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Human papillomavirus self-sampling with mRNA testing benefits routine screening

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Abstract

High risk human papillomavirus (hrHPV) based screening provides the possibility of vaginal self-sampling as a tool to increase screening attendance. In order to evaluate the impact and feasibility of opt-in self-sampling in the Finnish setting, we invited a randomized population of 5350 women not attending screening after age group invitation or after reminder, to attend HPV self-sampling-based screening in the autumn of 2018 in Helsinki. Out of those, 1282 (24.0%) expressed their interest and ordered the sampling package. Eventually 787 women (14.7% of the total invited population) took part in screening, 770 women by providing a vaginal sample within 2 months from invitation and 17 by providing a pap smear in the laboratory. Self-taken samples were collected in Aptima Multitest vials and tested using the Aptima HPV mRNA assay. A high proportion, 158/770 (20.5%) of the samples were positive in the Aptima HPV assay. One hundred and forty-one samples were further submitted to Aptima HPV Genotyping and extended genotyping by a Luminex based assay. Of those, 23 samples (16.3%) were HPV 16 positive and 7 (5.0%) were positive for HPV 18/45; extended genotyping revealed multiple high-risk and low-risk HPV genotypes. At follow-up seven cases of high-grade squamous intraepithelial lesion (HSIL) were diagnosed, which represents 4.4% of HPV positive women and 0.9% of screened women, whereas the rate was 0.5% in routine screening. Our findings suggest that self-sampling with HPV mRNA testing is a feasible approach to improve screening efficacy in a high-risk population among original nonattendees.

Abbreviations: AGC-FN, atypical glandular cells favor neoplastic; AGC-NOS, atypical glandular cells not otherwise specified; AHPV, Aptima HPV assay; AHPVgt, Aptima HPV genotyping assay; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; EFC, European Federation for Colposcopy; HPV, human papillomavirus; hrHPV, high-risk HPV; HSIL, high-grade squamous intraepithelial lesion; IFCPC, the International Federation of Cervical Pathology and Colposcopy; LHPVgt, Luminex HPV genotyping assay; LLETZ, large-loop excision of the transformation zone; lrHPV, low-risk HPV; LSIL, low-grade squamous intraepithelial lesion.

KEYWORDS
Aptima, HPV, mRNA, opt-in, self-sampling

What's new?

HPV-based screening for cervical cancer has many advantages, including that patients can collect samples themselves, at home. Here, the authors offered an HPV self-sampling option for women who did not obtain conventional screening. Of more than 5000 people invited, 787 took part. Using an mRNA-based assay, researchers detected high-risk HPV at more than double the rate detected by regular screening. Follow-up examinations revealed severe lesions in 0.9% of the screened women, compared to a rate of 0.5% in routine screening. HPV self-sampling appears to be a feasible approach to improve the uptake of cervical cancer screening.

1 | INTRODUCTION

In Finland, municipalities are legally obliged to offer cervical cancer screening to all 30- to 65-year-old women every 5 years. Most municipalities, including the capital Helsinki, invite 25-year-old women as well. Nationwide, traditionally cytology-based, organized screening since the 1960's has decreased cervical cancer incidence by as much as 80%.[1] However, since mid-1990's the incidence has increased among women below 40 years of age, and at present the incidence among those age groups is at the same level as before the screening era, in spite of active screening within and outside the screening program.[2] The overall attendance rate in Finland is around 70%, which, together with extensive opportunistic screening, produces an 87% coverage in 5 years among women at screening age.[2] All in all, a substantial proportion of cervical cancers are currently diagnosed in women who do not attend organized screening.[3]

Human papillomavirus (HPV)-based screening is gaining a firm foothold in many European countries due to the high sensitivity and excellent negative predictive value.[4] In Finland, HPV-based screening was first launched in a routine setting in the Tampere region in 2015,[5] gradually spreading to other municipalities, in 2019 also to the Helsinki and Uusimaa region. Currently in the regions offering primary HPV screening, hrHPV test followed by conventional cytology triage is performed among women aged 30 years and older; for 25-year-old women primary screening is done by conventional cytology.

HPV-based screening opens the possibility of vaginal self-sampling to increase screening attendance, and indeed equal or close to equal screening performance of self-collected vaginal samples as compared to provider-obtained samples has been shown in many studies.[6] In Finland, opt-out self-sampling has been shown to increase attendance among nonattendees to regular screening.[7,8] In opt-out self-sampling every invitee receives the sampling package together with the invitation and this approach, when tested side by side, has shown better attendance than opt-in self-sampling, where the invitation is sent first and the woman then actively chooses to order a sampling kit at the provided interface.[9] However, due to moderate or low overall attendance rates, the cost per attendee is higher in opt-out than in opt-in self-sampling.

