Significance of Human Papillomavirus (HPV) Analysis for the Detection of Precancerous Cervical Lesions

Impact of Self Sampling

KARIN SANNER
Abstract


Cervical cancer is the second most common cancer, with about 500 000 new cases per year among women worldwide. With a well-organized screening programme the number of cases can be reduced by more than 50%. In spite of having such a screening programme there are still around 450 new cases yearly in Sweden. The majority of these cases occur in non-attendees. There is thus a need to improve the Swedish cervical cancer screening programme in order to further reduce the number of cases of cervical cancer.

Cervical cancer and high-grade cervical dysplasia are caused by sexually transferred high-risk human papillomaviruses (HR-HPVs). In cases of persistent HR-HPV infection there is a risk of development of dysplasia and in some cases subsequent progress to cervical cancer. HR-HPV testing shows high sensitivity as regards the detection of cervical dysplasia. Self-sampling of vaginal fluid for the analysis of HR-HPV has many advantages, since a woman can perform the sampling herself in a private setting, whenever suitable, without the need to travel to a clinic.

Our studies have shown that sensitivity in the detection of precancerous lesions is about twice as great with the HR-HPV self-test compared with cytology-based tests. If a woman was HR-HPV-positive in two consecutive tests, the specificity of the HR-HPV test increased to about 98%. Among women with short-term persistent HR-HPV infection, the prevalence of CIN 2+ was over 40%. There was good concordance in sensitivity as regards the detection of CIN 2+ between self-obtained and physician-obtained samples, although self-sampling was associated with slightly lower specificity.

The prevalence of HR-HPV from day to day in premenopausal women was not influenced by hormonal changes during the menstrual cycle. Neither were there significant day-to-day changes in postmenopausal women. A single self-test thus provides reliable information on whether or not a woman has an HR-HPV infection.

In conclusion, self-sampling combined with the analysis of HR-HPV appears to be a powerful alternative as a primary screening method for the prevention of cervical cancer. Self-sampling for HR-HPV testing is a suitable, safe and accepted strategy for cervical cancer prevention among women.

Keywords: human papilloma virus, HPV, self-sampling, organized screening, cervical cancer screening

Karin Sanner, Uppsala University, Department of Women's and Children's Health, Akademiska sjukhuset, SE-751 85 Uppsala, Sweden.

© Karin Sanner 2013

ISSN 1651-6206
ISBN 978-91-554-8621-1
urn:nbn:se:uu:diva-196873 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-196873)
To Rakel, Gabriel and Thomas
List of Papers

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


Reprints were made with permission from the respective publishers.
Discussion ..................................................................................................... 40
Paper I ........................................................................................................ 40
Paper II ...................................................................................................... 41
Paper III ..................................................................................................... 43
Paper IV .................................................................................................... 44
Methodological considerations................................................................. 46
Conclusions .............................................................................................. 47

Future perspective ......................................................................................... 48

Sammanfattning på svenska .......................................................................... 50

Acknowledgements ....................................................................................... 53

References ..................................................................................................... 55
Abbreviations

ADC  Adenocarcinoma
AGC  Atypical glandular cells
AIS  Endocervical adenocarcinoma *in situ*
APC  Antigen-presenting cells
ASC  Atypical squamous cell
ASCUS Atypical squamous cells of unknown significance
ASC-H Aypical squamous cell – cannot exclude high-grade lesion
CC  Conventional cytology
CIN  Cervical Intraepithelial Neoplasia
DNA  Deoxyribonucleic acid
FDA  Food and Drug Administration (USA)
FTA  Flinders Technology Associates, Elute micro card
hc2  Hybrid capture 2
HCP  Healthcare provider
HIV  Human immunodeficiency virus
HMBS  Homo sapiens hydroxymethylbilane
HPV  Human Papillomavirus
HR-HPV High-risk human papillomavirus
HRT  Hormone replacement therapy
HSIL  High-grade squamous intraepithelial lesion
LBC  Liquid-based cytology
LEEP  Loop electrical excision procedure
LNG-IUD Levonorgestrel-releasing intrauterine device
LR-HPV Low-risk human papillomavirus
LSIL  Low-grade squamous intraepithelial lesion
NA  Non-attendees
Pap  Papanicolau
PCR  Poly chain reaction
SCC  Squamos cell carcinoma
SCJ  Squamocolumnar junction
SEK  Swedish krona
STI  Sexually transmitted infection
<table>
<thead>
<tr>
<th>TZ</th>
<th>Transformation zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIA</td>
<td>Visual inspection with acetic acid</td>
</tr>
<tr>
<td>VILI</td>
<td>Visual inspection with Lugols iodine</td>
</tr>
<tr>
<td>VLP</td>
<td>Virus-like particles</td>
</tr>
</tbody>
</table>
Introduction

Cervical cancer

Epidemiology

Cervical cancer is the second most common cancer among women worldwide. It affects about 15 per 100,000 women per year and kills about 8 per 100,000 per year. In 2008, cervical cancer was responsible for 275,000 deaths. Currently, almost 85% of cases occur in developing countries [1]. The explanation for this is probably lower availability of preventive programmes such as lack of, or inadequately organized gynaecological screening and education about sexually transmitted diseases and the social position of the women. Only 50 years ago the burden of cervical cancer was also high in developed countries, but here organized cervical screening has decreased the incidence of the disease and subsequent mortality [2]. There were 421 new cases of cervical cancer diagnosed in Sweden in 2011, which constituted 1.5% of all cancers among women [3]. Cancer in general is common in older people but cancer of the uterine cervix primarily affects younger women, with 50% of cases appearing between the ages of 30 and 55, when many women are actively involved in their careers or caring for their families [3]. Every year in the last few decades, around 150 women die of cervical cancer in Sweden, 60% of them being older than 65 years [4].

Clinical aspects of cervical cancer

Early manifestations and symptoms

Cervical cancer development is generally a process that takes several years and goes step by step. It is almost always induced by way of a persistent high-risk HPV (HR-HPV) infection that in some cases subsequently leads to asymptomatic precancerous lesions. Such lesions may then progress to invasive cancer [5]. These precancerous lesions are almost always asymptomatic. Early symptoms in some rare cases of precancerous lesions and micro-invasive disease include vaginal discharge, contact bleeding, and irregular or postmenopausal bleeding. These symptoms are not specific for cervical lesions and are therefore sometimes difficult to interpret for both patients and healthcare personnel. Both the costs and outcome of cervical cancer are strongly dependent on the stage at diagnosis. Cervical cancer at younger
ages has a much better prognosis and fewer societal costs than cervical cancer at older ages [6].

**Diagnosing cervical cancer**

The most effective way to minimize suffering and death due to cervical cancer is to diagnose and treat precancerous lesions, or cancer at an early (micro-invasive) stage. Organised screening is one way and an investigation in relation to early symptoms is another. It is impossible to diagnose precancerous lesions or micro-invasive cancer solely by colposcopy. Clinical suspicion of precancerous lesions in the cervix, or cervical cancer itself, requires a definitive histological diagnosis.

**Transformation zone and Histopathology**

The cervix is covered by both columnar and stratified non-keratinising squamous epithelia. Columnar epithelium covers the upper part of the cervix, the endocervix, and squamous epithelium covers the ectocervix and vagina. The junction between the two types of epithelia defines the squamo-columnar junction (SCJ). Through the influence of oestrogen during puberty, a process of metaplasia replaces the more fragile columnar epithelium with the more sturdy squamous epithelium, creating a "new" SCJ. The transformation zone (TZ) lies between the original SCJ and the new SCJ and almost every case of cervical neoplasia arises here [7-10] (Figure 1).

![Figure 1. Cervical SCJ and TZ in mid and later reproductive stage (30s age-range). Courtesy of Merck & Co., Inc.](image)

Cervical carcinogenesis develops in a stepwise process that is almost always induced by persistent infection with HR-HPV. Oncogenic HPV types have a molecular advantage in establishing a persistent infection that disrupts the apoptotic machinery of the cervical epithelial cell. This leads to disorganized, uncontrolled proliferation of cells and loss of normal maturation as they progress upwards through the epithelial cell layers. The lesions are termed carcinoma *in situ* or dysplasia and histologically sub-classified as cervical intraepithelial neoplasia (CIN) 1–3 (Figure 2).
Figure 2. Progress from normal epithelium to invasive cancer. From Comprehensive Cervical Cancer Control: a guide to essential practice. Integrating Health Care for Sexual and Reproductive Health and Chronic Diseases, World Health Organization, Figure 2.8, page 38 (2006). Reproduced with permission from World Health Organization.

CIN nomenclature divides cervical cancer precursors into CIN 1, CIN 2 and CIN 3, corresponding to mild, moderate and severe dysplasia. When cells of a lesion break through the epithelial basement membrane it is by definition invasive. The main forms of cervical cancer are squamous cell carcinoma (SCC) (80% of all cases of cervical cancer), adenocarcinoma (ADC) (15% of all cases) and a mixed form called adenosquamous carcinoma.

Causes and risk factors in the development of cervical cancer

Persistent HR-HPV infection is a prerequisite for the development of cervical neoplasia (described in the chapter about HVP). Not every HR-HPV infection develops into cancer. Most HR-HPV infection are cleared by the immune system without causing any lesions of the cervix. It is not fully understood what the conditions or cofactors are that lead to persistent HR-HPV infection and progress to cancer. The following factors have been discussed as risk factors of cervical cancer [11]:

- Viral type, more than one HR-HPV type and a high load of virus probably play a role in the risk of progression to cancer.
- People with immunodeficiency are more prone to develop persistent HPV infections and are at risk of a more rapid progression to a precancerous lesion and cancer.
- The increased risk of developing cervical neoplasia conferred by low age at first sexual intercourse, and high parity, is mainly related to the risk of acquiring HPV infection because of the vulnerable TZ during adolescence and pregnancy. A high lifetime number of
sexual partners of the woman or her partner is related to a higher risk of acquiring HPV infections [12].

