Self-sampling by elderly women for the detection of HPV and cervical dysplasia

RUTH S. HERMANSSON
Abstract


In Sweden, about 30% of the cervical cancer cases occur in women older than 60 and the mortality rate is as high as about 70% in this age group. There is a lack of knowledge concerning the prevalence of infection with oncogenic types of HPV, and cervical dysplasia in women of 60 years and older. Screening for oncogenic types of HPV is more effective than cytology in reducing the incidence of cervical cancer. It is established that self-collected samples are equally accurate as clinician-taken cervical samples when a validated PCR-based test is used for analysis.

Objectives: The overall aim was to gain knowledge about HPV infection and cervical dysplasia prevalence in elderly women and to evaluate the acceptability of repeated self-sampling at home for HPV testing.

Material and Methods: In Paper I, we investigated the prevalence of HPV and HPV-related cervical dysplasia in 1051 elderly women aged 60 to 89 attending an outpatient gynecology clinic. A gynecologist collected these samples. In Paper II, repeated self-sampling at home for HPV testing was offered to 375 women in each of the four age groups 60, 65, 70, and 75. In Paper III, we carried out a descriptive study with quantitative and qualitative methods to describe older women’s experiences of self-sampling. In Paper IV, we investigated the incidence of oncogenic HPV and HPV-related dysplasia among 632 women aged 65 to 80 years who five years earlier had a negative HPV test.

Results: The prevalence of HPV was just over 4% both when the samples were collected by a clinician (Paper I) and by self-sampling (Paper II). The majority of women positive in both the first and second HPV tests had dysplasia in histology. Of the women with dysplasia in histology, more than 80% had normal cytology. In Paper II, a self-collected sample was provided by 59.5% of the invited women. In Paper III, 97.2% of eligible women answered the survey, and 13 of 16 invited women participated in the interviews. Most of them reported that they prefer self-sampling because it was easy to perform, less embarrassing, and less time-consuming than a clinic visit. In Paper IV, the incidence of oncogenic HPV was 2.8% in the first test and 1.3% in the second test, and mild cervical dysplasia was found in 50% of women with persistent HPV infection.

Conclusions: A significant proportion of elderly women were found to have a persistent HPV infection. Among them, there was a high prevalence of dysplasia as diagnosed by histology. Cytology showed extremely low sensitivity. Self-sampling at home combined with repeat HPV testing was well accepted among older women.

Keywords: HPV, self-sampling, cervical dysplasia, elderly women.

Ruth S. Hermansson, Research group (Dept. of women’s and children’s health), Reproductive biology, Akademiska sjukhuset, Uppsala University, SE-751 85 UPPSALA, Sweden.

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"Id y enseñad a todos"

To my tribe.
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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<td>Adenocarcinoma</td>
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<tr>
<td>AGC-H</td>
<td>Atypical glandular cells, favor neoplastic</td>
</tr>
<tr>
<td>AGC-US</td>
<td>Atypical glandular cells of uncertain significance</td>
</tr>
<tr>
<td>AIS</td>
<td>Endocervical adenocarcinoma in situ</td>
</tr>
<tr>
<td>ASC-H</td>
<td>Atypical squamous cells that cannot rule out HSIL</td>
</tr>
<tr>
<td>ASC-US</td>
<td>Atypical squamous cells of uncertain significance</td>
</tr>
<tr>
<td>CC</td>
<td>Cervical cancer</td>
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<tr>
<td>CIN</td>
<td>Cervical intraepithelial neoplasia</td>
</tr>
<tr>
<td>CIS</td>
<td>Carcinoma in situ</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>FIGO</td>
<td>La Fédération Internationale de Gynécologie et d'Obstétrique</td>
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<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HPV</td>
<td>Human papillomavirus</td>
</tr>
<tr>
<td>HSIL</td>
<td>High-grade squamous intraepithelial lesion</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>IFCPC</td>
<td>International Federation for Cervical Pathology and Colposcopy</td>
</tr>
<tr>
<td>LBC</td>
<td>Liquid-based cytology</td>
</tr>
<tr>
<td>LEEP</td>
<td>Loop electrosurgical excision procedure</td>
</tr>
<tr>
<td>LSIL</td>
<td>Low-grade squamous intraepithelial lesion</td>
</tr>
<tr>
<td>NKcx</td>
<td>Nationella Kvalitetsregistret för Cervixcancerprevention</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative predictive value</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>pRb</td>
<td>Retinoblastoma protein</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SCC</td>
<td>Squamous-cell carcinoma</td>
</tr>
<tr>
<td>SCJ</td>
<td>Squamocolumnar junction</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>STI</td>
<td>Sexually transmitted infection</td>
</tr>
<tr>
<td>TZ</td>
<td>Transformation zone</td>
</tr>
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<td>WHO</td>
<td>World Health Organization</td>
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Introduction

Cancer is the second leading cause of death worldwide behind cardiovascular disease (1-3). With aging, tissues undergo a number of molecular changes similar to those observed in the early stages of carcinogenesis, including the formation of DNA adducts, DNA hypermethylation, and chromosomal translocation. This so-called proliferative senescence leads to resistance to apoptosis, which may predispose an individual to the development of cancer (3, 4). A progressive loss of cell-mediated immunity with age (known as immune-senescence) may result in more susceptibility to environmental carcinogens. Prevention of some cancers is possible as a result of the fact that cancer initiation and progression is a multistep process characterized by distinct events that provide opportunities to intervene in the carcinogenic process at several stages, and reverse the process in early stages. This is the case in cervical cancer, a disease that affects both young and older women.

In older women, cervical cancer is detected at later stages and prognosis is poor (5, 6). Taking into account the continuous increase in life expectancy and the fact that cancer risk increases with age, working strategies are desirable and necessary for the prevention, diagnosis, and treatment of cancer among elderly women.
Cervical cancer

Epidemiology

Cervical cancer (CC) is one of the leading causes of cancer death among women, representing the fourth most common malignancy diagnosed in women worldwide. Although CC is a highly preventable disease, the worldwide incidence in 2018 was 550,000 new cases, with 311,000 deaths (7). The highest incidence is observed in developing countries. In Europe, it is estimated that 58,373 women are diagnosed annually with CC, and 24,404 of them die due to the disease (8). In many high-income countries, the age-specific incidence of cervical cancer currently shows a double-peak pattern, with peaks at about 40 and 75 years of age (9, 10). Consequently, a higher proportion of cervical cancer cases is found in elderly women in those countries. In Sweden, around 550 new cases of CC are diagnosed annually, and the mortality rate due to CC is about 170 women per year. About 30% of cases of CC occur in women older than 60 and the mortality rate is about 70% in this age group (11, 12).

Etiology

Human papillomavirus

Cervical cancer in the vast majority of cases is a consequence of persistent infection with oncogenic human papillomavirus (HPV). HPV infection is common among both men and women. According to the World Health Organization (WHO), most sexually active individuals will acquire this sexually transmitted infection (STI), once or repeatedly in their lives (2). HPV infection is commonly short-lived, symptomless and often resolves naturally. Nevertheless, a persistent infection can cause precancerous lesions that left untreated can develop into cancer (Figure 1). HPV is one of the most powerful human carcinogens. There is evidence concerning the ability of HPV to cause anal, vulvar, vaginal and penile cancer as well as head-and-neck cancer (13, 14).

More than 200 different genotypes of HPV have been described, of which about 40 types infect the genital tract. These have been classified by the International Agency for Research on Cancer as “high-risk” HPV (oncogenic) (HR-HPV) and “low-risk” HPV (not oncogenic) (LR-HPV). Retrospective studies have shown that almost 100% of cervical cancer cases are HR-HPV-positive. HR-HPV types include HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and HPV-68 is considered as probably carcinogenic. Worldwide, the most common HPV types in CC are types 16 (57%), 18 (16%), 58 (5%), 33 (5%), 45 (5%), 31 (4%), 52 (3%), and 35 (2%) (14-16).

HPV infects the epithelial cells and infection depends on the differentiation pathway of epithelial cells to complete their lifecycles (17). Early gene ex-
pression is tightly controlled in the basal epithelial cells, with substantial amplification of viral DNA. Replication occurs in the suprabasal layer, whereas differentiating cells that are destined for maturity and senescence do not naturally express the replicative machinery that the virus depends on for survival. To elude this problem, HPV encodes two proteins, E6 and E7, which interact with central molecules in cell-cycle control. E6 proteins inhibit the p53-mediated DNA repair and apoptotic responses, resulting in tumor progression. This is known to be the most important event in HPV-associated carcinogenesis. The unscheduled cycle progression is further enhanced by E7 protein-mediated binding and inactivation of pRb (retinoblastoma protein). Cells lacking functional p53 and pRb are highly prone to a reduced apoptotic response, and increased genomic instability and proliferation rate, all of which are hallmarks of malignant transformation (18, 19).

The development of cancer depends not only on efficient negative regulation of cell-cycle control supporting the accumulation of genetic damage, but also on sophisticated techniques of immune evasion that enable the virus to be undetected for long periods. No cell-death, necrosis, or viremia phase exists that would trigger an inflammatory response (20).

![Figure 1. Pathogenesis of HPV in cervical cancer. Source: The Nobel Committee for Physiology or Medicine.](image)

**HPV prevalence**

The prevalence of HPV infection varies by country, region within country, population subgroup, and age (21-23). The prevalence of HPV worldwide is estimated to be around 11% throughout all age groups, but is considerably higher among younger women aged 20–24 years, with a prevalence of 20% (24, 25) and then a decrease with age. In some populations a second peak in HPV prevalence has been observed among women aged 55–59 years, with a rate of 10% (23). The cause of this second peak is not clear, but an explanation could be a new partner in middle age or reactivation of a latent HPV infection.
In elderly women a prevalence rate of around 4% has been reported (28). In the presence of precancerous lesions, HPV prevalence increases in proportion to the severity of the lesion, from 12% in women with normal cytology to 90% in women with HSIL (a high-grade squamous intraepithelial lesion) and invasive CC (25, 29). The prevalence of different HPV types varies somewhat in different regions of the world. However, HPV16 dominates worldwide and contributes to about half of all cases of cervical cancer, followed by HPV18, and together they contribute to around 70–76% of all invasive cervical cancers (14, 16). HPV prevalence at older ages has been shown to be a better predictor of CC incidence compared with its prevalence in women under 35 (30, 31).

**Persistent HPV infection**

The majority of HPV infections are transient; 70–75% clear within one year and approximately 90% clear within two years (32, 33). There is still no consensus concerning the definition of persistent HPV infection. Several studies have shown that intervals of ~ 6 months produce strong summative relative risks as regards the association between HPV and precancerous lesions (34, 35). It is impossible to know how long a woman with a first positive test result has been infected before the sample was taken. However, several reviews have shown that the association between HPV persistence and cervical neoplasia appears stronger among studies with two related characteristics: longer duration of HPV infection (>12 months) and wider HPV testing intervals (>6 months or >12 months). These reviews confirm that repeated HPV detection does indicate an increased risk of cervical cancer and its precursors, despite differences in HPV-persistence definitions, HPV-detection techniques, and testing intervals (34, 35). A Swedish study revealed that all women with persistent type-specific oncogenic HPV developed CIN 2+ (cervical intraepithelial neoplasia 2+) within six years of follow-up (36). The persistence of different HPV genotypes has been studied by Rositch and coworkers in a meta-analysis. The results showed that HPV-16 (54%; 95% CI: 48%, 60%), HPV-18 (48%; 95% CI: 40%, 56%) and HPV-33 (47%; 95% CI: 35%, 59%) were associated with the highest proportions of persistent infections at six months, while HPV-51 (30%; 95% CI: 19%, 42%) and HPV-66 (29%; 95% CI: 16%, 43%) were associated with the lowest proportions (35). Persistence also varies with age, and several studies have shown that younger women have a lower proportion of persistent infections compared with older women (37-39).

