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Human papillomavirus testing on self-sampled cervicovaginal brushes: An effective alternative to protect nonresponders in cervical screening programs

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Women not attending cervical screening programs are at increased risk of cervical cancer. We investigated in these nonresponders to what extent offering self-sampling devices for cervicovaginal brushes for high-risk human papillomavirus (hrHPV) testing would induce participation and, if so, what the yield of precursor (*i.e.* CIN2 or worse) lesions following self-sampling would be. In addition, we assessed screening history of participants and costs per detected high-grade CIN2 or worse ("CIN2+") lesion in comparison to the regular program in the Netherlands. Nonresponders received a device for hrHPV testing (self-sampling group, n = 2,546) or an extra recall for conventional cytology (control group, n = 284). The percentage of self-sampling responders were compared with responders in the recall group. hrHPV positive self-sampling responders were invited for cytology and colposcopy. CIN2+ yield and costs per detected CIN2+ were evaluated. Active response was higher in the self-sampling than in the control group (34.2 *vs.* 17.6%; $p < 0.001$). hrHPV positive self-sampling responders were less likely to have a prior screening history than screening participants ($p < 0.001$), indicating that they are regular nonresponders. hrHPV prevalence was similar (8.0 *vs.* 6.8%; p = 0.11), but CIN2+ yield was higher in self-sampling responders compared to screening participants (1.67 *vs.* 0.97%; OR = 2.93, 95% CI 1.48–5.80; p = 0.0013). Costs per CIN2+ lesion detected *via* self-sampling were in the same range as those calculated for conventional cytological screening (€8,836 *vs.* €7,599). Offering self-sampling for hrHPV testing in nonresponders is an attractive adjunct to effectively increase population coverage of screening without the adverse effect of markedly increased costs per detected CIN2+ lesion.

Key words: cervical screening program; human papillomavirus; self-sampling; nonresponders; cervical cytology

Screening programs have contributed to a decline of incidence and mortality of cervical cancer.[1–4] However, nonresponse remains an important problem of current screening programs.[5–7] In the Netherlands, women between 30 and 60 years of age are invited to cervical screening at 5-year intervals. The active participation rate is 63%. Of the remaining women, 9% respond by declining the invitation for various reasons such as pregnancy, breastfeeding, history of hysterectomy or smear having been taken by any other occasion, leaving 28% women who do not respond at all (hereafter referred to as "nonresponders").[8] Nonresponders in the screening program are at high risk for development of cervical cancer, since at least 50% of women diagnosed with cervical cancer in the United States, the UK and the Netherlands had no history of participation in cervical screening.[1,2,9–11]

Women who do not respond to invitations for conventional smears may be inclined to respond to a self-sampling technique,[12–18] but this has never been tested in the setting of a regular screening program. Although the sensitivity for high-grade cervical intraepithelial neoplasia (CIN) of cytological specimens obtained from self-sampled vaginal material is lower than the sensitivity of conventional cytology, studies have shown that self-sampled cervicovaginal specimens (SSVS) are highly representative for the high-risk human papillomavirus (hrHPV) DNA status of the cervix.[14–16,19,20] Since hrHPV infection has been established as the primary cause of cervical cancer in nearly all cases,[21,22] hrHPV detection on SSVS could be a valuable tool to identify nonresponder women at risk of cervical cancer.

In the present study, we investigated within the setting of a regular screening program to what extent offering hrHPV testing on SSVS leads to participation of nonresponder women. In addition, we compared the screening history of the hrHPV positive women who submitted a sample and compared this with that of age matched-, regular screening program participants. Finally, the yield of high-grade CIN as detected by hrHPV testing on SSVS in nonresponders against that found by conventional cytology in screening responders was evaluated as the costs per detected high-grade CIN lesion or cervical cancer (CIN2+) in both groups of women.

