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Difference Between Amplicor And Linnear Array Tests for Detecting Hpv- Induced Cervical Abnormalities

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ABSTRACT

The aim of the study was to morphologically analyse cervical samples prepared with liquid-based cytology using transport medium residual samples collected for the detection of hr-HPV, and to genotype the samples by the reverse hybridization strip assay in each specimen. For detection of viral infection and detailed genotyping the Amplicor assay and Linnear Array methods were used. Cytological diagnoses were made according to the Bethesda classification. Cell abnormalities were observed in HPV positive samples, but also in samples that tested HPV negative. Detection of HPV infection showed infection with one viral type but in most cases infection was caused by more viral types. Between those two tests, difference was noticed, reflecting on the frequency and on the appearance of existing low and high grade cervical lesion. The sensitivity of these tests has been observed to be somewhat different, though not statistically significantly.

KEYWORDS : HPV, genotyping, cervical lesion, cytology

INTRODUCTION:

An infection with human papillomaviruses (HPV) is very common, especially among young women and adolescents. In spite of the generally benign outcome of these infections, certain types of HPV have the potential to transform cells and are associated with mucosal precanceroses or malignancies. The evidence of the importance of persistent human papillomavirus infection in women with cervical abnormalities has become very convincing [1,2].

The physicians' understanding of the natural history of HPV disease has significantly improved, due to the development of molecular methods and techniques. Detection of the infection relies on proving the viral DNA and for that purpose a range of different commercial and molecular tests have been developed [3-5]. The most used assay is HC2 (hybrid capture test) – a ready-to-use assay for routine diagnostics and detection of HPV infection, regardless of HPV specific types. Advantage of this method are: reproducibility, reliability and there is no need for specialized employees for performing diagnostic methods and presenting results. But, for clinical management of HPV-induced precancerous lesions is very important to be able to distinguish an individual type of HPV. For that, polymerase chain reaction (PCR) - based methods are used and shortly become a "gold standard" for detection of HPV infection. However, these methods are expensive; a completely equipped laboratory is required as well as educated employees for performing molecular methods and still different PCR methods shows different sensitivity and specificity. Amplicor HPV test (Roche Amplicor Human Papilloma Virus Test, Roche Diagnostic, Switzerland) - one of the standardized PCR-based tests for detection high risk HPV genotypes (hr-HPV) has been commercialised for detecting 16 different, hr-HPV genotypes. Currently, mostly used HPV genotyping tests are based on reverse hybridization of amplified HPV products on a membrane-bound probes, such is Linnear Array HPV Genotyping Test (Linnear Array Test, Roche Diagnostic, Switzerland).

The detection of the oncogenic, hr-HPV genotypes as well as an individual HPV genotyping had consequently become an important part of cervical carcinoma screening and detection of precancerous lesions. Aim of this study is to emphasize the importance of genotypisation in women with the cytological abnormalities of the cervix and proven HPV infection.

EXAMINEES AND METHODS:

In this study, the cervical specimens from 339 patients were collected with the cervix brush (Cervex-Brush, Rovers Medical Devices) which was placed in the PreserveCyt vial (Cytyc Corporation, Hologic) and after making a monolayed smears with ThinPrep 2000 processor (Hologic), they were analyzed. In each of the patient HPV genotyping was done, the type of infection (mono- or multi-type infection) was determined, and the presence of particular stage of dysplasia was cytologically diagnosed using Bethesda classification.

In 269 patients a certain degree of cytological abnormalities were found, so supplementary smears were made from the vials of those patients. Cervical cytology specimens from 70 patients of the same age, collected at the same gynaecological out-patient clinic and during the same period without proven HPV infection and with normal cyctological results, were used as a control group.

Residual solution in the PreserveCyt vials were used for detecting viral DNA by Amplicor Test as well as for individual genotyping with Linnear Array test.

Amplicor test was preformed for detecting HPV infection in cervical cells collected in liquid media. It is a qualitative test that utilizes amplification of target DNA by the PCR and hybridization for the group detection of 13 hr HPV genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68, but does not allow identification of individual genotypes. The test uses biotynalated PGMY primers to define a sequence of nucleotides within polymorphic L1 region of the HPV genome that is aproximatelly 165 base pairs long designed to amplify HPV DNA from 13 hrHPV genotypes. Following PCR amplification and denaturation, detection occurs by hybridization of amplicons to probes coated in 96-microwells plates. Additionally, for detailed genotypisation, the Linnear Array test was used. Linnear Array test is qualitative test, as well, and it utilizes amplification of target DNA by the PCR and hybridization for the individually detection of 37 anogenital HPV genotypes. The test uses biotynalated PGMY primers to define a sequence of nucleotides within polymorphic L1 region of the HPV genome that is approximatelly 450 base pairs long and designed to amplify HPV DNA from 37 HPV genotypes. Following PCR amplification and denaturation, detection occurs by reverse hybridization of amplicons to immobilized membrane-bound probes.

All steps of the analysis were performed in accordance with the man-

ufacturer's protocols for both tests with additional primer targets the human beta-globin gene to provide control for cell adequacy, extraction and amplification.