**Latency**

The probability that a new HPV detection is due to a recently acquired infection is high in sexually active young women, and it decreases with age (40, 41). Although sex with new partners remains a risk factor of a new infection, rates of acquiring new partners also decline with age (42-44). These data sup-
port the idea that the proportion of cases of detectable HPV that can be attributable to prior versus new infection increases with age. It is uncertain whether an HPV infection that becomes undetectable on repeat testing has truly cleared, or whether the virus persists at undetectable levels or has entered a latent state. There is evidence from multiple studies of immune-compromised, older, less sexually active populations, with long-term follow-up, that supports the fact that HPV can establish a state of latency during which the virus remains undetectable but can reappear later (26, 40, 45).

In many high-income countries, the age-specific incidence of cervical cancer currently shows a double-peak pattern. The first peak in the incidence of CC is considered to be a consequence of HPV infections following the start of sexual activity. The second peak is more difficult to explain. It has been hypothesized to be associated with HPV infections from new sexual partners in mid-life and/or reactivation of latent HPV infection due to immune-system degeneration with age (27, 46, 47). Another possible explanation for the second peak, however, is that screening ends around the age of 60–64 and that there are new cases of CC due to persistent HPV infections that go undetected until the late stages of CC with symptoms.

Cofactors
It is not yet clear what conditions may cause persisting HPV infections, which represent a prerequisite for the development of CC. It is well accepted that cofactors are required for the development of invasive cancer. Several factors have been studied. Smoking has been described as an important independent cofactor in the development of CC (48). Other contributing factors are high-level parity, long-term use of oral contraceptives, genetic factors, inadequate immune function, genetic instability and co-infection with other STIs or HIV. Early age at first intercourse, and many sexual partners are considered to be risks of harboring an HPV infection (13).

It has also been suggested that there is an association between the vaginal microbiota composition, HPV infection and HPV-related disease (49, 50) with regard to certain lactobacilli species, such as *L. iners*, and certain non-lactobacilli species (51).
The Cervix

The cervix uteri (Latin, 'neck of the uterus') is the lower fibromuscular portion of the uterus (Figure 2). It is cylindrical or conical in shape and measures 3 to 4 cm in length, and about 2.5 cm in diameter and is located between the bladder and the rectum. The lower half of the cervix, called the portio vaginalis, protrudes into the vagina through its anterior wall, and the upper half remains above the vagina. The portio vaginalis opens into the vagina through an orifice called the external os.

Figure 2. The female reproductive system. Illustration: T. Winslow. With permission.

The histology of the cervix is complex. Overlying the fibrous stroma of the cervix is the cervical epithelium, a meshwork of cells. The epithelium is of two types: columnar (glandular) and stratified non-keratinizing squamous epithelia. The area where the two types of epithelia meet is called the squamo-columnar junction (SCJ). The SCJ is clinically important, as it is the site where over 90% of lower genital tract neoplasias arise. During childhood, the SCJ is located just inside the external os. Under the influence of hormones and the acidification of the vaginal environment during puberty, sub-columnar cells undergo metaplasia, a process of transformation. The metaplasia of these cells causes the SCJ to “rollout,” or evert, from its original position inside the external os to a position on the enlarged cervical surface (Figure 3). The columnar epithelium is also rolled onto the cervical surface, where it is exposed to vaginal secretions, irritants, and a changing hormonal milieu. The area between the original SCJ and the active SCJ is called the transformation zone (TZ) (52).
Precancerous lesions of the cervix

Cervical cancer is preceded by an interval of epithelial dysplastic changes, meaning the growth and progress of abnormal epithelial cells typically in the transformation zone, known as cervical intraepithelial neoplasia (CIN). Persistent infection with HPV is necessary for the development, maintenance and progression of CIN lesions. However, only a small proportion of infected women develop precancerous lesions and cancer.

Diagnosis of precancerous lesions

Precancerous lesions are detected within the framework of screening programs with the conventional Pap test or liquid-based cytology (LBC). Biopsy constitutes the basis of diagnosis of precancerous lesions of the cervix. The CIN system is used for diagnosis of histological cervical dysplasia; CIN I corresponds to mild dysplasia, CIN II to moderate dysplasia and CIN III to severe dysplasia or pre-invasive carcinoma, so-called Carcinoma In Situ (CIS). Classification of cytological pre-cancerous changes is based on the Bethesda system, where CIN 1 is characterized by low-grade squamous intraepithelial lesions (LSILs) and where CIN 2 and 3 (CIN 2+) are characterized by high-grade squamous intraepithelial lesions (HSILs). The Bethesda system also provides nomenclature for cells that cannot be classified as LSIL cells, such as atypical squamous cells of uncertain significance (ASC-US), atypical squamous cells that cannot rule out the possibility of HSIL (ASC-H), plus atypical glandular cells of uncertain significance (AGC-US), atypical glandular cells, favor neoplastic (AGC-H) and endocervical adenocarcinoma in situ (AIS) (3, 26).
Table 1. Correlation between dysplasia, cervical intraepithelial neoplasia (CIN) and Bethesda terminology. IARC (54).

<table>
<thead>
<tr>
<th>Dysplasia terminology</th>
<th>Original CIN terminology</th>
<th>Modified CIN terminology</th>
<th>The Bethesda system terminology (1991)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Within normal limits Benign cellular changes (infection or repair)</td>
</tr>
<tr>
<td>Atypia</td>
<td>Koilocytic atypia, flat condyloma, without epithelial changes</td>
<td>Low-grade CIN</td>
<td>ASCUS/AGCUS</td>
</tr>
<tr>
<td>Mild dysplasia or mild dyskaryosis</td>
<td>CIN 1</td>
<td>Low-grade CIN</td>
<td>LSIL</td>
</tr>
<tr>
<td>Moderate dysplasia or moderate dyskaryosis</td>
<td>CIN 2</td>
<td>High-grade CIN</td>
<td>HSIL</td>
</tr>
<tr>
<td>Severe dysplasia or severe dyskaryosis</td>
<td>CIN 3</td>
<td>High-grade CIN</td>
<td>HSIL</td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>Invasive carcinoma</td>
<td>Invasive carcinoma</td>
<td>Invasive carcinoma</td>
</tr>
</tbody>
</table>


Colposcopy
Following the finding of abnormal cytology, colposcopic examination is performed. Colposcopy remains the standard method for further investigation of screening-positive women. Through the colposcope, the colposcopist can see certain changes in cervical and vaginal tissues. The primary assessment in colposcopic examination covers evaluation of the TZ, identification of the location of abnormal cells, and targeting biopsy-taking (55). Complete and non-complete visualization of the TZ are described in the current ASCCP consensus management guidelines (56). The IFCPC terminology additionally uses “transformation zone type 1, 2 and 3” in the general assessment that refers to the grade of visibility of the TZ. A recent literature review demonstrated that the use of TZ type was not reproducible among clinicians, particularly TZ type 2, and there was no evidence that TZ type improves prediction or management of cervical disease (57-60). Colposcopy is a subjective examination. Indeed, colposcopic assessment depends on clinical experience and level of training. As a result, colposcopy is characterized by a wide range of sensitivity and specificity measures in the detection of CIN 2+ lesions, i.e., between 30–90% and 67–97%, respectively (61-63).

In elderly women the TZ becomes intracervical and is not visible in colposcopy. The cervical and vaginal epithelium becomes very thin, thereby allowing visualization of the sub-epithelial capillaries, which in turn may appear red and atypical. The use of acetic acid is not as effective in detecting premalignant changes in these cases (64). If there is any difficulty in assessing the postmenopausal cervix, it could be helpful to give a short course (3–4
weeks) of intravaginal estrogen (65). Colposcopically negative cases are not considered as truly negative without histological confirmation. However, it is impossible to take representative biopsy samples in cases with a TZ that is not fully visible. In order to obtain representative material, diagnostic conization might be appropriate to diagnose or exclude cervical dysplasia or cancer. Histopathology provides the final diagnosis, on the basis of which treatment is planned and serves as the gold standard for quality control of cytology and colposcopy (55). Findings in colposcopy are documented according to IFCPC nomenclature and the Swede score should be calculated (Table 2). The Swede score takes into consideration five characteristics. A score of 0, 1 or 2 is assigned to each of these characteristics; the scores for all five characteristics are added up to derive the final score. However, diagnosis and management should not be based solely on the Swede score. The colposcopist should consider the woman’s age, the screening-test results, and other risk factors when making a diagnosis.
Table 2. The Swede Score.

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aceto uptake</td>
<td>Zero or transparent</td>
<td>Shady, Milky (not transparent; not opaque)</td>
<td>Distinct, opaque white</td>
<td></td>
</tr>
<tr>
<td>Margins/Surface</td>
<td>Diffuse</td>
<td>Sharp but irregular, jagged, ‘geographical’ satellites</td>
<td>Sharp and even, difference in surface level, including ‘cuffing’</td>
<td></td>
</tr>
<tr>
<td>Vessels</td>
<td>Fine, regular</td>
<td>Absent</td>
<td>Coarse or atypical</td>
<td></td>
</tr>
<tr>
<td>Lesion size</td>
<td>&lt;5mm</td>
<td>5-15mm or 2 quadrants</td>
<td>&gt;15mm or 3-4 quadrants/ endocervically undefined</td>
<td></td>
</tr>
<tr>
<td>Iodine staining</td>
<td>Brown</td>
<td>Faintly or patchy yellow</td>
<td>Distinct yellow</td>
<td></td>
</tr>
<tr>
<td>Total score (maximum 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interpretation of Swede Score

<table>
<thead>
<tr>
<th>Overall Swede Score</th>
<th>Colposcopic prediction of probable histology</th>
</tr>
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<tbody>
<tr>
<td>0 – 4</td>
<td>Low grade/normal</td>
</tr>
<tr>
<td></td>
<td>CIN 1</td>
</tr>
<tr>
<td>5 – 6</td>
<td>High grade/non invasive cancer</td>
</tr>
<tr>
<td></td>
<td>CIN 2 +</td>
</tr>
<tr>
<td>7 - 10</td>
<td>High grade/suspected invasive cancer</td>
</tr>
<tr>
<td></td>
<td>CIN2 +</td>
</tr>
</tbody>
</table>

Treatment of precancerous lesions
There are different types of treatment of pre-cancerous lesions depending on case and availability. Ablative procedures include cryotherapy, cold coagulation and laser ablation. With these techniques lesions of the epithelial surface are eliminated but there is no sample for histopathological analysis. By using excisional techniques such as cold-knife conization, laser conization and the
Loop Electrosurgical Excision Procedure (LEEP), abnormal areas in the cervix are eliminated and a sample is also taken which can be sent for further analysis. Follow-up is recommended after the above-mentioned treatments in order to detect eventual remains of the lesion, or recurrence (3). In Sweden LEEP is the most commonly used technique.

Women under 25 years of age eliminate HPV efficiently and there is spontaneous regression in most cases of CIN within two years; 70–94% of low-grade squamous intraepithelial lesions (LSIL); 60–65% of high-grade squamous intraepithelial lesions (HSIL) (66). Excisional treatment of CIN reduces the risk of invasive cervical cancer by 95% (67). The optimal management of women with histological CIN1 is surveillance (56, 68). Swedish guidelines recommend excisional treatment of histological CIN 3, because of the high risk of progression to cancer. For histological CIN 2, Swedish guidelines recommend excisional treatment in women aged ≥25 years (68). However, the risk of progression to cancer in this group is lower than in cases of CIN 3, being only about 0.5% in two years (69). During the same time period, the regression rate of CIN 2 is about 50% and the progression rate is 18% in the overall population. However, in women aged >30 years the rates are 60% and 11% respectively (66) (69). Current knowledge of CIN regression rates in women over 60 years of age is poor.

