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## RANDOMIZED HEALTH CARE POLICY EVALUATION OF ORGANISED PRIMARY HPV SCREENING OF WOMEN AGED 56 - 60

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**RANDOMIZED HEALTH CARE POLICY EVALUATION OF ORGANISED****PRIMARY HPV SCREENING OF WOMEN AGED 56 - 60**

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**ABSTRACT**

Objective: To implement and reliably evaluate a new organized, population-based screening program using primary HPV screening.

Participants: Resident women in the Stockholm/Gotland region of Sweden, aged 56-60 years were randomized to either i) screening with cervical cytology (old policy) or ii) screening with HPV testing, followed by triage with cytology (new policy).

Outcome: The primary evaluation was the sensitivity for detection of cervical intraepithelial neoplasia grade II or worse (CIN2+).

Results: During January 2012 - May 2014, the organized screening program sent 42752 blinded invitations to the women in the target age group. 7325 women attended in the HPV policy arm and 7438 women attended in the cytology arm. In the new policy, the population HPV prevalence was 5.5%. There was thus a 94.5% decline in number of cytologies performed. HPV16 prevalence was 1.0% and HPV18 prevalence was 0.3%. In the HPV-policy arm, 78/405 (19%) HPV-positive women were also cytology positive and there were 19 cases of CIN2+ in histopathology. In the cytology policy, 153 women were cytology positive and there were 18 cases of CIN2+ in histopathology. Both the total number of cervical biopsies and the number of cervical biopsies with benign histopathology was much lower in the HPV policy (49 benign, 87 total versus 105 benign, 132 total).

Conclusion: primary HPV screening had, already before follow-up of HPV-positive women with new HPV test and referral of women with persistence, a similar sensitivity for CIN2+ as cytology-based screening.

**Strengths and limitations of this study**

- This study was performed within an organised cervical cancer screening programme.
- The randomized policy was evaluated with comprehensive registry linkages, providing population-based and complete data.
- The study contributes to the limited information available on experiences of real-life implementation of HPV screening.
- Limitations include a relatively short follow-up time, resulting in that most women with HPV persistence are not yet referred.
- How women perceive the information about HPV was not investigated and needs to be further evaluated.

## INTRODUCTION

Cervical screening using cytology has resulted in a marked reduction in cervical cancer incidence and mortality. Infection with oncogenic types of human papillomavirus (HPV) is a necessary risk factor for the development of cervical cancer and primary HPV DNA screening has a higher sensitivity for detection of the precursor lesions of cervical cancer than cytology<sup>1 2 3 4</sup>.

However, HPV DNA testing will also detect recent infections that are likely to clear spontaneously. Major trials of HPV-based screening have used triaging of HPV-positive women with cytology, follow-up of HPV+/Cyt- women with a new HPV test and referral of women who are HPV+/HPV+ (persistently positive for HPV)<sup>5</sup>. International guidelines recommend against referring HPV-positive women without triaging using cytology<sup>6</sup>.

Today, a large part of cervical cancers develop in women aged 60 and over, where no screening is offered. An HPV test before exiting the program is expected to give a longer lasting protection for cervical cancer than a cytology test. As HPV prevalences are age-dependent, with lower prevalence among the oldest, it is likely that the age group 56-60 could be a suitable age group for piloting of implementation of primary HPV screening as health gains are expected, but the numbers of HPV-positive women for work-up would be comparatively small.

Already in 2008, European guidelines recommended that HPV testing can be adopted as a primary screening tool, provided that it is implemented in a carefully controlled manner that can be evaluated<sup>6</sup>. The Randomized Healthcare Policy<sup>7</sup> was specifically mentioned as a recommended study design for a carefully controlled implementation that can be reliably evaluated<sup>6</sup>.

The primary objective of this study was to evaluate whether implementation of primary HPV screening in the organized screening program for cervical cancer has at least the same sensitivity (safety) as cytology-based screening when implemented in real life.

## MATERIALS AND METHODS

The cervical cancer screening program in the Stockholm-Gotland region of Sweden invites women to screening at three year intervals between ages 23 and 50 and five year intervals between ages 51 and 60 (age is defined by birth year). During January 2012 to May 2014 women aged 56 to 60 invited to their last screening were randomized to two different screening policies: i) primary cytology with HPV test as triage for women with ASCUS and CIN1/LSIL (old policy) or primary HPV test with triaging by cytology of HPV positive women (new policy) (figure 1, study population). Information about the randomized implementation of HPV testing in the organized screening program was sent out to all eligible resident women as part of the invitation to participate in the screening program. Before the new policy was piloted, the protocol had been discussed in both the regional committee of specialist physicians in gynecology and in a national hearing and found to be in accordance with the current scientific evidence. The RHP protocol was approved by the regional ethics committee (REC) in Stockholm (Decision number 2011/1298-31/3). The act of participating at the screening visit the woman had been invited to was, by the REC, regarded as consent for participation in the program. The RHP is registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (registration number NCT01511328).

During January 2012- May 2014, 42752 invitations were sent out to eligible resident women in the age group 56-60 years of age from the organized cancer screening program.

Randomization to the two different screening policies used the last control digit in the personal identification number (PIN) of the women. 7438 women attended primary cytology screening, whereas 7325 women attended primary HPV screening. The same invitation letter, informing about the randomized policy, was sent to all women.

Samples were collected by trained midwives at the same maternity care units as already being used as screening stations by the organized program. The cervical brush was put in a standard test tube filled with PreservCyt media (Thinprep®, Hologic, Boxborough, MA; USA).

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3 The samples were collected identically, regardless of which primary screening test that would  
4 be used.  
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8 HPV testing was performed using the Cobas® 4800 HPV Test (Roche Molecular Systems,  
9 Inc, South Branchburg, NJ, USA). The HPV types tested for were: 16, 18, 31, 33, 35, 39, 45,  
10 51, 52, 56, 58, 59, 66 and 68. The Cobas system reports the tests as HPV 16 positive, HPV  
11 18 positive and Other High Risk HPV positive. All HPV-positive samples (regardless of HPV  
12 type) were subjected to reflex cytology. Samples with invalid results were retested once, if  
13 still invalid a new sample was taken. The same notification letters about cytology results as  
14 already used by the program were used in this RHP. The notification letter to HPV+/Cyt-  
15 negative women reported that there was virus in the sample, but that the cytology was  
16 normal. It was further stated that this is not dangerous as long as the cytology is normal, but  
17 that an invitation to a repeat HPV and cytology test will follow after a year (or after 3 years,  
18 see below). In May 2013, new evidence from a joint European randomized trial indicated that  
19 cyt- women were adequately protected against cervical cancer for 3 years and the interval for  
20 re-testing of HPV+/Cyt- women was extended to 3 years.  
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35 In the cytology arm, the samples were prepared for the cytological examination as being  
36 currently in use by the program.  
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### 39 **HPV-policy**

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41 Women who were high-risk HPV positive and had abnormal cytology were followed up  
42 according to the already established guidelines for follow-up of cytology (same as in the  
43 cytology arm). Those that were HR-HPV positive with normal cytology were followed up with  
44 another HPV test at an extra visit 12 months later or, from May 2013, three years later. If  
45 HPV persistent (positive for the same HPV types at both visits) they were referred to  
46 colposcopy. For the whole Stockholm-Gotland region, management of women with HPV  
47 persistence was centralized to a single experienced gynecologist.  
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### Cytology policy

Women with CIN2+ in primary cytology were always referred to colposcopy. Those with ASCUS or LSIL (CIN1) in cytology were triaged with HPV-testing. If HPV-positive, they were referred to colposcopy. If HPV-negative they were invited one year later for a new smear.

We used the Swedish National Cervical Screening Registry to retrieve the information on all cytology and histology results of the participating women. The registry contains information on all cervical smears and all cervical biopsies taken in all counties in Sweden, resulting in that also smears and biopsies from relocating women are detected.



## RESULTS

There were 42752 screening invitations with appointments sent out, with about 35% of the invited women attending the appointment.

The HPV prevalence in the population was 5.5% (405 women/7325 attending women). The HPV16 prevalence was 1% and HPV18 prevalence was 0.3%. All 405 HPV-positive samples had a reflex cytology triage. 78/405 HPV-positive women (19.3%) had an abnormal cytology.

In the cytology policy, 153/7438 attending women had abnormal cytology. Out of these, 134 had ASCUS/LSIL and were tested for HPV. 43 women tested positive for HPV (7 were positive for HPV 16 and 4 for HPV18). Among all the women in the cytology arm, 132 women had a cervical biopsy with histology. In 18/132 biopsies there was a CIN2+ lesion (Tables 1 & 2).

There was a large number of cervical histopathologies taken from women who had had a normal cytology (about 1% of the women), almost always with benign histopathological diagnoses (Figure 1). This is a real-life study with comprehensive follow-up of all events occurring for all women in the RHP. The medical records of these women were reviewed. For 2 women, no explanation could be found why a cervical histopathology had been taken. For the other women, there had been an abnormal cytology registered at another bodily site (vulva, vagina or endometrium).