- Other genital infections such as Herpes simplex type 2, Chlamydia trachomatis and HIV.
- Hormone treatment (e.g. oral contraceptives)
- Tobacco smoking

Human Papillomavirus

The human papillomavirus (HPV) represents a genus of the family Papillomaviridae and is a common cause of infection among humans. HPV infects the epithelium of differentiating keratinocytes of the skin and the mucosa of the anogenital and oropharyngeal regions. About 200 types of papillomavirus have been identified and 120 of them isolated in humans. Approximately 35 HPV types are known to infect the human anogenital region.

HPV types can be subdivided into two categories: ‘low risk’ and ‘high risk’. Individuals infected with low-risk viruses, or non-oncogenic viruses, have a low risk of developing neoplasia. High-risk types can cause dysplastic lesions and invasive cancer at a high rate because these viruses have oncogenic properties. Thirteen anogenital HPV types are classified as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), some types as probable high-risk types (26, 53, 66, 73 and 82), and some as low-risk types (for example, in the genital tract, 6, 11, 42 and 44) [13]. Type 16 is the most common type in cervical cancer, and together with type 18 is generally acknowledged to cause about 70% of cases of cervical cancer [14]. HPV is identified in at least 99.7% of all cases of cervical cancer [15]. The incidence of HPV in other HPV-related cancers worldwide is 32% in the vulva and vagina, 25% in the penis and 83% in cancer of the anus [16].

HPV infection is the most common sexually transmitted infection. About 50–80% of men and women at sexually active ages are estimated to become infected with HPV at sometime in their lives [17]. Among women at sexually active ages the prevalence of HPV is about 10% worldwide, with variations between geographic areas. The prevalence of HR-HPV is higher in developing than in developed countries. In northern Europe a prevalence of 7.9% has been reported [18]. The incubation time is between 3–4 weeks to several years and most infections do not cause any symptoms [17]. Several studies have shown a mean duration of an HPV infection of between 4 to 10 months [19]. A minority of HPV infections persist, and individuals with persistent HR-HPV infections are at a substantial risk of developing precancerous cervical lesions (CIN 3). CIN 3 lesions are the targets of screening, because more than one-third of cases of persistent CIN 3 will progress to invasive cervical cancer within 10–20 years [20].
The HR-HPV can also cause cytological abnormalities and invasive cancer of the vulva, vagina, cervix, penis, anus and larynx [5, 21, 22]. LR-HPV can give rise to genital warts (condyloma acuminata) [23] and oropharyngeal papilloma [24, 25].

HPV causes cervical cancer
In the mid-nineteenth century Rigoni-Stern, an Italian physician, drew the conclusion that women affected by cervical cancer were married women and prostitutes, while nuns and virgins were not affected by the disease. A correlation was found between cervical cancer and sexual activity [26].

In the 1970s Zur Hausen associated HPV with cervical cancer [27] and in 1982 Green et al. linked DNA from HPV to cervical cancer [28]. In 1983 in Germany they were able to isolate a new type of HPV, called HPV 16, which was found in the majority of cervical cancer biopsy samples [29]. In 1984 it was discovered that HPV 16 was detected largely among cases of cervical intraepithelial neoplasia and also in a few cases of genital warts [30]. Furthermore, the carcinogenic properties of specific HPV types were discovered and these virus types were named high-risk, in contrast to low-risk viruses that were more common in benign lesions, like warts. With modern techniques it has been demonstrated that HPV is detected in virtually all cervical cancer cases [15].

HPV transmission and carcinogenesis
HPV is a small virus consisting of an 8000 base-pair-long circular double-stranded DNA structure wrapped in a protein shell that is composed of two capsid proteins, L1 and L2 (L = late.) Six early proteins known as E1, E2 and E4–7 are essential for virus replication, genome amplification, proliferation and oncogenesis [31]. Transmission requires intimate contact between skin and skin and/or mucosa, and the virus is primarily transmitted through sexual intercourse.

Microscopic damage to the skin takes the virus into the epithelium where it binds to the basement membrane by means of the capsid protein L1. By binding to the surface of keratinocytes, the virus-capsid is modified and L2 splits and binds to a co-protein which facilitates internalization into the host cell. After the introduction of HPV into stem cells in the basal layer of the epithelium, expression of viral non-structural proteins occurs. Under the regulation of these proteins, the dividing cell population expands vertically and epithelial cell differentiation is delayed and less complete. Viral proteins are expressed sequentially with differentiation and mature virions are produced only in the most superficial layers of the epithelium [31] (Figure 3). A persistent infection can, over time, cause genetic damage that leads to loss of control of the cell cycle and to mutations that cause malignant development.
Cervical cancer prevention

To prevent cervical cancer, there are three basic components:

**Primary prevention** means prevention of HPV infection and cofactors known to increase the risk of cervical cancer, and includes:

- education and awareness-raising of women and their partners, to reduce high-risk forms of sexual behaviour (several sex-partners lifetime, sexual debut at young age, getting other STIs)
- encouraging use of barrier methods (condoms and femidoms)
- HPV vaccine, currently against the oncogenic forms HPV 16 and 18
- efforts to discourage tobacco use (smoking is a known risk factor of cervical and other cancers).

**Early detection** of women at high risk of developing cervical cancer:

- organized screening programmes
- opportunistic screening

**Diagnosis and treatment** of precancerous lesions:

- histopathology for diagnosis
- conization with the intent to diagnose or treat [11]
Primary prevention

Using condoms
Condoms only offer partial protection against HPV transmission, because the virus can exist on body surfaces not covered by the condom, such as the perianal area and anus in men and women, the vulva and perineum in women, and the scrotum in men. Despite this, consistent and correct condom use has been shown to provide important benefits, since it reduces the risk of cervical precancer and cancer [12]. It allows faster HPV clearance in both men and women and increases regression of cervical lesions. It protects against other STIs, which are possible cofactors of cervical cancer. It protects against HIV infection, a known facilitator of both HR-HPV infection and progression to high-grade lesions [11].

HPV vaccine
Vaccines against HPV are prophylactic non-live vaccines, and contain purified virus-like particles (VLPs) of the recombinant major (L1) capsid proteins of different HPV types. These vaccines consist of viral protein without genetic material and the infection of cells or viral replication is therefore not possible. At present, two different HPV vaccines have been developed; a bivalent vaccine containing VLPs of HPV types 16 and 18 (Cervarix®) and a tetravalent vaccine containing VLPs of HPV types 6, 11, 16 and 18 (Gardasil®). Both include adjuvant and are recommended for use according to a three-dose schedule. The indication is based on the demonstration of efficacy in women aged 15–40 years and on the immunogenicity of the vaccine in girls and women aged 10–25 years. Both vaccines are safe and well-tolerated [32] and may prevent at least 70% of all cases of invasive cervical carcinoma [33]. The tetravalent vaccine has been shown to be associated with a decrease in genital warts [34, 35].

Early detection – organized screening programmes
The objective of screening for cervical cancer is to reduce mortality and incidence of the invasive disease. Screening is a public health intervention used on a population at risk, or target population. Screening is not undertaken to diagnose a disease, but to identify individuals with a high probability of having or of developing a disease [11]. Among all malignant tumours, cervical cancer is the one which can be most effectively controlled by screening [36].

Because cervical cancer has a typically slow progression, from atypical cells to cervical intraepithelial neoplasia and to invasive carcinoma, precancerous lesions can be treated and invasive cervical cancer prevented. The screening methods currently available in a wide range of settings include cytological smears, visual inspection with acetic acid (VIA) or Lugols iodine
(VILI), and HPV testing. The diagnosis can be confirmed by histological interpretation of biopsy samples.

The most extensive and long-term experience in cervical cancer screening is with cytology, which has been used in numerous countries since the 1950s. Cytology-based screening and treatment programmes have reduced cervical cancer incidence and mortality by as much as 80% in Canada, the USA and some Nordic countries, and by 50–60% in other European countries [11].

Organized screening in Sweden
The Swedish cervical cancer screening programme has been working since the end of the 1960s. Hitherto, screening in Sweden has been based on cytological smears. During the last few years liquid-based cytology (LBC) has been introduced in several counties but for decades the conventional method has been Papanicolau (Pap) smears. The introduction of organized gynaecological screening has resulted in a decrease of the prevalence of cervical cancer by at least 50% [37].

Age limits and intervals for invitation to cervical cancer screening are almost uniform among all 21 autonomous counties. In principle, every third year among women aged 23–50 years and every fifth year in those aged 51–60, invitations are sent out to have a cytological smear. The only major exception is Uppsala County, where all women aged 50–60 are offered an HPV test at 5-year intervals instead of a smear. In some counties a specific time and place for the test is issued in the invitation, while in others the women need to make their own reservations to see a midwife. In Sweden the fees for a cytological smear vary between 0 and 200 SEK. [38]

Screening tests
A good screening test should be accurate, reproducible, inexpensive, easy to perform and to follow up, acceptable and safe. The following tests meet the above criteria to a greater or lesser extent [11]:

Cytology
A sample of cells is taken from the uterine cervix and examined under a microscope by a cytotechnologist. The conventional cytology (CC) method is called a Pap smear after Dr. Papanicolau, who developed the method and described it in 1941 [39]. LBC was developed as an alternative cytological method. One advantage of LBC is that after preparation for cytological examination, supplementary analysis can be carried out, such as so-called reflex testing for HR-HPV. Use of LBC reduces the rate of inadequate samples [40-43]. Factors adversely affecting the quality of a cell sample include vaginal bleeding, vaginal inflammation or infection, severe genital atrophy (menopause) and pregnancy [44].
The Swedish classification system used to diagnose abnormalities of the cervix (Normal-CIN 3) can be translated into the Bethesda classification system [45], which is an attempt to standardize cervical cytological terminology [46].