Conization is still considered as the standard treatment for CIN at any age, when excisional management is indicated. In postmenopausal women the possibility of postoperative cervical stenosis should be considered. Vaginal estrogen use is associated with a low risk of stenotic complications; therefore, if possible, women should be advised to continue therapy for at least one year after conization (70).
Invasive disease

Invasive cervical carcinoma is cancer arising from the cervix. Squamous-cell carcinoma (SCC) is the cervical cancer with the highest incidence (about 80-85%), followed by adenocarcinoma (ADC) (about 15%), while other histological types such as neuroendocrine carcinoma are rare.

In the early stages, usually no symptoms are noticed. In later stages, symptoms may include abnormal vaginal bleeding, vaginal discharge, and pelvic or low-back pain. The presence of symptoms at diagnosis is associated with advanced disease and the prognosis is poor (17). Cervical cancer is further clinically staged in a system developed by the International Federation of Gynecology and Obstetrics (FIGO) (Figure 5). Since 2018, a revised classification has been available for use in CC staging where imaging and pathological findings can be used, when available, to supplement clinical findings with respect to tumor size and extent, at all stages (24).
FIGO staging of carcinoma of the cervix uteri (2018).

Stage I:
The carcinoma is strictly confined to the cervix uteri (extension to the corpus should be disregarded).

Stage II:
The carcinoma invades beyond the uterus, but has not extended onto the lower third of the vagina or to the pelvic wall.
Stage III:
The carcinoma involves the lower third of the vagina and/or extends to the pelvic wall and/or causes hydronephrosis or non-functioning kidney and/or involves pelvic and/or paraaortic lymph nodes.

Stage IV:
The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum and/or spread to distant organs.

Treatment
The treatment of cervical cancer is dictated by the FIGO stage. For patients with early CC stages, surgery is recommended. The gold standard management of patients with locally advanced cervical cancer includes external beam radiotherapy with concurrent cisplatin-based chemotherapy and brachytherapy (8, 16). Early stages of cervical cancer can be treated and usually have good prognosis, while stages III and IV are related to worse prognosis, often being incurable (6). Treatment of early-stage cervical cancer is also less complex, less expensive and more effective, with higher long-term survival rates and better quality of life (72).

Prognosis and survival rates
The 5-year survival rate (i.e. the percentage of women who are alive five years after their diagnosis) in cases of early-stage cancer can be over 90% in countries where women have access to timely diagnosis and good-quality treatment (73).

In Europe, with state-of-the-art staging and treatment, 3-year local control rates for patients with early-stage and advanced-stage cervical cancer are 87% to 95% and 74% to 85%, respectively (74). For all stages combined, the 3-year to 5-year survival rate from cervical cancer in many underdeveloped countries is <50% (75). Survival decreases with advancing age at diagnosis, from 81% for 16- to 44-year-old women to 34% for women aged ≥75 years (76). FIGO stage is one of the most important prognostic factors. Death from cervical cancer often involves local disease progression, resulting in significant suffering, including ureteral obstruction, hydronephrosis, pain, and fistulas (27).

Cervical cancer prevention
Primary prevention
The etiological association of HPV with CC has provided the background for the development of HPV vaccines and screening programs. Primary prevention of cervical cancer by immunization of young girls can prevent 95% of all
infections caused by HPV types 16 and 18 (3). There are currently three vac-
cines protecting against both HPV 16 and 18, which are known to cause at
least 70% of cervical cancers. One of the vaccines protects against additional
oncogenic HPV types 31, 33, 45, 52 and 58, which leads to a further 20%
reduction of cervical cancers. Given that the vaccines which are only protec-
tive against HPV 16 and 18 also have some cross-protection against other less
common oncogenic HPV types, the WHO considers the three vaccines equally
protective against cervical cancer. Two of the vaccines also protect against
HPV types 6 and 11, which cause anogenital warts.

Clinical trials and post-marketing surveillance have shown that HPV vaccines
are safe and effective in preventing infections with HPV. Vaccines work best
if administered prior to exposure to HPV. Therefore, the WHO recommends
vaccinating girls aged between nine and 14 years, preferably before the onset
of sexual activity, as the vaccine does not treat already existing infections or
lesions (3). Vaccines are available in most high-income countries as part of
the routine immunization program (7). In Sweden, as in many countries, the
vaccination program also includes boys.

Secondary prevention

Screening program

Screening is aimed at detecting women with high-grade precancerous
changes, which can then be treated, reducing the risk of cervical cancer.

The Swedish cervical screening program recommends sampling for cytol-
ogy every third year for women aged 23–29, sampling for an HPV test every
third year for women aged 30–49, with supplemental analysis for cytology for
women around 41 years old, and for women aged 51–64, sampling for
HPV test every seventh year. Self-sampling for HPV testing should be of-
fered to non-attenders.

Cytology

Cytology-based screening has been used for more than half a century and is
currently used by the majority of screening programs worldwide. It has un-
doubtedly proven its positive impact on reducing CC incidence, morbidity,
and mortality (55, 77). However, the low sensitivity of the technique, the high
costs to sustain the infrastructure, and the need for highly trained personal are
important issues that have brought primary cytology screening programs un-
der careful and critical observation during recent years (78, 79). Furthermore,
false-positive results due, for example, to immature squamous metaplasia, in-
flammatory atypia or atrophy have been a problem, since they often lead to
unnecessary new tests or colposcopy with biopsy. A consequence of this is
that a woman might be worried, and there are also health economic consequences (29). It is important to remember that cytology performs relatively poorly in postmenopausal women, in whom epithelial atrophy is very common, and the TZ is situated in the cervical canal. This makes the collection of material for cytological examination problematic as a result of poor accessibility, and the samples are also difficult to analyze, with a risk of inaccurate results. Another important fact to consider is the low capacity of cytology to detect cervical adenocarcinoma and AIS. The incidence of adenocarcinoma has not decreased despite an effective cytology program that has reduced the incidence of squamous-cell carcinoma (80, 81). Traditional cytology has been shown to have a sensitivity of 51% (30–87%) and specificity of 98% (86–100%). However, because of the subjective nature of the test, there is significant variation in the interpretation of cytology, which further contributes to its variable sensitivity and specificity rates (82, 83). Recent studies have shown that the specificity of cytology is decreasing in countries with high HPV coverage owing to the reduction of HSIL as a result of HPV vaccination (84).

HPV tests

HPV testing is based on the detection of HPV nucleic acids, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The development of clinically validated HPV tests that have shown higher sensitivity than cytology testing, has recently caused a paradigm shift. According to European Guidelines and the WHO, HPV testing is now proposed as the primary screening tool for CC prevention (85).

Several randomized controlled trials and a meta-analysis of randomized data have shown that HPV tests have substantially higher sensitivity and negative predictive value (NPV) in the detection of high-grade disease. HPV-based screening results in a 60–70% better protection rate against invasive cervical cancer in women over the age of 30 when compared with cytology (81, 86). European Guidelines recommend a five-year screening interval for HPV testing, which can be extended up to 10 years depending on the age and screening history of the woman (85). Partial HPV genotyping may be employed either as a part of primary screening or in triage. This approach would help to direct management of HPV-positive women, since more active management is probably needed for women positive for HPV types associated with the highest risks of dysplasia and cancer.

The major disadvantage of HPV testing is its very age-dependent lower specificity when compared with cytology, as the test can detect transient HPV infections without a true carcinogenic potential. Since many young women acquire HPV infections that most probably will resolve naturally, it is not cost-effective to perform HPV screening among women below the age of 30 (85, 87). European guidelines do not recommend primary HPV screening before the age of 30 and are in favor of screening starting at the age of 35. The guide-
lines suggest that primary HPV screening can stop at the same age as recommended for cytology, that is, at 60–65 years of age, provided that the most recent HPV test was negative (88). Nonetheless, it is important to note that the reason for this decision was based on the fact that cytology performs relatively poorly at those ages, owing to the fact that epithelial atrophy is very common and the TZ is situated in the cervical canal, making the collection of material for cytological examination less accessible and more difficult to analyze. Most studies also show that HPV testing as the sole primary screening method is more cost-effective than using it for co-testing with cytology (89, 90). A key driver of cost-effectiveness is the fact that HPV DNA testing allows a longer interval between screens without increasing the risk of cancer (91).

**Self-sampling for HPV testing**

HPV testing through self-collected vaginal specimens has gained attention because of its potential to increase screening participation. Self-sampling removes many of the barriers that prevent women from participation in regular screening programs (92). Randomized studies and meta-analyses have shown that clinically validated PCR-based assays are equally accurate when using self-collected samples versus clinician-taken cervical samples (93, 94).

In a meta-analysis, Arbyn et al. collected data from 36 studies (154,556 participants) to assess the clinical accuracy of HPV detection in vaginal self-samples versus cervical samples collected by a healthcare provider, in connection with detection of CIN 2+. The sensitivity of HPV detection in self-samples was similar to that in samples collected by a healthcare provider as regards detection of CIN 3+. For cytology, using LSIL as the threshold, self-sampling was 14% more sensitive in the detection of CIN 2+ (93). These data have been confirmed in other studies (95, 96).

Self-sampling has several advantages. The majority of women participating in self-sampling trials have reported a positive experience and prefer self-sampling rather than conventional sampling for diverse reasons such as it being less embarrassing, less uncomfortable and less time-consuming than a visit to a healthcare center (97-99). Most participants have been confident with the test results, although some have been concerned about performing the procedure correctly (100-102). Several studies have shown that women who self-collected their samples were motivated to undergo clinician examination and follow-up in the case of a positive HPV test (92, 103-105). Self-sampling results in increased response rates among non-attendees (95, 105-107), leading to increased coverage, which can also result in fewer and earlier detected CC cases, followed by cost savings. Thus, self-sampling at home for HPV testing is a more cost-effective alternative than clinic-based screening in this population (89, 108, 109).

Data about cost effectiveness in primary screening is limited. In a recent study it was reported that the total cost per woman participating in primary screening was 4.2 times higher in the Pap-smear arm than in the HPV self-
sampling arm (89). In addition, a self-collected sample can also be used for analysis of other biomarkers and/or vaginal microbiota, which can provide additional information about the significance of an HPV infection as well as vaginal health in general.

The emotional responses to a positive HPV test result have been studied and they have varied from feelings of shame and embarrassment to relief and/or reassurance, or they did not provoke any emotional reaction (110-112). The reasons for these variations include differences in the studied populations such as age, level of knowledge about HPV, and the presence or absence of dysplasia (110, 111). However, in a new era related to HPV vaccine, HPV screening and internet accessibility, knowledge about HPV infection, its significance and the need of follow-up may increase and contribute to better coping responses after having received notice of a positive HPV test result.

Self-sampling requires that individuals obtain a validated HPV test kit, they collect their own sample and send their specimen to a laboratory; the laboratory analyzes the self-collected specimen and returns the test result to the individual or the healthcare system. Self-sampling can be conducted either at a clinic or outside the healthcare system and can be initiated either by healthcare providers or by the women themselves. Accountability of and linkage to the healthcare sector is a very important consideration for the success of this self-care intervention, including providing adequate information and good-quality test kits, distributing kits equitably and ensuring follow-up after self-sampling test results.

**Triage for HPV-positive women**

HPV DNA testing has a very high negative predictive value, but it has low specificity (113). Appropriate protocols to manage HPV-positive women are essential in order to avoid over-referral and over-treatment. The results of HPV primary screening randomized control trials suggest that reflex cytology can be an appropriate option for triage of HPV-positive women (29, 91, 114, 115). However, challenges remain as regards how to manage women who are HPV-positive with a negative cytology result, especially in elderly women where cytology has limited sensitivity. An alternative approach is to triage with secondary biomarkers. Several biomarker options including detection of HPV E6/E7 mRNA, genotyping for HPV-16/18 and co-expression of p16INK4a/Ki-67 have been evaluated (116, 117).