The current study concerned cervical screening and cytologies & histopathologies taken outside of the cervix were thus not part of the study. As real-life cervical screening can result in that some smears from non-cervical sites are also taken, cervical biopsies can result from abnormalities in non-cervical smears. This activity appeared to be less common in the HPV policy, resulting in lower numbers of cervical biopsies in the HPV policy.

## DISCUSSION

The current study has implemented and evaluated primary HPV-based screening with triaging using cytology within a real-life organized cervical screening program. Primary HPV-based screening with triaging using cytology is nowadays the primarily recommended screening algorithm, from both the WHO, the EU and the Swedish National Board of Health and Welfare. In the age group where the new policy was first implemented (ages 56-60), we did not notice any adverse events that might have been expected (for example reduced adherence to follow-up induced by anxiety et c) and we conclude that the new policy was acceptable to the population.

The exact population-based HPV prevalences in this age group (with the HPV test used) was not known before, but the 5.5% prevalence was approximately as expected. There was thus a 94.5 % decrease in number of cytologies. The HPV test is performed using laboratory automation and the amount of personnel time required and costs was substantially reduced.

The number of primary referrals for gynecological follow-up was substantially reduced (to about half), but the yield of histopathology-verified CIN2+ was about the same, indicating an improved specificity of the screening program. HPV-screening is expected to result in an increased sensitivity for CIN2+, but only after cytology-negative women with HPV persistence have also been referred. As HPV+/Cyt- women are only re-tested for HPV after 3 years, the results of these referrals will not appear until about 2 years from now. HPV persistence after 3 years is reported to be about 30% and we can thus expect that about 100 women of the 305 HPV+/Cyt- women will ultimately be referred. The total amount of referrals induced by HPV-screening is thus expected to eventually be somewhat larger than, but comparable to, the amount of referrals induced by cytology-based screening. In this context, it should be noted that when a new screening program is first introduced there is a temporary increase in screen-detected lesions that were prevalent when the screening was started. Subsequent rounds of screening will to a larger extent detect lesions that occurred since the last

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3 screening round (incident lesions). The proportion of the lesions detected by HPV-based  
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5 screening, but not by cytology, that are prevalent or incident is not well known, although  
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7 estimates have been provided by some clinical trials that have run for several screening  
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9 rounds.

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11 The present study is investigating the effects of a real-life use of HPV-based screening. One  
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13 of the effects that is seen by a real-life study (but not by a randomized clinical trial, that are  
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15 typically double-blinded) is the effects that knowledge of the test results may have. It is for  
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17 example likely that cytotechnicians reading slides known to be HPV-positive may have an  
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19 increased attention, reducing the likelihood for false negative results (so-called reader error).  
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21 The fact that twice as many smears with ASCUS/LSIL diagnoses were detected in the HPV  
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23 screening arm may be a reflection of this. Conversely, it may seem surprising that the same  
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25 yield of CIN2+ is obtained in both arms, even though both arms used cytology results for  
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27 referral and the cytology arm performed 95% less cytologies – at least some CIN2+ lesions  
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29 would have been expected to be detected by reading HPV-negative smears. Possibly, the  
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31 effects of non-detection of HPV-negative CIN2+ and reduced reader error because of more  
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33 focused cytology readings may have been of about the same magnitude resulting in similar  
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35 yields.  
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39 The substantial amount of cervical histopathologies taken among women with normal  
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41 cervical cytology was largely attributable to prior cytologies with abnormal results, taken from  
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43 non-cervical sites. Screening for diseases of the vulva, vagina or endometrium is not an aim  
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45 of the cervical screening program. As for example endometrial cancer is known to be HPV-  
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47 negative, endometrial cancers will no longer be detected by the cervical screening program  
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49 when it is based on HPV detection. Evaluation of whether cervical screening has indeed had  
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51 any health benefit with regards to endometrial cancer therefore appears to be a research  
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53 priority.  
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3 The 2015 Swedish cervical screening guidelines recommend HPV-based screening for  
4 women 30-64 years of age. The current randomized health care policy finds that HPV-based  
5 screening is readily possible to implement, using the existing resources for the organized  
6 program and for the clinical follow-up.  
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12 This study was supported by the Swedish foundation for strategic research.  
13

### 14 15 **Competing interest**

16 None declared.  
17

### 18 19 **Contributors**

20 HL – aquired HPV testing data, coordinated the project and the writing of the paper.  
21

22 CE – assisted in planning of the project and its quality assurance.  
23

24 KME – assisted with registry linkages and interpretation of data.  
25

26 ACT – coordinated the sample taking stations and assisted in planning of the project.  
27

28 MH – performed registry linkages.  
29

30 KE – performed the clinical follow up of HPV-positive women, assisted with planning of the  
31 project.  
32

33 ST – coordinator of the screening programme, participated in project planning and  
34 responsible for implementation. Provided supervision.  
35

36 JD – designed the study and provided supervision.  
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38 All authors contributed to the writing of the manuscript by providing important intellectual  
39 input.  
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## Data sharing statement

Individual level data will be shared on request, to be sent to the corresponding author.

For peer review only

Table 1. Baseline characteristics of women in the Intervention and Control arm.

	Primary HPV Policy	Primary Cytology Policy
Invited women	21122	21630
Attending women	7325 (34.7%)	7438 (34.4%)
Referral rate to histopathology	59 (0.8%)	51 (0.7%)
Women with CIN2+ in histopathology	19	18
Lowgrade lesions	17	9
Benign Histopathologies	49	105

Table 2. Women attending primary HPV screening

Primary HPV	
Screened women	7325
HPV positive	405 (5.5%)
HPV positive with abnormal cytology	78
Histopathologies (HPV positive/All)	59/85
CIN2+ histopathologies (HPV positive/All)	19/19
Lowgrade lesions (HPV positive/All)	16/17
Benign histopathologies (HPV positive/All)	24/49

Table 3. Women attending primary cytology screening

Primary Cytology	
Screened women	7438
Women with abnormal cytology	153
HPV test of women with abnormal cytology	134
HPV positive women with abnormal cytology	43
Histopathologies (Abnormal cytology/All)	51/132
CIN2+ in histopathology (Abnormal cytology/All)	16/18
Lowgrade lesions (Abnormal cytology/All)	8/9
Benign histopathologies (Abnormal cytology/All)	27/105

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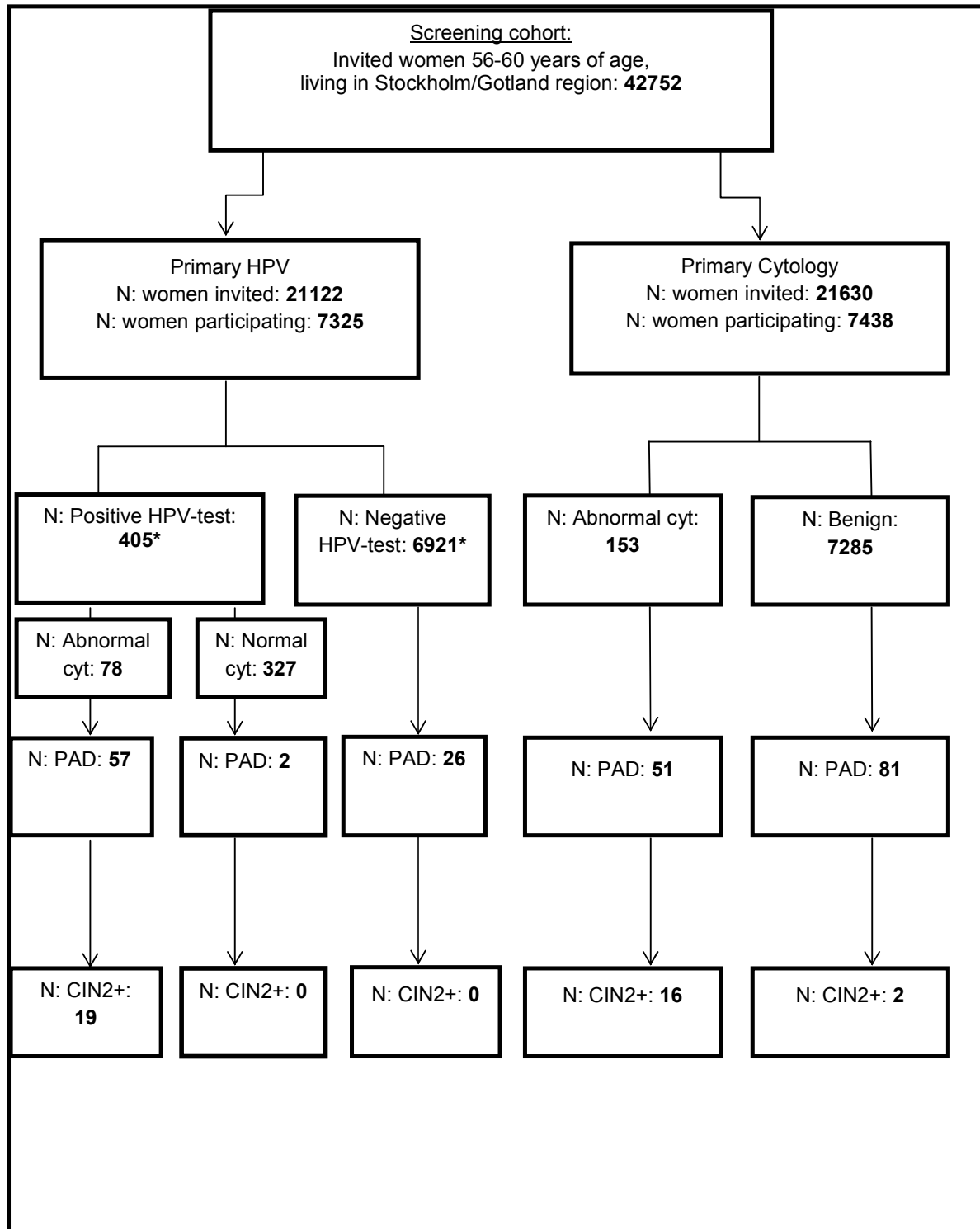


Figure 1: Study chart \*One woman had two samples taken, one negative and one positive for HPV.

