

Primary cervical cancer screening by self-sampling of human papillomavirus DNA in internal medicine outpatient clinics

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Background: We determined whether testing of self-collected vaginal swabs for human papillomavirus (HPV) DNA can be used to screen for cervical disease within internal medicine outpatient clinics.

Patients and methods: In this prospective study, 560 patients visiting two referral outpatient clinics for internal medicine were asked to take an HPV self-sample. Acceptability of self sampling, HPV prevalence and cervical intraepithelial neoplasia (CIN) detection rate were evaluated.

Results: 435 women (78%) performed HPV self-sampling; 1.5% considered self-sampling to be difficult. 134 women (31%) tested positive for high-risk type of HPV. There were significant differences between HPV-positive and -negative women with respect to the following: mean age (42 versus 46 years), number of women aged <16 years at first coitus (35% versus 23%) and history of drug abuse (8.3% versus 2.6%). Colposcopy could be performed for 70 HPV positive women: CIN 1–3 was identified in 24%. Two of 52 women with HPV-negative results undergoing colposcopy had biopsy-confirmed CIN 1. Test performance for detection of CIN 2–3 after correction for verification bias: sensitivity, 100%; specificity, 71%; negative predictive value, 100%; positive predictive value, 10%. HPV persistence was associated with a 5.7-fold risk of CIN 2–3 detection at follow-up.

Conclusions: Self-assessment for HPV DNA is an easy, feasible and well-accepted method for HPV testing and for cervical cancer screening in internal medicine outpatient clinics.

Key words: cervical cancer screening, HPV, self-sampling

Introduction

Cancer of the cervix uteri is the second leading cause of cancer death among women worldwide, with an incidence estimated at about 500 000 new cases each year [1]. The human papillomavirus (HPV) has been identified as the major causal agent of the disease [2]. Up to 99.7% of cervical cancers have been found to carry the HPV genome [3]. Cytology-based screening for cervical cancer has effectively reduced cervical cancer associated mortality. However, the majority of cervical cancer cases (60%) are still associated with absent or deficient screening [4]. Some possible explanations for not participating in cytology-based screening programs include the inconvenience, time and discomfort often associated with obtaining Pap smears, especially in older patients. In addition, errors in cervical sampling or smear interpretation are common, resulting in a considerable decrease of sensitivity in detecting cervical cancer and its precursors, the cervical intraepithelial neoplasia (CIN) [5]. There are many missed opportunities for cervical cancer screening. Many women developing invasive cervical cancer without the benefit of recent Pap smear screening were seen in primary care outpatient clinics prior to their diag-

nosis [6]. Considering only Internal Medicine and Family Practice clinic visits, 70% had been seen at least once and 42% had been seen three or more times in the 3 years preceding their diagnosis.

Previous studies have demonstrated the usefulness of adjunctive human papillomavirus DNA testing as a complement to cytology in primary screening [7–10]. Patient-obtained vaginal samples (self-sampling) for analysis of HPV DNA has a sensitivity for detection of high-grade cervical lesions and invasive cancer that is equivalent or even superior to that of a Pap smear [11, 12]. In addition, self-sampling has the advantage of not requiring a vaginal speculum examination, thus reducing the discomfort that may make screening unattractive even among women who have access to health care.

In our study we evaluated patient-obtained vaginal samples to determine the feasibility and effectiveness of self-sampling for HPV DNA as a screening tool for cervical cancer in tertiary referral internal medicine outpatient clinics. We analyzed the acceptability of self-sampling, the HPV prevalence and persistence within this population, important demographic and sexual characteristics, CIN detection rate and HPV self-sampling test performance.