Overexpression of the viral oncogenes E6 and E7 are necessary for malignant transformation. Detection of these oncogenes by the presence of their mRNA transcripts allows for better distinction between transient HPV infections and those persistent or active transforming infections that are likely to induce the development of pre-cancerous or cancerous lesions (118, 119).
Recently, methylation of particular genes has been found to be linked to high-grade pre-cancer and cervical cancer. Methylation of human genes is strongly associated with CIN and cancer. Several candidate genes have been shown to be consistently hypermethylated in cases of cervical cancer and high-grade CIN, most prominently CADM1, EPB41L3, FAM19A4, MAL, miR124, PAX1 and SOX119–21 (120-122). These markers show promise as triage markers for managing HPV-positive women, although published studies have been cross-sectional with short-term follow-up, in predominantly non-screening populations and conducted on cervical scrapes or self-collected samples (46-48).

Several studies have shown viral load to be an important quantifier of risk, particularly as regards HPV-16 (123) and also other HPV types showing different degrees of risk (124). Although data is available in connection with many HPV tests (relative light units for HC2 tests and CT scores for PCR-based tests), further studies should be carried out to ensure values are appropriately adjusted for the number of cells per sample.

**Screening and cervical cancer among older women**

Cervical screening programs in many countries stop at around the age of 65. However, there is no clear evidence of what is the appropriate age at which to stop screening (55). Diagrams that show age-specific incidence rates of CC in areas with established cervical screening programs have two peaks. One is around the age of 40 years and the other at around 75 years. In Sweden, this second peak has decreased only modestly with time (11, 12).

It has been suggested that as women leave a screening program they should be tested for HPV as an exit test and that surveillance should be continued in women who are HPV-positive, but evidence to support this strategy is limited (125). J.S. Smith and coworkers reported that HPV prevalence was strongly associated with age, although age curves of HPV infection differed notably across regions and countries. The shapes of the curves were declining in older ages, flat across age, or characterized by a U shape, with a relatively higher HPV prevalence in younger and older age groups. Overall HPV prevalence in most geographical regions consistently peaked in women aged 25 years, with a decrease in older age groups. In contrast, age curves of HPV prevalence among women in Central and South America and in Africa were characterized by an increased or stable HPV prevalence among women aged 45 years or older. Given that the second peak in HPV prevalence appears to be more pronounced and occurs at different ages within different geographical regions, these data suggest that the higher prevalence in older women is largely because of newly acquired HPV infections, primarily reflecting differences in sexual behavior across global regions. Alternatively, the U-shaped curves of
HPV by age may potentially be explained by reactivation of latent HPV infections in older women (126, 127).

In an IARC HPV prevalence survey an estimate of 10.41% (95% CI 10.2–10.7%) for the prevalence of HPV worldwide among women (of all ages) with normal cytology was reported. Age-specific prevalence rates showed higher proportions among the young age groups, a decline in young adults and a variable pattern afterward. In most countries, notably in the Americas, the prevalence increased again in the post-menopausal age groups. In Europe, the same pattern in the 40+ age group is maintained in most surveys, whereas in other high-prevalence countries in Asia the prevalence of HPV DNA remained fairly constant across all age groups (51). The substantial differences observed in age-specific curves of HPV prevalence between populations may have a variety of explanations. These differences, however, underline the fact that great caution should be used in inferring the natural history of HPV from age-specific prevalence rates (21). Recent research suggests that cervical screening of older women is associated with a considerable decrease in cervical-cancer incidence (128-130). Rustagi and coworkers estimated that cervical screening of all women aged 55–79 years in the United States could avert 630 deaths annually. These results provide a minimum estimate of the efficacy of human papillomavirus DNA screening, and a more sensitive test to reduce cervical cancer death among older women is needed (129).

Even if women know the relevant symptoms related to cervical cancer, age-related changes may mean older women do not detect them at an early stage. If sexual activity is reduced through declining libido or loss of a partner, symptoms such as pain or discomfort during intercourse, or bleeding afterward may not be identified. This results in a diagnosis at advanced stages of the disease, with high mortality (5, 131). Several studies have shown that survival increases if cervical cancer is diagnosed through screening rather than symptoms (132-134). The risk of CC in older women can be assessed differently between countries and regions with different approaches. In the United States, 10.6 CC cases per 100,000 women aged 65–74, and 8.2 cases in women older than 75 years have been considered to reflect a low risk, and screening ends at 65 years. This is the case if the woman does not have a history of moderate or severe abnormal cervical lesions or cervical cancer and has had either three negative Pap test results in a row or two negative co-test results in a row within the past 10 years, with the most recent test performed within the past five years (135).

In Australia, approximately 10.2–11 cases per 100,000 women aged 65 to 79, and 14.2 cases in women aged 80 years and older have been considered to reflect a high risk, and screening continues until 74 years of age. Women who are 75 years or older who have never had a cervical screening test, or have not had one in the previous five years, may request a test and can be screened (136).
Age in itself is not a reason for stopping screening. The problem, however, is how to obtain an adequate and accurate cervical cytological sample, a problem that increases with age. The problem includes difficulties with the examination itself, such as reduced mobility, pain or vaginal atrophy and difficulty of accessing the transformation zone, which is located higher in the endocervical canal in older women. However, these conditions vary from woman to woman, and for many healthy older women, 65 years is not a reason to deprive them of the benefit that screening brings. Furthermore, these problems have mainly been identified in connection with cytology and we are now in a new era in cervical screening whereby HPV testing is likely to become the primary screening tool (137), and the same problems may not be relevant. An alternative approach is to send older women a self-sampling kit for subsequent HPV testing.
Aims

The general aim of the current work was to increase knowledge of the prevalence and persistence of HPV infection and HPV-related dysplasia in elderly women.

Specific aims were to

I. investigate the prevalence of HPV and HPV-related cervical dysplasia in women of 60 years and older.

II. evaluate the acceptability of repeated self-sampling for HPV testing in elderly women.

III. explore and describe elderly women’s experiences of performing self-sampling at home.

IV. determine the incidence of oncogenic HPV and HPV-related dysplasia five years after a negative HPV test result among elderly women.
Materials and Methods

Ethics
All studies were approved by the Ethics Review Board, Uppsala, Sweden.

Study population

Paper I
The study was based on 1051 women aged 60–89 years (mean age 68) attending an outpatient gynecology clinic in Dalarna County, Sweden and having an HPV test as part of a gynecological examination. The study period was from September 2013 until June 2015. The Regional Ethics Committee in Uppsala approved the study (Dnr 2015/136).

Paper II
This study was conducted in Dalarna County, Sweden, between 2014 and 2016. Women were randomly selected from the population register, with 375 women in each of the four age groups (60, 65, 70 and 75). They were invited to perform repeated self-sampling at home for HPV testing. In brief, 893 out of 1500 invited women performed self-sampling. The study was approved by the Regional Ethics Committee in Uppsala (Dnr 2014/024).

Paper III
Women who had participated in Study II were invited to take part in Study III. All 893 women who provided a self-collected sample for HPV analysis were sent a survey by regular mail. All sixteen women in the self-sampling study whose first HPV test was positive and with a second test about four months later that was either negative or positive but without precancerous lesions in histology were invited to participate in an interview. Of those, thirteen women agreed to participate. The study was approved by the Regional Ethics Committee in Uppsala (Dnr.2014/024 and 2017/380).
Paper IV
The study population consisted of 804 women who had participated in a previous study on self-sampling for HPV testing (Study II) five years earlier and at that time without the presence of HPV infection. The women were invited to perform follow-up self-sampling at home for HPV testing, and self-sampling kits were sent through the postal service. The study period was from October 2019 until May 2020. The study was approved by the Swedish Ethics Review Authority (Dnr. 2019-02490).

Methods
Paper I
This retrospective study was based on 1051 women attending an outpatient gynecology clinic in Sweden and having an HPV test as part of a gynecological examination. Women lacking a cervix, and those with a previous negative Pap smear within two years were not included. Participants having a positive first test were re-examined after 3.5 months on average, including a second HPV test and a sample for liquid-based cytology (LBC). Those who were HPV-positive in the second test were referred to the outpatient clinic at the local hospital for colposcopy, biopsy and a second LBC examination. Women who tested negative in the second test had a third HPV test after one year. All LBC specimens were screened by cytotechnicians and those specimens considered abnormal were reviewed by a surgical pathologist. For cervical cytology, the Thin Prep Pap Test was used. Specialists in surgical pathology examined the cervical biopsy samples and cones for histological diagnosis. One senior pathologist re-evaluated all LBCs, cervical biopsy specimens and cones, focusing on glandular atypia and adenocarcinoma (ADC).

The HPV test was performed using a multiplex real-time PCR assay (HPVir) that has been clinically validated (138) and thus is one of the relatively few clinically validated HPV tests available today (139). A cervical sample was collected using a cytobrush. The sample was applied to a filter-paper matrix, an indicating FTA elute card, and allowed to dry before transportation and storage. The clinical material applied to this card is stable at room temperature and samples stored on FTA cards for one year have shown identical HPV typing results when compared with the use of liquid-based media (140, 141). The threshold for a positive HPV type was set to 10 copies per PCR. The DNA was obtained from the FTA cards, which detect the following HR-HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 (18 and 45 are detected together, and 33, 52 and 58 as one group) (26).

For statistical analysis, the Statistical Package for the Social Sciences (SPSS) version 22 for Windows, and Excel were used. A p-value of less than 0.05 was considered statistically significant. The age groups 60–64 years
(n=277), 65–69 years (n=394), 70–74 years (n=235), 75–79 years (n=95), and
80–84 years (n=50) were compared. For statistical significance testing be-
tween age groups, the chi-squared test in SPSS was used. Confidence intervals
(CIs) of proportions (Fleiss) were calculated using Excel.

Paper II

This prospective population-based longitudinal descriptive study was con-
ducted in Dalarna County, Sweden, between 2014 and 2016, and 1500 women
were invited to perform repeated self-sampling for HPV testing. The women
were randomly selected (by using the random generator in SPSS) from the
population register, with 375 women in each of the four age groups: 60, 65,
70 and 75. Written informed consent was obtained from women who agreed
to participate in the study, and self-sampling kits were sent via the regular
postal service. In brief, self-sampling was performed at home and the sample
was returned, in a prepaid envelope, to the laboratory for analysis of high-risk
HPV (Figure 6), as previously described (26, 29). Women with a positive first
HPV test were sent a new self-sampling kit four months after the first test was
done. The sampling kit and analysis was the same as described above (Paper
I). No reminders were sent to the women who did not respond to the invitation
or in cases when women agreed to participate but no sample was sent to the
laboratory.

Women who were repeatedly positive in the second HPV test were offered
examination by colposcopy, and sampling for histology and LBC. One of the
authors (RSH) performed the great majority of the colposcopies, cervical bi-
opsies, abrasions, and conizations for histological diagnosis. All LBC speci-
mens were screened by cytotechnicians, and specimens considered abnormal
were reviewed by a surgical pathologist. For cervical cytology, the Thin Prep
Pap Test was used. Cervical smears were collected with a plastic spatula and
a cytobrush. LBC specimens were placed in PreserveCyt solution and pro-
cessed in the Thin Prep 5000 processor (Hologic Cytyc Corporation, Box-
borough, Mass.).
**Figure 6.** Instructions on how to perform self-sampling.

Specialists in surgical pathology examined the cervical biopsy samples and cones for histologic diagnosis. One senior pathologist re-evaluated all LBC results, cervical biopsies and cones, focusing on glandular atypia and adenocarcinoma. All cytology and histology specimens were examined at the Department of Pathology and Cytology, Falun County Hospital, Falun, Sweden.

For statistical analysis, Excel and SPSS version 22 for Windows were used. The data was analyzed using both a per-protocol (PP) approach, including only women who complied with the protocol, and also with an intention-to-
treat (ITT) approach, including women who on their own initiative had a clinical second HPV test and follow-up. A p-value less than 0.05 was considered statistically significant. For statistical significance testing between age groups, the chi-squared test in SPSS was used. Confidence intervals (CIs) of proportions (Fleiss) were calculated using Excel.