In September 2018, while designing the launch of HPV based screening in Helsinki, we initiated an opt-in HPV self-sampling pilot study. Our purpose was to assess the feasibility and impact of opt-in self-sampling as an additional tool to increase attendance in organized screening in Finland. Further, we aimed to evaluate the potential of HPV genotyping in the triage of self-samples which are not eligible for cytology. Here we present the results of the self-sampling pilot, together with HPV-genotyping results and histological findings among the self-sampling participants.

2 | MATERIALS AND METHODS

2.1 | Identification of nonattendees, recruitment and invitation

Every calendar year the women entitled to screening that particular year are identified from the population registry by birth year and home municipality, and they receive a personal written invitation from the screening laboratory. In Helsinki, this invitation includes a prebooked appointment for sampling at the screening laboratory (which the woman can modify or cancel). If the woman does not attend, she receives a written reminder without prebooked appointment toward the end of the invitation year. Both prebooked appointments and written reminders have been shown to increase overall screening attendance.[10] If the invitee does not attend by the end of January next year, she will receive no further reminders and is considered a nonattendee.

In 2017, 46 372 women in Helsinki aged 25 to 69 were invited to age group screening or risk group screening due to previous borderline cytology or reported bleeding symptoms. To initiate this pilot study, in August 2018 we identified a total of 14 238 women who were considered nonattendees. Of those, 6000 were randomized to the self-sampling pilot (Figure 1). After randomization and rechecking, 20 women proved to have attended regular screening and were thus added to attendees of routine screening in the analysis. The resulting 5980 women randomized to self-sampling were subjected to address information update from the Digital and Population Data Services Agency. After excluding 630 individuals who had no current residence in Helsinki or had deceased, the final study cohort consisted of 5350 women. These women received personal invitation letters to self-sampling to their home address. The women were instructed to order self-sampling kits via a web interface specifically created for this pilot

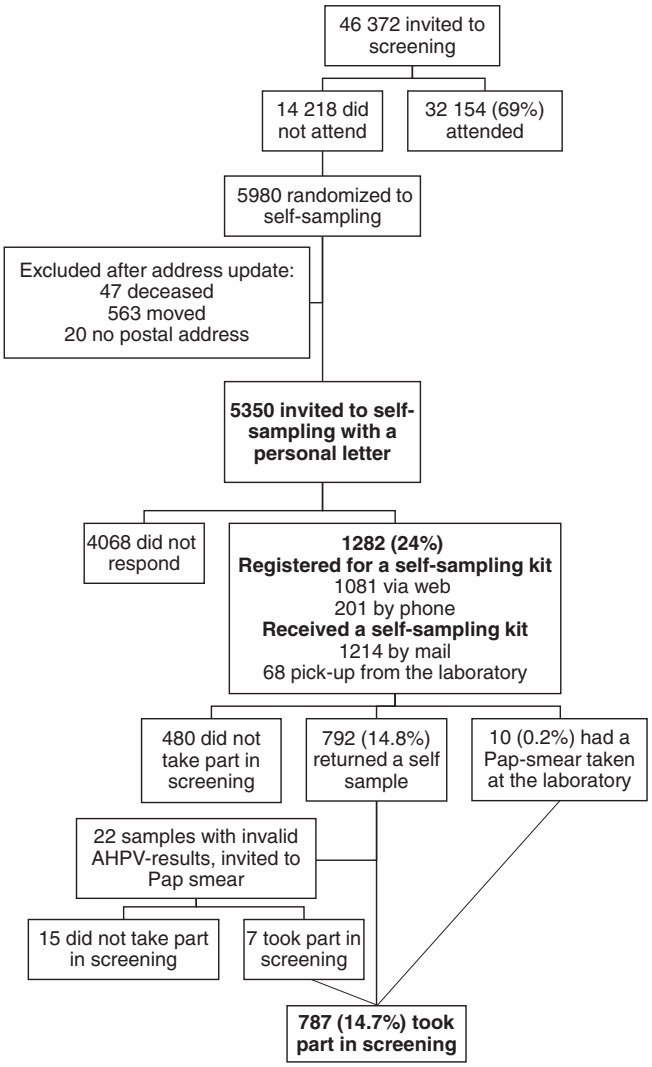

FIGURE 1 Scheme of invitations and attendance rates

study, or by telephone. They were further given the option to receive the sampling kit either by regular mail or by fetching it personally from the screening laboratory. The self-sampling kit consisted of the Aptima Multitest sampling kit (Hologic, Inc, Marlborough, MA) together with general information on screening, illustrated sampling instructions, and a preliminary information form for recording previous screening results and possible symptoms (in Finnish, Swedish and English). The women had the option to mail the self-taken vaginal sample to the screening laboratory in a prepaid envelope, or to bring it personally to laboratory.

A contact telephone number of the study nurse and a link to a web-based survey form were provided for further information and feedback.