PAD=Cervical histopathology



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# BMJ Open

## RANDOMIZED HEALTH CARE POLICY EVALUATION OF ORGANISED PRIMARY HUMAN PAPILLOMAVIRUS (HPV) SCREENING OF WOMEN AGED 56 - 60

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## ABSTRACT

Objective: To implement and reliably evaluate primary Human Papillomavirus (HPV) screening in an established and routinely running organized, large-scale population-based screening program.

Participants: Resident women in the Stockholm/Gotland region of Sweden, aged 56-60 years were randomized to either i) screening with cervical cytology, with HPV test in triage of low-grade cytological abnormalities (old policy) or ii) screening with HPV testing, with cytology in triage of HPV positives (new policy).

Outcome: The primary evaluation was the detection rate of cervical intraepithelial neoplasia grade II or worse (CIN2+).

Results: During January 2012 - May 2014, the organized screening program sent 42752 blinded invitations with a pre-booked appointment time to the women in the target age group. 7325 women attended in the HPV policy arm and 7438 women attended in the cytology arm. In the new policy, the population HPV prevalence was 5.5%, using an accredited HPV test (Cobas 4800). HPV16 prevalence was 1.0% (73/7325) and HPV18 prevalence was 0.3% (22/7325). In the HPV-policy arm, 78/405 (19%) HPV-positive women were also cytology positive. There were 19 cases of CIN2+ in histopathology, all among women who were both HPV-positive and cytology-positive. The PPV for CIN2+ in this group was 33.3% (19/57). In the cytology policy, 153 women were cytology positive and there were 18 cases of CIN2+ in histopathology. Both the total number of cervical biopsies and the number of cervical biopsies with benign histopathology was much lower in the HPV policy (49 benign, 87 total versus 105 benign, 132 total).

Conclusion: Primary HPV screening had a similar detection rate for CIN2+ as cytology-based screening, already before follow-up of HPV-positive, cytology-negative women with new HPV test and referral of women with persistence.

### Strengths and limitations of this study

- This study was performed within an organised cervical cancer screening programme.
- The randomized policy was evaluated with comprehensive registry linkages, providing population-based and complete data.
- The study contributes to the limited information available on experiences of real-life implementation of HPV screening.
- Limitations include a relatively short follow-up time, resulting in that most women with HPV persistence are not yet referred for clinical follow-up..
- How women perceive the information about HPV was not investigated and needs to be further evaluated.

## INTRODUCTION

Cervical screening using cytology has resulted in a marked reduction in cervical cancer incidence and mortality. Infection with oncogenic types of human papillomavirus (HPV) is a necessary risk factor for the development of cervical cancer and primary HPV DNA screening has a higher sensitivity for detection of the precursor lesions of cervical cancer than cytology<sup>1 2 3 4</sup>.

However, HPV DNA testing will also detect recent infections that are likely to clear spontaneously. Major trials of HPV-based screening have used triaging of HPV-positive women with cytology, follow-up of HPV+/Cyt- women with a new HPV test and referral of women who are HPV+/HPV+ (persistently positive for HPV)<sup>5</sup>. International guidelines recommend against referring HPV-positive women without triaging using cytology<sup>6</sup>.

Today, a large part of cervical cancers develop in women aged 60 and over, where no screening is offered. A negative HPV test before exiting the program is expected to give a longer lasting protection for cervical cancer than a negative cytology test. As HPV prevalences are age-dependent, with lower prevalence among the oldest, it is likely that the age group 56-60 could be a suitable age group for piloting of implementation of primary HPV screening as health gains are expected, but the numbers of HPV-positive women for work-up would be comparatively small.

Already in 2008, European guidelines recommended that HPV testing can be adopted as a primary screening tool, provided that it is implemented in a carefully controlled manner that can be evaluated<sup>6</sup>. The Randomized Healthcare Policy<sup>7</sup> (RHP) was specifically mentioned as a recommended study design for a carefully controlled implementation that can be reliably evaluated<sup>6</sup>.

The primary objective of this study was to evaluate whether implementation of primary HPV screening in the organized screening program for cervical cancer has at least the same CIN2+ detection rate (safety) as cytology-based screening when implemented in real life.

## MATERIALS AND METHODS

The cervical cancer screening program in the Stockholm-Gotland region of Sweden invites women to screening at three year intervals between ages 23 and 50 and five year intervals between ages 51 and 60 (age is defined by birth year). The population coverage of being tested is high: 74,4% of resident women in the target ages are tested according to recommendations. Women who do not attend the appointment in the invitation letter are invited again next year, resulting in that never-attending women will during their ages 23-64 have been invited >40 times. Thus, less than half of all invitations are “primary” invitations to regularly attending women and more than half of all invitations are reminder invitations to previously non-attending women. Population test coverage should thus not be confused with invitation attendance rate, which (because of the high proportion of reminder invitations to non-attenders) is about 35%.

There are no particular rules with regards to the last screening test – the rules for follow-up of that test are just the same as for all other tests. There is a globally a discussion on the usefulness of more stringent rules for the last, exit test, but this has not yet been implemented in our population.

During January 2012 to May 2014 the resident women aged 56 to 60 who were invited to their last screening were randomized to two different screening policies: i) primary cytology with HPV test as triage for women with low-grade cytology (ASCUS and CIN1/LSIL) (old policy) or primary HPV test with triaging by cytology of HPV positive women (new policy) (figure 1, study population). Information about the randomized implementation of HPV testing in the organized screening program was sent out to all eligible resident women as part of the invitation to participate in the screening program. Before the new policy was piloted, the protocol had been discussed in both the regional committee of specialist physicians in gynecology and in a national hearing and found to be in accordance with the current scientific evidence. The RHP protocol was approved by the regional ethics committee (REC)

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3 in Stockholm (Decision number 2011/1298-31/3). The act of participating at the screening  
4 visit the woman had been invited to was, by the REC, regarded as consent for participation in  
5 the program. The RHP is registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (registration number  
6 NCT01511328). The current report describes the pilot phase of the RHP, which was  
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8 designed to have a statistical power of 80% at a statistical significance level of 5%, assuming  
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10 a constant rate for actual CIN2+ incidence in each group and a similar sensitivity in each  
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12 group (80% actual sensitivity) and a target to show non-inferiority with a lower threshold of  
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14 80%.  
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19 During the study period, 42752 invitations were sent out to eligible resident women in the age  
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21 group 56-60 years of age from the organized cancer screening program. Randomization to  
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23 the two different screening policies used the last control digit in the personal identification  
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25 number (PIN) of the women. 7438 women attended primary cytology screening, whereas  
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27 7325 women attended primary HPV screening. The same invitation letter, informing about  
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29 the randomized policy, was sent to all women.  
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32 Samples were collected by trained midwives at the same, about 60, maternity care units as  
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34 already being contracted as screening stations by the organized program. All samples were  
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36 taken using the same liquid-based cytology system (Thinprep®, Hologic, Boxborough, MA;  
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38 USA), where the cervical brush of the system was put in a standard test tube filled with  
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40 PreservCyt media. The samples were collected identically, regardless of which primary  
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42 screening test that would be used.  
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45 HPV testing was performed using the Cobas® 4800 HPV Test (Roche Molecular Systems,  
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47 Inc, South Branchburg, NJ, USA). The HPV types tested for were: 16, 18, 31, 33, 35, 39, 45,  
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49 51, 52, 56, 58, 59, 66 and 68. The Cobas system reports the tests as HPV 16 positive, HPV  
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51 18 positive and Other High Risk HPV positive. All HPV-positive samples (regardless of HPV  
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53 type) were subjected to reflex cytology. Samples with invalid results were retested once, if  
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55 still invalid a new sample was taken. The same notification letters about cytology results as  
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3 already used by the program were used in this RHP. The notification letter to HPV+/Cyt-  
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5 negative women reported that there was virus in the sample, but that the cytology was  
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7 normal. It was further stated that this is regarded as a normal finding as long as the cytology  
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9 is normal, but that an invitation to a repeat HPV and cytology test would follow after a year (or  
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11 after 3 years, see below). In May 2013, new evidence from a joint European randomized trial  
12  
13 indicated that cyt- women were adequately protected against cervical cancer for 3 years and  
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15 the interval for re-testing of HPV+/Cyt- women was extended to 3 years.

