A HIGHLY EFFICIENT ASSAY FOR DETECTION OF HIGH-RISK HPV E7 PROTEINS IN CERVICAL SAMPLES

I. Koch[1], M. Kellner[1], M. Fleischhauer[1], S. Fehrmann[1], H. Pfister[1], C. Reichhuber[1], E. Boschetti[2], A. Pesic[2], I. Hagemann[3], A.M. Kaufmann[2], K. Chatzistamatiou[4], T. Agorastos[4], P. Jansen-Duerr[5], E. Soutschek[1], O. Böcher[1]
[1]Mikrogen GmbH, Neuried, Germany, [2] Charité-Universitaetsmedizin Berlin CBF, Clinic for Gynaecology, Berlin, Germany, [3]abts+partner, Kronshagen, Germany, [4]Aristotle University of Thessaloniki, Depts of Obstetrics and Gynecology Hippokrateio Hospital, Thessaloniki, Greece, [5]Leopold-Franzens-Universität Innsbruck, Institute for Biomedical Aging Research Innsbruck Austria AND Tyrolean Cancer Research Institute, Innsbruck, Austria

Objective

Cervical cancer is the fourth most common cancer in women worldwide. Human papillomavirus (HPV) is detected in 99.7% of cervical cancer cases. Nucleic acid-based cervical cancer screening methods frequently pick up HPV infections without underlying disease, thereby leading to low test specificity. Hence, implementation of a triage method to colposcopy for HPV-positive women in the screening algorithm is necessary. Different triage methods such as cytology, methylation patterns of host and viral DNA, and p16/Ki67 overexpression are under investigation.
Overexpression of HPV oncoproteins E6 and E7 during disease progression leads to loss of cell cycle control and neoplastic transformation. Therefore, these proteins are potential biomarkers for detection of persistent HPV infection. Here we describe the performance of a novel assay for detection of high-risk (hr) HPV E7 proteins. The assay was developed, validated, and clinically evaluated during the EU-PIPAVIR project.

Methods

An hrHPV E7 sandwich enzyme-linked immunosorbent assay (ELISA) – *recom*Well HPV 16/18/45 – was developed for detection of hrHPV types 16, 18, and 45. Suitable for measurement of E7 protein are liquid-based cytological samples in PreserveCyte.
Cervical samples were obtained from 2424 women aged 30-60 who participated in different sub-studies of the PIPAVIR project. Exclusion criteria were history of cervical intraepithelial neoplasia (CIN) (treated or not), and pregnancy. Samples were characterized by cytology and HPV-genotyping; E7 measurements were performed with *recom*Well HPV 16/18/45. Women positive for cytology [atypical squamous cells of undetermined significance or worse] or hrHPV DNA were referred to colposcopy followed by biopsy, when needed, and/or endocervical curettage. Data were recorded using a web-based data capturing system.

Results

Calculation of sensitivity, specificity, PPV and NPV across all studies

Sensitivity (CIN2+/CIN3+/CxCa: 36.1/58.3/85.7%), specificity (>98%), positive predictive value (PPV) (CIN2+/CIN3+/CxCa: 59.5/56.8/16.2%) and negative predictive value (NPV) (>97.5%) were calculated across all studies with 1573 clinical samples.

E7 ELISA for Triage of women positive for HPV 16/18 DNA

1473 samples were analyzed for validity of E7-based triage for HPV16/18-positive women. 282 women were positive for hrHPV DNA testing and further subjected to colposcopy. For the detection of CIN2+ for HPV16/18-positive women without further triage, sensitivity and PPV were 100.0% and 11.11%, respectively. No triage of HPV16/18 positive women required 9 colposcopies to diagnose one case of CIN2+. The sensitivity of *recom*Well HPV16/18/45 was 100.0% (meaning that no CIN2+ case was missed) and PPV was 19.75%. The *recom*Well HPV16/18/45 identified all 16 CIN2+ cases, requiring 43.75% less colposcopies than no triage of HPV16/18 positive women.

