Self-Collected Samples in Cervical Cancer Screening: Results of HPV and Pap Self-Collected Samples Compared to Physician-Obtained Specimens

Bernhard Reinhold Mangold

Zytolabor Ludwigsburg, Ludwigsburg, Germany

Keywords

Cervical cancer · Screening · Self-collected samples · HPV · Pap test

Abstract

Objective: In order to increase overall participation in cervical cancer screening, several investigators propose a concept of introducing self-tests. The study presented here compared test results of the Pap test and 4 different HPV test systems of self-collected and physician-collected vaginal specimens. **Study Design:** 208 patients of a colposcopy clinic had physician-taken and self-taken vaginal samples. All cell samples enabled a liquid-based Pap test and testing for carcinogenic HPV genotypes. In addition, all patients had a colposcopy with or without cervical biopsy and/or conisation. **Results:** 99 patients had the histological diagnosis of CIN2+. The HPV test sensitivity of self-collected samples differed significantly in this patient group depending on the test system performed. The sensitivity of the self-collected Pap test was significantly lower than HPV testing, but the positive predictive value of the Pap self-test was very high. **Conclusion:** The results of this study indicate that under the circumstances of self-testing HPV test systems differ in test

sensitivity and specificity. Self-collected Pap tests can provide a test result with a very high positive predictive value and introduce therapeutic strategies. In order to improve screening strategies, it could be an opportunity to combine HPV and Pap tests in self-taken vaginal samples, especially in countries with a low income level. In countries with a good medical infrastructure, self-testing has to be introduced with caution.

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Introduction

The discovery of the interrelation of HPV infections and carcinomas of the cervix together with the availability of standardized HPV tests has led to intense discussions about future cervical cancer screening strategies [1–3]. A number of established screening programs have already been supplemented with HPV testing, while in others the primary screening will be changed from cytologically based Pap testing to HPV testing [4–7].

Experts agree that the rate of participation plays a very important role in screening programs. Recent investigations have shown that the highest rate of cervical cancer

is found in the group of nonattenders [8, 9]. Therefore, in order to improve prevention strategies, several aspects of program organization have to be taken into account. For this purpose, invitation programs already exist in some countries [10]. Another suggestion is to implement modalities for the self-collection of samples [11–15]. A number of studies have demonstrated that HPV self-tests have a high sensitivity in the diagnosis of cervical (pre)cancer [16–18].

Even though the offer of the self-collection might motivate some people to participate in the screening program, the influence is limited. Some reasons for nonattendance, like anxiety about a cancer diagnosis, cannot be eliminated by self-collection of samples [19]. Furthermore, in case of a positive HPV test, the patients need a follow-up which is not possible without the direct contact to the practitioner. Sample self-collection raises the participant rate eventually only in the first step of the clinical diagnostic cascade.

This problem is tightened by the fact that HPV tests are very sensitive and detect also viral infections which will not require any treatment [20]. The low rate of the development of (pre)cancer may prevent patients with reservations to visit a practitioner also if they have a positive HPV test result. In addition, in some patients, it was even shown that the HPV infection of their vulva, vagina, and cervix differed in type and rate [21, 22]. This necessitates an exact sampling technique which is not possible with a self-sampling device [23, 24].

To further investigate the influence of the sample-taking method, this study compared results of self-collected and clinician-collected specimens. Samples of 208 patients from a colposcopy clinic were taken both by a clinician and by the women themselves. All cell samples were tested using different HPV tests and the cytological Pap test.

Materials and Methods

Attendees of a colposcopy clinic bear a high risk of having abnormal conditions of the cervix. After informed consent, 208 patients agreed to perform self-collection of a sample before their clinical investigation. The women were asked to insert a specially developed swab (Rovers® Viba-Brush) deep into their vagina without touching the labia. The swab was then diluted into fluid (PreservCyt®-Solution, Hologic) by a nurse. This procedure was followed by the investigation of the clinician. Under visual control (specula), the clinician collected cells from the cervix with a Rovers® Cervex-Brush with immediate dilution into PreservCyt®. This was followed by a colposcopy with iodine and acetic acid testing. If indicated, punch biopsies (PB) of the cervix were taken with

Table 1. Test results

	Self-test	Clinician-taken test
PAP test (ThinPrep®)	208	208
Punch biopsy		183
Cone biopsy		84
HPV		
Cervista®	208	208
Linear Array®	106	106
Aptima®	106	106
EUROArray®	106	106

Tischler-Morgan biopsy forceps. In some patients, a cone biopsy (CB) was performed after an interval, as a cold knife CB, a laser CB, or a large loop excision of the transformation zone.