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17 In the cytology arm, the samples were prepared for the cytological examination according to  
18  
19 current program standards. Clinical follow-up was exactly the same for both arms, both  
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21 regarding cytology (including the combination of low-grade cytology/HPV positivity) and  
22  
23 histopathology. The guidelines for clinical management were the same as routinely used in  
24  
25 Sweden. Briefly, there is judgement of the transformation zone for visibility, maturation and  
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27 epithelial changes. Application of 5% acetic acid assists in identifying undifferentiated  
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29 epithelia or inflammation as well as true CIN. The dimension and borderline of an abnormal  
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31 area is assessed by application of 5% potassium iodine to the ectocervix. All acetowhite  
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33 areas, including metaplasia (undifferentiated epithelium), inflammation and neoplasia are  
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35 biopsied. In case of incomplete colposcopy, several alternatives may be chosen, including  
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37 repeat cytology, endocervical cytology or diagnostic conization<sup>8</sup> As the cervical cancer-  
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39 preventive effect of the Swedish cervical screening program is among the best in the world<sup>6</sup>,  
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41 particularly for women who have actually participated, there is evidence to indicate that the  
42  
43 clinical management is adequate.

#### 44 45 46 **HPV-policy**

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48 Women who were high-risk HPV positive and had abnormal cytology were followed up  
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50 according to the already established guidelines for follow-up of cytology (same as in the  
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52 cytology arm). Those that were HR-HPV positive with normal cytology were followed up with  
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54 another HPV test at an extra visit 12 months later or, from May 2013, three years later. If  
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56 HPV persistent (positive for the same HPV types at b. Women positive for "Other HPV" in the  
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3 Cobas 4800 test had a second HPV test with complete HPV typing (Luminex) to determine if  
4 there was indeed type-specific persistence) they were referred to colposcopy. For the whole  
5 Stockholm-Gotland region, management of women with HPV persistence was centralized to  
6 a single experienced gynecologist.  
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### 10 11 **Cytology policy**

12 Women with CIN2+ in primary cytology were always referred to colposcopy. Those with low-  
13 grade cytological abnormalities (ASCUS (atypical squamous cell of uncertain significance) or  
14 LSIL (low-grade squamous intraepithelial lesion) (CIN1)) were triaged with HPV-testing. If  
15 HPV-positive, they were referred to colposcopy. If HPV-negative they were invited one year  
16 later for a new smear.  
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24 We used the Swedish National Cervical Screening Registry to retrieve the information on all  
25 cytology and histology results of the participating women. The registry contains information  
26 on all cervical smears and all cervical biopsies taken in all counties in Sweden, resulting in  
27 that also smears and biopsies from women who relocated are detected. The structure,  
28 operation and completeness of the registry has been described.<sup>9</sup> Please observe that, since  
29 the registry contains all data, a subsequent test could be taken for other reasons than the  
30 result of a previous test.  
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## RESULTS

There were 42752 screening invitations with appointments sent out to all resident women in the target area who did not yet have a smear taken according to recommendations (Table 1). As reminder invitations continue to be sent every year to non-attending women, the majority of invitations are reminder invitations sent to previously non-attending women. Thus, although the program has high population test coverage (74,4%) the proportion of invited women who attend each invitation is about 35% (Table 1).

The HPV prevalence in the population was 5.5% (405 women/7325 attending women)(Table 2). The HPV16 prevalence was 1% and HPV18 prevalence was 0.3%. All 405 HPV-positive samples had a reflex cytology triage. 78/405 HPV-positive women (19.3%) had an abnormal cytology (Table 2).

In the cytology policy, 153/7438 attending women had an abnormal cytology (Table 3). Out of these, 134 had ASCUS/LSIL and were tested for HPV. 43 women tested positive for HPV (7 were positive for HPV 16 and 4 for HPV18). Among all the women in the cytology arm, 132 women had a cervical biopsy with histology. In 18/132 biopsies there was a CIN2+ lesion (Figure 1; Table 3). The detection rate of CIN2+ was similar in the 2 arms (Odds Ratio 1.07), but the 95% confidence interval was wide (0.56-2.04) and completion of the final phase of the study will be needed to ascertain non-inferiority.

There was a large number of cervical histopathologies taken from women who had had a normal cytology (about 1% of the women), almost always with benign histopathological diagnoses (Figure 1). This is a real-life study with comprehensive follow-up of all cervical events occurring for all women in the RHP. The medical records of these women were reviewed. For 2 women, no explanation could be found why a cervical histopathology had been taken. For the other women, there had been an abnormal cytology registered at another bodily site (vulva, vagina or endometrium).

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3 The current study concerned cervical screening and cytologies & histopathologies taken  
4 outside of the cervix were thus not part of the study. But the study protocol included  
5 assessment of all cervical cytologies and histopathologies among randomized women,  
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7 regardless of the reason for taking the tests. As real-life cervical screening can result in that  
8  
9 some smears from non-cervical sites are also taken, cervical biopsies can result from  
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11 abnormalities in non-cervical smears. This activity appeared to be less common in the HPV  
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13 policy, resulting in lower numbers of cervical biopsies in the HPV policy.  
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## DISCUSSION

The current study has implemented and evaluated primary HPV-based screening with triaging using cytology within a real-life organized cervical screening program. Primary HPV-based screening with triaging using cytology is since 2015 the main recommended screening algorithm, from both the WHO, the EU<sup>6</sup> and the Swedish National Board of Health and Welfare ([www.socialstyrelsen.se](http://www.socialstyrelsen.se)). In the age group where the new policy was first implemented (ages 56-60), we did not notice any adverse events that might have been expected (for example reduced adherence to follow-up induced by anxiety et c) and we conclude that the new policy was acceptable to the population.

The exact population-based HPV prevalences in this age group (with the HPV test used) was not known before, but the 5.5% prevalence was approximately as expected. For example, in a large Danish cohort study, across all age groups, 898 of 4413 (20.3%) women with normal cytology were HPV DNA-positive by Cobas 4800. This proportion decreased from 33.9% in women aged 23–29 years to 18.6% at age 30–39 years and 6.0% at age 50–65 years<sup>10</sup> The HPV test is performed using laboratory automation and the amount of personnel time required and costs was substantially reduced.

The number of primary referrals for gynecological follow-up was substantially reduced (to about half), but the yield of histopathology-verified CIN2+ was about the same, indicating an improved specificity of the screening program. HPV-screening is expected to result in an increased sensitivity for CIN2+, but only after cytology-negative women with HPV persistence have also been referred. As HPV+/Cyt- women are only re-tested for HPV after 3 years, the results of these referrals will not appear until about 2 years from now. HPV persistence after 3 years is reported to be about 30% and we can thus expect that about 100 women of the 305 HPV+/Cyt- women will ultimately be referred. The total amount of referrals induced by HPV-screening is thus expected to eventually be somewhat larger than, but comparable to, the amount of referrals induced by cytology-based screening. In this context, it should be

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3 noted that when a new screening test is first introduced there is a temporary increase in  
4 screen-detected lesions that were prevalent when the screening was started. Subsequent  
5 rounds of screening will, to a larger extent, detect lesions that occurred since the last  
6 screening round (incident lesions). The proportion of the lesions detected by HPV-based  
7 screening, but not by cytology, that are prevalent or incident is not well known, although  
8 estimates have been provided by some clinical trials that have run for several screening  
9 rounds.

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11 The present study investigated the effects of a real-life use of HPV-based screening. One of  
12 the effects that is seen by a real-life study (but not by a randomized clinical trial, that is  
13 typically double-blinded) is the effect that knowledge of the test results may have. It is for  
14 example likely that cytotechnicians reading slides known to be HPV-positive may have an  
15 increased attention, reducing the likelihood for false negative results (so-called reader error).  
16 The fact that more abnormal cytologies (confer Tables 1 and 2) were detected in the HPV  
17 screening arm may be a reflection of this. Conversely, it may seem surprising that the same  
18 yield of CIN2+ is obtained in both arms, even though both arms used cytology results for  
19 referral and the cytology arm performed 95% less cytologies – at least some CIN2+ lesions  
20 would have been expected to be detected by reading HPV-negative smears. Possibly, the  
21 effects of non-detection of HPV-negative CIN2+ and reduced reader error because of more  
22 focused cytology readings may have been of about the same magnitude resulting in similar  
23 yields.

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25 The substantial amount of cervical histopathologies taken among women with normal  
26 cervical cytology was largely attributable to prior cytologies with abnormal results, taken from  
27 non-cervical sites. Screening for diseases of the vulva, vagina or endometrium is not an aim  
28 of the cervical screening program. As for example endometrial cancer is known to be HPV-  
29 negative, endometrial cancers will no longer be detected by the cervical screening program  
30 when it is based on HPV detection. Reduction of cervical biopsies taken after smears from  
31 non-cervical sites thus appears to be a likely benefit of the switch to HPV-based screening.