2.2 | HPV testing and genotyping

At the time of the study, none of the commercially available HPV assays had been validated for self-sampling by the assay manufacturer. Self-samples were collected in Multitest sampling vials according to the assay manufacturer's recommendation and tested by the Aptima HPV mRNA assay (AHPV; Hologic, Inc, Marlborough, MA).

The Aptima assay gives either "positive," "negative" or "invalid" result. All initially invalid samples were retested and, if needed, tested once more after dilution as recommended by the manufacturer. We report an "invalid" result for samples where the analytical output from the Aptima assay remained invalid even after two retesting rounds. No leaking or insufficient sample quantity was observed.

HPV testing was performed at the Department of Virology, Diagnostic Center of the Helsinki University Hospital, in the Panther instrument (Hologic) similarly to diagnostic Aptima HPV samples. Initially, a considerable proportion of the samples were found invalid in HPV testing. Similar problems had emerged at the Skåne Labmedicin laboratory in Lund, Sweden, where a protocol of heating the samples at +90°C for 1 hour before testing, had consequently been established and the rate of invalid samples had dropped to acceptable levels.[11] This approach was applied to all self-samples in our study as well.

Aptima runs were performed in a dedicated laboratory space, where no other laboratory procedures take place. In each Aptima assay run, positive and negative calibrators are run in triplicate, and positive and negative controls on single tubes. To monitor the hybridization, amplification and identification steps of the assay, each sample is spiked with an internal control during the assay. The laboratory participates in four external quality assessment rounds from two different providers annually.

All adequate HPV positive samples were further subjected to partial genotyping at the Department of Virology using the Aptima HPV Genotyping Assay (AHPVgt), which provides partial genotyping for HPV 16 (HPV 16) and HPV 18 or HPV 45 (HPV 18/45) and a pooled result for the other 11 hrHPV types covered by AHPV. AHPV positive samples were further subjected to extended genotyping using a method based on PCR amplification and Luminex detection (LHPVgt) at Karolinska Hospital, Sweden. The LHPV assay covers HPV genotypes 6, 11, 16, 18, 26, 30, 31, 33, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 67, 68, 69, 70, 73, 74, 81, 82, 83, 86, 87, 89, 90 and 91.[12,13]

2.3 | Follow-up of women with a positive HPV test result from a self-taken sample

After a negative AHPV test result women at any age were informed of a normal screening result. All AHPV positive women were invited to conventional cytology triage: those below 35 years of age to the screening laboratory, and women aged 35 years or older to the Department of Obstetrics and Gynecology of Helsinki University Hospital. The criteria for colposcopy referral in cytology triage were low-grade squamous intraepithelial lesion or more severe squamous finding (≥LSIL), atypical glandular cells favor neoplastic (AGC-FN) or atypical glandular cells not otherwise specified (AGC-NOS) on the pathologist's recommendation. AHPV positivity with normal cytology, atypical squamous cells of undetermined significance (ASC-US) or

TABLE 1 Rate of invited and attended women in regular screening vs HPV self-sampling by age group in 2017 in Helsinki, Finland

	Regular screening			Self-sampling		
Age group	Invitees	Attendees	Attendance %	Invitees	Attendees	Attendance %
25-29	6867	4083	59.5	924	117	12.7
30-34	6991	4525	64.7	886	113	12.8
35-39	6015	4028	67.0	748	105	14.0
40-44	5006	3528	70.5	585	103	17.6
45-49	4064	2918	71.8	474	76	16.0
50-54	4788	3611	75.4	467	55	11.8
55-59	4543	3402	74.9	441	79	17.9
60-64	4067	3034	74.6	440	73	16.6
65-69	4031	3025	74.0	385	66	17.1
Total	46 372	32 154	69.3	5350	787	14.7

AGC-NOS without colposcopy referral resulted in a call to risk group screening after 12 months.

Colposcopy was performed according to routine protocols by certified colposcopists. Colposcopic diagnosis was set according to the International Federation of Cervical Pathology and Colposcopy (IFCPC) and the European Federation for Colposcopy (EFC) criteria.[14] Colposcopy directed biopsies were taken if colposcopic Grade 1 or 2 lesions were found. Large loop excision of the transformation zone (LLETZ) was performed at the first visit if colposcopy showed Grade 2 (high grade) lesions and high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H) or AGC-FN cytology, or Type 3 transformation zone and HSIL, ASC-H or AGC-FN cytology. New samples for cytology and HPV test were taken if the index sample results were over 3 months old. Cytological and histological samples were processed according to routine protocols and interpreted at the Department of Pathology of the Helsinki University Hospital.