Material and methods

Study participants

The study was initiated as an intervention trial in addition to the regular population-based cervical screening program. The protocol was approved by the multicenter research ethics committees of the Erasmus University Medical Center, Rotterdam and VU Medical Center, Amsterdam. We selected 2,830 nonresponder women between 30 and 50 years of age at invitation, who according to the Regional Health Council Database (in the area Amstelland/de Meerlanden and Kennemerland) had neither responded to the regular invitation nor to the first 6 months reminder between January 2003 and April 2004. Upper age limit of 50 was chosen because of low hrHPV positivity in women aged 50 or more. The time from the last 6 months reminder to the start of the study was at least 5 and at most 24 months. Women were assigned to either the self-sampling cohort or the control group at a 9 to 1 ratio, based on their randomly assigned invitational-procedure number in the database. The 9:1 ratio was chosen because the control group was used only for comparison of response rates, whereas the study

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group size was based on our estimate of the expected yield of CIN2+ lesions. Since an initial small pilot study ($n = 100$) revealed that 30% of nonresponders submitted a SSVS (unpublished data), we calculated that at least 2,500 women should be included in the self-sampling group to detect CIN2+ with a standard error of 0.5%. For comparison of CIN2+ yield and viral positivity with screening participants, data were compared with those of age-matched women ($n = 6{,}208$) who participated in a population-based screening (*i.e.* POBASCAM) trial in the same region. The POBASCAM trial is a randomized prospective cohort trial that was conducted within the regular screening population in the same area (1998 to 2002).[23]

Women of the self-sampling group received a self-sample kit with instructions and an explanatory letter, whereas those of the control group received an extra recall for regular cytology with an explanatory letter. The kit for SSVS consisted of a Viba-brush® (Rovers Medical Devices, Oss, The Netherlands), a collection tube containing 5 ml Universal Collection Medium (UCM; kindly provided by Digene Corporation, Gaithersburg, USA), instructions (written and drawn) and a return envelop. UCM is a preservation medium with universal properties that allows not only liquid-based cytology but also several DNA-based assays such as hybrid capture 2 and PCR. The latter has been tested in a recent study.[24] Women were asked to return the SSVS collection tube (with the brush) within a padded envelope to the laboratory for hrHPV testing. As outcome measure for the response rate, we included all women who responded within 6 months after sending the kit, or, in case of the control group, who responded within 6 months after having received their second reminder.

Procedures

Upon arrival in the lab, SSVS samples were vortexed for 10 sec and the brush removed, after which the sample was concentrated to 1 ml by spinning down for 10 min at 3,000*g* and removing 4 ml of the supernatant. For PCR purposes, 150 μl of the concentrated sample was taken and centrifuged in a 1.5-ml reaction tube for 10 min at 3,000*g*. The supernatant was subsequently removed and the pellet resuspended in 1 ml 0.01 M Tris-HCL pH 7.4. This suspension was frozen for at least 1 hr at −80°C, thawed and then boiled at 100°C for 10 min. After centrifugation for 1 min at 3,000*g* followed by a short vortexing step, 10 μl of this crude suspension was ultimately used in the PCR. Testing for hrHPV was conducted by the consensus primer GP5+/GP6+-PCR with an enzyme immunoassay, read-out using a cocktail oligoprobe mix for 14 hrHPV types (HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), as described before.[25] A β-globin PCR was performed for quality assessment of the samples.

All women received a written test result and explanation. In case of an invalid sample (*i.e.* β-globin PCR negative material), women were asked to repeat the self-sampling test. All hrHPV positive women were invited for additional cytology, colposcopy and biopsy.

Cervical smears were classified according to the CISOE-A classification, the standard classification in the Netherlands for cervical cytology, which can be easily translated into Bethesda nomenclature.[26] Histology results were classified in a 5-tiered classification system consisting of the following categories: no dysplasia (CIN 0), mild dysplasia (CIN 1), moderate dysplasia (CIN 2), severe dysplasia or carcinoma *in situ* (CIN 3) and invasive carcinoma.

During the entire study period, a telephone number was available for questions about hrHPV infection, cervical cancer and the self-sampling test. After receiving kits, some 20 telephone calls were received. This amount almost doubled after sending out results of the hrHPV test. Main questions were about acquisition of the virus and perceived gravity of the situation.