	CIN2+	CIN3+	CIS / CxCa
Sensitivity	36.1%	58.5%	85.7%
	22/61	21/36	6/7
Specificity	99.0%	99.0%	98.0%
	1504/1519	1528/1544	1542/1573
PPV	59.5%	56.8%	16.2%
	22/37	21/37	6/37
NPV	97.5%	99.0%	99.9%
	1504/1543	1528/1543	1542/1543

Tab. 1: Calculation of sensitivity, specificity, PPV and NPV of *recom*Well HPV 16/18/45

	Sensitivity	Specificity	PPV	NPV
	n/N (%) 95% CI	n/N (%) 95% CI	n/N (%) 95% CI	n/N (%) 95% CI
	Exact Binomial test p-values	*McNemar test p-values*	*Weighted generalized score statistic p-values*	*Weighted generalized score statistic p-values*
HPV 16/18	16/16	–	16/144	–
	100.0%		11.11	
	(100.0-100.0)		(5.97-16.24)	
recom-Well HPV 16/18/45	16/16	63/128	16/81	63/63
	100.0	49.21	19.75	100.0
	(100.0-100.0)	(40.55-57.87) $^{*}p_{2v3}$, $^{*}p_{2v5}$	(11.08-28.42)	(100.0-100.0)

P-values less than 0.05 refer to statistically significant difference between different screening modalities (e.g. p_{2v4} refers to difference between screening modality 2 versus 4)

Tab. 2: Performance of E7 detection as a triage test for HPV 16/18 positive women to colposcopy (CIN2+ threshold).

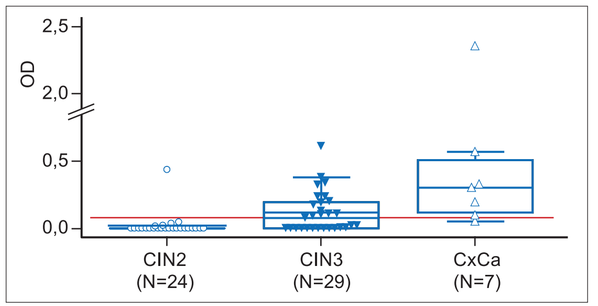

Fig. 1: E7 positivity in different disease stages of HPV 16, 18, or 45 positive samples

Discussion

Low specificity has proven to be an inherent disadvantage of HPV DNA testing: It leads to an increased number of colposcopies since HPV positivity does not reflect disease (i.e. CIN2+) but rather defines a high-risk population. In order to minimize this effect, it is necessary to implement an appropriate triage method for women tested positive for HPV to identify among them those with an already existing precancerous cervical lesion or cancer. Further triage using E7 positivity reflecting – subsequent to HPV infection – steps in cervical carcinogenesis may be beneficial. Indeed, it was shown that further triage lead to a 43.75% decrease in colposcopy referral for that group of women without any losses in sensitivity, i.e. without missing any CIN2+ cases.

Conclusion

E7 detection of hrHPV by ELISA is a feasible method to detect hrHPV infection and dysplasia. As there is evidence to suggest that E7 expression is up-regulated in high-grade dysplasia, the *recom*Well HPV 16/18/45 may offer great potential for detection of dysplasia with a higher specificity for disease than HPV DNA- or RNA-based tests, and could be a means of molecular triage in reflex testing of hrHPV positive screening results with HPV 16/18 genotyping. Our results support the detection of HPV E7 oncoprotein as a method of triage to colposcopy for HPV16/18 positive women (instead of no triage) in the framework of a screening program based on primary HPV screening with HPV 16/18 genotyping.

Literature

A New Sandwich ELISA Test Simultaneously Detecting E7 Proteins of HPV-16,18 and 45 in Cervical Smears. Metzger *et al.* Clin Microbiol 2016; 5:260
Human papillomavirus E7 protein detection as a method of triage to colposcopy of HPV positive women, in comparison to genotyping and cytology. Final results of the PIPAVIR study. Agorastos *et al.* (in review)

ECCMID 2017, April 22 – 25, 2017, Vienna