Both of the tests have CE mark approval for use as the diagnostic tests in Europe.

RESULTS:

In the population studied, cell abnormalities were cytological diagnosed in 77.05% of HPV positive samples, but cellular abnormalities were also found in 25.8% of the samples that tested HPV negative.

Genotypisation by Linnear Array test showed all together 15 different genotypes of HPV found in this study. Infection with HPV type 16 proved to be the most common (30.6%), followed in descending order of frequency by HPV- 51, -52, -31, -18, -58, -56, -45 and type 73 in 20.1%, 16.8%, 15.9%, 9.7%, 7.9%, 7.8%, 5.3% and 2.6% respectively. Mono-type infection (infection with single type of virus) was proven with Linnear Array test in 30.4% (82/269) cases, but in most patients - 69.5% (187/269), infection was caused by multi-type infection.

"Figure 1. about here?.

From each patient, samples were used for Amplicor test as well as for Linnear Array test. Between the tests used, difference was noticed in 44 of all samples –(12.9%; 44/339), as is shown in table 1. It is visible, that in spite of negative Amplicor result, in the 32 samples (72.2%; 32/44) certain types of HPV were found by Linnear Array Test. Seventeen of them were in high risk group, and 15 of them were of low risk types.

In twelve samples no viral type was found in spite of a positive Amplicor Test.

"Table 1. about here?.

Among Linnear positive and Amplicor negative patients, ten patients had cytological abnormalities (low grade lesion as a cut-off value for abnormality): eight of them had low grade epithelial abnormalities (LSIL) and two had high grade lesions (HSIL-CIN2 and HSIL-CIN3) proven by histology.

"Table 2. about here?.

Among Amplicor positive samples, 27.2% (12/44) of them were false positive, since in 12 samples none of the HPV types was detected by Linnear Array Test.

Adding the cytological data to the results of the patients with the proven HPV infection by Amplicor Test revealed 27.2% (53/195) of HPV positive patients in group with no cell abnormalities. In the group of patients with cellular abnormalities, HPV infection was confirmed among ASCUS, LSIL, HSIL and carcinoma cases in the samples, in 82.9% (39/47); 88% (103/117), 94.5% (52/55) and 100%, respectively. Results using Linnear Array test are slightly different, as is shown in table 2.

"Table 3. about here?.

The sensitivity as well as specificity of these tests has been observed to be somewhat dissimilar, though not statistically significantly ($p>0,05$).

"Picture 2. About here?.

DISCUSSION:

Ever since G. Papanicolaou described a method for detecting cancer cells in the cervical smears in the first half of the 20th century, this method called the Pap test, has been established as a gold standard for early detection of the precancerous and cancer lesions and soon has become a method of choice for cervical cancer screening programs all over the world [6]. There are lot of provide evidence that cervical lesions are associated with persistent infection with oncogenic types of HPV. Screening programs provide a way to identify the potential for disease in apparently asymptomatic patients, and since infection with HPV is completely asymptomatic, screening for the associated disease is of an extreme importance.

Currently, the recommendations for HPV DNA testing for cervical cancer screening include triaging patients with equivocal or low-grade cytological abnormalities [7], co-testing in combination with cytology in patients over 30 years of age to guide refferal for colposcopy, and it's recommended for prediction of the therapeutic outcome after treatment of cervical intraepithelial neoplasia (CIN) lesions [8,9].

Two HSIL, eight LSIL and four ASCUS lesions were found in the present analysis, among patients with discrepancy between the used tests. Similar results were found by other authors, as the Linnear Test has been used as a control test in cases of discrepancies between commercial HC2 (Hybrid Capture 2) test and Amplicor test [10,11]. The difference between the tests may be explained by the difference in the number of viral copy that has been used by tests as well as determinated positive diagnostic rate[12].

In this study, HPV infection was found in 27% of the patients with no cytology abnormalities. Prevalence of high risk HPV types was reported by many authors and is up to 10% of women in the general screening population with no cervical abnormalities, but results showed a lot of diversities. Correct result in detection of the HPV infection varies and depends on several variables like: exact patients population involved in the study [13], cytological status of the selected population, their age, occupation, and among others things -on the assay used for the HPV discovery [10,14-18].

After epidemiologic classification of HPV types associated with cervical cancer, made by Munoz et al. [18], many of performed meta-analyses confirmed these results all over the world, and revealed five most common genotypes as follows: type 16, 18, 45, 31 and 33 [14,17-20].

Analyses also exposed slight differences in the geographical distribution of hr-HPV genotypes around the globe, and the existence other oncogenic types of HPV which are responsible for cervical diseases [19,20]. Despite of those differences, there are far more similarities. Worldwide distribution of HPV types 16 and 18 was found in almost 70% of the cervical cancers, as well as in precancer lesions (HSIL)[21].

Recent studies by Snijders et al. have shown that HPV testing is about 45% more sensitive than cytology alone in detecting high-grade lesions, so it has been concluded that this is a good enough reason to use HPV testing alone for primary cervical screening [22].