**Paper III**

This is a descriptive study using quantitative and qualitative methods. Data on experiences of self-sampling at home for HPV testing were collected from a questionnaire sent to all women who participated in a self-sampling intervention study (Paper II). All 893 women who provided a self-collected sample for HPV analysis were sent a survey by regular mail. The questionnaire included 20 closed-ended questions concerning various aspects of the women’s health, early gynecological disease, lifestyle and two specific questions about their concerns of performing self-sampling. For this study, only the questions about self-sampling were analyzed. The survey also included an open-ended question concerning their opinions, experiences or advice on how the self-sampling could be improved.

Interviews were done with the women who tested positive in the first HPV test and either were negative in their second HPV test or were positive in their second HPV test but without precancerous lesions or cancer. Individual interviews were undertaken during the winter of 2017–2018 according to a semi-structured interview protocol. The protocol contained open-ended questions designed to elicit responses to the research questions of the study. The interviews were audio-recorded and transcribed verbatim by a research assistant. The main subjects in the interview concerned: a) a woman’s experiences of the pros and cons of performing self-sampling at home as compared with sampling by a healthcare provider, b) a woman’s knowledge about HPV infection and the relationship between HPV and cervical cancer, and c) a woman’s experiences when receiving notice of having a positive HPV test result and the feelings such information brought.

The survey data were entered into an Excel database. For statistical analysis, SSPS version 22 was used. The interview data were analyzed using qualitative content analysis with a deductive approach and undertaken in two steps. First, the text files of the interview were read as soon as they had been transcribed in order to gain an overview of the material. Next, three of the authors performed the data analysis by reading the text again and identifying meaningful units in the text relating to the main subjects in the interviews. The meaningful units were grouped in categories according to three predefined subjects of interest, and codes in each category were identified. Any discordance was resolved by discussion.
This prospective longitudinal descriptive study was conducted in Dalarna County, Sweden, between autumn 2019 and spring 2020. In brief, self-sampling was performed at home and the sample was returned, in a prepaid envelope, to the laboratory for analysis of high-risk HPV, as previously described. Women with a positive HPV test were sent a new self-sampling kit four months after the previous test was done. The sampling kit and analysis was the same as described above.

Women who were repeatedly positive in the second HPV test were offered examination by colposcopy, and sampling for histology and LBC. Two of the authors performed the colposcopies, cervical biopsies, and abrasions for histological diagnosis.

All LBC specimens were analyzed as described above (Study II) and statistical analysis was also the same as described for Study II.

Results

Paper I

All 1051 women offered an HPV test accepted one, and all samples were considered adequate and analyzed (Figure 7). All HPV-positive women but one were followed for 22 to 43 months. Forty-three women (4.1%, 95% CI 3.0–5.5%) were positive for HPV in their first test. In the second test, on average 3.5 months later, 27 women (2.6%, 95% CI 1.7–3.8%) were still positive. All HPV-positive women (n=43) had a cervical smear for LBC collected at the same time as the second sample for HPV testing was collected. Of the women with a second positive HPV test result, 81.5% (22/27) had dysplasia in histology, where four had CIN 2, and 18 had CIN 1. Women with HPV-related dysplasia, CIN 1–2, in the study population thus represented 2.1% (95% CI 1.3–3.2%) and those with CIN 2 alone represented 0.4% (95% CI 0.1–1.0%) of the study population.

Of the 27 women that were positive for HPV in the second test, four had either ASC-US or CIN 1 in cytology. One of them had benign histology and became HPV- and cytology-negative after one year. Of the 22 women with dysplasia in histology, 19 (86.4%) had normal cytology while three (13.6%) also had dysplasia (1 ASC-US, 2 CIN 1) in cytology. Of the women who were HPV-negative in the second HPV test (37.2%; 16/43), all had normal cytology and were scheduled for a follow-up HPV test after one year.

In the third HPV test of 15 women after one year (one woman had died as a result of non-gynecological disease), two (13.3%) were HPV-positive with the same HPV type as in the first test. Cytology and histology showed CIN 1 in one woman and normal results in the other. No atypia of glandular cells, adenocarcinoma in situ or ADC was found. In screening history (1986 and
of the 43 women that had a positive first HPV test, 16 (37.2%) had a history of dysplasia (10 CIN 1, two CIN 2, two CIN 3, two others). Ten of these women (23.3%) had dysplasia before 60 years of age and six women when older than 60.

Figure 7. Flow chart showing study design, HPV and dysplasia occurrence.

All HPV types tested for were found (Figure 8). Multiple infections were found in three women in the first test, and one of them was HPV-negative in the second test. In the second test, one of these women had multiple infection with the same HPV types. Two other women had a single-type infection in the first test but multiple infection in the second test. Of all 27 (62.8%) who tested positive in the second HPV test, 7.4% (n = 2) had shifted HPV type from the first to the second test.
At colposcopy of 26 of the 27 women who were also HPV-positive in the second HPV test, the transformation zone (TZ) was not visible in 17 (65.4%) and partly visible in nine (34.6%) of the cases. No-one had a fully visible TZ. Vaginal lesions were found in colposcopy in three cases and biopsy for histological evaluation showed vaginal intraepithelial neoplasia (VAIN I) in all three cases. All three women with VAIN I also had CIN 1.

There were no differences in the prevalence of HPV or dysplasia between age groups (five years and 10 years), and there were no HPV-positive women above the age of 80 (n = 50). The most prevalent HPV types were HPV 16 (27.9%; 12/43), followed by HPV 33/52/58 (20.9%) and HPV 31 (14.0%). The distribution of HPV types in patients with dysplasia is shown in Figure 9. Conization was performed in 20 HPV-positive women, and of these, 15 of 19 (78.9%) were HPV-negative at follow-up, on average 15 months (9 ± 36 months) after surgery.
Paper II

Of the 1500 invited women, 940 (62.7%) agreed to participate in the study and received a self-sampling kit. Of these, 893 women sent a sample to the HPV laboratory (Figure 10). The overall participation rate was 59.5%, with a lower participation rate in the older age groups (p = 0.006). The participation rates in each age group were as follows: 62.9% (236/375) at age 60, 63.5% (238/375) at age 65, 59.5% (223/375) at age 70 and 52.3% (196/375) at age 75. Five of the women’s samples contained insufficient material for the HPV assay and those women received a new self-sampling kit for resampling.

Samples from all 893 women were finally analyzed.

Overall, 39 women (4.4%, 95% CI 3.2–6.0%) were HPV-positive in the first test and 22 (2.5%, 95% CI 1.6–3.8) in the second test, i.e., 22/39 (56.4%) remained positive in the second test collected on average 5.5 months after the first test. There was no significant difference in HPV prevalence in the different age groups (Table 3) and the prevalence of HPV-16 was 1.1% in HPV test 1. Multiple infections were found in three women in the first test (3/893, 0.3%).

Table 3. HPV prevalence in the different age groups.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>HPV pos (n)</th>
<th>HPV neg (n)</th>
<th>Total (n)</th>
<th>HPV pos (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>10</td>
<td>226</td>
<td>236</td>
<td>4.2</td>
</tr>
<tr>
<td>65</td>
<td>12</td>
<td>226</td>
<td>238</td>
<td>5.0</td>
</tr>
<tr>
<td>70</td>
<td>10</td>
<td>213</td>
<td>223</td>
<td>4.5</td>
</tr>
<tr>
<td>75</td>
<td>7</td>
<td>189</td>
<td>196</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>854</td>
<td>893</td>
<td>4.4</td>
</tr>
</tbody>
</table>

All HPV types tested for were found. Thirty-four women performed a second self-sample, followed by an HPV test and were included in the per-protocol (PP) approach, and 19 of these women (55.9%) had a positive second HPV test result. Of these, 17 underwent colposcopy followed by histological examination (one woman had her cervix resected and one was lost to follow-up) and 76.5% (13/17) had dysplasia (six CIN 1 and seven CIN 2). As the colposcopic findings were inadequate and none of the women had a fully visible transformation zone, sampling for histology by cervical abrasion and random biopsies was performed. Vaginal dysplasia was found in biopsy samples from iodine-negative areas in the vagina. Five women did not perform second self-sampling according to the study protocol and they were included in the ITT group. Four of them underwent assisted HPV testing (using the same PCR assay type [hpVIR]), at a clinic and three of them were HPV-positive. One of them had CIN 1 and one CIN 2 in histology. One woman also included in the
ITT arm underwent a second test and she had CIN 3 disease in histology. In total, in the ITT arm, 16 women had dysplasia in histology (seven CIN 1, eight CIN 2 and one CIN 3). Cytological results (LBC) were normal in all except one in the PP arm (ASCUS), and two in the ITT arm (ASCUS and ASC-H, respectively).

Among the women in the PP arm, 68.4% (13/19) with a positive second HPV test had dysplasia in histology. Out of all 893 women, 16 (1.8%) had CIN, i.e., 0.8% (7/893) had CIN 1 and 1.0% (9/893) had CIN 2+ (Figure 10). In one woman with CIN 1 a vaginal lesion was found in colposcopy, and biopsy sampling for histological evaluation showed vaginal intraepithelial neoplasia, VAIN 2.

Figure 10. Flow chart showing study design. HPV and dysplasia occurrence.

No glandular atypia was diagnosed. The positive predictive value (PPV) for any CIN (CIN 1+) was 41.0% (16/39) after the first HPV test and 68.4%
(13/19) after the second HPV test in the PP arm, and 68.2% (15/22) in the ITT approach. The PPV for CIN 2+ was 23.1% (9/39) after the first HPV test and 36.8% (7/19) after the second HPV test in the PP approach, and 36.4% % (8/22) in the ITT arm.

**Paper III**

Of 893 eligible women, 868 (97.2%) answered the survey. The sociodemographic characteristics of the participants are shown in Table 4. The survey contained two specific questions about women’s experiences of self-sampling. To the question about how easy or difficult it had been to collect the sample at home, 49.2% (427/868) answered “very easy”, 46.8% (406/868) answered “easy” and 2.0% (18/868) answered “not easy”. Two percent of the women (17/868) did not answer this question. To the question “Do you prefer self-sampling compared with sampling by a healthcare provider?” 59.0% (511/868) answered that they preferred self-sampling, 16.0% (143/868) said that they would prefer a sample collected by a healthcare provider and 24.0% (205/868) were uncertain. One percent of the women (8/868) did not answer this question.

**Table 4.** Demographic characteristics of the participants (n = 868).

<table>
<thead>
<tr>
<th>Age, years</th>
<th>60–75</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-positive, n (%)</td>
<td>36 (4.2)</td>
</tr>
<tr>
<td>HPV-negative, n (%)</td>
<td>832 (95.8)</td>
</tr>
<tr>
<td>Nulliparous, n (%)</td>
<td>66 (7.6)</td>
</tr>
<tr>
<td>Parity (1–7), n (%)</td>
<td>799 (92.4)</td>
</tr>
<tr>
<td>Never smokers, n (%)</td>
<td>757 (87.9)</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>85 (9.8)</td>
</tr>
<tr>
<td>Earlier smokers, n (%)</td>
<td>19 (2.2)</td>
</tr>
<tr>
<td>Single, n (%)</td>
<td>203 (23.4)</td>
</tr>
<tr>
<td>Partners, n (%)</td>
<td>664 (76.6)</td>
</tr>
<tr>
<td>Sexually active, n (%)</td>
<td>380 (43.8)</td>
</tr>
<tr>
<td>Not sexually active, n (%)</td>
<td>453 (52.2)</td>
</tr>
<tr>
<td>Sexual activity not reported, n (%)</td>
<td>34 (4.0)</td>
</tr>
</tbody>
</table>

Thirteen women aged 60 to 75 years participated in the interviews. The results of content analysis of the interviews according to each of the three main categories are summarized in Table 5 (and in further detail below) with quotations marked in *italics*. 
**Table 5.** Categories and examples of codes and meaning units in each category.