3 | RESULTS

3.1 | Attendance rate

Among 46 372 women invited to screening in 2017 in Helsinki, 32 154 (69.3%) took part in routine screening. In this pilot study, of the 14 218 women who did not attend screening after two invitations, a subgroup of 5350 women were sent invitations to attend HPV self-sampling in October-November 2018. Out of them 1282 (24.0%) opted for self-sampling either via the web-based interface (N = 1081; 84.3%) or by phone (N = 201; 15.7%), and by ordering sampling devices by regular mail (N = 1214; 94.7%) or by fetching them personally (N = 68; 5.3%) from one of the two laboratories in Helsinki where they were made available. A total of 792 women returned a self-taken sample to the laboratory. Of the self-taken samples 22 (2.8%) gave an invalid result in the AHPV-assay even after repeated testing. These women were sent an invitation to a Pap smear in the screening laboratory, and seven of them participated. Ten women attended by providing a Pap sample in the laboratory. The final overall participation rate among all self-sampling invitees was 14.7% (787/5350). A scheme of invitations and attendance rates is presented in Figure 1.

The attendance rates by age group in regular screening and in self-sampling are shown in Table 1. In regular screening the participation rate was lowest (59.5%) among women aged 25 to 29 years and highest (75.4%) among women aged 50 to 54 years. Also among self-sampling invitees the participation rate was lowest among the youngest age groups.

In routine screening the participation rate among those invited to age-group screening (71.3%) was slightly higher than among risk-group screening invitees (69.1%) (data from the laboratory information system). In self-sampling the difference was more pronounced: the participation rate was 25.2% among the original nonattendees of age-group screening and 13.7% among the original nonattendees of risk-group screening.

3.2 | Practical issues from invitee perspective

A contact telephone number of the study nurse and a link to a web-based survey for feedback were included in the information sent to invitees together with the sampling supplies. There were only a few patient calls to the study nurse. The link to the feedback form was available only in the printed invitation letter, which probably explains the scarce use of the form. The most commonly raised issue altogether was the difficulty in holding the small sampling tube in upright position.

3.3 | HPV detection and genotyping

Aptima HPV mRNA assay and genotyping results by age group are shown in Table 2. Out of the 770 self-taken samples with a valid AHPV-result, 158 (20.5%) were high-risk HPV positive. The positivity rates ranged between 15.0% and 25.2% in the different 5-year age

TABLE 2 High-risk HPV positivity rates and HPV genotypes in the different age groups of 792 women providing self-collected HPV samples

	N	AHPV positive		Subjected to AHPVgt	HPV 16 positive		HPV 18/45 positive		Invalid
Age group	N	N	%	N	N	%	N	%	0
25-29	112	25	22.3	23	5	21.7	1	4.3	0
30-34	111	28	25.2	27	6	22.2	2	7.4	1
35-39	104	21	20.2	20	5	25.0	0	0.0	0
40-44	103	18	17.5	16	3	18.8	0	0.0	0
45-49	76	14	18.4	12	1	8.3	0	0.0	1
50-54	55	11	20.0	8	0	0.0	1	12.5	0
55-59	80	12	15.0	10	2	20.0	2	20.0	3
60-64	77	17	22.1	16	1	6.3	0	0.0	5
65-69	74	12	16.2	9	0	0.0	1	11.1	12
Total	792	158	20.0	141	23	16.3	7	5.0	22

Note: For comparison, the percentage of hrHPV positive in regular HPV-based screening in 2019 is given.
Abbreviations: AHPV, Aptima HPV assay; AHPVgt, Aptima HPV genotyping Assay.

groups with no clear trend by decreasing or increasing age. The rate of invalid samples was considerable in the oldest age group of 65- to 69-year-old women, where 12/74 samples (16.2%) were invalid.

Of the 158 initially AHPV positive samples 141 were sufficient for AHPV genotyping for HPV 16 and HPV 18/45 (Table 2). The rate of HPV 16 (23 samples, 16.3%) or HPV 18/45 (seven samples, 5.0%) was rather low, 21.3% of all tested AHPV positive samples. Two valid AHPV positive samples (1.4%) gave invalid result in AHPVgt. Based on AHPVgt results, the remaining 109 of the 141 samples (77.3%) harbored other hrHPV types.

The 141 AHPV positive samples available for genotyping were further subjected to extended HPV genotyping using a Luminex-based assay (LHPVgt). HPV was detected in 122 (86.5%) of these 141 samples, and in 19 samples no HPV was found in the Luminex based assay (Supplementary Table 1a). The rates of HPV genotypes detected in LHPVgt are shown in Supplementary Table 1b.

Assuming concordance if both genotyping assays detected either HPV 16, HPV 18/45, or other hrHPV type, also together with other HPV genotypes, and "other hrHPV" meaning those covered by AHPV, 83 of 139 samples (59.7%) genotyped with both assays gave concordant results and 56/139 (40.3%) gave discordant results (genotyping data for individual samples not shown). Among samples found to contain genotypes other than HPV 16, 18 or 45 in AHPVgt, half (54/109) gave discordant result in LHPVgt: either HPV 16, 18 or 45 was detected (16 samples), non-AHPV genotype(s) was detected (20 samples), or no HPV DNA was detected (18 samples).