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3 Evaluation of whether cervical screening has indeed had any health benefit with regards to  
4 endometrial cancer appears to be a research priority.

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7 Strengths of the study: This study was performed within an organised cervical cancer  
8 screening programme with high population coverage and the results are therefore  
9 generalizable to a real-life population-based cervical screening program, including exactly  
10 the same strategy for reaching non-attending women (repeat reminder invitations). The  
11 attending women will therefore constitute the same mix of women attending “primary”  
12 invitations and women attending reminder invitations as seen in the real-life screening  
13 program.  
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22 The randomized policy was evaluated with comprehensive registry linkages, providing  
23 population-based and complete data. Because Sweden has a population-based system for  
24 registration of smears and biopsies, all results are included even if women chose to attend  
25 healthcare to which they were not referred (e.g. private healthcare). This reduces the risk for  
26 selection biases incurred by losses to follow-up.  
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32 Limitations of the study: Limitations include a relatively short follow-up time, resulting in that  
33 most women with HPV persistence are not yet referred. Although longer follow-up to include  
34 the results of referrals of women with HPV persistence (but normal cytology) is expected to  
35 increase sensitivity of HPV screening, studies nested in a real-life screening program (where  
36 continued invitations and testing is taking place) will face increasing difficulties in  
37 ascertaining exactly which smear induced which biopsy. From a program perspective,  
38 providing the overall results from the first 2 years of organised HPV screening is therefore  
39 considered more useful than providing the ultimate sensitivity estimates. Furthermore, these  
40 estimates are well known from many previous studies in the research setting <sup>25 11-15</sup>  
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50 Another limitation is that we did not follow-up how women perceived the information about  
51 HPV. This needs to be further evaluated, but a conclusion of the current study is that  
52 population-based HPV screened appeared to be generally acceptable to the population.  
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3 Comparison with others: The study contributes to the limited information available on  
4 experiences of real-life implementation of HPV screening. Although a very large number of  
5 studies have evaluated HPV screening in the research setting, also in longitudinally followed  
6 randomized controlled trials with invasive cancer as endpoint <sup>5 15</sup> , there are only few studies  
7 reporting on HPV screening in a real-life program. An RHP in Finland had a rather similar  
8 study design <sup>16</sup> except that a broad implementation in all relevant ages was introduced at the  
9 outset. We chose a more cautious implementation with a relatively small RHP restricted only  
10 to a small age group. A study on the introduction of HPV screening in central Italy reported a  
11 doubled increase in detection rates and a fourfold increase in referral rates, but with a  
12 protocol where HPV-positive, cytology-negative women were retested after 1 year<sup>17</sup>. The  
13 authors suggest that more conservative protocols are needed. Indeed, Dijkstra et al reported  
14 that HPV-positive, cytology negative women can safely be referred to late retesting 5 years  
15 later<sup>18</sup>.

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29 Our study implies that HPV screening will have similar safety as cytology-based screening in  
30 the first years after introduction, even before referring HPV-positive cytology-negative  
31 women. The issue of how to manage this group of women may depend on available  
32 resources, but it seems clear that aggressive management is not required from a safety  
33 perspective.

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40 The difference between a real-life study and a research study is clearly exemplified by the  
41 noteworthy number of screened women who are referred for clinical follow-up, but never  
42 have a biopsy taken (confer Figure 1). In the research setting, there is typically near-  
43 complete adherence to guidelines. In the real-life setting, there are often deviations from  
44 guidelines. For example, the most recent assessment found that there are annually about  
45 160 women in Sweden with a high-grade abnormality in cytology, but no subsequent biopsy <sup>9</sup>  
46 which confers a very high risk for cervical cancer development.



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3 Caution should be exerted in interpreting our experience that the total cost of screening was  
4 reduced with HPV screening. Costs of testing are highly dependent on purchasing, efficiency  
5 of laboratory organization and testing volumes used, which may be different in other settings.  
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9 In conclusion, the current randomized health care policy finds that HPV-based screening is  
10 readily possible to implement, using the existing resources for the organized program and for  
11 the clinical follow-up. Primary HPV screening had, even before follow-up of HPV-positive,  
12 cytology-negative women with new HPV test and referral of women with persistence, a  
13 similar detection rate for CIN2+ as cytology-based screening. The 2015 Swedish cervical  
14 screening guidelines ([www.socialstyrelsen.se](http://www.socialstyrelsen.se)) recommend HPV-based screening for women  
15 30-64 years of age and the results of the current study support an extension of HPV  
16 screening using the protocol of this study to the entire target group 30-64 years of age.  
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27  
28 This study was supported by the Swedish Foundation for Strategic Research.  
29  
30

## 31 **Competing interest**

32  
33 None declared.  
34  
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36

## 37 **Contributors**

38  
39 HL – acquired HPV testing data, coordinated the project and the writing of the paper.  
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41

42  
43 CE – assisted in planning of the project and its quality assurance.  
44  
45

46  
47 KME – assisted with registry linkages and interpretation of data.  
48  
49

50  
51 ACT – coordinated the sample taking stations and assisted in planning of the project.  
52  
53

54  
55 MH – performed registry linkages.  
56  
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58  
59 KE – performed the clinical follow up of HPV-positive women, assisted with planning of the  
60 project.

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2  
3 ST – coordinator of the screening programme, participated in project planning and  
4 responsible for implementation. Provided supervision.  
5  
6

7 JD – designed the study and provided supervision.  
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10 All authors contributed to the writing of the manuscript by providing important intellectual  
11 input.  
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### 14 15 16 17 18 **Data sharing statement** 19

20 Individual level data will be shared on request, to be sent to the corresponding author.  
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Table 1. Baseline characteristics of women in the Intervention and Control arm.

	Primary HPV Policy	Primary Cytology Policy
Invited women	21122	21630
Attending women	7325 (34.7%)	7438 (34.4%)
Referral rate to histopathology	59 (0.8%)	51 (0.7%)
Women with CIN2+ in histopathology	19	18
Lowgrade lesions (CIN1) in histopathology	17	9
Benign Histopathologies (any histopathological diagnosis of lower severity than CIN 1).	49	105

Table 2. Women attending primary HPV screening

<b>Primary HPV</b>	
Screened women	7325
HPV positive	405 (5.5%)
HPV positive with abnormal cytology	78
Histopathologies (HPV positive/All)	59/85
CIN2+ histopathologies (HPV positive/All)	19/19
CIN1 histopathology (HPV positive/All)	16/17
Benign histopathologies (HPV positive/All)	24/49

Table 3. Women attending primary cytology screening

<b>Primary Cytology</b>	
Screened women	7438
Women with abnormal cytology	153
HPV test of women with abnormal cytology	134
HPV positive women with abnormal cytology	43
Histopathologies (Abnormal cytology/All)	51/132
CIN2+ in histopathology (Abnormal cytology/All)	16/18
CIN1 histopathology (Abnormal cytology/All)	8/9
Benign histopathologies (Abnormal cytology/All)	27/105

## FIGURE LEGEND

## Figure 1: Study chart

\*One woman had two samples taken, one negative and one positive for HPV. PAD=Cervical histopathology \$ Constitutes 11 women with CIN3, 6 with CIN2, 1 woman with CIN2/3 (exact grading of given) and 1 woman with invasive cervical cancer. #Constitutes 8 women with CIN3 and 8 women with CIN2. @ 2 women with invasive cervical cancer.

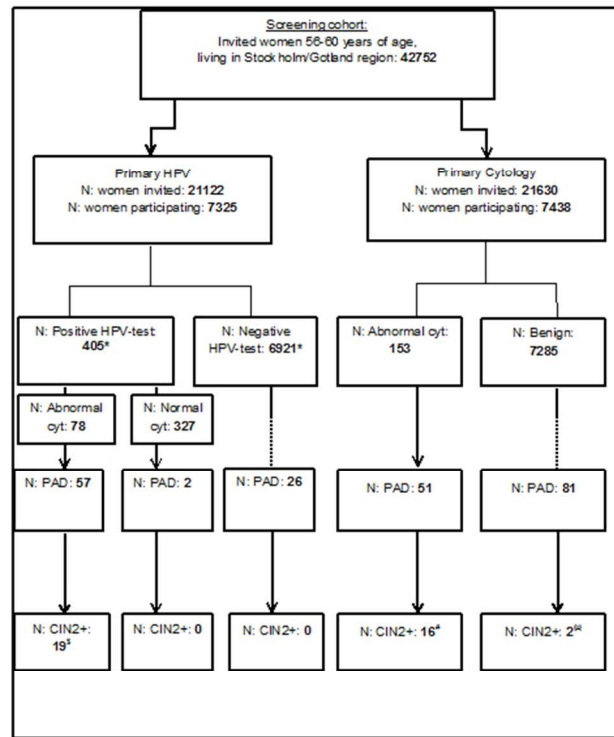
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## FIGURE LEGEND

Figure 1: Study chart

\*One woman had two samples taken, one negative and one positive for HPV. PAD=Cervical histopathology \$ Constitutes 11 women with CIN3, 6 with CIN2, 1 woman with CIN2/3 (exact grading not given) and 1 woman with invasive cervical cancer. #Constitutes 8 women with CIN3 and 8 women with CIN2. @ 2 women with invasive cervical cancer.