Data from the ATENA study, introduced and clinically validated the Cobas HPV Test (Roche Molecular System. Inc) as a potential test for primary screening in women over 21 years. This study showed that risk of HSIL-CIN 2 or higher lesion in the population of HPV-type 16 positive women with no cervical abnormalities is up to 13.6%, and that among hr HPV negative women, estimated risk of developing HSIL is 0.3% and 0.8% for CIN 2 and CIN 3, respectively [23-25].

The ATENA study is based on an earlier US study by Khan et al. where the authors provide an option for managing patients ≥30 years with negative cytology and positive HPV type 16/18 [26]. These patients should be reffered to colposcopy, while those women positive for one of the other high risk types should be retesting within 12 months with cytology and another HPV testing.

In the Europe, Netherlands will probably be the first European country to adopt HPV testing as the primary tool for organised cervical screening. The Dutch Council for Health Care has advised the Ministry of Heath Care to introduce primary HPV testing in the cervical cancer screening program while cytology remains the option of choice for triage and management of high risk HPV positive patients [27].

In conclusion, it can be generally recommended that HPV genotyping should be included in the cervical cancer screening program in every country because the precise data on hr-HPV genotype distributions have implications for follow-up protocols in screening programs but also in achieving the highest sensitivity and specificity possible for diagnostic tests used for screening and for assessing the impact of an vaccine program.

Consensus on the number and types of HPV should exist in every country and has not been reached yet, hence further and extensive investigation is recommended.

TABLES AND PICTURES:

FIGURE 1. Genotype distribution and type of HPV infection in the study

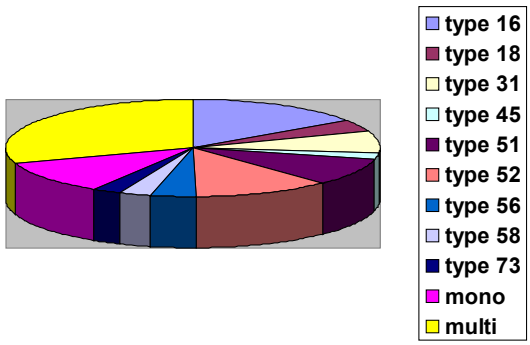


TABLE 1. Difference between Amplicor and Linnear Array Tests

	Linnear Array Test				
Amplicor test	High-risk type	Low-risk type	Total positive	Total negative	TOTAL
Positive	145	23	168	12	180
Negative	17	15	32	57	89
TOTAL			200	69	269

Se=84%; Sp=82,6%; PPV= 93,3%; NPV= 64% for Amplicor test

**Se= 93,3%; Sp=64%; PPV= 84%; NPV= 82,6% for Linnear Array test

(Se =sensitivity;Sp=specificity;PPV=positive predictive value; NPV= negative predictive value)

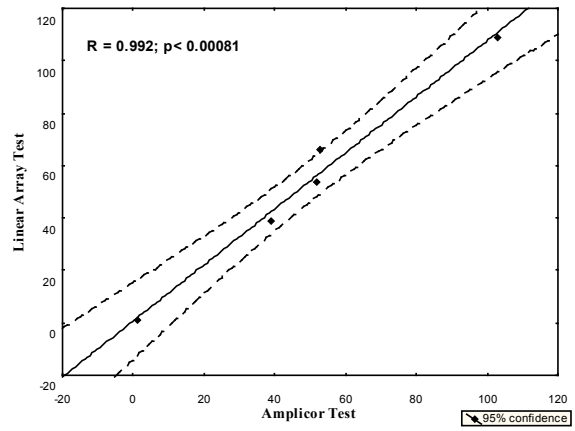
TABLE 2. Relationship between cytology data and HPV results with Amplicor Test and Linnear Array Test

	Amplicor test			Linear Array test		
Cytology result	Positive	Negative		Positive	Negative	TOTAL
No abnormalities	53	66		66	53	119
TOTAL No abnormalities	53	66		66	53	119
ASCUS	39	8		39	8	47
LSIL	103	14		109	8	117
HSIL (cin2+3)	52	3		54	1	55
Ca	1	--		1	--	1
TOTAL with abnormalities	195	25		203	17	220

TABLE 3. Results of Odds ratio test and Chi-square test for cytology data between Amplicor Test and Linear Array Test

Cytology result	Odds ratio test		Chi-square test		
	Odds ratio	95 % CI	95 % CI	c^2	P
No abnormalities	0.64	0.39 - 1.08	-7.63 to 23.03	0.599	0.4391
ASCUS	1.00	0.34 - 2.93	-	-	-
LSIL	0.54	0.22 - 1.34	-4.91 to 11.51	0.318	0.5728
HSIL (cin2+3)	0.32	0.03- 3.19	-3.59 to 10.79	0.220	0.6391
Ca	-	-	-	-	-
TOTAL (with abnormalities)	0.65	0.34 - 1.25	-	-	-

FIGURE 2. Results of linear correlation obtained for cytology data between Amplicor Test and Linear Array Test



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