Statistical analysis

Response rates of the self-sampling and control group were compared with the χ^2 test. Differences in hrHPV prevalences and in CIN2+ detection rates between the self-sampling group and the POBASCAM cohort of age-matched responders were assessed using Cochran–Mantel–Haenszel testing. Regarding CIN2+ detection, women in the POBASCAM cohort were directly referred for colposcopy in case of a cytological reading of moderate dyskaryosis or worse (which is equivalent to HSIL according to the Bethesda classification). Since for these women hrHPV status was irrelevant for direct referral, the blinded and unblinded groups were pooled.

hrHPV-positive women in the self-sampling group who had been invited for at least one prior screening round were tested for differences in historical screening attendance. A difference in attendance at the previous screening round 5 years earlier was assessed by Cochran–Mantel–Haenszel testing. Women who had been invited for at least 2 screening rounds were also tested on a difference in attendance at any of the previous 2 screening rounds. Attendance at the previous screening round or at any of the 2 previous screening rounds (regular interval is 5 years) was assumed if a smear was taken within the last 7 or 12 years, respectively.

Cost-effectiveness calculations

We compared the costs and effects of conventional cervical cancer screening in the POBASCAM cohort to the additional costs and effects of offering self-sampling to nonresponders. The effects were measured by the number of detected CIN2+ lesions found in the POBASCAM and self-sampling study group at baseline. Calculated costs per medical procedure included direct medical costs and indirect costs of travelling and production loss.[27] Cost calculations involved summing the costs of the screening procedures, colposcopies, biopsies, CIN treatments and follow-up after treatment. To assess the impact of self-sampling on the country level, total costs and detected CIN2+ were rescaled to the whole population in the Netherlands where 750,000 women are yearly invited and 63% attend screening.[28]

Results

Response rates

A total of 2,546 SSVS packages were sent to nonresponder women belonging to the self-sampling group, whereas a second reminder (consisting of a regular invitation form plus a letter explaining the importance of attending the screening program) was sent to 284 women in the control group. A flowchart of the study design is given in Figure 1.

Of the self-sampling group, 194 (7.6%) women responded by returning a prepaid postcard indicating their reason for not participating, which included breastfeeding/pregnancy, previous hysterectomy, treatment by a gynecologist or other reasons. Of the remaining 2,352 women of the SSVS group, 70 (3.0%) responded to the self-sampling package by visiting the general practitioner for conventional cytology (without using the SSVS kit), whereas 736 women (31.3%) returned SSVS samples to the lab; 1,546 (65.7%) women did not respond. From the 736 samples received at the laboratory, 17 had a β-globin PCR negative test result and the corresponding women received a second SSVS kit. Eight of them resubmitted a second SSVS (all of them being hrHPV negative), whereas 9 women did not respond the second time, yielding ultimately a total of 727 valid tests. Twelve (4.2%) women in the control group responded by returning a prepaid postcard. Of the remaining 272 control women, 48 responded to the second recall for regular cytology (17.6%). Active participation in the SSVS self-sampling group (including 3.0% conventional cytology) was statistically significantly higher than that in the second recall group (34.2 *vs.* 17.6%, $p < 0.001$).

hrHPV test results on self-sampled vaginal specimens

hrHPV DNA was detected in 58 (8.0%) of 727 women with a valid hrHPV SSVS test (Fig. 1). The overall percentage of hrHPV DNA positivity in age-matched women of the POBASCAM