**Epithelial cell abnormalities (Bethesda system) [11]:**

**Squamous cells**
- Atypical squamous cell (ASC):
  - of undetermined significance (ASCUS);
  - cannot exclude high-grade lesion (ASC-H).
- Low-grade squamous intraepithelial lesion (LSIL), encompassing CIN 1.
- High-grade squamous intraepithelial lesion (HSIL), encompassing CIN 2 and CIN3.
- Squamous cell carcinoma (SCC).

**Glandular cells**
- Atypical glandular cells (AGC) (specify endocervical, endometrial, or not specified).
- Atypical glandular cells, favour neoplastic (specify endocervical or not specified).
- Endocervical adenocarcinoma *in situ* (AIS).
- Adenocarcinoma (ADC).

**HPV DNA-based screening method**
HPV DNA-testing is introduced at different levels of screening programmes starting with the triage of ASCUS and CIN 1 and follow-up after treatment of CIN 2-3 and AIS [47].

HPV testing as primary screening method was recently introduced in Uppsala County for women aged 50–60 years at 5-year intervals. Women in Uppsala County who had not attended the organized screening programme for 6 years or longer have been offered self-sampling for HR-HPV analysis since 2007 in a project funded by Uppsala County Council.

**Visual methods**
Abnormalities are identified by inspection of the cervix with or without magnification, after application of dilute acetic acid (vinegar) (VIA) or Lugol’s iodine (VILI). Because they do not rely on laboratory services, VIA and VILI are promising alternatives to cytology where resources are limited, for example in developing countries. The sensitivity of visual methods is low [11].
Diagnosis and treatment

All biopsy-confirmed CIN 2 and 3 lesions should be treated, because around one third of them persist and have the potential to progress to invasive cancer [20]. Treatment of CIN 2+ involves removal or destruction of the neoplastic cervical cells, in Sweden by cervical conization, mostly by way of the loop electrical excision procedure (LEEP) or laser cauterization. CIN 1 is more likely to resolve spontaneously and these patients can be followed up every 6 months until the lesion regresses to normal, or there is evidence of progression of the abnormality. If progression is noted, or in cases where women over 35 years old have a positive HR-HPV infection together with abnormal cytology, treatment should be considered.

Strengths and limitations of organized screening

Screening for cervical cancer by cytological examination (the Pap test) has reduced the incidence of invasive cervical cancer by 50% in Sweden [37]. In younger women, cases of invasive carcinoma are detected at an earlier invasive stage [6]. Nevertheless, there are around 450 new cases of cervical cancer in Sweden each year and around 150 of these women will die from their disease.

Pap smear screening and cervical cancer

A nationwide audit (2008) showed that around 65% of cervical cancer cases in Sweden occur in women not attending a screening programme. Another 25% are found in women participating in screening, but with a series of normal cytological specimens preceding tumour diagnosis [48]. In women aged 60 years and older, who have been invited to screening for the last time, most cancer cases are diagnosed as a result of clinical symptoms leading to admission to a gynaecological surgery for examination [48]. The proportion of women with abnormal smears before cancer development significantly decreases with age [49]. The benefit of Pap smear screening is limited in older women in spite of a high cancer incidence in that age group [50].

Screening coverage

Coverage in the cervical screening programme in Sweden was 79% in 2011, with great variation between counties. Uppsala County showed the lowest coverage in the screening programme (66%) and Dalarna County showed the highest (92%) [51]. In Uppsala County, coverage among women aged 23–50 was 62% and for women aged 51–60 it was 73%, which means that the younger women had lower coverage, which is the trend for most counties. Coverage has changed marginally during the last few years [51].
Non-attendees in Uppsala County

A population-based case-control study in 1998 with 430 non-attendees (NA) and 514 attendees at Pap smear screening in Uppsala County showed that non-attendance was more common among women who had not used oral contraceptives, who had not taken their own initiative to undergo a Pap smear, who had visited different gynaecologists, and who had visited a physician very often or not at all. Regular condom use, living in rural/semirural areas, and not knowing the recommended screening interval were all associated with non-attendance, while socioeconomic status was not. Multivariate analysis also showed that non-attendance was more likely among women who did not perceive cervical cancer to be as severe as other malignancies, who did not see the benefit of a Pap smear, who had time-consuming and economic barriers, and who did not feel anxious about the test result or cervical cancer. The results were strengthened with increasing time since the last smear or if self-reported attendance status was used instead of true attendance. NA also held harder to their preferences than attendees, stating that they would not participate if their preferences were not met and they were less likely to intend to participate in future screening. Among the NA, 57% underestimated the time lapse since the last smear [52, 53].

Cytology vs. HPV-test in primary screening

It is well known that Pap smear screening has a relatively low sensitivity [54]. This means that a proportion of women will develop cancer in spite of a number of previous normal Pap smears. In some women, progression to an invasive cancer stage has already occurred when an abnormal Pap smear is recognized [6]. HR-HPV testing has been shown to be twice as sensitive as regards detecting precancerous lesions as screening by way of a Pap smear [54] and the premalignant lesions are recognized earlier [55, 56]. In postmenopausal women, the difference in sensitivity between cytology and HPV typing may be even greater, with testing for HR-HPV being three times more sensitive than cytological screening as regards detecting women at risk of tumour development [57].

This high sensitivity translates into a very high negative predictive value of the HPV detection assay, allowing for extended screening intervals for test-negative women [56] of up to 6 years [58]. A single HR-HPV test has the disadvantage of lower specificity than a cytological test [54].

Self-sampling

During the last decade much attention has been paid to the possibility of using self-collected (cervico-) vaginal material as an alternative to cervical samples collected by a healthcare provider (HCP), as a part of cervical cancer screening programmes [59, 60]. Self-collected vaginal samples are not
suitable for cytological assessment as a result of the risk that there will be no or too few cells from the transformation zone. However, vaginal self-sampling is an appropriate option for HPV testing, since the results are fully comparable with those from samples collected by an HCP [61, 62].

**Self-sampling devices**

Samples that a woman collects herself consist of vaginal material instead of the cervical scrapes collected by an HCP. Brushes [60], swabs, tampons [63], or vaginal lavage devices [64] have been used for self-sampling. Urine sampling for HPV analyses has also been evaluated [65]. It is clear from these studies that women are able to collect appropriate samples themselves and then send them to a laboratory for analysis. Depending on the sampling device, the sample is either stabilized in a liquid medium [62] or dried on some type of dry medium [59, 66].

**Self-collected versus HCP-collected samples**

There is explicit concordance in HR-HPV detection rates in connection with self-collected and HCP-collected samples. Petignat *et al.* found that there was no difference in HPV prevalence between self- and physician-obtained samples, whatever the sampling device or method for HPV analysis [67]. HPV self-sampling is thus a suitable method for studies on HPV transmission and in vaccine trials.

There are conflicting data concerning sensitivity and specificity when comparing self- and HCP-collected samples. The reason for such an outcome might depend on the method used for analysis. The results of most previous studies indicate that sensitivity is lower with self-collected rather than HCP-collected samples [68]. In one study with 13 140 screened women in a low-resource setting in China it was found that self-sampling was associated with lower sensitivity as regards the detection of CIN 2 and CIN 3 compared with physician-obtained samples (86.2% and 97% respectively). Both methods showed greater sensitivity than liquid-based cytology (80.7%), and lower specificity (80.7%, and 82.7% versus 94.0% for cytology) [69]. Concerning sensitivity, the use of HR-HPV testing is in itself more important than the type of sampling device used or whether the sample is collected by the woman herself or by an HCP [70].

**Women’s attitudes to self-sampling**

There are many advantages of self-sampling, since a woman can perform it herself in a private setting [71] whenever suitable, without the need to travel to a clinic or other medical department [72, 73]. Other reasons for preferring self-sampling include the fact that women do not have to see an HCP and have a gynaecological examination [74]. Most women with experience of self-sampling have found the procedure easy, or very easy to follow [71, 75, 76], regardless of the self-sampling method. A small number of women have
reported concerns about whether or not they had performed the self-sampling properly [67, 74, 77]. It is known that the majority of cervical cancer cases are found among women who have not attended a cervical cancer screening programme [78]. NA therefore represent an important target population when attempting to reduce the incidence of cervical cancer. It is well known that self-sampling is well tolerated among women not attending an organized screening programme [68]. In several studies the response rate among NA was significantly increased by offering self-sampling for HR-HPV testing, compared with recall for regular Pap smears [64, 79, 80]. A majority of women normally attending a screening programme also prefer self-sampling [77]. Most likely, self-sampling for HPV testing would contribute to increased attendance in cervical screening programmes in low-, mid- and high-resource settings [64, 74, 79-82], and as a consequence a reduced incidence of cervical cancer.
Aims

The general aim of the present work was to evaluate if self-sampling for the analysis HR-HPV is an efficient, safe and suitable screening method for routine use in a cervical cancer prevention programme.

The specific aims of the individual studies were:

I. To evaluate whether the use of self-sampling in combination with testing for HR-HPV increases the participation rate in organized screening and if this strategy increases the detection rate of premalignant cell alterations (CIN 2–3) in comparison with a single cytological smear.

II. To see if repeat testing for HR-HPV using self-sampling of vaginal fluid can be used to increase specificity in screening for cervical cancer in women aged 30–65 years.