203x162mm (96 x 96 DPI)



# BMJ Open

## RANDOMIZED HEALTH CARE POLICY EVALUATION OF ORGANISED PRIMARY HUMAN PAPILLOMAVIRUS (HPV) SCREENING OF WOMEN AGED 56 - 60

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3 **RANDOMIZED HEALTH CARE POLICY EVALUATION OF ORGANISED**  
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5 **PRIMARY HUMAN PAPILLOMAVIRUS (HPV) SCREENING OF WOMEN AGED 56 - 60**  
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**ABSTRACT**

Objective: To implement and reliably evaluate primary Human Papillomavirus (HPV) screening in an established and routinely running organized, large-scale population-based screening program.

Participants: Resident women in the Stockholm/Gotland region of Sweden, aged 56-60 years were randomized to either i) screening with cervical cytology, with HPV test in triage of low-grade cytological abnormalities (old policy) or ii) screening with HPV testing, with cytology in triage of HPV positives (new policy).

Outcome: The primary evaluation was the detection rate of cervical intraepithelial neoplasia grade II or worse (CIN2+).

Results: During January 2012 - May 2014, the organized screening program sent 42752 blinded invitations with a pre-booked appointment time to the women in the target age group. 7325 women attended in the HPV policy arm and 7438 women attended in the cytology arm. In the new policy, the population HPV prevalence was 5.5%, using an accredited HPV test (Cobas 4800). HPV16 prevalence was 1.0% (73/7325) and HPV18 prevalence was 0.3% (22/7325). In the HPV-policy arm, 78/405 (19%) HPV-positive women were also cytology positive. There were 19 cases of CIN2+ in histopathology, all among women who were both HPV-positive and cytology-positive. The PPV for CIN2+ in this group was 33.3% (19/57). In the cytology policy, 153 women were cytology positive and there were 18 cases of CIN2+ in histopathology. Both the total number of cervical biopsies and the number of cervical biopsies with benign histopathology was much lower in the HPV policy (49 benign, 87 total versus 105 benign, 132 total).

Conclusion: Primary HPV screening had a similar detection rate for CIN2+ as cytology-based screening, already before follow-up of HPV-positive, cytology-negative women with new HPV test and referral of women with persistence.

### Strengths and limitations of this study

- This study was performed within an organised cervical cancer screening programme.
- The randomized policy was evaluated with comprehensive registry linkages, providing population-based and complete data.
- The study contributes to the limited information available on experiences of real-life implementation of HPV screening.
- Limitations include a relatively short follow-up time, resulting in that most women with HPV persistence are not yet referred for clinical follow-up..
- How women perceive the information about HPV was not investigated and needs to be further evaluated.

## INTRODUCTION

Cervical screening using cytology has resulted in a marked reduction in cervical cancer incidence and mortality. Infection with oncogenic types of human papillomavirus (HPV) is a necessary risk factor for the development of cervical cancer and primary HPV DNA screening has a higher sensitivity for detection of the precursor lesions of cervical cancer than cytology<sup>1 2 3 4</sup>.

However, HPV DNA testing will also detect recent infections that are likely to clear spontaneously. Major trials of HPV-based screening have used triaging of HPV-positive women with cytology, follow-up of HPV+/Cyt- women with a new HPV test and referral of women who are HPV+/HPV+ (persistently positive for HPV)<sup>5</sup>. International guidelines recommend against referring HPV-positive women without triaging using cytology<sup>6</sup>.

Today, a large part of cervical cancers develop in women aged 60 and over, where no screening is offered. A negative HPV test before exiting the program is expected to give a longer lasting protection for cervical cancer than a negative cytology test. As HPV prevalences are age-dependent, with lower prevalence among the oldest, it is likely that the age group 56-60 could be a suitable age group for piloting of implementation of primary HPV screening as health gains are expected, but the numbers of HPV-positive women for work-up would be comparatively small.

Already in 2008, European guidelines recommended that HPV testing can be adopted as a primary screening tool, provided that it is implemented in a carefully controlled manner that can be evaluated<sup>6</sup>. The Randomized Healthcare Policy<sup>7</sup> (RHP) was specifically mentioned as a recommended study design for a carefully controlled implementation that can be reliably evaluated<sup>6</sup>.

The primary objective of this study was to evaluate whether implementation of primary HPV screening in the organized screening program for cervical cancer has at least the same CIN2+ detection rate (safety) as cytology-based screening when implemented in real life.

## MATERIALS AND METHODS

The cervical cancer screening program in the Stockholm-Gotland region of Sweden invites women to screening at three year intervals between ages 23 and 50 and five year intervals between ages 51 and 60 (age is defined by birth year). The population coverage of being tested is high: 74,4% of resident women in the target ages are tested according to recommendations. Women who do not attend the appointment in the invitation letter are invited again next year, resulting in that never-attending women will during their ages 23-64 have been invited >40 times. Thus, less than half of all invitations are “primary” invitations to regularly attending women and more than half of all invitations are reminder invitations to previously non-attending women. Population test coverage should thus not be confused with invitation attendance rate, which (because of the high proportion of reminder invitations to non-attenders) is about 35%.

There are no particular rules with regards to the last screening test – the rules for follow-up of that test are just the same as for all other tests. There is a globally a discussion on the usefulness of more stringent rules for the last, exit test, but this has not yet been implemented in our population.

During January 2012 to May 2014 the resident women aged 56 to 60 who were invited to their last screening were randomized to two different screening policies: i) primary cytology with HPV test as triage for women with low-grade cytology (ASCUS and CIN1/LSIL) (old policy) or primary HPV test with triaging by cytology of HPV positive women (new policy) (figure 1, study population). Information about the randomized implementation of HPV testing in the organized screening program was sent out to all eligible resident women as part of the invitation to participate in the screening program. Before the new policy was piloted, the protocol had been discussed in both the regional committee of specialist physicians in gynecology and in a national hearing and found to be in accordance with the current scientific evidence. The RHP protocol was approved by the regional ethics committee (REC)

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3 in Stockholm (Decision number 2011/1298-31/3). The act of participating at the screening  
4 visit the woman had been invited to was, by the REC, regarded as consent for participation in  
5 the program. The RHP is registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (registration number  
6 NCT01511328). The current report describes the pilot phase of the RHP, which was  
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8 designed to have a statistical power of 80% at a statistical significance level of 5%, assuming  
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10 a constant rate for actual CIN2+ incidence in each group and a similar sensitivity in each  
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12 group (80% actual sensitivity) and a target to show non-inferiority with a lower threshold of  
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14 80%.  
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19 During the study period, 42752 invitations were sent out to eligible resident women in the age  
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21 group 56-60 years of age from the organized cancer screening program. Randomization to  
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23 the two different screening policies used the last control digit in the personal identification  
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25 number (PIN) of the women. 7438 women attended primary cytology screening, whereas  
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27 7325 women attended primary HPV screening. The same invitation letter, informing about  
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29 the randomized policy, was sent to all women.  
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32 Samples were collected by trained midwives at the same, about 60, maternity care units as  
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34 already being contracted as screening stations by the organized program. All samples were  
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36 taken using the same liquid-based cytology system (Thinprep®, Hologic, Boxborough, MA;  
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38 USA), where the cervical brush of the system was put in a standard test tube filled with  
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40 PreservCyt media. The samples were collected identically, regardless of which primary  
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42 screening test that would be used.  
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45 HPV testing was performed using the Cobas® 4800 HPV Test (Roche Molecular Systems,  
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47 Inc, South Branchburg, NJ, USA). The HPV types tested for were: 16, 18, 31, 33, 35, 39, 45,  
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49 51, 52, 56, 58, 59, 66 and 68. The Cobas system reports the tests as HPV 16 positive, HPV  
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51 18 positive and Other High Risk HPV positive. All HPV-positive samples (regardless of HPV  
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53 type) were subjected to reflex cytology. Samples with invalid results were retested once, if  
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55 still invalid a new sample was taken. The same notification letters about cytology results as  
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3 already used by the program were used in this RHP. The notification letter to HPV+/Cyt-  
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5 negative women reported that there was virus in the sample, but that the cytology was  
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7 normal. It was further stated that this is regarded as a normal finding as long as the cytology  
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9 is normal, but that an invitation to a repeat HPV and cytology test would follow after a year  
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11 (or after 3 years, see below). In May 2013, new evidence from a joint European randomized  
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13 trial indicated that cyt- women were adequately protected against cervical cancer for 3 years<sup>5</sup>  
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15 and the interval for re-testing of HPV+/Cyt- women was extended to 3 years. The European  
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17 trial did not specify duration of adequate protection for cytology-negative women by age  
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19 group<sup>5</sup> and sampling from the transformation zone may be more difficult among older  
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21 women. However, the duration of protection of cytology-negativity is well known to be at least  
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23 as long in older age groups, as for example reflected in the fact that many countries have  
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25 longer screening intervals for older women (Sweden for example: [www.socialstyrelsen.se](http://www.socialstyrelsen.se)),  
26  
27 and Sweden therefore considered it to be conservative (safe) to use a 3-year interval in all  
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29 age groups.