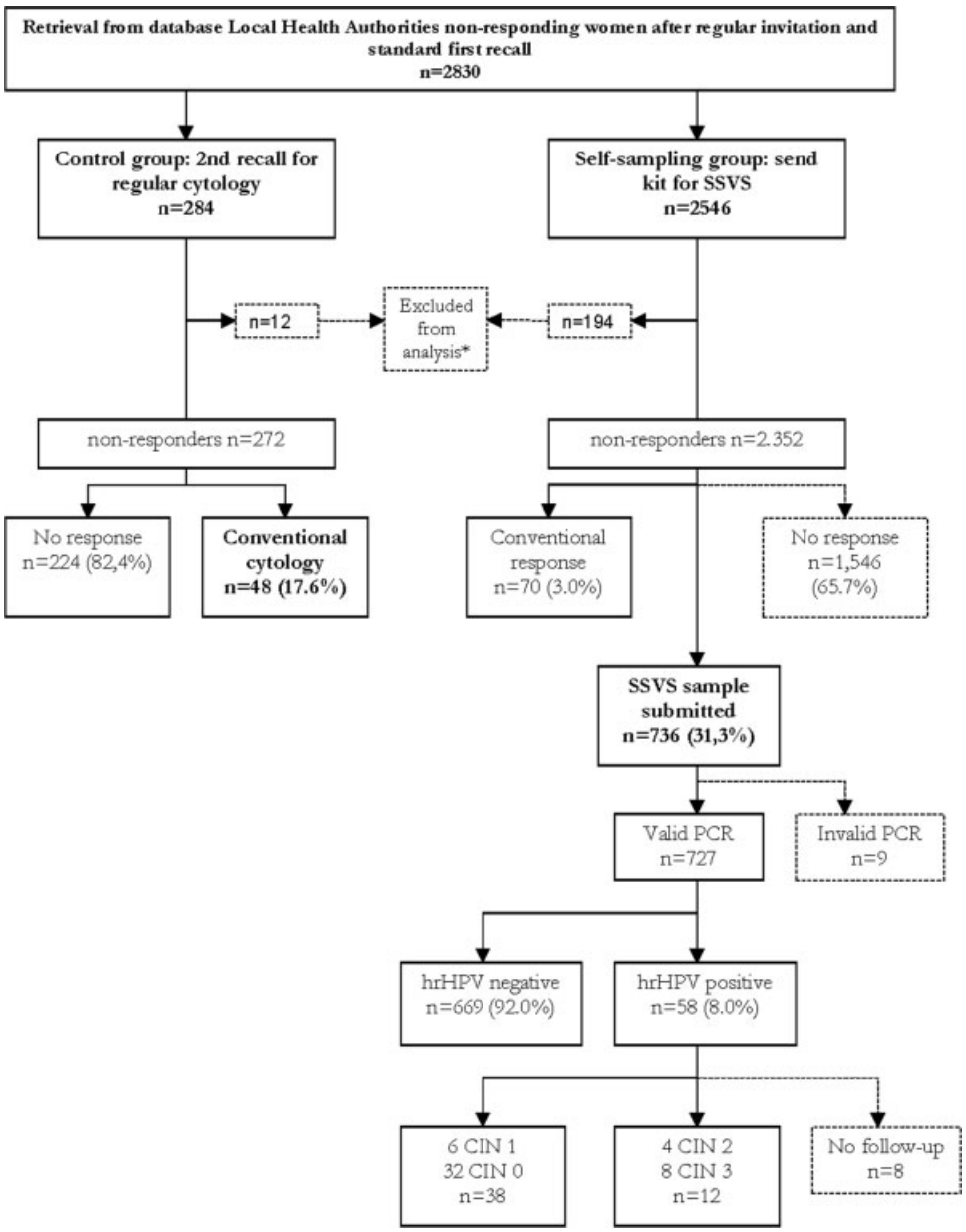

Figure 1 – Flowchart of the intervention trial for nonresponders in cervical screening program. *Excluded from analysis were women who responded by returning a form, indicating the reason for not taking a self-sample test or responded to a second recall for cytology because of one the following reasons: pregnancy, breast feeding, prior hysterectomy, treatment by gynecologist or "other reasons" (*e.g.*, emigration, deceased, other illness, *etc*.).

cohort was 6.8% (*i.e.* 422 of 6,208 women). The prevalence of hrHPV was slightly higher in the self-sampling group than that in the POBASCAM responder cohort but the observed difference was not statistically significant (OR_{MH} 1.28; 0.93–1.76; 95% CI; $p = 0.11$).