Patients and methods

Five hundred and sixty women attending two internal medicine outpatient clinics (main focus: oncology, hematology and gastro-enterology) of the

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University Hospital in Munich, Großhadern, were invited by a nurse to participate in this study. The single inclusion criterion was no history of hysterectomy. After written informed consent was obtained, for HPV self-assessment the participating women were asked to insert a sterile cytobrush about 5 cm into the vagina and to place it into a specimen collection tube. Written instructions consisted of a one-page leaflet. HPV DNA self-sampling was approved by the ethical committee of the medical faculty. HPV testing was performed with the Hybrid Capture System II (Digene, Gaithersburg, MA, USA), which detects 13 different high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). The test was run according to the manufacturer's protocol. HPV determination was quantitative, and women with samples producing readings of 1 or more times the positive control (1 pg/ml HPV DNA) were considered as HPV test positive.

Participants were informed about their HPV test results and the possible clinical significance by letter. The option of an appointment in our colposcopy clinic was offered to all participants when desired by the women.

Follow-up consisted of a postal questionnaire and a thorough gynecological examination including colposcopy: to all participants a questionnaire with 35 items was sent that included questions on demographic characteristics, sexual behavior (Table 1) and acceptability of self-sampling (Table 2). If no response was obtained after 1–2 months, a reminder letter was sent. Analysis of the questionnaire was performed with regard to the HPV status (HPV-positive versus HPV-negative). Analysis of the questions with regard to the acceptability of self-sampling differentiated between women <35 and ≥35 years of age. All HPV-positive participants were contacted by telephone and recalled for a gynecological examination and colposcopy. To enable calculations on test performance (sensitivity, specificity, positive and negative predictive value) of HPV self-sampling, an appropriate number of randomly selected (blinded to demographic characteristics) HPV-negative women were recalled as well ($n = 52$).

The gynecological examination was done at the colposcopy clinic at the Department of Obstetrics and Gynecology, University Hospital Munich, Großhadern, and followed a specific sequence: two Pap smears were obtained, one from the ectocervix (cotton tip swab) and one from the endocervix (cytobrush). A HPV DNA sample was obtained from the cervix with a cytobrush (HPV-doctor). Standard colposcopy was performed with acetic acid (3%) by experienced colposcopists. Directed biopsies were taken from presumed low- and high-grade lesions (CIN 1 or worse). In order to check the concordance between the HPV results taken by the physician (HPV-doctor) and those taken by the patient (HPV-patient), 52 consecutive patients were asked prior to the gynecological examination to take an additional HPV self-sample.

Cervical smears were classified according to the cytological classification most widely used in Germany (Münchener Nomenklatur) [13]: I, normal cytology; II, mild to moderate inflammatory, metaplastic or degenerative changes; III, squamous or glandular cells of unclear significance; IIID, mild to moderate dysplasia; IVa, severe dysplasia or carcinoma *in situ*; IVb, carcinoma *in situ*, invasion cannot be ruled out; V, invasive carcinoma. Histology was classified as CIN: CIN 1, mild dysplasia; CIN 2, moderate dysplasia; CIN 3, severe dysplasia/carcinoma *in situ*.

Statistical analysis

The sensitivity, specificity, positive and negative predictive value of the hybrid capture II results (self-assessment) were calculated using three different cut points for being test positive: (i) cytological smear ≥IIID or CIN ≥1; (ii) CIN 1/2/3; and (iii) CIN 2/3. As gold standard cytology results and colposcopy directed biopsy results were used. As probability for verification by gold standard differed between HPV-positive and -negative women, the correction for verification bias was performed [14]. Proportions were compared using the Chi-squared tests or Fisher's exact test when appropriate. The *t*-test or Mann-Whitney *U*-test were used for continuous variables. The agreement between HPV-doctor, HPV-patient and HPV test results at recruitment and at follow-up

was calculated using the κ statistic reflecting the overall agreement beyond chance. The relative risk (RR) for CIN in women with HPV persistence versus no persistence was determined. For proportions and relative risks, 95% confidence intervals (95% CI) were calculated.

