



# Prevalence of HPV and pathological changes among women 70 years of age, 10 years after exclusion from the Swedish cervical cancer screening program

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## Abstract

**Purpose** Örebro County introduced an updated screening program 2016 with primary HPV test for women over 30 years and prolonged screening, increasing the cut-off age from 56–60 to 64–70. The aim of this study was to investigate the prevalence of HPV genotypes and their correlation to histological changes in women, 10 years after exclusion from the screening program, due to an eventual implementation of a catch-up program including all women aged 60–70.

**Methods** All women in Örebro County, born 1,946 ( $n = 1,968$ ), were invited to a liquid-based cell sample with primary HPV screening. Samples were analyzed for hrHPV mRNA and positive samples were genotyped. hrHPV positive women were offered to do a conization.

**Results** Out of 809 participants, 31 (3.8%) were hrHPV positive, of these 22 did a conization. Histologically, 5/22 (23%) had LSIL and 5/22 (23%) had HSIL. Normal histology was found in 12/22 (55%). The most prevalent genotypes were HPV 16, 33, 52, 56, and 68. Of the women with HSIL, one case of cervical cancer was confirmed in a recone biopsy after 4 months.

**Conclusion** The study showed considerable prevalence of hrHPV and histologically confirmed LSIL/HSIL. These data led to catch-up screening for women between 60 and 70 years when overlapping two screening strategies.

**Keywords** HPV prevalence · Cervical cancer · Screening · HPV genotypes · Older women

## Introduction

The prevalence of infection with oncogenic types of human papillomavirus (HPV) and cervical dysplasia in women of 60 years of age and older, has not been extensively studied. A prevalence of 5.5% of high-risk HPV (hrHPV) has been reported for our region [1]. Different studies, all with quite small numbers, show similar prevalence figures, 8% in a Danish study among women > 65 [2], 5% in the Athena trial among 70-year-old women or older [3], and also 5% in an Australian study [4].

Systematic cervical cancer screening has led to a significant decrease in cervical cancer cases in Sweden [5, 6], as

well as worldwide [7]. However, the incidence has increased in recent years in Sweden with around 100 cases per year and in 2017, 559 new cases of cervical cancer were diagnosed in Sweden, and 149 died of the disease [8]. About 30% of the cases of cervical cancer occur in women older than 60 and the mortality rate is around 70% in this age group [9, 10].

The current screening policy that uses primary HPV screening in the ages 30–64, and samples only analyzed with cytology as a triage if found hrHPV positive, was issued in 2015 by the National Board of Health and Welfare [11]. Implementation of the new policy is ongoing, but is not yet nationwide. Recommended from 30 years is primary HPV screening, whereas cytology as primary screening continues between 23 and 29 years. This is a recommended regime from both WHO, EU, and the Swedish National Board of Health and Welfare since persistent hrHPV infection is a prerequisite for development of precancerous lesions as well as manifest cervical cancer [12, 13]. The new recommendation includes women up to 64 years of age, and recommend screening tests every third year from 23 to 49, and

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every seventh year 50–64 years of age. With a last sample after turning 64, some women will be 70 years of age at last screening round.

The shift to a new screening strategy demands resources as well as several organizational changes in both the clinic and the laboratory. The screening algorithm with screening up to 60 years of age was interpreted as stopping at 60. This has led to a group of women that have not been tested since 55–60 years of age, which is a considerable difference from a last sample at 64–70. In Örebro County, HPV as primary screening has been offered since September 2016. Challenges with a group of women in between two screening strategies occurred at this point. Cost, effectiveness, and the capacity to include all these women who were not screened with HPV nor cytology for 10–14 years, were taken under consideration. The reduction of cervical cancer in countries with an existing cervical screening program is mainly among women in age 30–60 years and not to the same extent among women above this age cohort [6, 14]. Data show that over 60% of the newly diagnosed cervical cancers in Sweden are found among women over 65 years of age and/or women who have not participated in the screening program for the last 7 years [15, 16].