Comparison of screening history in self-sampling group and screening cohort

As the invitational database of the municipal health council functions since 2003 and is restricted to invitational smears only (*i.e.*, containing no smears on other grounds), we obtained screening history concerning all smears for both test and control group from the National Pathology Registry (PALGA). However, owing to tight legislation in the Netherlands concerning privacy, records obtained from PALGA became fully anonimized for both test and control group, thus impeding any proper group-allocation (*i.e.*, control group *vs.* HPV-negative group *vs.* nonparticipants in this study). We were unable, therefore, to obtain insight into screening histories of women who had not responded by submitting a self-sampled specimen. Only for HPV positive women who had undergone further cytological and histological testing, full screen history could be compared to the Pobascam control group. In addition, since invitational screening starts in the Netherlands at 30, we could compare screening histories of hrHPV positive women who were at least 35 years of age (from either self-sampling group or age-matched responder group). These women were chosen because, in contrast to younger women, they had been invited for at least one prior screening round. Given the known attendance rate of women aged 30–34 in the population-based screening program in the Netherlands, it can be expected that about 80% of the women aged 35 or more have been screened at least once before.

TABLE I – hrHPV POSITIVE WOMEN IN SELF-SAMPLING GROUP HAVE LESS SCREENING HISTORY THAN hrHPV POSITIVE WOMEN IN AGE-MATCHED COHORT (HISTORICAL CONTROL)

Screening history[1]	hrHPV positive women in self-sampling cohort	hrHPV positive women in POBASCAM cohort	Odds ratios
At least one screening smear $\leq$7 years ago[2]	13/37 (35%)	279/356 (78%)	OR 0.102 (95% CI: 0.042–0.247); $p < 0.001$
At least one screening smear $\leq$12 years ago[3]	6/14 (43%)	20/26 (77%)	OR 0.118 (95% CI: 0.021–0.666); $p = 0.026$

[1]For this analysis, women belonging to age groups 31 and 35 were excluded, since these women had not been invited for an earlier screening round.–[2]Only women who had been invited for at least 1 previous screening round.–[3]Only women who had been invited for at least 2 previous screening rounds.

TABLE II – COSTS PER CIN2+ DETECTED IN CONVENTIONAL PROGRAM COMPARED TO SSVS AS "ADJUNCPROGRAM"

	Conventional procedure	Self sample test per CIN2+
Cost per CIN2+ detected[1]	€7,599 (95% CI €5,910–10,532)	€8,836 (95% CI €5810–18472)

[1]Specification per medical procedure is given as follows (index 2005; indirect costs of travelling and production loss included; see text[27] for further details. Conventional response form[28]). Costs are assessed on organization invitation, costs involved with testing and costs for diagnosis and treatment. Screening organization (organization-invitation) €15/smear (similar for both conventional and ss test and dependent on response). Costs involved with testing: Smear taking at general practitioner and cytological evaluation €39/smear. Self-sample material and high-risk HPV testing € 2/invitation + €33/test. Adjunct smear taking at general practitioner and cytological evaluation €43/smear. Costs involved with treating: diagnosis/treatment/and follow-up $\geq$CIN2 €1,889; diagnosis/treatment/follow-up CIN1 €1,434; diagnosis/follow-up CIN0 €335/colposcopy without biopsy €171.

Indeed, the screening history of hrHPV positive women of the POBASCAM cohort was comparable to this figure: 279 of 356 (78%) of these women had at least one screening smear taken $\leq$7 years earlier (Table I). In contrast, only 10 out of 37 (27%) women in the self-sampling group had at least one smear taken within the last 7 years (OR_{MH}: 0.09; 95% CI: 0.03–0.23; $p < 0.001$), indicating a significantly lower rate of prior screening in the self-sampling group.

This difference between both cohorts remained when screen history was surveyed over a longer period, *i.e.* over at least 12 years of women who were old enough for having a long screening history (*i.e.* 41 years and older). In the self-sampling group, only 6 out of 14 (43%) women had at least one screening smear $\leq$12 years earlier, which was significantly less than that in the POBASCAM cohort where the attendance rate within the last 12 years was 20/26 (77%) (OR_{MH}: 0.12; 95% CI: 0.02–0.67; $p = 0.026$; Table I).

Cytology and histology results of women with hrHPV positive SSVS

Eight (14%) of the 58 women who had a hrHPV positive SSVS did not respond to the written (and reminded) alerts on their SSVS hrHPV positive test result. From the remaining 50 hrHPV positive women, all underwent cervical cytology.