Results

Of 560 women, 435 (78%) agreed to participate in the study and performed HPV self-sampling. Figure 1 shows the flow of participants through the study and the reasons for ineligibility and refusal. In 134 women (31%), the test for high-risk type of HPV was positive. Three hundred and thirty-nine (78%) questionnaires were collected for analysis of demographic parameters, which are summarized in Table 1. There were statistically significant differences between HPV-positive and -negative women with respect to mean age ($P < 0.001$), number of women with first coitus at ≤16 years of age ($P = 0.029$), age of menarche ($P = 0.047$) and history of drug abuse ($P = 0.018$). For history of cancer, the *P* value was 0.085. HPV-positive and -negative women did not differ significantly with respect to tobacco use or history of number of different lifetime coital partners. Mean number of Pap smears within the last 3 years was 2.9 for all women.

The acceptability of self-sampling was very good (Table 2): none of the women aged <35 years and only 1.9% of the women >35 years of age considered self-sampling to be difficult. Ninety-seven per cent of all patients were willing to perform self-sampling at home. Twenty-four per cent of patients aged >35 years would prefer self-sampling over a gynecological examination.

There was good agreement between HPV-doctor and HPV-patient for 52 consecutive participants at the follow-up visit ($\kappa = 0.71$; Table 3). Nine women were HPV-positive in the self-taken sample and six were positive in the doctor's sample. HPV DNA test results were concordant in 48 of 52 women (92%). Six were positive and 42 were negative on both tests. One woman testing negative by her self-obtained sample tested positive with the doctor's sample. Three women had a positive self-attained HPV sample and a negative doctors' sample.

Analysis of agreement between HPV test results at recruitment (self-sample) and at the follow-up visit (doctors' sample) was performed in 119 participants (Table 4). There was fair agreement between the HPV test results comparing the two time points ($\kappa = 0.24$). Sixty-seven (56%) women were HPV DNA positive at recruitment, and only 20 (17%) at follow-up. Forty-eight (72%) of the 67 formerly HPV-positive participants were HPV-negative at the follow-up visit, and only one woman (1.9%) that was HPV-negative at recruitment was later HPV-positive. HPV DNA test results were concordant in 70 of 119 women (59%). Nineteen were positive and 51 were negative in both tests.

The HPV test at follow-up was positive for four of 12 participants with CIN 1, three of five with CIN 2 and both women with CIN 3. Diagnosis of CIN 2–3 at follow up (mean 5.5 ± 2.5 months) was significantly associated with HPV persistence defined as a positive HPV test result at both time points (at recruitment and at follow up; relative risk, 5.7; 95% CI 2.9 to 11.3; $P = 0.001$). This translates into a risk fraction of 83% attributable to HPV persistence in women with CIN 2–3.

Table 1. Demographic characteristics of study population (questionnaire)