All histological diagnoses of PB were evaluated in the same pathology laboratory. The histological diagnoses of CB specimens were evaluated independently in different pathology laboratories. Histological classification of intraepithelial neoplasia followed the WHO guidelines (WHO 2003; CIN1–3) in all cases [25].

From all self- and clinician-taken cell samples, a liquid-based cytology specimen (ThinPrep®2000 Prozessor; Hologic Inc., San Diego, CA, USA) was produced. This was followed by different HPV tests (Table 1). In a first group of 102 patients, a simple target hybridization (non-NAAT)-based method was used, followed by signal amplification (Cervista®; Hologic). The Cervista test targets the HPV gene regions L1/E6/E7 and offers qualitative detection of DNA from 14 high-risk (hr) HPV types. It cannot determine the underlying specific HPV type. In a second group of 106 patients, Cervista together with 3 other HPV tests based on target amplification (NAAT) were performed. The first was the Linear Array® HPV genotyping test (Roche Diagnostics GmbH, Mannheim, Germany). The Linear Array test is a qualitative PCR-based method (target region L1) which detects, in a type-specific manner, the DNA of 37 hr- and low-risk (lr)-HPV types. The second was the Aptima® HPV test (Hologic Inc.). The Aptima test is a transcription-mediated amplification-based method (target region E6/E7) for the qualitative detection of E6/E7 mRNA from 14 hr-HPV types. This test cannot determine the underlying specific HPV type. And the third was the EUROArray HPV (Euroimmun AG, Lübeck, Germany). The EUROArray HPV is a qualitative PCR-based method (target region E6/E7) which detects, in a type-specific manner, the DNA of 30 hr- and lr-HPV types. Based on the publication of Muñoz et al. [26] the detection of one or more of the 18 prescribed hr- and probable-hr-HPV types was counted as a HPV-hr-positive result.

The liquid-based cytology slides of 206 patients were screened and interpreted according to the nomenclature of Munich 2 and 3 used in Germany. The slides of the first 102 patients were interpreted by 2 cytologists independently, one cytologist was completely blinded. The nomenclature of Munich 2 and 3 was transformed into Bethesda criteria in the following manner: Munich group I/II = Bethesda negative, Munich group IIID/IIID1 = Bethesda LSIL, Munich group III = Bethesda ASCUS, Munich group IID2/IVa/IVb = Bethesda HSIL.

Fig. 1. HPV-hr test negativity in 106 patients with 4 different HPV tests.

Fig. 2. HPV-hr test positivity in 106 patients with 4 different HPV tests.

χ^2 tests were used to determine statistically significant differences between the self-taken and physician-taken samples. For the HPV testing, a contingency table of 2×2 was used, and for cytology a contingency table of 2×3 . If a number amounted to 0, the delta option was applied. With a significance level of $p < 0.05$, the difference between the clinician-taken and self-taken samples was considered significant.

Results

Histology

In this study, 183 of 208 patients (88%) had a PB of the cervix, and in 84 patients (40%) a CB was performed; 83 (40%) patients underwent both histological examination of a PB specimen and CB. In 18 cases (22%), the result of the PB (negative/CIN1 vs. CIN2+) differed from the histological result of the CB (7 cases with PB worse, 11 cases with CB worse); 99 (48%) patients had the diagnosis of CIN2+ in PB and/or CB, and 47 patients (23%) had the independent diagnosis of CIN3+ (1 invasive cancer) in PB and CB simultaneously.

HPV

Except for Euroimmun, all other HPV tests showed a lower rate of HPV-hr positivity in the self-test group than

in the clinician-taken test samples. Differences between test positivity (HPV-hr) and test negativity were higher in the self-test group (Fig. 1, 2). In clinician-taken test samples, there was a complete concordance of the 4 HPV-hr test results in 83 of 106 patients (78%). In the self-test samples, a concordance of the 4 test results was only found in 72 patients (68%).