The aim of the study was to distinguish the prevalence of HPV and the relations to histological findings among women of 70 years of age, at least 10 years after inclusion in a former cervical cancer screening program. The result of this study was useful in making legitimate decisions on how to implement HPV-based screening program and invitations for older women in Örebro County.

## Materials and methods

### Study population

All women born 1946 in Örebro County, were invited by letter April 2016 to April 2017,  $n = 1968$ . These women were no longer included in the organized screening program for cervical cancer and exited the program at least 10 years ago. The invitation contained an offer about professional screening for cervical cancer, sampled by midwives at Örebro University hospital. All women who had undergone a hysterectomy ( $n = 204$ ) were excluded and all non-responders after a first invitation, were reminded by a new invitation. Informed consent was obtained from all individual participants included in the study. The Regional Ethical Review Board, Uppsala, Sweden, approved this study (Dnr 2014/121).

### HPV screening and genotyping

Samples were collected using liquid-based cytology (LBC) (ThinPrep, Hologic, Marlborough, MA, USA). The samples

were collected from May 2016 to May 2017. All samples were analyzed with Aptima HPV Assay (Hologic, Marlborough, MA, USA) which is a qualitative nucleic acid amplification test that detects HPV E6/E7 mRNA from 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in LBC samples. The test results in hrHPV positive or negative without discrimination between genotypes. Therefore, samples positive for any of the 14 hrHPV were subjected to DNA extraction and genotyping with multiplex real time PCR test Anyplex<sup>TM</sup> II HPV28 (Seegene, Seoul, Korea).

### Cytology

All hrHPV positive LBC specimens were screened by one certified cytotechnician and assessment followed the international Bethesda classification system with atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells, cannot exclude high-grade lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), squamous cell carcinoma, atypical glandular cells (AGC), adenocarcinoma in situ (AIS), or adenocarcinoma.

### Cone biopsy and histology

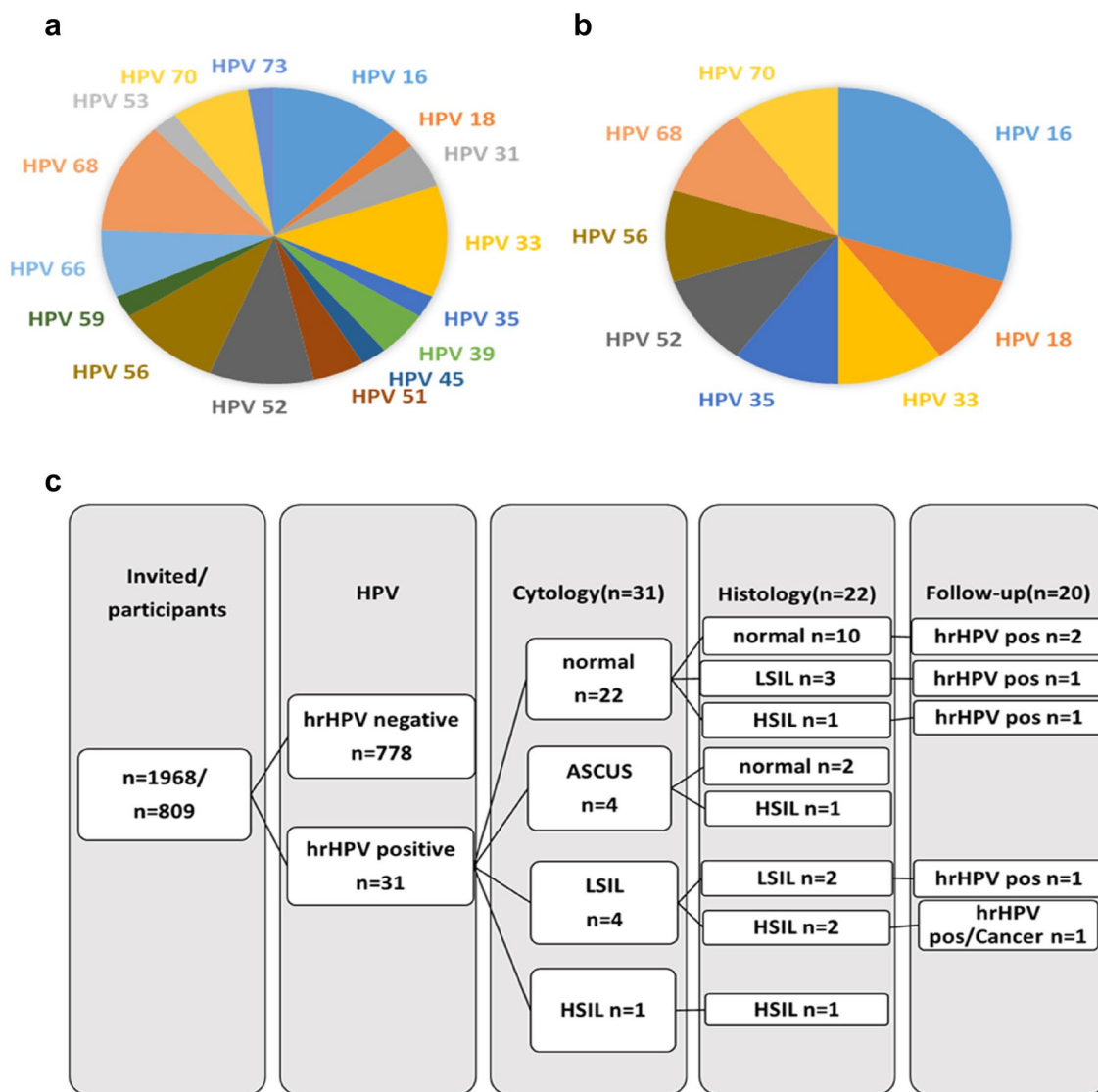
The women with samples positive for hrHPV mRNA were offered an appointment to a senior gynecologist (author LB) for electrosurgical cone biopsy, in local anesthesia.