<table>
<thead>
<tr>
<th>Categories</th>
<th>Codes in each category</th>
<th>Examples of meaning units</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV self-sampling compared with sampling by a healthcare provider.</td>
<td>- Easy to perform self-sampling at home</td>
<td>- It was very good, I mean, that you could do it at home and then just send it.</td>
</tr>
<tr>
<td></td>
<td>- Easy to understand the instructions</td>
<td>- Very easy, it was great!</td>
</tr>
<tr>
<td></td>
<td>- Preference of self-sampling compared with sampling by a healthcare professional at a clinic.</td>
<td>- It was actually very nice to take it myself instead of lying down in the chair.</td>
</tr>
<tr>
<td>Knowledge and concerns about the relationship between HPV infection and risk of CC.</td>
<td>- Little knowledge about HPV</td>
<td>- Now I know a little more after reading the information, but before I didn't know so much.</td>
</tr>
<tr>
<td></td>
<td>- Little knowledge about the relationship between HPV infection and CC</td>
<td>- Yes... to start with I didn't actually know anything...but later I read a bit that it can cause cancer...</td>
</tr>
<tr>
<td></td>
<td>- Little knowledge about CC screening and prevention</td>
<td>- No, I do not know so much. But I know there is a vaccine for little girls.</td>
</tr>
<tr>
<td>Experiences and feelings about an HPV-positive result.</td>
<td>- Worries of having an HPV-positive result</td>
<td>- I was not worried! A little surprised, maybe.</td>
</tr>
<tr>
<td></td>
<td>- No feelings of shame about having an HPV-positive result</td>
<td>- I was a little frightened. I didn't know much about it. I wondered if I had cancer.</td>
</tr>
<tr>
<td></td>
<td>- Need for more information</td>
<td>- I did not think so much about this! I have been married to the same man for more than 30 years.</td>
</tr>
</tbody>
</table>

CC = cervical cancer
In the survey, there was an opportunity for the participants to leave comments or an opinion about self-sampling. There were comments from 176 women; 75 confirmed that self-sampling was easy and uncomplicated, 26 women reported that they felt uncertain as to whether the sampling was performed correctly, 11 women reported that the brush was hard and uncomfortable and two of these women had minor bleeding after sampling.

Paper IV

The numbers of women included and excluded at each stage of the study, as well as the numbers of end diagnoses in cytology and histology, are presented in Figure 11. Of the 804 invited women, 634 agreed to participate in the study and received a self-sampling kit. Of these, 632 women sent a sample to the HPV laboratory. The participation rate in each age group was as follows: 93.3% at age 65, 74.0% at age 70, 80.7% at age 75 and 64.6% at age 80 (Table 6). Four of the women’s samples contained insufficient material for the HPV assay and those women received a new self-sampling kit for resampling. Samples from all 632 women were finally analyzed. Overall, 18 women (2.8%) were HPV-positive in the first test and eight (1.3%) in the second test collected on average 5.4 months after the first test.
Sixteen women performed a second self-sample for HPV testing and were included in the per-protocol (PP) approach, and seven of these women had a positive second HPV test result. Among these seven women, colposcopy was performed and histology carried out (one woman underwent cervical resection). None of the women had a fully visible transformation zone, and sampling for histology by cervical abrasion and random biopsies was performed. No-one showed vaginal changes at the time of colposcopy. Two women performed second self-sampling after 1.2 and 1.6 months respectively, and not according to the study protocol; hence they were included in the ITT group.
One of them had a positive result in the second HPV test. Both showed benign cytology and histology. No glandular atypia was diagnosed.

There was no significant difference in HPV prevalence in the different age groups (Table 6). The most prevalent HPV type in both HPV tests was HPV-51 (Table 7).

Table 6. HPV prevalence in the different age groups.

<table>
<thead>
<tr>
<th>Age, years</th>
<th>HPV-negative five years previously</th>
<th>Performed self-sampling (%)</th>
<th>HPV test 1 negative (%)</th>
<th>HPV test 1 positive (%)</th>
<th>HPV test 2 positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td>210</td>
<td>196 (93.3)</td>
<td>189 (96.4)</td>
<td>7 (3.6)</td>
<td>3 (1.5)</td>
</tr>
<tr>
<td>70</td>
<td>219</td>
<td>162 (74.0)</td>
<td>159 (98.1)</td>
<td>3 (1.9)</td>
<td>2 (1.2)</td>
</tr>
<tr>
<td>75</td>
<td>197</td>
<td>159 (80.7)</td>
<td>155 (97.5)</td>
<td>4 (2.5)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>80</td>
<td>178</td>
<td>115 (64.6)</td>
<td>111 (96.5)</td>
<td>4 (3.5)</td>
<td>2 (1.7)</td>
</tr>
<tr>
<td>Total</td>
<td>804</td>
<td>632 (78.6)</td>
<td>614 (97.2)</td>
<td>18 (2.8)</td>
<td>8 (1.3)</td>
</tr>
</tbody>
</table>

Table 7. HPV type, cytology, and histology in women testing HPV-positive in follow-up HPV tests (n = 8).

<table>
<thead>
<tr>
<th>Age</th>
<th>HPV test 1</th>
<th>HPV test 2</th>
<th>Cytology</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>31</td>
<td>31</td>
<td>Benign</td>
<td>benign</td>
</tr>
<tr>
<td>65</td>
<td>51</td>
<td>51</td>
<td>Benign</td>
<td>benign</td>
</tr>
<tr>
<td>70</td>
<td>51</td>
<td>51</td>
<td>LSIL</td>
<td>LSIL</td>
</tr>
<tr>
<td>75</td>
<td>51</td>
<td>51</td>
<td>Benign</td>
<td>LSIL</td>
</tr>
<tr>
<td>80</td>
<td>56</td>
<td>56</td>
<td>Benign</td>
<td>benign</td>
</tr>
<tr>
<td>80</td>
<td>18/45</td>
<td>18/45</td>
<td>Ascus</td>
<td>benign</td>
</tr>
<tr>
<td>65</td>
<td>33/52/58</td>
<td>33/52/58</td>
<td>Ascus</td>
<td>LSIL</td>
</tr>
<tr>
<td>65</td>
<td>33/52/58</td>
<td>33/52/58</td>
<td>Ascus</td>
<td>LSIL</td>
</tr>
</tbody>
</table>

LSIL: low-grade squamous intraepithelial lesion; ASCUS: atypical cells of undetermined significance; PP: per-protocol approach; ITT: intention-to-treat approach.
Knowledge of HPV biology in older women is scarce. To reduce the incidence of cervical cancer in this group it is necessary to learn about the prevalence, persistence, and latency of HPV infection. Few studies have been focused on older women who have not been screened after leaving the regular screening program. Data are mainly from studies on younger women where older people have also been included.

In our studies HPV prevalence was shown to be relatively low in older women, but if they had a second positive HPV test result, the risk of cervical dysplasia was high. In Studies I and II the prevalence rate of HPV was just over 4%. This result is in line with that in a study from Denmark that showed an HPV prevalence of 4.3% in elderly women with a mean age of 74.6 years (10). The most common HPV type in study I and II was HPV 16 with a prevalence of about 30% follow by HPV 33/52/58 and HPV 18/45 that together represented around 60% of all HPV infections. These results are in line with what de San Jose and coworkers presented in a meta-analysis where the five most common HPV types in HPV-positive women worldwide were HPV16, HPV18, HPV31, HPV58, and HPV52, representing 50% of all HPV infections (22).

In Paper II, self-sampling for HPV testing was used and well accepted among elderly women, where 59.5% of those invited participated in the study. There was a high participation rate, though no reminder was sent to the women who did not respond to the invitation, or in cases when women agreed to participate but no sample was sent to the laboratory.

A high participation rate might be explained by the fact that older women have a higher awareness of cancer, and are also aware that they are no longer invited to the screening program. The HPV prevalence in this group (mean age 67 years) was similar to that found when samples were collected from the cervix (average age 68 years) by a gynecologist (142). It is well known that vaginal self-sampling for HPV testing is fully comparable with sampling by a healthcare provider, as long as a sensitive and validated PCR-based method is used for analysis of the samples (93). In our studies the HPV test was performed using a multiplex real-time PCR assay (HPVir) that has been clinically validated according to the guidelines for HPV test requirements for cervical-cancer screening (138), and all samples could be analyzed.

It has previously been shown that an HPV infection persists longer in women older than 30 years compared with younger women (143). In Studies
I and II we found that the rate of loss of detection of HPV between tests one and two was around 40% when the second HPV test was carried out on average five months after the first test. If this is the result of clearance or a latent undetectable infection is difficult to determine. According to the results of Study I, two women out of 15 (13%) were positive with the same HPV type in the third HPV test as in the first test, one year after a negative second test. The number of cases was very low and firm conclusions can of course not be drawn, but the data might indicate the magnitude of the problem. The two positive samples are most probably explained by being a latent infection with no, or limited viral replication, or it could be that the result of the HPV test was below the threshold of positivity when the second HPV test was done.

Korostil and coworkers demonstrated that about one in 10 women who appear to have cleared their HPV infection may be latently infected (144). In a previous study, on women aged 30–65, the loss of detection was 41% with 2.7 months between tests one and two (145). In another study, on women aged 30–49, the loss of detection was 29% with 4.4 months between tests one and two (94, 96). These data indicate that the loss of detection seems to be at least as high in older women when compared with younger women. However, the loss of detection in young women probably corresponds to true clearance, and in older women, it might be more common with a latent undetectable infection.

It is known that the great majority of cases of high-grade dysplasia is the result of a persistent infection with oncogenic HPV. Our results show a prevalence rate of HPV-related dysplasia of around 2%. The strategy of repeating the HPV test results in substantially fewer women requiring investigation by means of colposcopy and biopsy, and higher specificity in the identification of CIN 2+ (94). We found a CIN 2+ prevalence rate of 1.0% in women aged 60–75 years. This is slightly lower than that which has been reported in the Swedish screening population aged 23–60 years, with a CIN 2+ prevalence rate of 1.3–1.4% (146). It was recently reported that the prevalence of CIN 2+ was 1.1% when screening was based on cytology, but 2.0% when based on HPV testing (96). In the first study, in women with cervical dysplasia all oncogenic HPV types were identified except HPV types 56 and 59. In the group of women with dysplasia the most prevalent HPV types were 33/52/58, 16 and 39 in that order. It should be noted that in the group with persistent HPV 16, 42% presented cervical dysplasia and in the group with persistent HPV 39, 75% presented dysplasia. Despite the small size of the group this may point that in elderly women the progression to cervical dysplasia might differ from younger women where HPV 16 and 18 are the main causes of cervical dysplasia.

In line with previous studies (142, 147) we also found a poor correlation between cytology and histology. In the first study only 18.8% of women with dysplasia in histology had abnormal cytology, and in the second study only 13.6% of women with dysplasia had abnormal cytology. The role of cytology
for screening older women is thus very limited. As early as in 1985 Robert and coworkers reported that Pap-test false-negative rates are higher in this age group because atrophic cellular changes can mimic neoplastic changes. False-negative results may be obtained as a result of difficulty in sampling the squamocolumnar junction, which has often retracted into the endocervix (148). In a recent study on women aged > 55 years with diagnosed CC it was reported that cervical cytology was negative in 84.6% in screening samples collected during the five years preceding cancer diagnosis (149). Therefore, cytology does not seem to be an appropriate method for screening women older than 60. Colposcopy in elderly women is also of limited usefulness due to difficulties in visualizing the TZ. Almost half of the women had a nonvisible TZ and none was fully visible. Biopsy samples lacking the TZ are not reliable and thus diagnostic conization should be offered to women with persistent HPV infection and negative biopsies, especially if other risk factors are present. Adequate management of cervical dysplasia in elderly women is not established. In younger women, CIN 2+/HSIL is the treatment threshold, but in older women, this might not be optimal, owing to inability to predict the individual probability of progression of LSIL to HSIL or cervical cancer.