3.4 | Primary screening results among women providing a Pap smear

All 17 women who were invited to self-sampling but attended screening by having a Pap screening sample taken in the laboratory (10 by individual choice, 7 due to invalid HPV test result) had normal results.

3.5 | Cytological and histological findings in follow-up of high-risk HPV positive women

Altogether 770 attendees were tested for high-risk HPV with valid AHPV results. Follow-up compliance and results among self-sampling participants is shown in Table 3. Of participants below 35 years of age, 53/230 (23.0%) were hrHPV positive and were invited to cytology triage performed in the screening laboratory. Of them 42 (79.2%) followed the invitation. Among the participants aged 35 years or older, 105/557 (18.9%) were hrHPV positive and were referred to cytology triage at the Department of Gynecology with slightly higher compliance to follow-up of 90.5% (95 women). Overall, loss to follow-up among self-sampling participants was 13.3% of HPV-positive women.

Among <35-year-old hrHPV positive women, 4/53 (7.5%), or 1.7% of the 10-year age group, were diagnosed with histological HSIL. Among ≥35-year-old hrHPV positive women, HSIL detection rate was 3/105 (2.9%), corresponding to 0.5% of all attendees of this age. The overall HSIL detection rate among original screening nonattendees who took part in screening after self-sampling offer was 0.9% (Table 3), while it was 0.5% among participants in routine screening (Supplementary Table 2).

Of all HSIL cases, 2 were HPV 16 positive, 1 was HPV 18/45 positive, and 4 were positive for other hrHPV types based on AHPV and AHPVgt assays. The HPV genotypes detected in all HSIL and LSIL cases by AHPVgt or LHPVgt are shown in Supplementary Table 1c.

4 | DISCUSSION

In the present study, opt-in HPV self-sampling using the Aptima HPV mRNA assay was carried out among a randomized subset of nonattendees to a cytology-based screening program as a third intervention after primary invitation and a reminder letter. Out of all women

TABLE 3 Cytological and histological findings of hrHPV positive women attending self-sampling presented by age group

Age group	Participants, total	AHPV+	Follow-up results[a]											
			Borderline cytology[b], no histology			Histological LSIL			Histological HSIL			Lost to follow-up		
		N	N	% of AHPV+	% of all attendees	N	% of AHPV+	% of all attendees	N	% of AHPV+	% of all attendees	N	% of AHPV+	% of all attendees
25-34	230	53	3	5.7	1.3	0	0.0	0.0	4	7.5	1.7	11	20.8	4.8
35-44	208	39	4	10.3	1.9	1	2.6	0.5	1	2.6	0.5	1	2.6	0.5
45-54	131	25	2	8.0	1.5	5	20.0	3.8	1	4.0	0.8	1	4.0	0.8
55-64	152	29	0	0.0	0.0	2	6.9	1.3	1	3.4	0.7	7	24.1	4.6
65-69	66	12	0	0.0	0.0	1	8.3	1.5	0	0.0	0.0	1	8.3	1.5
Total	787	158	9	5.7	1.1	9	5.7	1.1	7	4.4	0.9	21	13.3	2.7

Abbreviations: AGC-NOS, atypical glandular cells not otherwise specified; AHPV, Aptima HPV assay; ASC-US, atypical squamous cells of undetermined significance; hrHPV, high-risk HPV; HSIL, high-grade squamous intraepithelial lesion; LSIL, ow-grade squamous intraepithelial lesion.

[a]Includes women with only Pap-smear follow-up, only colposcopy follow-up with histological samples or both, with normal results.

[b]Includes ASC-US, AGC-NOS and LSIL cytological findings with recommendation to cytological follow-up in 12 months, no histological follow-up.

invited to self-sampling, 24.0% registered to the pilot and were provided sampling kits, and the final participation rate was 14.7%. As attendance after routine invitations was 69%, extrapolating the self-sampling participation rate to all nonattendees would correspond to a roughly 5 percentage point increase in screening attendance, yielding a ca 74% total attendance rate in our setting.

Self-sampling studies have been carried out in many countries with the aim to increase screening attendance for health profit, albeit only a few of them using HPV mRNA testing.[6] In a large opt-in study carried out in Denmark, a participation rate of 20.6% was reached.[15] In opt-out setting where no activity is required from nonattendees to receive self-sampling kits, higher attendance rates are typically reached.[9,16] In our previous opt-out studies carried out in the Finnish screening setting, the participation rate in self-sampling was 32% if self-sampling was used as a first reminder and 20% to 24% when it was used as a second reminder to nonattendees to routine screening.[7,10,17,18] Opt-out self-sampling studies carried out in other Nordic countries among long-time nonattendees to screening have achieved participation rates of 13% to 18%.[19,20] Self-sampling is currently offered as an option parallel to provider-collected sampling, for example, in the Netherlands.[21]

In an opt-in setting, the practicalities related to sampling device distribution need to be easy and accessible for the women for optimal participation rates. For the purpose of our study, a simple, user-friendly web-based interface for registration and ordering of sampling kits was established, and it was used by five out of six participants. Although web-based registration and choice of sample logistics by regular mail were in majority, alternative options were also chosen by some and thus may help increase acceptability and attendance. Based on the low number of contact calls to the study nurse, the detailed instructions for sampling were most likely clear and adequate. Occasional negative feedback was most commonly related to the small size of the sampling tube, as some participants reported that it was difficult to hold the tube in upright position without a separate holder. Approximately 38% of the ordered kits were wasted (no sample returned), which, depending on the overall costs of self-sampling, might be considered acceptable.