The cone specimens were formalin-fixed, paraffin-embedded and sections (4  $\mu$ m) were stained with hematoxylin and eosin and evaluated according to current WHO classification [17], by one senior pathologist. The last follow-up sample in the study was 6 months after the cone biopsy. If the women had findings, clinical follow-up continued as the national screening program stipulated [18].

## Results

Of the 1968 women that were invited to this study, 809 accepted to participate. 204 were excluded after invitation due to a previous hysterectomy (10%) and the rest were non-responders (Fig. 1).

When analyzing the LBC samples, hrHPV positivity was seen in 31/809 specimens, a prevalence of 3.8%. Among the hrHPV positive samples, 2/31 (7%) were negative with the DNA-based genotyping test. The most common HPV genotypes were HPV 16, 33 and 68, with a prevalence of 0.6% each for the whole study population (Fig. 1a). Samples positive for more than one genotype were seen in 7/31 (23%), and several HPV genotypes were only positive in multi-infections, 35, 45, 51, 53, 59, 66, and 73.



**Fig. 1** **a** Distribution of genotypes in all positive samples, **b** distribution of genotypes in samples with dysplasia, **c** flow chart of HPV, cytology, and dysplasia occurrence

All hrHPV-positive samples were analyzed with cytology and 9 (29%) had cytological abnormality, 4 (13%) with ASCUS, 4 (13%) LSIL and 1 (3%) with HSIL (Fig. 1c). These women either accepted to do a cone biopsy or were followed according to the current guidelines at the time when carrying through with the study [18].

Of the women with hrHPV positive test results, 22/31 (71%) did a cone biopsy, and of these 10/22 (45%) had histological abnormalities on examination, 5 LSIL and 5 HSIL. Of these 10 dysplasias, 6 had aberrant cytology on earlier LBC specimens (Fig. 1c).

After the conization, a new screening sample was taken on all women 4–6 months after the cone and results showed that 6/20 women still had hrHPV positive samples. One of the HSIL was found to be squamous cell carcinoma IA at

follow-up after 4 months, and proceeded to surgery as stipulated per guidelines (Fig. 1).