Characteristic	HPV high risk					
	Positive	VA (<i>n</i> = 134)	Negative	VA (<i>n</i> = 301)	All women	VA (<i>n</i> = 435)
Age, years; mean ± SD; <i>P</i> <0.001	42 ± 12.3	125	46.3 ± 11.9	274	45 ± 12.2	399
Gravidity, <i>n</i> ; mean ± SD	2.1 ± 1.1	64	2.3 ± 1.4	168	2.3 ± 1.3	232
Parity, <i>n</i> ; mean ± SD	1.6 ± 0.9	64	1.5 ± 0.9	169	1.6 ± 0.9	233
Age at first pregnancy, years; mean ± SD	23.1 ± 4.4	64	25.0 ± 5.3	166	24.5 ± 5.1	230
Menarche, years; mean ± SD <i>P</i> = 0.047	13.1 ± 1.5	94	13.5 ± 1.5	233	13.4 ± 1.5	327
Age of first coitus, years; mean ± SD	18.1 ± 4.2	96	18.4 ± 2.9	231	18.3 ± 3.3	327
Age of first coitus <16 years, <i>n</i> (%); <i>P</i> = 0.029	34 (35)	96	54 (23)	231	88 (27)	327
Lifetime partners, <i>n</i> (%)						
1–2	30 (32)	95	90 (39)	230	120 (37)	325
3–5	34 (36)	95	73 (32)	230	107 (33)	325
6–9	18 (19)	95	40 (17)	230	58 (18)	325
>9	13 (14)	95	27 (12)	230	40 (12)	325
Education, <i>n</i> (%)						
Low	26 (27)	96	80 (34)	237	116 (32)	333
Medium	41 (43)	96	82 (35)	237	123 (37)	333
High	29 (30)	96	75 (32)	237	104 (31)	333
Tobacco use, <i>n</i> (%)						
Current	22 (23)	96	44 (19)	236	66 (20)	332
Ever	31 (42)	74	77 (40)	191	108 (41)	265
Never	43 (58)	74	114 (60)	191	157 (59)	265
Ever on oral contraception, <i>n</i> (%)	84 (88)	96	196 (83)	235	280 (85)	331
History of an abnormal cytology result, <i>n</i> (%)	10 (11)	93	16 (6.8)	237	26 (7.9)	330
History of condyloma, <i>n</i> (%)	3 (3.2)	93	7 (3.0)	236	10 (3.0)	319
History of sexually transmitted diseases, <i>n</i> (%)	7 (7.6)	92	30 (13)	236	37 (11)	328
Genital herpes virus, <i>n</i>	1	–	10	–	11	–
Gonorrhea, <i>n</i>	1	–	3	–	4	–
Other, <i>n</i>	5	–	14	–	19	–
History of cancer, <i>n</i> (%); <i>P</i> = 0.085	19 (20)	95	29 (12)	235	48 (15)	330
History of drug abuse, <i>n</i> (%); <i>P</i> = 0.018	8 (8.3)	96	6 (2.6)	235	14 (4.2)	331
History of transplantation, <i>n</i> (%)	5 (5.3)	94	10 (4.2)	237	15 (4.5)	331
Cancer screening on a regular basis, <i>n</i> (%)	87 (91)	96	199 (84)	238	286 (86)	334
No. of smears within the last 3 years, <i>n</i> ; mean ± SD	3.1 (1.7)	90	2.8 (1.4)	233	2.9 (1.5)	323
History of laser treatment of the portio, <i>n</i> (%)	5 (5.6)	90	5 (2.2)	232	10 (3.1)	322

Overall return rate of the questionnaires, 78% (*n* = 339); return rate for HPV (+) women, 72.4% (*n* = 97); return rate for HPV (–) women, 80.4% (*n* = 242).

VA, no. of valid answers for each item.

P values (first column) are given only for characteristics when HPV-positive women were significantly different from HPV-negative women.

Detection rate for CIN

Adequate follow-up with regard to the gynecological examination and colposcopy (and directed biopsies if required) could be performed in 70 (52%) of 134 HPV-positive women (Figure 1). There were no differences between women with and without follow-up with regard to any of the demographic characteristics listed in Table 1, with the exception of number of smears within the last 3 years (2.9 with follow-up versus 3.5 without follow-up; *P* = 0.038).

Fifty-two (17%) of the 301 HPV-negative women served as a negative control group and were assessed by colposcopy in the same way as HPV-positive women. In the HPV-positive group (*n* = 70), a biopsy confirming CIN of any grade was identified in 17 women (24%), CIN 2/3 in seven women (10%) and CIN 1 in 10 (14%). Cytology grade ≥IIIID was identified in 16 (23%) women. In the HPV-negative group (*n* = 50), only two histologically confirmed cases of CIN 1 (3.8%) were identified, both of them

Table 2. Acceptability of self sampling

	Assessment group					
	<35 years (<i>n</i> = 72)	VA	≥35 years (<i>n</i> = 261)	VA	All women (<i>n</i> = 333)	VA
Practicability, <i>n</i> (%)						
Easy	65 (90)	72	122 (85)	261	287 (86)	333
Moderate	7 (9.7)	72	34 (13)	261	41 (12)	333
Difficult	0 (0)	72	5 (1.9)	261	5 (1.5)	333
Willing to perform self-sampling at home, <i>n</i> (%)	72 (100)	72	250 (96)	261	322 (97)	333
Preference of self assessment over gynecological examination, <i>n</i> (%)						
Yes	14 (20)	69	60 (24)	249	74 (23)	318
No	8 (12)	69	36 (15)	249	44 (14)	318
Both	47 (68)	69	153 (61)	249	200 (63)	318
Willing to pay for HPV self-test if available over the counter (£15–50), <i>n</i> (%)	39 (55)	71	141 (57)	246	180 (57)	317

VA, no. of valid answers for each item.