The importance of CIN 1 in elderly women has not been studied. The risk of CC in older women, as in younger women, is most probably strongly related to HPV infection, but whether the natural course of the disease is the same is still uncertain. In a Swedish study it was reported that women with a history of CIN 3 are considered to require long-term follow-up as a result of the risk of CC development (150).

In Study I, there was a high proportion of cases of HPV negativity after conization in short-term follow-up. Yet the optimal strategy, in terms of preventing cervical cancer in elderly women, will depend on the degree to which precancerous changes progress to cancer or spontaneously regress, and this has not been studied in this age group. However, it might be that cervical dysplasia carries a higher risk of cancer development in elderly women, in relation to their postmenopausal status, immune deficiencies due to other diseases, medication, and old age, which can all have an impact on the risk of progression of HPV-related dysplasia in this group.

The focus in Paper III was older women’s experiences of performing self-sampling at home with unsupervised collection of a vaginal sample. The rate of participation in the survey was high (97%), giving greater credibility to the results. Among surveyed women, the great majority of participants responded that it was very easy or easy to take a self-sample. Only 2% indicated that self-sampling was not easy. To the question about the preference of self-sampling compared with sampling by a healthcare provider, more than half of the women reported that they prefer self-sampling. We found high acceptability for self-sampling, which is consistent with the results of previous studies on younger women (46-48). Nelson and coworkers reported in a review that self-
sampling for HPV testing is generally well accepted by women not attending a screening program, and is preferred to clinician-based sampling (151). There was no difference in acceptability between the age groups included in our study.

The interviews were aimed at capturing women’s experiences and concerns about self-sampling, their knowledge of HPV and their experiences concerning notice of receiving a positive HPV test result. The women had almost no knowledge of HPV testing and no one was familiar with self-sampling before participation in the current study.

The participants generally had a positive attitude to self-sampling at home. They could see advantages such as it being easy, relatively comfortable, and private, and it was also less time- and resource-consuming than an appointment at a clinic. These results are surprisingly similar to those found in earlier studies performed among younger women (107, 152). Most women were satisfied with performing self-sampling and had confidence in the accuracy of the test result. Nobody was worried about using regular mail to send the sample and all seemed to be confident with the analysis. Similar results were shown in a Finnish study conducted on non-attendees who performed self-sampling, where more than 80% felt confident with the self-sampling and a similar proportion trusted the test results (107).

A lack of knowledge about the relationship between HPV infection and CC development, or an underestimation of the risk of CC was found, and this could be one reason for the low level of worry and concern about a positive HPV test result. During the interview, it was clear that women with better knowledge about HPV were more worried about being HPV-positive.

Most of the interviewed participants had a stable relationship. We found that no one reported feelings of shame or anxiety about being diagnosed with HPV, and no one reported that this knowledge hurt their relationship. These findings are not in agreement with those in other somewhat older studies, where it was found that women were anxious about being HPV-positive, since an HPV infection is a sexually transmitted disease that could have a negative impact on their relationship (110, 153). The reason behind the present more relaxed attitude is not clear. It might be connected to the fact that the women in the current study were much older, or that many of them had limited knowledge of HPV.

In our study, the majority were comfortable and satisfied with the information and instructions that they received in the study invitation. This outcome has several practical implications; for example, including adequate and balanced information about HPV and the significance of an HPV infection, in order to prepare the woman for the coming test result and possible further examination.

We also found a high demand and intention to use self-sampling in the future. Women would use self-sampling if it was available and most of them asked when the next time for sampling is. Our findings are important, because
in Sweden life expectancy for women is high and about one-third of the CC cases occur in women above the age of 60 (154). Self-sampling thus constitutes a superior alternative for providing screening to elderly women. Moreover, self-sampling has the potential to further reduce costs, as it eliminates the need for an initial clinical encounter in the screening process (155, 156).

The significance of a newly detected HPV infection among older women is not well understood. Aging itself is a risk factor of cancer and most chronic diseases (3, 157). There are controversies concerning when to stop screening and the significance of a negative exit test (55). Screening practices in post-menopausal women are especially challenging, since both cytology tests and colposcopies are less accurate among these women. Consequently, the assurance that a negative test result provided among this group is not well established. A recent study showed that a persistent HPV infection needs to be monitored despite normal Pap smears, since 6/40 (15%) women older than 40 years were found to have undiagnosed CIN 2+ when LEEP was performed (158).

In Study IV the detection rate of HPV was low at follow-up of women that were HPV-negative five years earlier. We were unable to determine whether these newly detected infections resulted from a new sexually acquired HPV infection or a reactivated latent infection acquired many years earlier. More than half of the women were HPV-negative at repeat testing after an average of five months, which is somewhat more than which has been found in other studies, with HPV negativity among 35–40% of women in repeat testing (159, 160). This could be an expression of latent infection, low viral load or test results below the cut-off level. In fact, the HPV infections detected at the 5-year follow-up examination may constitute a mixture of newly acquired and persistent infections.

The most frequent HPV type in both the first and second tests was HPV-51, a finding which is in line with the results of other studies. Nielsen and coworkers showed that HPV-51 was common in both LSIL and HSIL lesions (18.0 and 13.3%, respectively) in a population of 11,617 women with one sample each and a mean age of 36.4 years. However, it is unclear whether a woman's age influences her risk of HSIL or cervical cancer after the detection of HPV. The meaning of persistent HPV with or without LSIL has not been studied in older age groups. It is not certain that data on young women are representative of postmenopausal women, especially elderly women. Also, the contribution of changes in local immune defense, blood perfusion, tissue atrophy, and vaginal microbiota to persistent HPV infection has not been evaluated in elderly women. In this study the histological results were based on biopsy samples and in some cases on cervix abrasions, which is a limitation, since the transformation zone was not completely visible on the cervix. In these cases, it might have been advisable to perform diagnostic conization.
Conclusions

In the current four studies it has been shown that:

HPV prevalence was relatively low in older women, but if they had a second positive HPV test result, the risk of cervical dysplasia was high. An HPV prevalence rate of 4.4% after self-sampling of vaginal fluid is similar to the rate of 4.1% when the sample was collected from the cervix by a gynecologist. The strategy of repeating the HPV test leads to substantially fewer women requiring investigation by means of colposcopy and biopsy, and greater specificity as regards the identification of CIN 2+.

Since dysplasia was not detected by cytology in the vast majority of cases, cytology does not seem to be an appropriate method for screening women older than 60. Colposcopy is also of limited use in this group, since no one had a fully visible TZ. In this group histology is mandatory, but in many cases not enough. Better biomarkers for diagnosis and triage are necessary.

Self-sampling at home for HPV testing is fully feasible for elderly women, with a similar degree of dysplasia detection as with sampling by a clinician. Elderly women generally had a positive attitude to self-sampling. They identified advantages such as it being easy, relatively comfortable, private, and time-saving. In this group no one reported feelings of shame or anxiety about being diagnosed with HPV, and no one reported that this information hurt their relationship. These facts are important and contribute to the acceptability of self-sampling in this group.

The incidence of HPV positivity in previously HPV-negative elderly women was low, but low-grade dysplasia was common in those with repeat positive tests results. Additional research is needed to determine whether elderly women with one negative HPV test result are sufficiently protected against cervical cancer.
Future perspectives

Cervical cancer prevention in older women can be especially challenging, since there are no specific guidelines, and both cytology and colposcopy are of limited accuracy in this group. Elderly women have an incidence of cervical dysplasia that only is slightly lower than that in women of screening age, and about one third of CC cases and two thirds of deaths from CC strike women older than 65 years. Therefore, further efforts need to be established to reduce the number of CC cases in the growing group of elderly women.

The age-specific risks of cervical pre-cancer and cancer among older women with HPV infection are not known. A clearer understanding of HPV persistence versus latency is essential. Cohort studies with long-term follow-up involving more sensitive measures of type-specific viral load, will determine the incidence of a truly persistent infection, the characteristics of this infection and its contribution to carcinogenesis. Age-related changes in the patterns of viral genome methylation are also of interest owing to the possibility that HPV can persist in an epigenetically regulated latent state (161). In older women the most prevalent HPV genotype is not the same as in young women and therefore triage with HPV-16/18 is not sufficient for adequate diagnosis and treatment in this group. Furthermore, type-specific differences in the natural history of HPV infection that affect the exposure-disease relationship are not well studied. More accurate biomarkers are also necessary to identify HPV-positive women with major risks of dysplasia and invasive disease.

The Netherlands was, in 2016, the first country in Europe to switch from cytology-based to HPV-based cervical-cancer screening, with cytology triage for those with positive HPV test results. The program includes sending self-sampling devices to non-attenders (162). With the growing evidence that screening for oncogenic HPV infection is more effective than cytology in reducing the incidence of cervical cancer, other countries have followed this trend. Self-sampling for HPV testing has been shown to be a convenient and cost-effective method to increase screening participation among hard-to-reach women (95, 156).

During the coronavirus disease 2019 (COVID-19) pandemic, prevention, screening and diagnosis of cancer have all been affected by the disruption of health services in many countries (163-166). With the knowledge that the accuracy of HPV testing based on self-sampling is excellent when combined with a clinically validated PCR-based assay, The Swedish Board of Health and Welfare incorporated (although temporarily) self-sampling as a primary
screening option to continue the screening program (167). This represents an opportunity to study the feasibility and outcome of self-sampling as the primary screening method in Sweden. In the future, self-sampling for HPV testing might become the primary method of screening for cervical cancer.

Today, discontinuation of screening is based merely on age, despite the heterogeneity of the older female population. It would be more appropriate if it were to be based on contextual analysis of various risk factors of future CC development. These could include screening history, previous dysplasia treatment, immunosuppressive diseases or treatment, and other comorbidities. In other words, a more individualized analysis is needed before making the decision as to who can leave the screening program and who needs to continue.

Self-sampling combined with repeated HPV testing should be an adequate and cost-effective strategy for screening elderly women.

Persisterande infektion med cancerassocierade (onkogena) typer av humant papillomavirus (HPV) är orsak till nästan all livmoderhalscancer. HPV-testning har högre känslighet för upptäckt av cellförändringar än vanlig cellprov, vilket resulterar i effektivare skydd mot livmoderhalscancer. Fördelen med HPV-test jämfört med cellprov ökar med ålder. Detta är grunden för de nuvarande europeiska riktlinjerna, som rekommenderar HPV-testning för primärscreening för kvinnor äldre än 30 år med ett provintervall på 5 år. Denna rekommendation sträcker sig till 65 års ålder eftersom uppgifter om screening saknas till stor del för kvinnor äldre än 65 år. Hos postmenopausala kvinnor, (efter den fertila åldersperioden), gör hormonella förändringar med sänkt östrogennivå, att det område där förstadier till cancer utvecklas flyttas från ett synligt område på livmodertappens yta upp i livmoderhalskanalen. Detta innebär att vanligt cellprov och även mikroskopisk undersökning för biopsitagnin har mycket begränsat värde jämfört med hos kvinnor i fertil ålder. Vaginal självprovtagnin för HPV-testning har i ett flertal studier visat resultat som är jämförbara medprov tagna av sjukvårdspersonal (barnmorska eller läkare) när en validerad PCR-metod används för analys av HPV. Det saknas kunskap om hur vanligt det är med HPV-infektion hos äldre, betydelsen av persisteraande HPV-infektion, samt om hur vanligt det är med latent infektion hos äldre kvinnor.
Syftet med denna avhandling var att öka kunskapen om HPV-infektion och cellförändringar hos äldre kvinnor, samt att utvärdera acceptansen av upprepad självprovtagning i hemmet för HPV-testning.