Cervical cytology was normal in 30 (60%). Sixteen women with normal cytology declined colposcopy-directed biopsy and thus had no histology. They opted for follow-up by cytology. The remaining 14 women with normal cytology underwent colposcopy-directed biopsy, which resulted in 1 CIN 3, 3 CIN 2, 2 CIN 1 and 8 CIN 0 cases.

Cytological abnormalities were found in the remaining 20 (40%) women who had hrHPV positive SSVS. Fourteen of them had borderline or mild dyskaryosis (BMD) and 6 of them had moderate dyskaryosis or worse (>BMD). Of the 14 women with BMD, 3 women refused colposcopy-directed biopsy and opted for cytological follow-up. Eleven remaining women with BMD underwent colposcopy-directed biopsy, yielding 1 CIN 3, 1 CIN 2, 4 CIN 1 and 5 CIN 0 cases. All 6 women with >BMD had CIN 3. In total, 12 of 50 hrHPV positive women presented with an underlying high-grade CIN lesion.

Comparison of CIN2+ detection rates in self-sampling group and regular screening program (POBASCAM cohort)

Together, 12 of 727 (1.6%) women with a valid SSVS test had underlying high-grade CIN. We compared this figure with the total yield of histologically confirmed CIN2+ diagnoses in 6,208 age-matched women participating in the POBASCAM trial, who were immediately referred for colposcopy upon a cytological test result of >BMD. The overall detection rate of CIN2+ was higher in the self-sampling group than in the POBASCAM cohort (61/6208 = 0.97%)(OR_{MH} 2.59, 95% CI 1.31–5.12; $p = 0.0047$). The CIN2+ detection rate was still increased in the self-sampling group, although not statistically significantly, after including histology data of women with BMD in the POBASCAM cohort who were referred for colposcopy when having an abnormal smear upon repeat cytology at 6 or 18 months (OR_{MH} 1.68 (0.88–3.21; 95% CI; $p = 0.11$; data not shown), as is conventionally done in the Dutch national program. There was no association between odds ratio and age ($\chi^2 = 0.45$; $p = 0.93$), *i.e.* there was no significant difference between any of the age strata.

Cost-effectiveness of offering SSVS

The detection rate of 0.97% CIN2+ lesions found by immediate colposcopy after >BMD in the POBASCAM cohort can be translated into an absolute figure of 4,567 (95% CI 3,295–5,872) CIN2+ lesions in the Netherlands (*i.e.* 4,567 CIN2+ lesions = 750,000 invited women × 63% screening response × 0.97% detection rate). Based on our figures, when offering self-sampling to nonresponders, the number of detected CIN2+ lesions would increase by 1,085 (95% CI 519–1,650; 1,085 CIN2+ lesions = 750,000 × 28% screening nonresponse × 31.3% SSVS response × 1.65% $\geq$CIN 2 detection rate). The total direct and indirect costs of offering conventional cervical screening (including diagnosis and eventual treatment of CIN after >BMD) in the Netherlands was calculated at €34,703,000 annually and the total costs of offering SSVS to nonresponders was calculated at €9,587,000. The resulting costs per detected CIN2+ lesion were in the same range for cytological screening and self-sampling, *i.e.* €34,703,000/4,567 = €7,599 (95% CI €5,910–10,532) for conventional screening *versus* €9,587,000 /1,085 = €8,836 (95% CI €5,810–18,472) for self-sampling (Table II).

Discussion

Half of the cases of cervical carcinoma is found in women who do not attend regular cervical screening. Our results show that offering self-sampling of vaginal specimens for hrHPV testing led to a higher response rate than a second recall in the group of nonresponders of the nation-wide cervical screening program. The hrHPV positive women who responded by submitting a SSVS

were also likely to have refrained from participation in previous screening rounds, and consequently can be considered regular nonresponders. The relevance of these findings are emphasized by the observation that hrHPV testing on SSVS is highly effective in detecting CIN2+. We found that the detection rate of CIN2+ lesions was significantly higher in the hrHPV positive SSVS group than in age-matched women participating in a regular screening program that were referred immediately because of >BMD. Importantly, the costs per CIN2+ detected *via* hrHPV testing on SSVS in the nonresponders are in the same range as those calculated for conventional cytological screening (€8,836 *vs.* €7,599). Thus, offering self-sampling for hrHPV testing to recruit nonresponders is likely to increase the effectiveness of the screening program markedly.