III. To evaluate if self-sampling of cervicovaginal fluid using a Vibabrush and an indicating FTA Elute cartridge can be used for reliable HPV typing, when compared with analyses of samples obtained by a physician using a cytobrush and an indicating FTA Elute Micro card, and with cervical biopsy sample analysis.

IV. To determine the prevalence of HR-HPV from day to day in pre- and postmenopausal women, and to see if the prevalence is influenced by hormonal changes during the menstrual cycle.
Material and Methods

Patients

During 2008–2010, women from Uppsala County, between the ages of 30–65 years old who had not attended organized cytological screening for 6 years or longer, according to the register of the screening programme, were offered self-sampling of vaginal fluid at home, for subsequent analysis concerning HR-HPV. Women that chose to participate sent a vaginal fluid sample for HR-HPV analysis.

Women positive for HR-HPV in the self-sampling test were invited for a follow-up visit at our gynaecological outpatient clinic. At the visit a physician collected a sample for a second HR-HPV test, took a smear and carried out cervical biopsies. This visit took place on average 3 months after the initial HPV test. The first two studies in this thesis are based on data from this group of patients. For Studies III and IV women in the same cohort were asked to participate.

Ethical considerations

The data used in the studies were from samples collected at home and at routine follow-up visits at the gynaecological outpatient clinic at the Akademiska University Hospital in Uppsala. The samples were collected as part of routine clinical management for follow-up of patients with HR-HPV. All data were de-identified prior to analyses. Projects including data presented in Papers I and II were evaluated by the Ethics Committee at Uppsala University (Dnr 2004:M-202 and 2009/001). Paper III was a quality study of the current HPV method in clinical practice in Uppsala County and was included in the ethical evaluation above. The study described in Paper IV was approved by the Ethics Committee at Uppsala University, Sweden (Dnr 2011/215). Informed consent was obtained from all women included in the study.
Analyses
Tests for the detection of HR-HPV

Qvintip device and Digene hybrid capture 2
After self-sampling, one method used for the detection of HR-HPV was Qvintip (Aprovix AB, Uppsala Sweden), which is based on a method involving hybrid capture 2 (hc2; Qiagen AB, Solna, Sweden). This method detects the presence of any of 13 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). The Digene hc2 technique can detect HPV DNA concentrations over 1 pg ml⁻¹, which is proportional to the light emission of the positive control and corresponds to 5000 HPV genomes per specimen in the well (Papers I and II).

HpVIR
Another HPV test used (in connection with follow-up visits (Studies I and II) and in Studies III and IV) was the hpVIR method, which detects 12 different 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)). The physician used a standard cytobrush to take a sample at the opening of the cervix. A Rovers® Viba-brush (Rovers Viba-brush; Rover Medical Devices B.V., Oss, the Netherlands) was used when the women collected the vaginal samples themselves. The cervico-vaginal fluid was applied to an indicating FTA® elute micro card (GE Healthcare, United Kingdom) (Figure 4) and investigated by using a real-time PCR assay. This assay detects and quantifies the housekeeping gene HMBS (Homo sapiens hydroxymethylbilane synthase; GenBank accession no. M95623.1). In order to determine if a sample contains a sufficient amount of material for an HPV test to be considered informative, a threshold of 10 copies of the HMBS housekeeping gene per PCR was used. For the HPV test itself, the sample had to contain a minimum of 10 HPV copies to be considered positive [83, 84].

Cytological investigation
Samples for cytological investigation (Pap staining) were collected from three standard sites, vaginal fornix, portio vaginalis cervicis, and endocervical cervix. The cells were placed on a glass slide (cytology smear), fixed in 95% ethanol and stained with Pap stain before examination. The smears were examined by a cytotechnologist and grouped into normal, ASCUS, CIN 1, CIN 2 and CIN 3 (Paper I).
Histological evaluation

Biopsy samples were collected at colposcopy from at least four sites where there were visual abnormalities on the portio, or from the transformation zone if the portio appeared normal. In addition there was histological evaluation of cone biopsy samples.

The biopsy specimens were fixed in 10% formalin, embedded in paraffin, and 4-µm sections were stained with haematoxylin-eosin. The sections were examined by specialists in surgical pathology, under a light-microscope and classified as normal, CIN 1, CIN 2 or CIN 3 (Papers I, II and III).

In some cases 10-µm sections of cervical biopsy samples were also collected for subsequent detection of HR-HPV (Paper III).

![Figure 4. Vaginal fluid self-sampled with Vibabrush and applied to an indicating FTA elute micro card. (Illustration from I Gustavsson)](image)

Methods

Paper I

During 2008 we contacted 2829 women in Uppsala County (30–58 years old) who had not attended the organized screening programme for 6 years or longer according to the cytological screening register. They received an informative letter and a form where they could order a test for self-sampling of vaginal fluid at home. The collected material was sent to the laboratory and the hc2 method for detection of HR-HPV was used.

The results of the HR-HPV analyses were communicated to the participating women within 2–3 weeks after the arrival of the vaginal samples at the laboratory. Women testing positive for HR-HPV were offered attendance at the gynaecological outpatient clinic at the Akademiska University Hospital in Uppsala for a follow-up examination within 1–3 months.
At the follow-up visit, the physician performed colposcopy and collected biopsy samples from the portio as well as material for cytology and a repeat HR-HPV test. The second HPV test was performed using the hpVIR method.

Paper II

During 2008–2010, 7331 women from Uppsala County, between the ages of 30–65 years who had not attended organized cytological screening for 6 years or longer according to the screening register, were offered self-sampling of vaginal fluid at home using the Qvintip device. Women who agreed to participate sent a vaginal fluid sample to the Aprovix laboratory in Uppsala for HR-HPV analysis. The samples were analysed using the hybrid capture 2 (hc2) method. Women testing positive for HR-HPV were offered attendance at the gynaecological outpatient clinic at the Akademiska University Hospital in Uppsala for a follow-up examination within 1–3 months.

At the follow-up visit, the physician performed colposcopy, collected biopsy samples from the portio and sampled cervical fluid for repeat HR-HPV analysis. The latter HPV test was performed using the hpVIR method.

Women with two consecutive positive HR-HPV test results, and either abnormal cytology (ASCUS–CIN 3) and/or CIN 1–3 in histopathological analysis, were recommended further follow-up, or in the case of CIN 2+ in the biopsy sample or Pap smear, were treated by way of surgical cone resection. The cervical biopsy samples were examined by specialists in surgical pathology. Diagnostic conization was performed among women older than 30 in cases of CIN 1 in histopathology when it not was possible to visualise the transformation zone. The end point of the study was identification of CIN 2+ in cervical biopsy tissue or cone resection. CIN 2+ corresponds to HSIL according to the Bethesda definition of premalignant cell alterations in the cervix.

Paper III

This study included 50 women who had not attended an organized screening programme for ≥ 6 years. The women had earlier been invited to perform a self-sampling test at home for detection of HR-HPV and were all found to be HR-HPV-positive at that time. At their follow-up visit to the gynaecological outpatient clinic at the Akademiska University Hospital in Uppsala they were invited to participate in this study. The women received verbal and written instructions on how to collect a vaginal sample and apply this to a cartridge. They carried this out at the visit. After self-sampling they were examined by colposcopy and samples for a cervical smear and a repeat HPV test were collected. In addition, a biopsy sample was collected for both histology and HPV analysis.
This study was a prospective cohort study where 25 HR-HPV-positive women performed repeated daily self-sampling. Both postmenopausal and premenopausal women were asked to participate in the study, and all of them had at least one positive HR-HPV test result before they were included. The postmenopausal women collected samples for 28 consecutive days while the premenopausal women collected samples each day from the first to the last bleeding-free day over one menstrual cycle. Most (20/25) of the participating women had not attended an organized Pap-smear programme for $\geq 6$ years. One of the premenopausal women had amenorrhea as a result of having a levonorgestrel-releasing intrauterine device (LNG-IUD) and a second woman used oral contraceptive pills (desogestrel continuously), while none of the others used any hormonal contraception or other hormonal treatment. One of the postmenopausal women used hormone replacement therapy (HRT). The majority of the women reported a stable relationship with a male partner. There was a trend towards a higher number of lifetime sexual contacts among the premenopausal women. The frequency of smokers (60%) was high compared with that in the general population, which is about 22% for women at this age (Table 1, paper IV) [85].

The sampling period for the premenopausal women was divided into oestrogen-dominated and progesterone-dominated periods in order to test whether the level of vaginal HR-HPV differed between the proliferative and secretory menstrual cycle phases. The woman using a LNG-IUD and the woman using oral contraceptive pills (desogestrel continuously) were not included in this part of the study.

The women were instructed to perform vaginal self-sampling using a Viba-brush and FTA card. All women received written instructions describing the sampling procedure, and those who had not performed self-sampling before also received verbal instructions. The FTA cards were numbered according to the day in the cycle and sent for analysis.

During the period when daily samples were collected, the women were asked to complete a diary with information on vaginal intercourse, barrier protection, genital or systemic infection, vaginal discharge or bleeding.

Cervical biopsy samples for histology were obtained from every woman in the study before daily sampling. Women with abnormalities in their histological results and positive for HR-HPV, were treated by means of cervical conization, performed after the sampling period (data not shown).

Statistical analysis
Wilcoxon’s matched pair signed rank test was used to determine if the menstrual phases were associated with different HR-HPV levels. Spearman’s rank correlation coefficients were used to estimate the associations between
the number of virus copies and the sampling day for each woman, and for the groups of postmenopausal and premenopausal women.
Results

Paper I

The self-sampling device was ordered by 1609 women (57% of 2829) and 1107 (39%) performed sampling of vaginal fluid, which was sent to the laboratory for testing for HR-HPV. An HPV-positive reaction was obtained in 6.7% (74 out of 1107) of the samples. There was no significant age difference with regard to participation rate. However, the prevalence of HPV decreased with age. It was 11.1% in women of 30–39 years old and 2.9% in women aged 50 years or more (Table 1).