Table 3. Crosstable analysis: agreement of HPV status between sample taken by the doctor (HPV-doctor) and self-sample (HPV-patient) for 52 consecutive participants at follow-up

	HPV patient		
	Positive	Negative	All together
HPV doctor, <i>n</i> (%)			
Positive	6 (86)	1 (14)	7 (100)
Negative	3 (7)	42 (93)	45 (100)
All together	9 (100)	43 (100)	52 (100)

belonged to the cytological group IIID. There were no women with CIN 2 or 3 in the HPV-negative group. Table 5 shows the test performance before and after correction for verification bias. These values adjust for different probabilities of cytological and/or histological verification in HPV-positive versus HPV-negative women, and reflect the values that would be expected if follow-up had been performed in all women.

Comment

The mean age of all women participating in our study was 45 years. This reflects the older population attending clinics for internal medicine with the main focus on hematology, oncology and gastro-enterology. In our study we found a surprisingly high prevalence of high-risk type HPV (31%). The HPV prevalence in the general population is usually reported to be as high as 5–7% of women >32 years of age [5, 15]. The high HPV prevalence could partly be explained by demographic features of our population that differ from the general population: 15% had a history of cancer, 4.5% a history of organ transplantation and 4.2% a history

Table 4. Crosstable analysis: agreement between HPV test results at recruitment (self sample) and at the follow-up visit (doctors' sample); *n* = 119

	HPV status at follow-up		
	Positive	Negative	All together
HPV status at recruitment, <i>n</i> (%)			
Positive	19 (28)	48 (72)	67 (100)
Negative	1 (2)	51 (98)	52 (100)
All together	20 (17)	99 (83)	119 (100)

of drug abuse. HPV-positive women as compared with HPV-negative participants, however, were younger, more likely to have first sexual intercourse at an age <16 years, be younger at menarche, and more often have a history of cancer and of drug abuse. These are known risk factors for HPV infection and cervical cancer. Persistence of HPV infection is associated with high-risk HPV types and is more frequent in women aged >30 years than among those aged <24 years, indicating that older HPV-positive women may represent a subset of women with difficulty in clearing the infection [16]. About one-quarter of the HPV-positive women that have been followed up with colposcopy had histologically proven CIN. The high prevalence of high-risk type HPV DNA and CIN within our population demonstrates that women attending tertiary referral clinics for internal medicine should be considered as a high-risk population with regard to HPV DNA status and cervical neoplasia. HPV persistence was associated with a 5.7-fold risk of detection of CIN 2/3 at follow up. This means that in about four of five women with CIN 2/3, their histological result can be attributed to HPV persistence. These findings are in agreement with results of other studies demonstrating that persistent infection with high-