In this pilot study, a remarkably high hrHPV test positivity rate of 20.5% among self-sampling attendees was detected. This is 2.3 times the rate of 8.9% among women who attended regular screening in 2019, the first year of HPV based screening in the region (Finnish Cancer Registry at https://stats.cancerregistry.fi, updated May 12, 2021). The same AHPV test is used in routine screening as in the pilot study, albeit with a different preprocessing protocol for the samples. We cannot completely rule out the possibility that aerosols or fumes emerging during the additional heating step on a heat block might have caused occasional contamination and consequently false hrHPV positive results in the laboratory, leading to excessive invitations to additional sampling and follow-up. However, with regard to histological screening results in this self-sampling pilot, altogether seven women were diagnosed with HSIL. These cases represented 4.4% of the AHPV positive population and 0.9% of the total participant population. This is almost twice the 0.5% rate of ≥HSIL lesions among women attending routine screening in Helsinki or the whole

Finland in 2017 (https://stats.cancerregistry.fi). Despite differing primary screening test modalities in routine and self-sampling settings, the population we were able to reach by offering HPV self-sampling thus seems to be a high-risk population, and health benefit could be gained by improving screening attendance among this population. Due to the higher sensitivity of HPV testing, the rate of precancers is increased in HPV based screening as compared to cytology based screening.[5] Our study population consisted of nonattendees to cytology based screening, and the added value obtained by HPV self-sampling may thus appear higher than it would have been, had the routine screening been based on HPV testing.

In previous opt-out self-sampling studies conducted among nonattendees in Finland, lower hrHPV positivity rates of 6% to 13% using the Hybrid Capture 2-test were found.[7,8,17] Importantly, as compared to the present study, the rate of ≥HSIL in the previous study was even higher, 6% of HPV positive women (0.7% of all attendees).[8] When sampling is performed by a professional, the sensitivities of the Aptima and Hybrid Capture 2 assays are claimed to be similar or even slightly higher for the Hybrid Capture 2 assay,[22] suggesting that different assay sensitivities would not explain the difference in hrHPV detection rates. Additionally, the Hybrid Capture 2 assay is known to have some cross-reactivity with low-risk HPV genotypes,[23] and thus it cannot be ruled out that some Hybrid Capture 2 positive samples may have harbored low-risk HPV. Albeit addressing only DNA based assays, a metaanalysis by Arbyn et al suggests that signal amplification assays, as exemplified by Hybrid Capture 2, may be inferior to target amplification assays on self-collected samples.[6]

Aptima HPV assay has been successfully used for self-sampling in previous studies.[19,20,24-26] High-risk HPV testing using self-collected vaginal samples and Aptima HPV assay has somewhat lower sensitivity but similar specificity than provider-collected cervical HPV samples.[6] In our setting, initial testing yielded a high rate of invalid test results, as compared to the ca 0.3% invalidity rate among our regular provider-taken AHPV samples, independent of the age of the screening attendee. Adding a preheating step helped reduce the high initial rates of invalid results, as has been shown in other studies.[11] Apparently a lot of mucus was present in self-collected vaginal samples as opposed to cervical samples taken by a trained nurse in regular screening, and the heating step helped to overcome this source of invalid results. However, the rate of invalid samples remained high, 16.2%, in the oldest age groups. One explanation could be scanty samples due to postmenopausal atrophy resulting in too cautious sampling, and thus optimizing the sampling instructions might help overcome this problem. Still, the rate of invalid samples was notably higher than in other studies on AHPV-testing on self-taken samples among older age-groups, also among women above screening age.[19,25]

The launch of self-sampling requires careful assessment of triage options to optimize health benefits and cost-efficacy. In previous opt-out self-sampling studies carried out in Finland, loss to follow-up was quite high, 20% and 36% of women with an HPV positive result in a self-taken sample.[8,17] Among the self-sampling participants in the present study, 13.3% of the women who were referred to pap triage did not attend. Unfortunately, we do not have access to data concerning possible follow-up visits outside organized screening. Our study invitees were not sent reminders to register or to return the self-taken sample as has been done in some opt-in self-sampling studies.[27] Neither were they reminded to attend cytology triage at a nurse's appointment. Our findings suggest that once awakened to the importance of screening, most of the women are committed to participate. Reminders may further improve health impact.