The 74 women with HR-HPV were admitted to the gynaecological outpatient clinic at the Akademiska University Hospital in Uppsala for follow-up examination. However, at that time seven women had moved out of the county and four had chosen to visit midwives. A repeated test for HR-HPV and a cervical biopsy were carried out and a cytological smear was obtained in 60 of the 63 remaining women. Persistent HR-HPV infection was seen in 73% (44 out of 60) of the women, and among them 43% (19 out of 44) showed CIN 2–3 alterations in the cervical biopsy samples (Table 1).

Table 1. Acceptance to perform self-sampling and prevalence of HR-HPV infection and histological diagnose in biopsy samples in relation to age.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Total number of women</th>
<th>No of women performing self-sampling (%)</th>
<th>No of HR-HPV-positive women (%)</th>
<th>No of women with persistent HR-HPV infection (%)</th>
<th>No of women with CIN 2–3 lesions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30–39</td>
<td>984</td>
<td>373 (38)</td>
<td>42 (11.1)</td>
<td>24/33 (73)</td>
<td>11/24 (46)</td>
</tr>
<tr>
<td>40–49</td>
<td>968</td>
<td>386 (40)</td>
<td>22 (5.7)</td>
<td>15/18 (83)</td>
<td>7/15 (47)</td>
</tr>
<tr>
<td>50–58</td>
<td>877</td>
<td>343 (39)</td>
<td>10 (2.9)</td>
<td>5/9 (56)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>Total</td>
<td>2829</td>
<td>1107 (39)</td>
<td>74 (6.7)</td>
<td>44/60 (73)</td>
<td>19/44 (43)</td>
</tr>
</tbody>
</table>

The prevalence of CIN 2–3 in women with persistent HR-HPV infection was not associated with any particular age group. It was 40% (8 out of 20) in women over 40 years of age and 46% (11 out of 24) in women younger than 40.

A biopsy sample was obtained from 63 of the 74 HR-HPV-positive women and 22 samples showed histological CIN 2–3 lesions, corresponding to 2.0% (22 out of 1107) of the total number of women performing self-sampling. The prevalence of CIN 2–3 was 2.9% among participating women under 40 years of age and 1.1% among women over 40.
The cytological smears taken concomitantly with the cervical biopsy samples at the gynaecological examination were normal in 75% (45 out of 60) of the cases and various kinds of dysplasia (ASCUS–CIN 3) were observed in 25% (15 out of 60). Among the 19 women with CIN 2–3 in their biopsy samples, cytological smears were normal in 47% (9 out of 19) of these cases. Only four (21%) of the cytological smears revealed CIN 2–3 (Table 2).

<table>
<thead>
<tr>
<th>Histology</th>
<th>Normal</th>
<th>ASCUS</th>
<th>CIN 1</th>
<th>CIN 2–3</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>21</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>24 (40)</td>
</tr>
<tr>
<td>CIN 1</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>17 (28)</td>
</tr>
<tr>
<td>CIN 2–3</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>19 (32)</td>
</tr>
<tr>
<td>Total</td>
<td>45 (75%)</td>
<td>7 (12%)</td>
<td>3 (5%)</td>
<td>5 (8%)</td>
<td>60 (100)</td>
</tr>
</tbody>
</table>

Table 2. Relationship between cytology and the histological diagnose in cervical biopsy samples in 60 women admitted to a gynaecological surgery due to a previous positive HR-HPV test.

Paper II

In total, 39% (2850/7331) of the invited women performed self-sampling of vaginal fluid at home. No difference in the participation rate was seen among the different age groups (data not shown). In all, 6.6% (188/2850) of the participants were positive for HR-HPV in the self-obtained sample. The prevalence of HR-HPV decreased with age, from 11.5% in women aged 30–39 years to 4.7% in women aged 50–65 years (Table 3).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>HPV positive, N</th>
<th>HPV positive, %</th>
<th>Women, N</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>63</td>
<td>11.5</td>
<td>546</td>
</tr>
<tr>
<td>40-49</td>
<td>59</td>
<td>6.6</td>
<td>896</td>
</tr>
<tr>
<td>50-65</td>
<td>66</td>
<td>4.7</td>
<td>1408</td>
</tr>
<tr>
<td>All</td>
<td>188</td>
<td>6.6</td>
<td>2850</td>
</tr>
</tbody>
</table>

Table 3. Prevalence of HR-HPV infection in primary screening in relation to age

Of the 188 women who were positive for HR-HPV in the self-obtained sample, 168 underwent a follow-up examination, giving a compliance rate of 89% (168/188). Twenty women had moved from the County of Uppsala or were examined outside the place of study, leading to incomplete data. Eleven of the 168 women chose to visit a midwife for cervical smear sampling for cytological screening and HR-HPV analysis. These women were not further included in the analysis. In three additional women, cervical biopsy samples were collected but no sample for HR-HPV analysis was obtained, and these women were also excluded from further analyses.
In the remaining 154 women, cervical biopsies were performed in combination with sampling for repeat HPV testing at a gynaecological surgery by the same physician (KS). Five HPV tests were regarded as invalid because of a low amount of cells in the sample. Finally, there were 149 women with two valid consecutive HR-HPV tests, of which 88 were positive and 61 negative, and the presence of CIN 2+ and CIN 3 lesions was evaluated (Table 4). The mean time between the first and second HPV test was 2.7 months, ranging from 4.0 months for women in the age group of 30–39 years to 1.9 months for women in the age group of 50–65 years.

Repeated HR-HPV positivity was found in 67% of women aged 30–39 years, in 64% of women aged 40–49 years and in 47% of women aged 50–65 years. The difference between age groups was not statistically significant (p=0.350). Among the 88 women with a persistent HPV infection, 36 (41%, 95% CI 31–51) showed CIN 2+ in their histology. The prevalence of CIN 2+ in women with short-term persistent HPV infection varied from 49% in women aged 30–39 years to 24% in women aged 50–65 years (Table 4).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>HPV positive, n (%)</th>
<th>Women, n</th>
<th>CIN2+, n (%)</th>
<th>CIN3, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>35 (67)</td>
<td>52</td>
<td>17 (49)</td>
<td>9 (26)</td>
</tr>
<tr>
<td>40-49</td>
<td>28 (64)</td>
<td>44</td>
<td>13 (46)</td>
<td>6 (21)</td>
</tr>
<tr>
<td>50-65</td>
<td>25 (47)</td>
<td>53</td>
<td>6 (24)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Total</td>
<td>88 (59)</td>
<td>149</td>
<td>36 (41)</td>
<td>18 (20)</td>
</tr>
</tbody>
</table>

Information on the infecting HPV type was obtained for 88 women participating in the second HPV test. HPV16 was the most prevalent type (32%), followed by HPV18/45 (19%) and the group HPV 33/52/58 (19%). Among other types, HPV 31 occurred in 9%, HPV 35 in 3%, HPV 39 in 3%, HPV 51 in 3%, HPV 6 in 4% and HPV 59 in 10% of women positive in the second HPV test. The prevalence of a HPV 16 positivity decreased with age, from 34% in premenopausal women to 23% in women of 50 years and older (p<0.02).

The specificity of a single HPV test in comparison with two consecutive HPV tests for detection of histologically defined CIN 2+ is shown in Table 5. A single HR-HPV test in primary screening showed a specificity of 95.9%, whereas applying a repeat HR-HPV test increased the specificity to 98.1% (p<0.0001). The specificity of both primary and repeat HR-HPV testing increased with age and women aged 50–65 showed a specificity with a repeat test of 98.6%, which is significantly higher than the specificity associated with women aged 30–39 (p=0.0021). Specificity was calculated from the number of cases of CIN 2+ among the 149 women followed up (Table 5).
Table 1 in original Paper II, also includes data on histological diagnoses of women who were followed up after the study was closed. The data were initially included in the calculations for specificity (see Table 3 original Paper II), but for the current calculation they were removed. Table 5 shows specificity calculated from reanalysed data. The current calculation on data from the 149 women generally gave a higher specificity for one HR-HPV test and a marginally higher specificity for two consecutive tests. Even with this short-term repeated HPV testing, there was a small numerical increase in specificity.

Table 5. Specificity (%) for identification CIN 2+ lesions in relation to women’s age. The women had a HR-HPV infection in primary screening and in the short-time consecutive positive HPV tests.

<table>
<thead>
<tr>
<th>Age(years)</th>
<th>Primary test positive</th>
<th>Repeat test positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>93.2</td>
<td>96.5</td>
</tr>
<tr>
<td>40-49</td>
<td>96.4</td>
<td>98.3</td>
</tr>
<tr>
<td>50-65</td>
<td>96.6</td>
<td>98.6</td>
</tr>
<tr>
<td>All</td>
<td>95.9</td>
<td>98.1</td>
</tr>
</tbody>
</table>

Paper III

All samples contained sufficient amounts of genomic DNA for HR-HPV typing. The samples the women collected themselves contained on average 3.5 times more nuclear genomic DNA (mean = 7000 copies of genomic DNA) than samples collected by a physician (mean = 2000 copies of genomic DNA). Self-sampling resulted in 68% (34/50) HR-HPV-positive samples, compared with 56% (28/50) in connection with the physician-obtained samples and 42% (21/50) of the biopsies. The HPV types found in all HPV-positive samples are shown in Table 1 of Paper III.