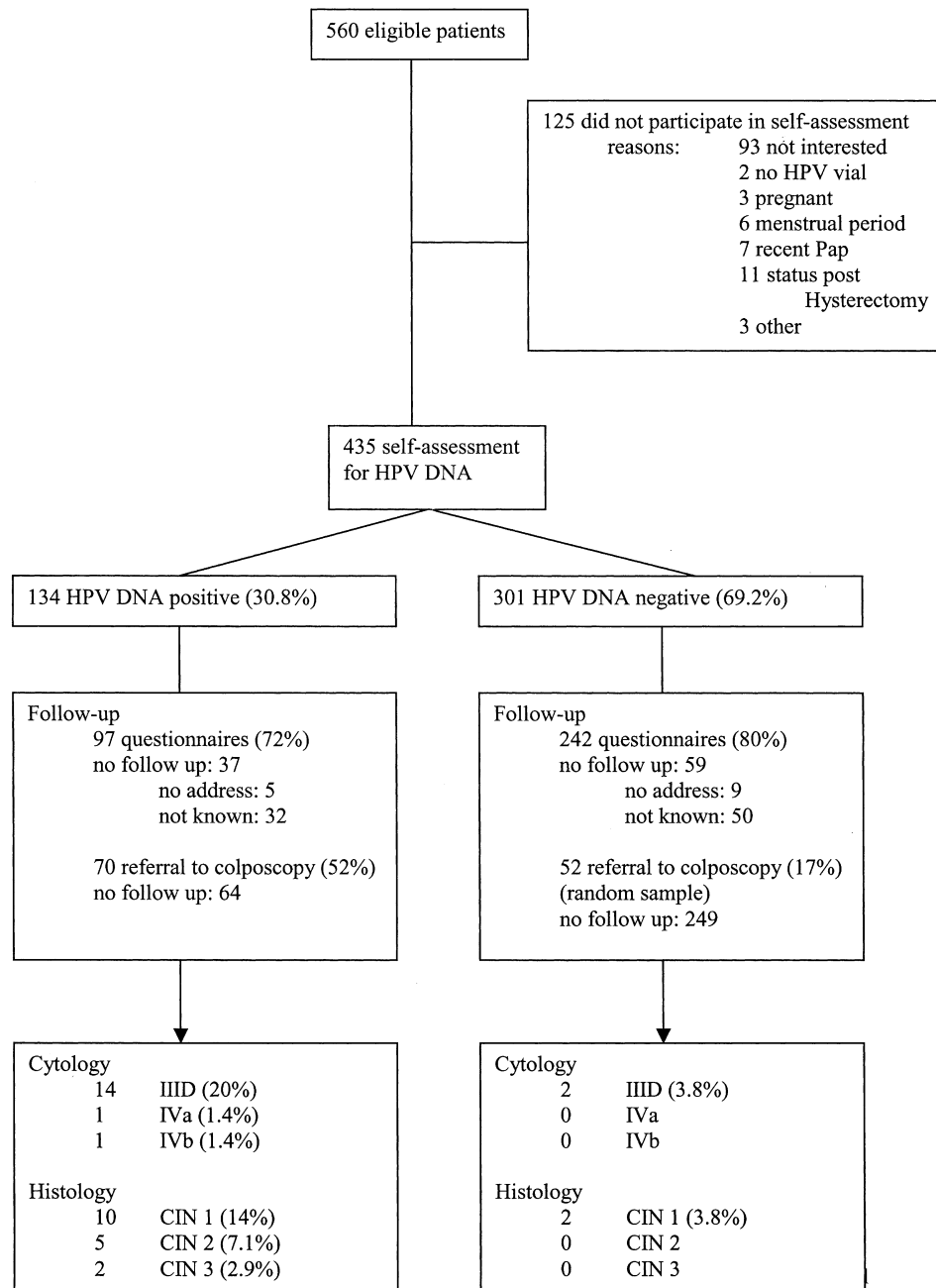


Figure 1. Flow of 560 patients through recruitment and screening.

risk human papillomavirus is an important factor for development and maintenance of CIN 3 [17].

On average, women participating in our study had one Pap smear per year, indicating that a well screened population had been analyzed in the care of an established health service. This is consistent with reports that 57% of women with invasive disease had a normal cytology result within 5 years prior to diagnosis, and that 47% of patients <70 years of age diagnosed with invasive cancer greater or equal to FIGO stage IB1 disease had a history of adequate cytology-based screening [18, 19].