There were also differences in test results between self- and clinician-taken test samples within the test systems. The highest rate of discordance between self- and clinician-taken test (HPV-hr positive/negative) was found in Aptima (14%). Euroimmun and Cervista had a discordance rate of 10 and 9%; the lowest rate of discordance was found in the Linear Array with 7%. The PCR-based test systems of Roche and Euroimmun allowed a comparison of test results not only in respect of HPV-hr/lr positivity but also in a type-specific manner. The Linear Array showed a discordance of HPV-hr types in self- and clinician-taken test samples in 20 cases (19%); under consideration of hr and lr types, the rate increased to 26%. The Euroimmun showed a discordance of HPV-hr types in 32 cases (30%); under consideration of hr and lr types, the rate increased to 47%.

A histological diagnosis of CIN2+ (PB and/or CB) had 99 of the 208 patients. The test sensitivity of the performed

Fig. 3. Differences between HPV-hr test positivity in 99 patients with CIN2+.

Fig. 4. Pap test result in 47 patients with CIN3+ (PB and CB, 1 invasive cancer).

Fig. 5. Pap self-test: LSIL.

HPV tests differed in this patient group significantly. Cervista and Aptima had in self-test samples a lower sensitivity than in clinician test samples (Cervista self-test sensitivity: 76%, clinician test: 92%; Aptima self-test sensitivity: 82%, clinician test: 96%). The Linear Array and Euroimmun tests had altogether a higher sensitivity and did not show a difference between self-test and clinician test samples with an overall sensitivity of 96% (Fig. 3).

Cytology

The overall Pap test results of the two different cytologists (102 patients) showed a concordance of test results (negative versus LSIL/ASCUS/HSIL) in the clinician test samples of 92% and in the self-test samples of 85%. A very high rate of concordance was found in the clinician test samples of patients with the histological diagnosis of CIN2+ (50 patients) with 98%.

The rate of abnormal Pap test results in all patient (208 patients) was in the clinician test samples 28% higher than in the self-test samples (73 vs. 45%). In the group of patients with the histological diagnosis of CIN3+ in PB and CB (47 patients), the concordance of the Pap test re-

Fig. 6. Pap self-test: HSIL.

Fig. 7. Self-test immunocytology (p16/Ki67 CINtec plus®): positive.

sult of the clinician test samples and histology was very high (91% HSIL). Even in the self-test sample group there was a concordance between cytology and histology of 45% HSIL. In addition, 23% of these self-test samples were diagnosed as LSIL or ASCUS (Fig. 4).

Discussion

The impact of the sampling technique differed between the test modalities investigated. Regarding test sensitivity, the highest difference between self and clinician testing was found in Pap tests (45 vs. 91% HSIL in the detection of CIN3+). It is well known that Pap test sensitivity varies dramatically, and this result emphasizes once more the importance of an optimal sampling technique in cytological screening.

The effect of the sampling technique on HPV test sensitivity depends on the test system. Cervista and Aptima (Hologic) showed a significant difference between self and clinician testing in this investigation. The PCR-based Linear Array (Roche) and Microarray (Euroimmun) did not show a difference in test sensitivity, even though the comparison of HPV-type-specific test results (I_r and/or hr) showed a difference up to 47%. Especially in healthy persons without (pre)cancer this is a problem, because test specificity will be lowered. Therefore, in a screening

based on HPV self-testing, several facts have to be taken into account. The sensitivity depends on the test system and reaches not in all test systems the sensitivity of clinician testing. On the other hand, highly sensitive test systems demand special skills in the technique of self-sampling [27]. An uncontrolled sampling technique (e.g., at home or with a vaginal lavage) bears the risk of measuring HPV infections not correlated to the cervix uteri and interacts negatively with test specificity.

Beside the aspects of different test systems and sampling techniques, the question of how to motivate patients for self-sampling arises. Just mailing sampling devices to patients will not be sufficient. However, advertising self-sampling bears the risk to influence negatively the habits of visiting a gynecologist. Alternatively, primary care providers could detect nonattenders and provide self-sampling devices under control. Older patients in particular neglect cervical cancer screening but visit physicians because of other health problems.

Finally, we need a strategy to manage patients with a positive HPV-hr self-test result. A useful method to increase the positive predictive value of the method would be to perform a Pap test within the available self-test samples (Fig. 5–7); 45% of patients with the diagnosis of CIN3+ had in this investigation a HSIL result in Pap self-testing. All patients with a HSIL result in Pap self-testing had the diagnosis of CIN2+.

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Statement of Ethics

The patients have given their informed consent.

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