I delstudie I som pågick under perioden september 2013 till oktober 2015 samlades prover från 1051 kvinnor i åldern 60 till 89 år för HPV-test. Proverna togs i samband med besök på en gynekologisk öppenvårdsmottagning i region Dalarna. En gynekolog tog prov från livmoderhalsen för HPV-testning. Kvinnor med ett positivt resultat i första testet provtogs en andra gång efter i genomsnitt 3,5 månader för HPV-test och vätskebaserat cellprov. De med ett positivt andra HPV-test undersöktes med kolposkopi och vävnadsprov. Förekomsten av HPV var 4,1%, vid det första testet och vid det andra testet förblev 2,6% positiva. Majoriteten av kvinnor som var positiva i båda HPV-testerna hade cellförändringar, 81,5% (22/27). HPV-relaterad cellförändring hittades hos 2,1% av de 1051 kvinnorna.

I delstudie II gjorde 1500 kvinnor i region Dalarna upprepad självprovtagning i hemmet för HPV-test. Totalt 375 kvinnor i var och en av de fyra åldersgrupperna 60, 65, 70 och 75 år erbjöds deltagande. Kvinnor med två på varandra följande positiva HPV-tester remitterades till två av regionens sjukhus och undersöktes av gynekolog med provtagnings av både cellprov och vävnadsprov. Ett självtaget prov skickades till laboratoriet av 59,5% (893/1500) av de inbjudna kvinnorna. Den totala prevalensen av HPV var 4,4% i det första testet och 2,5% i det andra testet som togs i genomsnitt 5,5 månader senare. Cellförändringar hittades hos 1,8% (16/893) och allvarligare cellförändringar hittades hos 1,0% (9/893) av alla kvinnor. Av de 16 kvinnorna med cellförändringar i vävnadsprovet hade endast 3 (18,8%) ett avvikande cellprov.

Syftet med delstudie III var att beskriva äldre kvinnors (60 till 75 år) erfarenheter av självprovtagning. Kvantitativa och kvalitativa metoder användes. Enkätdata samlades in via en enkät som skickades till de kvinnor som deltog i självprovtagningsstudien (Studie II). Individuella intervjuer gjordes med kvinnor som testade positivt i det första självprovtagning och var antingen negativa i sitt andra HPV-test eller var positiva i sitt andra HPV-test, men utan cellförändringar eller cancer. Av 893 tillfrågade kvinnor svarade 868 (97,2%) på enkäten. Bland de tillfrågade kvinnorna rapporterade 49,2% att det var mycket lätt att utföra självprovtagning, 46,8% svarade att det var lätt och endast 2,0% svarade att det inte var lätt. En majoritet, 58,9% svarade att de föredrog självprovtagning, 16,5% att de föredrog provtagning av en vårdgivare medan 23,7% hade ingen preferens. I intervjuerna deltog 13 av 16 inbjudna kvinnor. De flesta rapporterade att de föredrog självprovtagning eftersom det var lätt att utföra, mindre pinsamt och mindre tidskrävande än ett besök på en klinik. Majoriteten av kvinnorna rapporterade att de inte blev oroliga när de informerats om att HPV-testet var positivt.

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I studie IV var målet att studera förekomsten av HPV hos kvinnor som var HPV-negativa fem år tidigare. Studien genomfördes i Region Dalarna. Studiepopulationen bestod av 804 kvinnor (65 till 80 år) som fem år tidigare deltog i delstudie II (självprovtagning för HPV-testning) och vid den tiden var HPV-negativa. Kvinnorna blev nu igen erbjudna att utföra självprovtagning för HPV-testning. Kvinnor med ett positivt resultat i prov ett utförde ett upprepat HPV-test efter genomsnitt 5 månader. De med ett positivt andra HPV-test undersöktes av gynekolog med både cellprov och vävnadsprov. Av de 804 inbjudna kvinnorna, skickade 632 ett prov till HPV-laboratoriet. Alla 632 proverna analyserades. Totalt var 2,8 % av kvinnorna HPV-positiva i det första provet och 1,3 % i det andra provet som samlades i genomsnitt 5,4 månader efter det första. Lätta cellförändringar hittades hos hälften av de som vara HPV positiva i både prov ett och två.

Summary in Spanish.

Automuestreo en adultas mayores para la detección de VPH y displasia cervical

El cáncer de cuello uterino es la cuarta forma más común de cáncer en mujeres en el mundo. En Suecia, esta forma de cáncer ha aumentado durante los últimos 10 años después de una fuerte disminución desde la década de 1960 cuando se inició el cribado. Cada año, unas 550 mujeres suecas enferman de cáncer de cuello uterino, de las cuales 150 mueren anualmente a causa de la enfermedad. Aproximadamente el 30% de las mujeres que desarrollan cáncer de cuello uterino tienen más de 60 años y la tasa de mortalidad es de aproximadamente el 70% en este grupo. El cáncer de cuello uterino en mujeres mayores de 65 años se diagnostica con mayor frecuencia en etapas avanzadas y, por lo tanto, tienen un peor pronóstico. Durante el último siglo, la esperanza de vida media de las mujeres en Suecia ha aumentado de 55 a 84 años y muchas mujeres mayores de 65 años gozan de buena salud, trabajan y tienen una vida sexual activa. El programa sueco actual de cribado cubre a las mujeres hasta los 64 años. En la mayoría de países las mujeres mayores de 65 años no suelen estar incluidas en los programas de detección del cáncer de cuello uterino.

La infección persistente con tipos oncogenicos de virus del papiloma humano (VPH) es la causa de casi todos los cánceres de cuello uterino. En las mujeres posmenopáusicas (después del período de edad fértil), los cambios hormonales con niveles reducidos de estrógeno hacen que el área donde se desarrollan los precursores del cáncer se mueva de un área visible en la superficie del cuello uterino hacia un área más profunda en el canal cervical. Esto significa que las muestras de citología convencionales y también el examen microscópico (colposcopia) para la toma de biopsia tienen un valor muy limitado en comparación con las mujeres en edad fértil.

La prueba del VPH tiene una alta sensibilidad en todas las edades y, si se aplica como prueba repetida, también tiene una alta especificidad. La ventaja de la prueba del VPH sobre la citología aumenta con la edad. Ésta es la base de las directrices europeas actuales, que recomiendan la prueba del VPH para la detección primaria en mujeres mayores de 30 años con un intervalo de muestreo de 5 años. Esta recomendación se extiende hasta los 65 años, ya que en las mujeres mayores de 65 años se carece en gran medida de datos sobre tamizaje. El auto muestreo vaginal para la prueba del VPH ha demostrado ser
completamente comparable con las muestras tomadas por los profesionales de la salud (parteras o médicos) cuando se utiliza un método de PCR validado.

El nivel de conocimiento sobre la infección por VPH, la persistencia y la prevalencia de la infección latente en mujeres mayores es escaso.

El propósito de esta disertación fue aumentar el conocimiento sobre la infección por VPH y los cambios dysplásicos cervicales en mujeres mayores. Así como evaluar la aceptación al automuestreo repetido en casa para la prueba del VPH.

En el subestudio I, que duró desde septiembre de 2013 hasta octubre de 2015, se realizó la prueba del VPH en 1051 mujeres de entre 60 y 89 años cuando visitaron una clínica ginecológica ambulatoria en la región de Dalarna. Una ginecóloga tomó las muestras del cuello uterino. Las mujeres con un resultado positivo se sometieron a una segunda prueba del VPH y una muestra de citología después de 3,5 meses en promedio. Aquellas con una segunda prueba de VPH positiva fueron examinadas con colposcopia y muestras de tejido. La incidencia de VPH fue del 4,1%, en la primera prueba y en la segunda prueba el 2,6% se mantuvo positivo. La mayoría de las mujeres que dieron positivo en ambas pruebas de VPH presentaron displasia cervical, 81,5% (22/27). Displasia cervical se encontró en el 2,1% de las 1051 mujeres.

En el subestudio II, se ofreció a 1500 mujeres de la región de Dalarna un auto muestreo repetido en el hogar para la prueba del VPH. Cuatro grupos de edad (60, 65, 70 y 75 años) fueron identificados con 375 participantes en cada grupo. Las mujeres con dos pruebas positivas consecutivas fueron derivadas a dos de los hospitales de la región y fueron examinadas por ginecologo, con citología, colposcopia y biopsias. El 59,5% (893/1500) de las mujeres invitadas proporcionó una muestra de recolección propia. La prevalencia general del VPH fue del 4,4% en la primera prueba y el 2,5% fue persistentemente positiva en la segunda prueba, que se realizó en promedio 5,5 meses después de la primera. Se encontraron cambios displásticos en el 1,8% (16/893) y se encontraron cambios displásticos severos en el 1,0% (9/893) de todas las mujeres. De las 16 mujeres con cambios celulares en la muestra de tejido, 13 (81,2%) tenían una muestra de citología normal.

El propósito del subestudio III fue describir las experiencias relacionadas al auto-muestreo en las mujeres mayores (60 a 75 años). Se utilizaron métodos cuantitativos y cualitativos para recopilar datos de una encuesta a las mujeres que participaron en el auto muestreo para la prueba del VPH. Se realizaron entrevistas individuales con mujeres que fueron encontradas positivas en la primera autopregunta y luego negativas en su segunda prueba de VPH o fueron positivas en su segunda prueba de VPH, pero sin displasia cervical ni cáncer. De 893 mujeres invitadas a participar, 868 (97,2%) respondieron la encuesta. Entre las mujeres encuestadas, el 49,2% refirió que fue muy fácil realizar el auto muestreo, el 46,8% respondió que fue fácil y solo el 2,0% respondió que no fue fácil. La mayoría, el 58,9% respondió que prefería el auto muestreo, el 16,5% que prefería el muestreo por un proveedor de salud, el 23,7% no tenía
preferencia y el 0,9% no respondió la pregunta. Trece de las 16 mujeres invi-
tadas participaron en las entrevistas. La mayoría informó que prefería el auto
muestreo porque era fácil de realizar, menos vergonzoso y consumía menos
tiempo que una visita a una clínica. La mayoría de las mujeres informaron que
no se preocuparon cuando se les informó sobre una prueba de VPH positiva.
Y ninguna de las participantes reportó que la información de una prueba po-
sitiva afectó negativamente la relación con su pareja.

En el subestudio IV, el objetivo fue estudiar la incidencia del VPH en mu-
jeres que eran VPH negativas cinco años antes. El estudio se realizó en la
región de Dalarna, Suecia. La población de estudio consistió en 804 mujeres
(65 a 80 años) que cinco años antes participaron en el subestudio II (auto-
muestreo para la prueba del VPH) y en ese momento eran VPH-negativas.
Ahora se invitó a las mujeres a realizar un nuevo auto muestreo para la prueba
del VPH. Las mujeres con un resultado positivo realizaron una segunda prueba
después de un promedio de 5 meses. Aquellas con una prueba repetida positiva
fueron examinadas por un ginecólogo con muestras para citología, colposco-
pia y muestras de tejido. De las 804 mujeres invitadas, 632 enviaron una mues-
tra al laboratorio. Se analizaron las 632 muestras. En total, el 2,8% de las mu-
jeres resultaron VPH positivas en la primera prueba y el 1,3% en la segunda
prueba. Se encontraron cambios displásticos leves en el 50% de las mujeres
con infección persistente por VPH.

Con estos cuatro estudios, hemos demostrado que una proporción signifi-
cativa de mujeres mayores tiene una infección persistente por VPH. Entre el-
las, se encontró una alta incidencia de cambios displásticos cervicales que se
diagnosticaron principalmente con muestras de tejido. Las muestras de citolo-
gía mostraron una sensibilidad extremadamente baja. Además, el auto mues-
treo en el hogar en combinación con pruebas repetidas del VPH fue bien acep-
tado entre las mujeres mayores. Por último, la prevalencia del VPH en mujeres
mayores previamente negativas al VPH fue baja, pero entre las que dieron
positivo en una prueba repetida, los cambios celulares fueron comunes.

Investigación adicional es necesaria para determinar si las mujeres mayores
con una sola prueba de VPH negativa están suficientemente protegidas contra
el cáncer de cuello uterino.
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)