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31 In the cytology arm, the samples were prepared for the cytological examination according to  
32  
33 current program standards. Clinical follow-up was exactly the same for both arms, both  
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35 regarding cytology (including the combination of low-grade cytology/HPV positivity) and  
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37 histopathology. The guidelines for clinical management were the same as routinely used in  
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39 Sweden. Briefly, there is judgement of the transformation zone for visibility, maturation and  
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41 epithelial changes. Application of 5% acetic acid assists in identifying undifferentiated  
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43 epithelia or inflammation as well as true CIN. The dimension and borderline of an abnormal  
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45 area is assessed by application of 5% potassium iodine to the ectocervix. All acetowhite  
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47 areas, including metaplasia (undifferentiated epithelium), inflammation and neoplasia are  
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49 biopsied. In case of incomplete colposcopy, several alternatives may be chosen, including  
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51 repeat cytology, endocervical cytology or diagnostic conization<sup>8</sup> As the cervical cancer-  
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53 preventive effect of the Swedish cervical screening program is among the best in the world<sup>6</sup>,  
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2 particularly for women who have actually participated, there is evidence to indicate that the  
3 clinical management is adequate.  
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### 6 7 **HPV-policy**

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9 Women who were high-risk HPV positive and had abnormal cytology were followed up  
10 according to the already established guidelines for follow-up of cytology (same as in the  
11 cytology arm). Those that were HR-HPV positive with normal cytology were followed up with  
12 another HPV test at an extra visit 12 months later or, from May 2013, three years later. If  
13 HPV persistent (positive for the same HPV types at b. Women positive for “Other HPV” in the  
14 Cobas 4800 test had a second HPV test with complete HPV typing (Luminex) to determine if  
15 there was indeed type-specific persistence) they were referred to colposcopy. For the whole  
16 Stockholm-Gotland region, management of women with HPV persistence was centralized to  
17 a single experienced gynecologist.  
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### 28 **Cytology policy**

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30 Women with CIN2+ in primary cytology were always referred to colposcopy. Those with low-  
31 grade cytological abnormalities (ASCUS (atypical squamous cell of uncertain significance) or  
32 LSIL (low-grade squamous intraepithelial lesion) (CIN1)) were triaged with HPV-testing. If  
33 HPV-positive, they were referred to colposcopy. If HPV-negative they were invited one year  
34 later for a new smear.  
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41 We used the Swedish National Cervical Screening Registry to retrieve the information on all  
42 cytology and histology results of the participating women. The registry contains information  
43 on all cervical smears and all cervical biopsies taken in all counties in Sweden, resulting in  
44 that also smears and biopsies from women who relocated are detected. The structure,  
45 operation and completeness of the registry has been described.<sup>9</sup> Please observe that, since  
46 the registry contains all data, a subsequent test could be taken for other reasons than the  
47 result of a previous test.  
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For peer review only

## RESULTS

There were 42752 screening invitations with appointments sent out to all resident women in the target area who did not yet have a smear taken according to recommendations (Table 1). As reminder invitations continue to be sent every year to non-attending women, the majority of invitations are reminder invitations sent to previously non-attending women. Thus, although the program has high population test coverage (74,4%) the proportion of invited women who attend each invitation is about 35% (Table 1).

The HPV prevalence in the population was 5.5% (405 women/7325 attending women)(Table 2). The HPV16 prevalence was 1% and HPV18 prevalence was 0.3%. All 405 HPV-positive samples had a reflex cytology triage. 78/405 HPV-positive women (19.3%) had an abnormal cytology (Table 2).

In the cytology policy, 153/7438 attending women had an abnormal cytology (Table 3). Out of these, 134 had ASCUS/LSIL and were tested for HPV. 43 women tested positive for HPV (7 were positive for HPV 16 and 4 for HPV18). Among all the women in the cytology arm, 132 women had a cervical biopsy with histology. In 18/132 biopsies there was a CIN2+ lesion (Figure 1; Table 3). The detection rate of CIN2+ was similar in the 2 arms (Odds Ratio 1.07), but the 95% confidence interval was wide (0.56-2.04) and completion of the final phase of the study will be needed to ascertain non-inferiority.

There was a large number of cervical histopathologies taken from women who had had a normal cytology (about 1% of the women), almost always with benign histopathological diagnoses (Figure 1). This is a real-life study with comprehensive follow-up of all cervical events occurring for all women in the RHP. The medical records of these women were reviewed. For 2 women, no explanation could be found why a cervical histopathology had been taken. For the other women, there had been an abnormal cytology registered at another bodily site (vulva, vagina or endometrium).

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3 The current study concerned cervical screening and cytologies & histopathologies taken  
4 outside of the cervix were thus not part of the study. But the study protocol included  
5 assessment of all cervical cytologies and histopathologies among randomized women,  
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7 regardless of the reason for taking the tests. As real-life cervical screening can result in that  
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9 some smears from non-cervical sites are also taken, cervical biopsies can result from  
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11 abnormalities in non-cervical smears. This activity appeared to be less common in the HPV  
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13 policy, resulting in lower numbers of cervical biopsies in the HPV policy.  
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## DISCUSSION

The current study has implemented and evaluated primary HPV-based screening with triaging using cytology within a real-life organized cervical screening program. Primary HPV-based screening with triaging using cytology is since 2015 the main recommended screening algorithm, from both the WHO, the EU<sup>6</sup> and the Swedish National Board of Health and Welfare ([www.socialstyrelsen.se](http://www.socialstyrelsen.se)). In the age group where the new policy was first implemented (ages 56-60), we did not notice any adverse events that might have been expected (for example reduced adherence to follow-up induced by anxiety et c) and we conclude that the new policy was acceptable to the population.

The exact population-based HPV prevalences in this age group (with the HPV test used) was not known before, but the 5.5% prevalence was approximately as expected. For example, in a large Danish cohort study, across all age groups, 898 of 4413 (20.3%) women with normal cytology were HPV DNA-positive by Cobas 4800. This proportion decreased from 33.9% in women aged 23–29 years to 18.6% at age 30–39 years and 6.0% at age 50–65 years<sup>10</sup> The HPV test is performed using laboratory automation and the amount of personnel time required and costs was substantially reduced.

The number of primary referrals for gynecological follow-up was substantially reduced (to about half), but the yield of histopathology-verified CIN2+ was about the same, indicating an improved specificity of the screening program. HPV-screening is expected to result in an increased sensitivity for CIN2+, but only after cytology-negative women with HPV persistence have also been referred. As HPV+/Cyt- women are only re-tested for HPV after 3 years, the results of these referrals will not appear until about 2 years from now. HPV persistence after 3 years is reported to be about 30% and we can thus expect that about 100 women of the 305 HPV+/Cyt- women will ultimately be referred. The total amount of referrals induced by HPV-screening is thus expected to eventually be somewhat larger than, but comparable to, the amount of referrals induced by cytology-based screening. In this context, it should be

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3 noted that when a new screening test is first introduced there is a temporary increase in  
4 screen-detected lesions that were prevalent when the screening was started. Subsequent  
5 rounds of screening will, to a larger extent, detect lesions that occurred since the last  
6 screening round (incident lesions). The proportion of the lesions detected by HPV-based  
7 screening, but not by cytology, that are prevalent or incident is not well known, although  
8 estimates have been provided by some clinical trials that have run for several screening  
9 rounds.  
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17 The present study investigated the effects of a real-life use of HPV-based screening. One of  
18 the effects that is seen by a real-life study (but not by a randomized clinical trial, that is  
19 typically double-blinded) is the effect that knowledge of the test results may have. It is for  
20 example likely that cytotechnicians reading slides known to be HPV-positive may have an  
21 increased attention, reducing the likelihood for false negative results (so-called reader error).  
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23 The fact that more abnormal cytologies (confer Tables 1 and 2) were detected in the HPV  
24 screening arm may be a reflection of this. Conversely, it may seem surprising that the same  
25 yield of CIN2+ is obtained in both arms, even though both arms used cytology results for  
26 referral and the cytology arm performed 95% less cytologies – at least some CIN2+ lesions  
27 would have been expected to be detected by reading HPV-negative smears. Possibly, the  
28 effects of non-detection of HPV-negative CIN2+ and reduced reader error because of more  
29 focused cytology readings may have been of about the same magnitude resulting in similar  
30 yields.  
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44 The substantial amount of cervical histopathologies taken among women with normal  
45 cervical cytology was largely attributable to prior cytologies with abnormal results, taken from  
46 non-cervical sites. Screening for diseases of the vulva, vagina or endometrium is not an aim  
47 of the cervical screening program. As for example endometrial cancer is known to be HPV-  
48 negative, endometrial cancers will no longer be detected by the cervical screening program  
49 when it is based on HPV detection. Reduction of cervical biopsies taken after smears from  
50 non-cervical sites thus appears to be a likely benefit of the switch to HPV-based screening.  
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3 Evaluation of whether cervical screening has indeed had any health benefit with regards to  
4 endometrial cancer appears to be a research priority.