All women that were positive for HR-HPV in the biopsy specimens were also positive in samples obtained by vaginal self-sampling and cervical samples obtained by a physician. The overall agreement for the detection of HR-HPV in samples collected by the women themselves and those obtained by a physician was estimated to 88% (kappa = 0.75, 95% confidence interval 0.56–0.94). Four women had additional multiple HPV infections solely in the self-sampling material. In another six women an HR-HPV infection was only detected by self-sampling and in all these cases the HPV copy numbers were close to the cut-off for positivity in the HPV typing assay used [84, 86]. Among the women that were HR-HPV-positive in samples obtained by both self-sampling and by a physician, there was also positivity in the biopsy samples in 75% (21/28) of the cases.
The results of histological evaluation of the cervical biopsy samples revealed CIN 2–3 in 22% (11/50) of the women, CIN 1 in 40% (20/50) and 38% (19/50) showed normal histology (Table 2, Paper III). Among women with CIN 2–3, 91% (10/11) were HR-HPV-positive.

Among women with CIN 1, 65% (13/20) were HR-HPV-positive in self-obtained samples, compared with 55% (11/20) in connection with the physician-obtained samples, and 45% (9/20) in the biopsy samples. Women with normal histology were positive for HR-HPV in 58% (11/19) of samples obtained by self-sampling, 37% (7/19) of samples obtained by a physician and 11% (2/19) of the biopsy samples, and had the lowest HPV concordance between the three sample sets. The three sample sets showed 100% concordance in HPV positivity among women diagnosed with high-grade lesions, CIN 2–3 (Table 2, Paper III), (Figure 5). Six women were HR-HPV positive only in samples obtained by self-sampling, of whom 4 had normal histology and 2 had CIN 1.

A sample from a woman with CIN 2 in histology showed negativity for HR-HPV in the hpVIR test in the three sample set. Her samples were further analysed using the GP5/6+ PCR system followed by Sanger sequencing [87]. This analysis showed that the biopsy sample contained HPV 81 and/or 84. HPV 81 was also found in the physician-obtained sample, while the self-obtained sample contained insufficient amounts of material for analysis.

![Figure 5. Detected HR-HPV infection in three sample set in women with histological diagnoses from cervical biopsy samples. (Illustration from I Gustavsson)](image)

**Paper IV**

Repeated sampling was performed by 25 women with a mean age of 49 (range: 30–61) years. Of these, 13 (52%) were postmenopausal, with a mean age of 57 (range: 52–61) and the remaining 12 (48%) were premenopausal, with a mean age of 40 (range: 30–46).
The premenopausal women collected samples on average for 22 days (range: 17–28) and all postmenopausal women, the woman with the LNG-IUD and the woman using oral contraceptive pills (desogestrel continuously) collected samples for 28 days. The time interval between a woman’s only or last HR-HPV-positive sample and the start of her sampling period ranged from 2 to 19 weeks (median 5 weeks).

There were consistent typing results throughout the sampling period for 19 women, with either a daily presence of virus (14 women) or no virus at all (5 women) (Table 6). Among the 14 women that were consistently positive during the sampling period, 11 were infected with only one type of HR-HPV. The amount of this single virus was relatively stable, with high copy numbers detected throughout the sampling period (Figure 6, ID no. 3). The three other women who were consistently positive for HR-HPV showed daily presence of two or more HR-HPV types, and at least one HPV type was detected in each sample (Figure 7, ID no. 17). In six women the HPV typing results were not completely consistent throughout the sampling period. Three of them had a majority (89–90%) of negative samples during the sampling period, but showed an occasional HR-HPV-positive sample, with HPV copy numbers just above the threshold for positivity. In two women, the majority of samples during the sampling period were HR-HPV-positive (89% and 96% of the samples), but with a low copy number that was sometimes under the threshold used for positivity (Figure 8, ID no. 11). Finally, one woman was positive for HPV 51 during the majority of the sampling period (70%), but typed negative during the last quarter of the period (Figure 9, ID no. 12).

When assessing the consistency of the typing results, we combined all samples from all women except for case no. 12 (shown in Figure 9) where we suspected clearance of infection. Of the 607 samples obtained from the 24 women, 596 were consistently positive or negative during the sampling period. Among the remaining 11 samples, 7 were positive in a series of negative samples and 4 samples were negative in women that otherwise typed positive. The 11 inconsistent samples constituted 2% of all samples (Table 3, paper IV).

The viral load (estimated as HPV copies per sample) during a sample period varied substantially between individual women. Among the women who were consistently HR-HPV-positive throughout the study period, the mean number of copies varied from $10^2$ to $10^5$. In contrast, the three women who were mostly HR-HPV-positive but had a few HPV-negative samples had viral loads of between 1,600–5,400 copies.

The variation in HPV copy numbers between sampling days in individual women was considerable. The copy numbers during a sampling period ranged from $10^3$ to $10^5$ copies. One woman (Figure 7, ID no. 17) was infected with 4 different types of HR-HPV and these varied by more than two million copies/day between some sampling days. Interesting in this context
may be the fact that this woman was being treated with azathioprine, an immuno-suppressive drug, because of Crohn’s disease.

There were no significant differences in viral load between the oestrogen-dominated follicular phase and the gestagen-dominated luteal phase (p = 0.0625) and no significant correlation between number of copies and sampling day among pre- and postmenopausal women (p = 0.68). Whether unprotected vaginal intercourse influenced HPV copy numbers could not be determined as a result of the relatively limited number of occasions reported.

Table 6. Distribution and consistence of the HPV result during each woman’s sampling-period. a HPV types 18 and 45 are typed together as one group and 33, 52 and 58 are detected together as a second groups. The other types are presented as single types.

<table>
<thead>
<tr>
<th>ID number</th>
<th>HR-HPV typea</th>
<th>Premenopausal (M)</th>
<th>Postmenopausal (P)</th>
<th>Consistent result (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative</td>
<td>M</td>
<td>M</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>16, 33/52/58, 51, 56</td>
<td>M</td>
<td>M</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>M</td>
<td>M</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>Negative</td>
<td>M</td>
<td>M</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>Negative</td>
<td>M</td>
<td>M</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>M</td>
<td>M</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>M</td>
<td>M</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>16, 33/52/58</td>
<td>M</td>
<td>M</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>Negative</td>
<td>M</td>
<td>M</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>Negative</td>
<td>M</td>
<td>M</td>
<td>90</td>
</tr>
<tr>
<td>11</td>
<td>51</td>
<td>M</td>
<td>M</td>
<td>89</td>
</tr>
<tr>
<td>12</td>
<td>51</td>
<td>M</td>
<td>M</td>
<td>70</td>
</tr>
<tr>
<td>13</td>
<td>Negative</td>
<td>P</td>
<td>P</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>16</td>
<td>P</td>
<td>P</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>59</td>
<td>P</td>
<td>P</td>
<td>100</td>
</tr>
<tr>
<td>16</td>
<td>Negative</td>
<td>P</td>
<td>P</td>
<td>100</td>
</tr>
<tr>
<td>17</td>
<td>18/45, 31, 33/52/58, 56</td>
<td>P</td>
<td>P</td>
<td>100</td>
</tr>
<tr>
<td>18</td>
<td>16</td>
<td>P</td>
<td>P</td>
<td>100</td>
</tr>
<tr>
<td>19</td>
<td>31</td>
<td>P</td>
<td>P</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>33/52/58</td>
<td>P</td>
<td>P</td>
<td>100</td>
</tr>
<tr>
<td>21</td>
<td>51</td>
<td>P</td>
<td>P</td>
<td>100</td>
</tr>
<tr>
<td>22</td>
<td>31</td>
<td>P</td>
<td>P</td>
<td>100</td>
</tr>
<tr>
<td>23</td>
<td>16</td>
<td>P</td>
<td>P</td>
<td>100</td>
</tr>
<tr>
<td>24</td>
<td>18/45, 33/52/58</td>
<td>P</td>
<td>P</td>
<td>96</td>
</tr>
<tr>
<td>25</td>
<td>Negative</td>
<td>P</td>
<td>P</td>
<td>89</td>
</tr>
</tbody>
</table>
Figure 6. A woman (ID no. 3) with a single HR-HPV 56 infection (grey line) during the entire sampling period. The green line shows the amount of measured DNA. The red line is the threshold for an HR-HPV-positive sample (≥10 HPV copies).

Figure 7. Stable infection with multiple HR-HPV types (ID no. 17). At least 3 types of HR-HPV were detected during the sampling period. The group consisting of HPV 33, -52- 58 (blue line) showed very low copy numbers and did not reach the threshold for positivity (red line, ≥10 copies) every day during the sampling period.
Figure 8. A woman (ID no. 11) with an HR-HPV51 infection (pink line). The infection was above the threshold for positivity on the majority of sampling days, but below the level on sampling days 9 and 27. On day 13 the HPV copies were just on the threshold line. The green line shows the amount of DNA. The red line is the threshold for an HR-HPV-positive sample (≥10 HPV copies).

Figure 9. Infection with HR-HPV51 (ID no. 12). The woman showed a declining number of copies during the first week and the copy number was below the threshold for positivity at sampling day 6. The number of copies then recovered and stabilised, later to decline again below the detection limit at the end of the period. The green line shows the amount of DNA. The red line is the threshold for an HR-HPV-positive sample (≥10 HPV copies).
Discussion

Paper I

This descriptive study showed high sensitivity for HPV self-sampling compared with cytology for the detection of CIN 2+ among women not attending a cytological screening programme for cervical cancer, but with a high rate of participation in our study.

In countries with organised gynaecological screening, the majority of cervical cancer cases occur among women who have not attended screening (collection of a cervical smear) [48]. Thus, to reduce the number of women with cervical cancer, an improvement in participation rate is an important issue. One means to overcome the problem of NA is to offer women the possibility of performing self-sampling of vaginal fluid at home and sending in the sample to a laboratory for HPV analysis. Several international studies have shown that many women not taking part in regular screening participate in self-sampling, indicating that this is an efficient way to increase screening coverage [79]. Several studies have shown participation rates for self-sampling among NA of 8.7% [74] to 31.5% [88].