With regard to detection of CIN 2 or 3 via self-sampling, our study demonstrated both an excellent sensitivity and negative

predictive value (each 100%). We used a cytobrush for self-sampling that has demonstrated a good sensitivity (92%) in detecting CIN 2–3 in an earlier study [12]. Other techniques (cervicovaginal lavage, tampon, Dacron swab) have shown lower sensitivities [11, 20]. Self-sampling with a cotton tip swab even missed 50% more cancers than did physician sampling, indicating that the cotton tip technique is not a safe method for the collection of samples aimed at primary cervical cancer screening [21]. The high negative predictive value associated with a high sensitivity supports the suggestion that screening intervals for women negative for high-risk HPV could safely be lengthened [5]. This offers possible cost-saving effects that could compensate for the increased screening

Table 5. Test performance of HPV self collection ($n = 122$)

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Crude analysis (no correction for verification bias)				
Smear \geq IIID or CIN 1/2/3 ^a	88.00 (67.66–96.85)	50.52 (40.24–60.75)	31.43 (21.15–43.76)	94.23 (83.08–98.50)
CIN 1/2/3 ^b	89.47 (65.46–98.16)	48.54 (38.65–58.54)	24.29 (15.17–36.26)	96.15 (85.67–99.33)
CIN 2/3 ^c	100.00 (56.09–100)	45.22 (36.01–54.75)	10.00 (4.45–20.10)	100.00 (91.43–100.00)
Analysis corrected for verification bias				
Smear \geq IIID or CIN 1/2/3 ^a	70.8	76.8	31.4	94.2
CIN 1/2/3 ^b	73.8	74.0	24.3	96.2
CIN 2/3 ^c	100	71.4	10	100

Women with the following results were regarded as having a positive result.

^aSmear \geq IIID or CIN \geq 1 on histological examination.

^bOnly CIN \geq 1 on histological examination.

^cOnly CIN 2 or CIN 3 on histological examination.

All other women were regarded as having a negative result, respectively.

expenditures caused by HPV testing. Such cost-saving effects have been demonstrated recently by several investigators [22–25].

Analysis of the participant's answers with regard to acceptability of self-sampling by a cytobrush reflected ease of use: only 1.5% of 333 women considered self-sampling to be difficult, 97% were willing to perform self-sampling at home and 57% of the participants would pay for the HPV self-test if it was available over the counter. The high acceptance of self-sampling could further decrease cervical cancer mortality, bearing in mind that most cases of cervical cancers are associated with absent or deficient screening [4].

This study has several limitations. Not all women received verification by histology or cytology; however, a substantial fraction of verified results were available in both groups and a formal correction for verification bias could be performed. In the HPV-positive group the only difference between women with and without follow-up was the higher number of smears within the last 3 years in the group not followed up. This indicates that the groups that have been followed up are representative for the whole study population. The results of this study, however, cannot easily be generalized to the normal population, because in our study screening took place in tertiary centers. Positive and negative predictive values are dependent on the prevalence of CIN, which is expected to be higher in the study population compared with the general population. Therefore, PPV may be lower and NPV higher in the general population compared to the reported values.

For HPV (self-)testing to be cost effective in primary screening, it is necessary to develop an effective policy for the management of women who test positive for high-risk HPV types.

Taking into account our and related findings [26] we would like to propose the following algorithm for cervical cancer screening: for women between 25 and 30 years of age, cytology screening every second year should be the preferred screening method, because HPV testing in this age group would detect too many transient HPV infections that are not associated with high-grade CIN. For women over 30 years of age we recommend HPV testing every 3 years. This can be accomplished by a gynecologist or,

alternatively, as self-sampling. In case of a test result positive for a high-risk HPV type it should be repeated after 6 months. Again, surveillance reduces the detection of transient HPV infections, and of low-grade lesions, which have a high spontaneous regression rate. If the second HPV test also detects a high-risk HPV type then colposcopy with cytology and directed biopsies is indicated. One aim of cervical cancer screening is to include as many women as possible. Regarding this, HPV self-sampling could contribute to the improvement of our screening. However, these suggestions have to be tested in future studies with regard to costs and outcomes.

Our data show that self-sampling offers the chance of cervical cancer screening not only in gynecological office practices, but also in other medical fields such as internal medicine. Self-assessment for HPV DNA is an easy, feasible and well-accepted method of testing for HPV and for cervical cancer in tertiary referral internal medicine outpatient clinics.

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