSc) treated with background mycophenolate mofetil (MMF). In this 52 week, investigator-initiated, single-center, double-blind, placebo-controlled, pilot study, 20 patients with dcSSc started on MMF and were randomized 1:1 to also receive belimumab at 10 mg/kg intravenously or placebo. The authors assessed safety, efficacy, and differential gene expression. In the belimumab group, the median modified Rodnan skin thickness score (MRSS) decreased from 27 (interquartile range [IQR] 26.5, 31) to 18 (IQR 11, 23) (P = 0.039). In the placebo group, the median MRSS decreased from 28 (IQR 22, 28) to 21 (IQR 14, 25) (P = 0.023). The median change in MRSS was -10 (IQR -13, -9) in the belimumab group and -3.0 (IQR -15, -1) in the placebo group (P = 0.411). There were no significant differences between the groups in the number of adverse events. A significant decrease in expression of B cell signaling and profibrotic genes and pathways was observed in patients with improved MRSS in the belimumab group but not in the placebo group. Patients in both treatment groups experienced significant improvements in MRSS. The median difference was greater in the belimumab group but did not achieve statistical significance in this small pilot study. Adverse events were similar between the groups. Changes in gene expression were consistent with mechanism of action and showed that clinical response to treatment with belimumab is associated with a significant decrease in profibrotic genes and pathways. Additional studies are needed to determine the role of belimumab in the treatment of dcSSc.

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