Besides cytology, HPV genotyping is another triaging option for HPV-positive women. In our study population, the rate of the highest risk HPV genotypes, HPV 16 and HPV 18/45, was lower than expected. Altogether only in 3 out of 7 HSIL cases either HPV 16 or 18/45 was detected by AHPVgt, and 4 cases were positive for other high-risk HPV genotypes covered by the AHPV assay. Among these 4 cases, the LHPVgt assay detected HPV 16 and HPV 33 in one sample each, HPV 18, 45 and 52 in one sample, and one sample remained HPV negative. Our study was not powered to explore triaging options, but these results may suggest that in the Finnish setting, a broader selection of genotypes is needed in triage to detect all HSIL cases. Extended genotyping revealed that the HPV genotype distribution in our study population differs to some extent from the distribution in other European countries, in agreement with previous reports from Finland.[28] Although the 5-year age groups were small in our study population, younger women tended to have more HPV 16 findings than older women, and the highest rate of HPV 16 was detected among 35- to 39-year-old women. Similar results were shown in a previous study from Finland.[28]

Together with the expanding use of hrHPV based cervical cancer screening, vaginal self-sampling becomes one possible option to increase attendance. The feasibility of HPV mRNA testing in self-sampling was further reinforced in the present study. Implementation will require careful cost-benefit calculations in local settings. The launch of self-sampling into the screening program in Finland should be considered as a secondary option for nonattenders. This opt-in study confirms the findings in the previous opt-out studies in Finland, that self-sampling is a feasible alternative to improve attendance, if sufficient rates cannot be reached by other means.

AUTHOR CONTRIBUTIONS

Conceptualization: Eeva Auvinen, Pekka Nieminen, Jukka Pellinen, Jussi Tarkkanen, Anni Virtanen; *Formal analysis*: Eeva Auvinen, Anni Virtanen; *Funding acquisition*: Eeva Auvinen; *Investigation*: Eeva Auvinen, Pekka Nieminen, Anni Virtanen; *Methodology*: Eeva Auvinen, Anni Virtanen; *Project administration*: Eeva Auvinen, Jukka Pellinen; *Resources*: Eeva Auvinen, Jukka Pellinen, Anni Virtanen; *Writing—original draft*: Eeva Auvinen, Pekka Nieminen, Anni Virtanen; *Writing—review & editing*: Eeva Auvinen, Pekka Nieminen, Jukka Pellinen, Joakim Dillner, Jussi Tarkkanen, Anni Virtanen. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to claim.

DATA AVAILABILITY STATEMENT

Detailed research data can be made available upon reasonable request.

ETHICS STATEMENT

Research permission for the study has been granted by the Helsinki University Hospital and the City of Helsinki. Collection of human samples in the context of statutory cervical cancer screening organized by the City of Helsinki does not require ethical permission.