**Discussion**

HPV-based screening has the potential to prevent additional cases of cervical cancer in women over 65 years of age. However, the clinical management of HPV positivity in older age groups is not well established. In this study, the prevalence of histological verified dysplasia among the 70-year-old women with positive hrHPV was 45%. This is high, considering that during the same time interval, in the same county, 36% of 50-year-old women with screening samples showing either LSIL or hrHPV had histological

verified dysplasia, 3 LSIL, and 1 HSIL (data from local registries).

The prevalence of hrHPV in our study population was 3.8%, which is in good agreement with another Swedish study where 4.1% of the women (mean age 68) were hrHPV positive [19]. Different studies show a prevalence that differ worldwide. Among European studies, HPV prevalence among women between 55 and 65 differ from just below 5% to just over 10%, but data from 70-year-old women are to our knowledge lacking [2, 20–23]. The difference in HPV prevalence may be due to if an organized screening program is in practice, as well as a how frequent women undergo hysterectomy in a population.

The number of hysterectomised women in this cohort was 10% (204 of 1,968 responding to the study). This is consistent with other studies for example in Denmark, where 11.9% of women of 55–64 years had done surgery [24], whilst another Danish study showed a prevalence of hysterectomy among women above 65 to be  $\geq 16\%$  [25]. Both studies were focusing on attendance rate in the screening program. The exact number of hysterectomised women among the non-responders to this study is unknown though.

In Örebro County, Sweden, there has been an organized screening program in place since the 1960s, which means that the study population can be considered well screened. During the years 2003–2005, this age cohort had a screening attendance rate of around 87–89%. This may explain our rather low prevalence results. In this study, there is no information about whether the participants are the women that actually have participated regularly in the screening program, nor if they had normal screening results between 50–60 years of age.

Data from Wang et al. [14] show the importance of screening up to older ages, if the woman has abnormal screening history or is unscreened with cytology or HPV [26]. This fact, together with the results showing that almost half of the hrHPV positive women had dysplasia indicates even more obviously the advantages of including all women up to 70 years of age, when switching to primary HPV screening strategy. Evidence also show that there is lower performance of cervical cytology as a screening tool in this particular population [27]. This together with the fact that hrHPV is a necessary cause of cervical cancer [12] prove that all women should now be included in the new screening program based on hrHPV before exclusion from the screening program.

We also strongly advise conization directly after a positive hrHPV together with LSIL on cytology, for women who have not attended the screening program for a long time, 10 years or more. In addition, a cone biopsy is suggested regardless of cytology results on only one hrHPV positive test if the woman is older than 64 and HPV status is unknown for many years, since our data show 45% dysplasia

rate. This is in consistence with data from Elfgrén et al. [28] as their results show evidence of very high long-term risk of HSIL among women with continuous HPV persistence, as well as data on HPV infection, which more often tend to become persistent in menopausal women [22].

The strength of this study is that the study population equals the target group in case that the health care organization makes a decision to perform a catch-up screening program. The limitations of the study are the small study group and low take-up, 46%, as well as the fact that there could not be a control group. The screening coverage in this region in Sweden among women aged 51–60 has been around 70–75% for several years, and further back even higher, and the general impression is that women generally are interested in being screened.

The prevalence of hrHPV and the high proportion of histological occurrences in the biopsies (45%) in this study, also including diagnosis of an early cancer, urged the County Council to extend the screening program to include all women up to 70 years of age, regardless of screening tests that were taken before the implementation. This catch-up program will be evaluated and reported, to get more data on HPV prevalence among women between 60–70 years of age. Since to our knowledge, evidence about this transition phase between two methods are not well analyzed, and since Örebro County, in the Swedish context, was early in changing to HPV screening strategies, this study can be of great value clinically and for local screening organizations. Almost all countries with organized screening are now considering transition to primary HPV testing, if it is not already completed.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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