The participation rate of self-sampling among NA described in this study was unexpectedly high, 39.1%. Reasons for the different participation rates between studies could include logistics and the means of offering the self-sampling procedure. The women in our study received reminders by post, as regards both ordering the device and performing the sampling. The test was even free of charge, which probably favourably affected the participation rate. The low coverage of cytological cervical cancer screening in Uppsala County could be another reason for the high participation rate.

A previous randomized study in Uppsala County with a similar study protocol that included reminder letters, showed similar results for participation in self-sampling, with 39.0% attendance. In comparison, a reminder letter for cytology increased the attendance rate by only 8.0% [82]. Those women not taking part in organised screening have an increased prevalence of HR-HPV infections [89] and an increased incidence of cervical cancer [90, 91]. A high participation rate in self-sampling for the analysis of HPV among NA means that a large proportion of women at an increased risk of development of cervical cancer can be included in screening programmes.

Testing for HR-HPV in primary screening results in greater sensitivity in detecting high-grade lesions, in comparison with Pap-smear screening [55,
The fact that a single cytological examination has a low sensitivity is obvious from our results, where 47% of the women with biopsy-verified CIN 2–3 lesions on the cervix showed normal cytological smears collected on the same occasion. Only 21% of cases of CIN 2+ show CIN 2+ in Pap smear analyses. This could be one reason why as many as a quarter of all cases of cervical cancer occur among women regularly participating in organised screening, with repeated normal cytological results [48].

HPV testing leads to identification of around twice as many CIN 2+ lesions per 1000 examinations as regular Pap-smear screening [54]. It was shown that 43% of the women with a persistent HPV infection also had histological CIN 2–3 lesions in the cervix. There was no obvious age difference, although the number of women with CIN 2–3 was slightly higher in women of <50 years. In total, 2.0% of all the participating women in the study showed histological CIN 2–3 lesions. In comparison, 0.9% of all cytological smears in organised gynaecological screening in Sweden show CIN 2+ and the number of histologically verified CIN 2+ lesions is about the same. This means that around twice as many CIN 2–3 lesions are detected with self-sampling combined with testing for HR-HPV in the group of non-attending women, compared with cytological screening among women in the general population who participate in a screening programme. The higher prevalence of HR-HPV in the group of non-attending women can partly be explained by more CIN 2–3 in this group. The higher sensitivity of the HPV test compared with the cytology test is another reason.

In addition to increased coverage, the women are offered a method that is more relevant in identifying those at an increased risk of developing cervical cancer as a result of a short-time persistent HR-HPV infection.

**Paper II**

This study showed that two consecutive samples for HR-HPV analysis with a few months of each other resulted in greater specificity than a single HR-HPV sample, when associating the results with the detection rate of CIN 2+.

HR-HPV sampling in primary screening provides increased protection against cervical cancer in comparison with Pap-smear screening [54, 56]. The introduction of HR-HPV testing in primary screening is facilitated by its high sensitivity [56, 92, 93] and by its high negative predictive value, indicating that screening intervals can be prolonged [55, 94, 95]. However, it has the disadvantage of lower specificity compared with cytology-based screening [54].

A number of studies have shown that repeat testing for HPV leads to greater specificity than a single test in primary screening [96-99]. Further, women with aberrant cytological results have a lower clearance rate of HR-HPV in comparison with women showing normal results [100]. A meta-
analysis showed that persistent HPV infection is consistently associated with a higher risk of CIN 2+/HSIL [101].

By repeating the HR-HPV test within a few months after the primary test, we improved specificity for the entire spectrum of women of 30–65 years of age in comparison with a single HR-HPV test. The specificity of the HPV analysis increased with age, concomitant with a decreasing prevalence of persistent HPV infections. In agreement with the results of previous investigations [102], the prevalence of HR-HPV decreased with age. Women under the age of 40 had a prevalence of HR-HPV of 11% and the corresponding figure for women over 50 was less than 3%. In addition, a large proportion of postmenopausal women with abnormal smears are HPV-negative [103]. It has been reported that the efficiency of cytological screening decreases with age [50, 57, 104] and consequently, the difference in sensitivity between HPV typing and cytology increases with age [57]. This means that at a defined age the HPV test is both more sensitive and more specific than the cytological examination.

It was assumed that all women who were HR-HPV-negative in the first sample did not have CIN 2+. These women (2659), however, were not examined by means of colposcopy and no biopsy samples were collected to confirm this assumption. Instead, we followed up these women in the organized screening register 3–5 years after the HR-HPV-negative result in regard to any abnormalities in Pap smears or cervical histology. One woman who was HR-HPV-negative in 2008 had SCC diagnosed in 2011, but otherwise there were no diagnosed premalignant abnormalities in the other 2658 women. In a previous study it was shown that HR-HPV-negative women have a cumulative incidence of CIN 3+ of 0.27% at follow-up six years later [58], but that of course depends on what method is used for the HPV analyses.

The time interval between the initial and repeat HPV test was similar to that used in the follow-up of women with ASCUS and CIN 1 alterations in routine cytological screening. As transient HPV infections usually remain for around 6–24 months, it means that some of the women testing positive in the two consecutive HPV tests are likely to have cleared the virus later. Thus, our study design leads to underestimation of the specificity of repeat HPV testing. However, as information concerning a HR-HPV infection may cause anxiety, we reduced the time span between a first positive sample and the second sampling.

We noted that there was a lower prevalence of CIN 2+ in women with short-term persistent HPV infections in the higher age group, i.e. 50 years or older. This could be a result of the shorter follow-up time in this age group, only 1.9 months compared with that in women aged 30–39 years, where the interval was 4 months. Another explanation for a lower prevalence of CIN 2+ is that the transformation zone in menopausal women is hidden in the
cervical canal and causes problems when collecting appropriate biopsy samples during colposcopy.

As expected, HPV 16 and 18/45 were the most common virus types, constituting 51% of the HPV-positive samples. The prevalence of HPV16 decreased with age from 34% in premenopausal women to 23% in postmenopausal women. This observation may have some significance with respect to the long-term effect of human HPV vaccination.

**Paper III**

Self-sampling in connection with HPV testing has been shown to be a suitable alternative to physician-obtained samples [105], and the prevalence of carcinogenic HPV types has been found to be similar in vaginal and cervical specimens [106].

In this study we compared the use of the Viba-brush and the FTA cartridge for cervicovaginal self-sampling for subsequent HPV typing with physician-obtained samples. Previous investigators have evaluated the use of Dacron swabs [105], tampons [63], cervicovaginal lavage [75] and cytobrushes [107] for self-sampling, often in combination with different forms of liquid-based storage and transport media. The use of these often hazardous solutions is not recommended for self-sampling at home, and these liquid samples are not suitable for sending by regular mail. An important consideration is the cost of extraction of DNA from the clinical samples, since many HPV tests are DNA-based. The indicating FTA elute card provides a suitable collection medium for cervical epithelial cells and allows a very convenient and cost-effective protocol for DNA extraction [83].

The results showed that sufficient amounts of DNA were obtained by the Viba-brush in combination with the FTA cartridge, and that DNA yield was on average 3.5 times higher in the vaginal smears obtained by self-sampling compared with cervical smears obtained by a physician. The brush used for sampling has to be soft enough not to rip the surface of the FTA filter paper but at the same time it should allow easy release of the sample. Both Lenselink et al. [108] and our group have shown that the Viba-brush and FTA cartridge represent convenient collection devices. Use of the Viba-brush must also be acceptable to the women, as reported by Bais et al. [79].

There was good concordance in HPV positivity between self-sampling and physician-obtained sampling. Similar results were also shown in a recent study [108] in which a self-obtained sample applied to an FTA cartridge was compared with a physician-obtained liquid-based sample. In the present study all HR-HPV-positive physician-obtained samples were also positive in self-sampling. The HPV types found by self-sampling and physician-obtained sampling were identical, with the exception of 6 women that were HPV-positive solely in the self-sample. These results indicate that women
may carry several HPV types in the vagina that are not present in the endocervix. The number of infecting HPV types was further reduced in the biopsy samples. When comparing the HPV typing result with histological diagnosis it was clear that the additional HPV infections detected only in the vagina were not associated with >CIN 2 lesions, since four of the samples showed normal histology and two showed CIN 1, similar to the results found in a study by Belinson et al. [109]. Vaginal samples will probably be associated with lower specificity than endocervical samples, but no clinically important HR-HPV infections should go undetected. Self-sampling will most probably increase the screening coverage and more high-risk infections will be detected [62, 75, 110, 111], which subsequently will lead to a decreased incidence of cervical cancer.

**Paper IV**

The major finding in this prospective cohort study was the high degree of consistency concerning positivity and negativity of HR-HPV in vaginal fluid during a sustained period of daily self-sampling. Viral copy numbers were generally sufficient for the detection of HR-HPV throughout the sampling period, with the exception of a few test days in a few women where the number of virus copies dropped under the threshold for positivity. There were fluctuations in viral load from day to day in all infected women and an explanation for that could be variation in HR-HPV DNA replication, which is not necessarily associated with a specific external factor. These fluctuations may in some cases represent resolution and acquisition of HR-HPV infections. A few days of negative test results in otherwise clearly HR-HPV-positive women could be associated with ongoing resolution of an infection. Such a pattern was seen in case no. 12 (Figure 9), where we observed several negative samples at the end of the sampling period. Ongoing resolution of HPV infection might also have been the case for patient no. 11 (Figure 8), where there were several days of negativity. This is supported by the histological results, where case no. 11 had CIN 1 in the biopsy samples and normal histology in the cone, and case no. 12, with CIN 2 in the biopsy samples and CIN 1 in the cone.