The high response rate to offering SSVS in nonresponders may be attributed to the fact that these women prefer this self-sampling procedure above visiting a general practitioner. Indeed, in a questionnaire filled in by the women referred to the colposcopy clinic ($n = 30$), 29 women marked that the self-sampling procedure was easy and 25 of them indicated that they would prefer this test to conventional cytology. These data warrant further epidemiological investigation of nonresponder women into reasons for declining the invitation of the regular screening program.

Nonresponders in screening programs are considered to be a high-risk group for cervical cancer.[1,2,9–14] In line with this, an increased OR for prevalent CIN2+ in hrHPV positive nonresponder women was evident, confirming that these women represent a group with a higher risk of CIN2+ than regular screening responders. Although there was a reasonable increase in hrHPV detection rate in nonresponders compared with regular screening participants (7.8% *vs.* 6.7%), this increase did not reach statistical significance, which may be attributed to the fact that the sensitivity of detecting hrHPV is somewhat lower on self-collected samples compared to classical cervical scrape samples.[21] Furthermore, the ratio between clinically relevant *versus* clinically irrelevant hrHPV infections may be increased in nonresponders of the screening program *versus* responders of the screening program, due to program effect.

It should be kept in mind that we compared the yield of CIN2+ in nonresponders following hrHPV testing on SSVS with corresponding age-matched POBASCAM responders who were directly referred for colposcopy because of a smear with >BMD cytology. However, POBASCAM responders with BMD (2.4% of the screened population) have 2 repeat smears (after 6 and 18 months) and are referred for colposcopy if any of the 2 repeat smears show cytological abnormalities. In 10% of these indirectly referred POBASCAM responders, CIN2+ is detected. When BMD women with CIN2+ diagnosed following cytologically abnormal repeat smears at 6 and 18 months were added to the CIN2+ cases detected after direct referral of women with >BMD, the overall detection of CIN2+ in the hrHPV positive self-sampling responders appeared as good as that in the conventional screening program ($p = 0.11$).

Thus, our study underscores the potential value of SSVS for hrHPV testing in cervical screening programs as a method for enhancing the effectiveness of the screening program.[16–20] If we extrapolate our results to the situation in the Netherlands where the annual nonresponse involves 210,000 women (about 300 of which contain cervical carcinomas), offering self-sampling for hrHPV testing could result in the early detection of 1,085 extra CIN2+ lesions, leading to ~100 cervical cancers being prevented or detected earlier. This is a substantial figure if we consider that annually about 700 cervical carcinomas are diagnosed in the Netherlands.[29] Therefore, hrHPV testing on SSVS clearly merits further attention, although efforts to improve the response remain certainly mandatory, since still 69% of the nonresponder women did not participate at all.

Moreover, if SSVS for hrHPV testing of nonresponders were to be considered as an adjunct in a cervical screening program, a clear follow-up strategy must be adhered to limit redundant colposcopic referral of hrHPV positive women with a negative test result in reflex cytology. Given our experience, we presently advocate a protocol in which hrHPV positive women with abnormal cytology are directly referred for colposcopy-directed biopsy. hrHPV positive women with normal cytology could be invited for repeat hrHPV and cytological testing after 6 months and referred for colposcopy when one or both tests are positive. The costs of detecting CIN2+ in self-sampling women could even be decreased when follow-up of those with hrHPV positive normal smears would be done solely by hrHPV testing.

In summary, offering hrHPV testing on SSVS is an attractive adjunct to offer protection to women, which are not reached by the cervical screening program. SSVS can increase the effectiveness of the regular cervical screening program significantly at nearly the same costs per detected CIN2+ lesion. Finally, in the era where prophylactic HPV vaccination is likely to be offered to young adolescent women in the near future, HPV testing on SSVS might also be highly attractive for these women, since it allows them to control the effectiveness of vaccination and their risk of cervical lesions by themselves without intervention by the general practitioner.

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