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7 Strengths of the study: This study was performed within an organised cervical cancer  
8 screening programme with high population coverage and the results are therefore  
9 generalizable to a real-life population-based cervical screening program, including exactly  
10 the same strategy for reaching non-attending women (repeat reminder invitations). The  
11 attending women will therefore constitute the same mix of women attending “primary”  
12 invitations and women attending reminder invitations as seen in the real-life screening  
13 program.  
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22 The randomized policy was evaluated with comprehensive registry linkages, providing  
23 population-based and complete data. Because Sweden has a population-based system for  
24 registration of smears and biopsies, all results are included even if women chose to attend  
25 healthcare to which they were not referred (e.g. private healthcare). This reduces the risk for  
26 selection biases incurred by losses to follow-up.  
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32 Limitations of the study: Limitations include a relatively short follow-up time, resulting in that  
33 most women with HPV persistence are not yet referred. Although longer follow-up to include  
34 the results of referrals of women with HPV persistence (but normal cytology) is expected to  
35 increase sensitivity of HPV screening, studies nested in a real-life screening program (where  
36 continued invitations and testing is taking place) will face increasing difficulties in  
37 ascertaining exactly which smear induced which biopsy. From a program perspective,  
38 providing the overall results from the first 2 years of organised HPV screening is therefore  
39 considered more useful than providing the ultimate sensitivity estimates. Furthermore, these  
40 estimates are well known from many previous studies in the research setting <sup>25 11-15</sup>  
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50 Another limitation is that we did not follow-up how women perceived the information about  
51 HPV. This needs to be further evaluated, but a conclusion of the current study is that  
52 population-based HPV screened appeared to be generally acceptable to the population.  
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3 Comparison with others: The study contributes to the limited information available on  
4 experiences of real-life implementation of HPV screening. Although a very large number of  
5 studies have evaluated HPV screening in the research setting, also in longitudinally followed  
6 randomized controlled trials with invasive cancer as endpoint <sup>5 15</sup> , there are only few studies  
7 reporting on HPV screening in a real-life program. An RHP in Finland had a rather similar  
8 study design <sup>16</sup> except that a broad implementation in all relevant ages was introduced at the  
9 outset. We chose a more cautious implementation with a relatively small RHP restricted only  
10 to a small age group. A study on the introduction of HPV screening in central Italy reported a  
11 doubled increase in detection rates and a fourfold increase in referral rates, but with a  
12 protocol where HPV-positive, cytology-negative women were retested after 1 year<sup>17</sup>. The  
13 authors suggest that more conservative protocols are needed. Indeed, Dijkstra et al reported  
14 that HPV-positive, cytology negative women can safely be referred to late retesting 5 years  
15 later<sup>18</sup>.

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29 Our study implies that HPV screening will have similar safety as cytology-based screening in  
30 the first years after introduction, even before referring HPV-positive cytology-negative  
31 women. The issue of how to manage this group of women may depend on available  
32 resources, but it seems clear that aggressive management is not required from a safety  
33 perspective.

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40 The difference between a real-life study and a research study is clearly exemplified by the  
41 noteworthy number of screened women who are referred for clinical follow-up, but never  
42 have a biopsy taken (confer Figure 1). In the research setting, there is typically near-  
43 complete adherence to guidelines. In the real-life setting, there are often deviations from  
44 guidelines. For example, the most recent assessment found that there are annually about  
45 160 women in Sweden with a high-grade abnormality in cytology, but no subsequent biopsy <sup>9</sup>  
46 which confers a very high risk for cervical cancer development.

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3 Caution should be exerted in interpreting our experience that the total cost of screening was  
4 reduced with HPV screening. Costs of testing are highly dependent on purchasing, efficiency  
5 of laboratory organization and testing volumes used, which may be different in other settings.  
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9 In conclusion, the current randomized health care policy finds that HPV-based screening is  
10 readily possible to implement, using the existing resources for the organized program and for  
11 the clinical follow-up. Primary HPV screening had, even before follow-up of HPV-positive,  
12 cytology-negative women with new HPV test and referral of women with persistence, a  
13 similar detection rate for CIN2+ as cytology-based screening. The 2015 Swedish cervical  
14 screening guidelines ([www.socialstyrelsen.se](http://www.socialstyrelsen.se)) recommend HPV-based screening for women  
15 30-64 years of age and the results of the current study support an extension of HPV  
16 screening using the protocol of this study to the entire target group 30-64 years of age.  
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27  
28 This study was supported by the Swedish Foundation for Strategic Research.  
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## 31 **Competing interest**

32  
33 None declared.  
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## 37 **Contributors**

38  
39 HL – acquired HPV testing data, coordinated the project and the writing of the paper.  
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42  
43 CE – assisted in planning of the project and its quality assurance.  
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46  
47 KME – assisted with registry linkages and interpretation of data.  
48  
49

50  
51 ACT – coordinated the sample taking stations and assisted in planning of the project.  
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55 MH – performed registry linkages.  
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59 KE – performed the clinical follow up of HPV-positive women, assisted with planning of the  
60 project.

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3 ST – coordinator of the screening programme, participated in project planning and  
4 responsible for implementation. Provided supervision.  
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7 JD – designed the study and provided supervision.  
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10 All authors contributed to the writing of the manuscript by providing important intellectual  
11 input.  
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### 14 15 16 17 18 **Data sharing statement** 19

20 Individual level data will be shared on request, to be sent to the corresponding author.  
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Table 1. Baseline characteristics of women in the Intervention and Control arm.

	Primary HPV Policy	Primary Cytology Policy
Invited women	21122	21630
Attending women	7325 (34.7%)	7438 (34.4%)
Referral rate to histopathology	59 (0.8%)	51 (0.7%)
Women with CIN2+ in histopathology	19	18
Lowgrade lesions (CIN1) in histopathology	17	9
Benign Histopathologies (any histopathological diagnosis of lower severity than CIN 1).	49	105

Table 2. Women attending primary HPV screening

<b>Primary HPV</b>	
Screened women	7325
HPV positive	405 (5.5%)
HPV positive with abnormal cytology	78
Histopathologies (HPV positive/All)	59/85
CIN2+ histopathologies (HPV positive/All)	19/19
CIN1 histopathology (HPV positive/All)	16/17
Benign histopathologies (HPV positive/All)	24/49

Table 3. Women attending primary cytology screening

<b>Primary Cytology</b>	
Screened women	7438
Women with abnormal cytology	153
HPV test of women with abnormal cytology	134
HPV positive women with abnormal cytology	43
Histopathologies (Abnormal cytology/All)	51/132
CIN2+ in histopathology (Abnormal cytology/All)	16/18
CIN1 histopathology (Abnormal cytology/All)	8/9
Benign histopathologies (Abnormal cytology/All)	27/105

## FIGURE LEGEND

## Figure 1: Study chart

\*One woman had two samples taken, one negative and one positive for HPV. PAD=Cervical histopathology \$ Constitutes 11 women with CIN3, 6 with CIN2, 1 woman with CIN2/3 (exact grading of given) and 1 woman with invasive cervical cancer. #Constitutes 8 women with CIN3 and 8 women with CIN2. @ 2 women with invasive cervical cancer.

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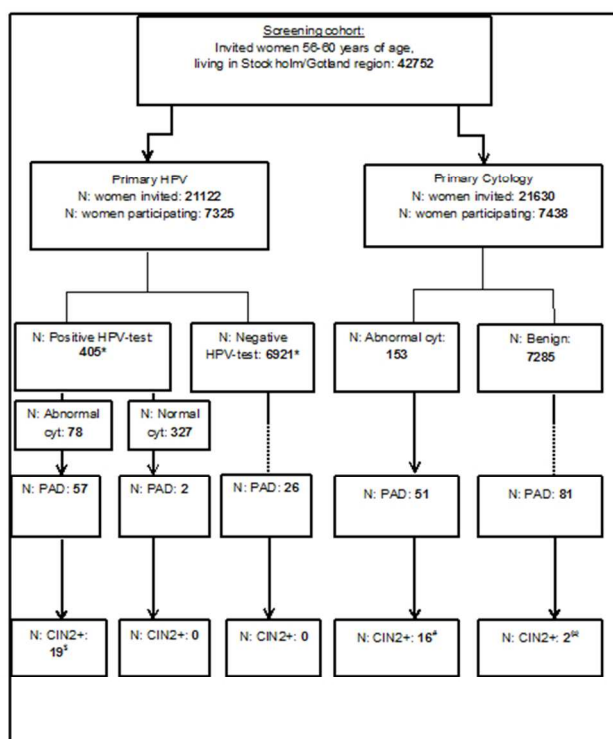


FIGURE LEGEND

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\*One woman had two samples taken, one negative and one positive for HPV. PAD=Cervical histopathology \$ Constitutes 11 women with CIN3, 6 with CIN2, 1 woman with CIN2/3 (exact grading ot given) and 1 woman with invasive cervical cancer. #Constitutes 8 women with CIN3 and 8 women with CIN2. @ 2 women with invasive cervical cancer.

203x162mm (96 x 96 DPI)