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REFERENCES

1. Anttila A, Nieminen P. Cervical cancer screening programme in Finland. *Eur J Cancer*. 2000;36:2209-2214.
2. Salo H, Nieminen P, Kilpi T, et al. Divergent coverage, frequency and costs of organised and opportunistic Pap testing in Finland. *Int J Cancer*. 2014;135:204-213.
3. Lönnberg S, Anttila A, Luostarinen T, Nieminen P. Age-specific effectiveness of the Finnish cervical cancer screening programme. *Cancer Epidemiol Biomarkers Prev*. 2012;21:1354-1361.
4. Maver PJ, Poljak M. Primary HPV-based cervical cancer screening in Europe: implementation status, challenges, and future plans. *Clin Microbiol Infect*. 2020;26:579-583.
5. Veijalainen O, Kares S, Kujala P, et al. Human papillomavirus test with cytology triage in organized screening for cervical cancer. *Acta Obstet Gynecol Scand*. 2016;95:1220-1227.
6. Arbyn M, Smith SB, Temin S, Sultana F, Castle P, Collaboration on Self-Sampling and HPV Testing. Detecting cervical precancer and reaching underscreened women by using HPV testing on self samples: updated meta-analysis. *BMJ*. 2018;363:k4823. doi:10.1136/bmj.k4823
7. Virtanen A, Nieminen P, Luostarinen T, Anttila A. Self-sample HPV tests as an intervention for nonattendees of cervical cancer screening in Finland: a randomized trial. *Cancer Epidemiol Biomarkers Prev*. 2011;20:1960-1969.
8. Virtanen A, Anttila A, Luostarinen T, Malila N, Nieminen P. Improving cervical cancer screening attendance in Finland. *Int J Cancer*. 2015;136:E677-E684.
9. Giorgi Rossi P, Marsili LM, Camilloni L, et al. The effect of self-sampled HPV testing on participation to cervical cancer screening in Italy: a randomised controlled trial (ISRCTN96071600). *Br J Cancer*. 2011;104:248-254.
10. Virtanen A, Anttila A, Luostarinen T, Nieminen P. Self-sampling versus reminder letter: effects on cervical cancer scteening attendance and coverage in Finland. *Int J Cancer*. 2011;128:2681-2687.
11. Borgfeldt C, Forslund O. Increased HPV detection by the use of a pre-heating step on vaginal self-samples analysed by Aptima HPV assay. *J Virol Methods*. 2019;270:18-20.
12. Lagheden C, Eklund C, Kleppe SN, Unger ER, Dillner J, Sundström K. Validation of a standardized extraction method for formalin-fixed paraffin-embedded tissue samples. *J Clin Virol*. 2016;80:36-39.
13. Schmitt M, Bravo IG, Snijders PJF, Gissmann L, Pawlita M, Waterboer T. Bead-based multiplex genotyping of human papillomaviruses. *J Clin Microbiol*. 2006;44:504-512.
14. Redman CWE, Kesic V, Cruickshank ME, et al. On behalf of the European Federation for Colposcopy and Pathology of the lower genital tract (EFC) and the European Society of Gynecologic Oncology (ESGO). *Eur J Obstet Gynecol Reprod Biol*. 2021;256:57-62.
15. Lam JUH, Elfström KM, Ejegod DM, et al. High-grade cervical intraepithelial neoplasia in human papillomavirus self-sampling of screening non-attenders. *Br J Cancer*. 2018;118:138-144.
16. Giorgi Rossi P, Fortunato C, Barbarino P, et al. Self-sampling to increase participation in cervical cancer screening: an RCT comparing home mailing, distribution in pharmacies, and recall letter. *Br J Cancer*. 2015;112:667-675.
17. Karjalainen L, Anttila A, Nieminen P, Luostarinen T, Virtanen A. Self-sampling in cervical cancer screening: comparison of a brush-based and a lavage-based cervicovaginal self-sampling device. *BMC Cancer*. 2016;16:221. doi:10.1186/s12885-016-2246-9
18. Virtanen A, Anttila A, Nieminen P. The costs of offering HPV-testing on self-taken samples to non-attendees of cervical cancer screening in Finland. *BMC Womens Health*. 2015;15:99. doi:10.1186/s12905-015-0261-7
19. Ernstson A, Urdell A, Forslund O, Borgfeldt C. Cervical cancer prevention among long-term screening non-attendees by vaginal self-collected samples for hr-HPV mRNA detection. *Infect Agent Cancer*. 2020;15:10. doi:10.1186/s13027-020-00280-0
20. Ernstson A, Forslund O, Borgfeldt C. Promotion of cervical screening among long-term non-attendees by human papillomavirus self-sampling. *J Cancer Prev*. 2021;26:25-31.
21. Loopik DL, Koenjer LM, Siebers AG, Melchers WJG, Bekkers RLM. Benefit and burden in the Dutch cytology-based vs high-risk papillomavirus-based cervical cancer screening program. *Am J Obstet Gynecol*. 2020;224:200.e1-200.e9. doi:10.1016/j.ajog.2020.08.026
22. Iftner T, Becker S, Neis KJ, et al. Head-to-head comparison of the RNA-based Aptima human papillomavirus (HPV) assay and the DNA-based hybrid capture 2 HPV test in a routine screening population of women aged 30 to 60 years in Germany. *J Clin Microbiol*. 2015;53:2509-2516.
23. Castle PE, Solomon D, Wheeler CM, Gravitt PE, Wacholder S, Schiffman M. Human papillomavirus genotype specificity of hybrid capture 2. *J Clin Microbiol*. 2008;46:2595-2604.
24. Asciutto KC, Ernstson A, Forslund O, Borgfeldt C. Self-sampling with HPV mRNA analyses from vagina and urine compared with cervical samples. *J Clin Virol*. 2018;101:69-73.
25. Ernstson A, Asciutto KC, Sturesson J, Norén J, Forslund O, Borgfeldt C. Detection of HPV mRNA in self-collected vaginal samples among women at 69-70 years of age. *Anticancer Res*. 2019;39:381-386.
26. Hellsten C, Ernstson A, Bodelsson G, Forslund O, Borgfeldt C. Equal prevalence of severe cervical dysplasia by HPV self-sampling and by midwife-collected samples for primary HPV screening: a randomised controlled trial. *Eur J Cancer Prev*. 2021;30:334-340.
27. Broberg G, Gyrd-Hansen D, Jonasson JM, et al. Increasing participation in cervical cancer screening: offering a HPV self-test to long-term non-attendees as part of RACOMIP, a Swedish randomized controlled trial. *Int J Cancer*. 2014;134:2223-2230.
28. Aro K, Nieminen P, Louvanto K, et al. Age-specific HPV type distribution in high-grade cervical disease in screened and unvaccinated women. *Gynecol Oncol*. 2019;154:354-359.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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