Most of the women who were considered HR-HPV-negative also showed consistent results, with negativity throughout the sampling period. There were, however, a few exceptions where some samples in some women were slightly above the threshold for positivity. This pattern might be the result of an infection that was in the process of being cleared and the few positive samples might thus have been remnants of the infection.

Whether or not HR-HPV is detected depends on the sensitivity of the test method and the quality of the sample. Self-sampling with the devices used in our study has recently been found to be as reliable as sampling carried out by
a physician [112]. The PCR assay detects very low levels of HR-HPV, and a sample has to contain a minimum of 10 HPV copies to be considered as positive. The low threshold for positivity of the test increases consistency throughout the sampling period in infected women. The real-time PCR-based assay used in this study gives a measure of the quantity of a detectable virus in terms of number of copies of each HPV type. It is thus possible to study each virus in women infected with single or multiple types of HR-HPV, and our results show relatively large fluctuations in numbers of copies of several HPV types throughout the sampling period.

The study design, involving frequent sampling in all cases, could be suspected to lead to artefacts and false results as regards virus detection and viral load. The question of whether or not frequent sampling of cervical samples may affect the detectability of HPV viruses has been discussed [113]. A consequence of this would be a risk of false negatives, but such an association has not been found [114]. In our study, with daily sampling, we did not find any significant correlation between copy numbers and sampling day. The fact that the women in our study performed the test day after day may contribute to less careful sampling, which thus would hamper the test result with false negatives or false positives. This may explain the observation of a single negative sample in an otherwise HR-HPV-positive woman (ID no. 24, Table 6).

Variability in the number of viral copies was considerable, in both the pre- and postmenopausal women. There are several studies concerning whether or not hormonal changes during the menstrual cycle affect the prevalence of HR-HPV, but the results are contradictory [113-118]. In spite of the large number of samples in our study, we found no particular change in the numbers of HPV copies during the menstrual cycle. It thus seems that changes in circulating oestrogen and progesterone levels do not affect the number of viral copies in vaginal smears.

This study shows that a single self-test provides reliable information on whether or not a woman has an HR-HPV infection. Our data show a small risk of missing a clinically relevant infection that may progress to precancerous lesions or cancer, since the vast majority of samples from women with a clinically relevant HR-HPV infection test positive. One reason for negative results in some cases might be that there is ongoing clearance of the virus, which significantly reduces the risk of development of precancerous lesions. "False-positive" samples can probably also be explained by clearance of the virus. By repeating the HR-HPV test after 3–6 months, it can be decided whether or not the infection was transient and had cleared, or if it remains positive and the woman should be further investigated.
Methodological considerations

Study populations

The study populations involved in Studies I–III and the vast majority of patients in Study IV included women who had not taken part in organised gynaecological screening in Uppsala County for six years or more. It has been reported that women not attending an organised cytological screening programme have an increased prevalence of HR-HPV infections compared with women regularly participating [89], but in our studies the prevalence of HR-HPV among NA shows the same figure as in normal population.

The aims with Studies I and II were to see whether or not women not participating in screening would accept self-sampling as a preferable alternative to a Pap smear collected by a physician. Therefore, the study population consisted of NA. These studies also concerned comparison of the results of HR-HPV self-sampling with cytology, with respect to the detection of CIN 2+. It must be remembered that the use of NA is not necessarily representative of the screening population. In our studies, however, we compared self sampling for HR-HPV analysis with Pap smears in the same population, which is an advantage in that the risk of selection bias is small and the women are their own controls. Comparison of self-sampling followed by HR-HPV analysis, and Pap smear results, does not depend on the type of study cohort. It has been shown that the sensitivity of HR-HPV testing compared with cytology tests for detecting CIN 2+ does not differ between referral- or screening populations [68]. In Studies III and IV it was an advantage, and practically a necessity, to include women with a strong likelihood of HR-HPV infection, so we recruited women during their follow-up after one or two HR-HPV-positive samples. It can be concluded that it seems as if self-sampling for the analysis of HR-HPV is a reliable method in cervical cancer prevention programmes. However, there are still several knowledge gaps that need to be addressed before self-sampling can be used in clinical routine.

HPV methods

We used two different HR-HPV tests in the work presented in Papers I and II. Although the hpVIR test is favoured because of its ability to be type-specific, the self-sampling device Qvintip has been validated in combination with the FDA-approved hc2 method [89] [81]. The two methods give similar but not absolutely identical results with regard to the identification of smears harbouring HR-HPV [86]. The hpVIR assay does not detect HPV 68. The lack of HPV 68 in the hpVIR assay could have resulted in women being classified as having a transient rather than a persistent infection in Study II. This would have led to overestimation of the increase in specificity after
repeat sampling. Given the relatively low frequency of this HPV type, the effect is likely to be limited.

The hc2 method is reported to lead to cross-hybridization of at least 15 low-risk HPV types but it cannot be used to determine the specific HPV type present. This may have contributed to a higher prevalence of HPV in the first sampling in Studies I and II. This means a falsely higher prevalence of HR-HPV and this may contribute to an underestimation of the specificity of self-sampling for HR-HPV analysis.

Conclusions

Self-sampling for HR-HPV testing is a suitable, safe and accepted method for cervical cancer prevention among women not taking part in a regular cervical cancer screening programme. There is a relatively large number of women who are unable or unwilling to visit an HCP to take part in a cervical screening programme and the concept of self-sampling will therefore most probably significantly increase coverage in cervical cancer prevention programmes.

Repeat testing for HR-HPV represents a screening method with higher sensitivity and specificity than cytological screening, especially among middle-aged and older women. Primary screening based on HPV tests is therefore clearly favourable in middle-aged and older women.

Self-sampling, using the Viba-brush and the indicating FTA elute cartridge for HPV typing, seems to be as sensitive as sampling by an HCP. One single self-test seems to provide reliable information concerning whether or not a woman has an HR-HPV infection and it also seems as if the risk of missing a clinically relevant HPV infection seems to be very small.

Thus, self-sampling for the analysis of HR-HPV appears to be a powerful alternative as a primary screening method for the prevention of cervical cancer.
Self-sampling combined with HR-HPV testing will most probably become an important component in future cervical cancer prevention programmes. This test strategy has high sensitivity and specificity and the sampling can be performed by the woman herself in a private setting whenever suitable, without the need to travel to a clinic or other medical department.

In a new European guideline concerning cervical cancer prevention, to be presented in April 2013, HR-HPV testing is recommended as the method of choice in primary screening programmes for women aged 30–35 and older. In this guideline it is concluded that there is a need for more data before self-sampling can be recommended as a routine method. Self-sampling for HR-HPV analyses has been used in several studies around the world and is considered safe, reliable and is also well accepted among women. Some previous studies have shown that samples collected by self-sampling are associated with lower sensitivity compared with samples collected by a physician, but this needs to be further studied. There is also a need to study self-sampling combined with HR-HPV testing on a larger scale, as part of routine screening, and to compare outcomes such as sensitivity, specificity, effects on cervical cancer incidence and health economy, with screening based on HR-HPV testing in samples collected by physicians.

Cervical cancer is a common disease associated with high mortality in many low-resource countries. There are no organized cervical cancer prevention programmes in such countries as a result of lack of knowledge, equipment and resources [1]. Self-sampling combined with HR-HPV testing might be suitable in low-resource settings [119], since sampling does not require any intervention by a HCP and there is no need for a gynaecological examination. Analysis of HR-HPV is also easy to perform and does not require a cytotechnologist. A sampling device such as the indicating FTA elute micro card has the advantage of easy handling and no liquid transport or storage medium, and it seems not to be sensitive to moisture environments and a warm climate [120].

Women of today are familiar with self-testing in connection with many medical issues such as pregnancy, Chlamydia infection or determination of ovulation. To perform a self test contributes to a feeling of independence and freedom, and there is increased privacy compared with the need to have a sample collected by a HCP. There are several reasons why women do not go to a clinic to have a cervical or vaginal sample collected. One reason might
be that screening for cervical cancer prevention occurs in a phase of a woman’s life when she is in the midst of career and family life and it is just not a priority.
Livmoderhalscancer orsakas av ett sexuellt överförft virus, HR-HPV (HögRisk- Humant PapillomVirus). Det är ett vanligt förekommande virus över hela världen och de flesta infektioner läker ut av sig själva. När HR-HPV-infektionen blir kvarstående, en så kallad persistierande infektion, kan den ge upphov till cellförändringar i livmodertappens transformationszon och dessa kan övergå till cancer. Cellförändringarna kan indelas i olika svårighetsgrader där CIN 1 är en mild förändring som ofta är övergående medan CIN 2 och CIN 3 är allvarliga cellförändringar som måste behandlas. Om de inte behandlas finns risk för att de utvecklas till cancer. Insjuknandet i livmoderhalscancer kan minskas bland annat genom regelbunden gynekologisk hälsovård (organiserad screening). Trots att denna metod är känd sedan länge insjuknar i världen ungefär 500 000 kvinnor årligen i livmoderhalscancer.

Organiserad screening infördes i Sverige i slutet av 1960-talet och detta har bidragit till att antalet cancerfall halverats i vårt land. Screeningen går ut på att hitta allvarliga cellförändringar och behandla dessa för att förhindra att cancer utvecklas. Sedan screeningen infördes har barnmorskor och läkare tagit cellprov från livmoderhalsen hos kvinnor. Cellprovet har sedan tolkats av cytotekniker som bedömt cellerna i mikroskop och graderat provet som normalt eller med cellförändringar enligt de olika svårighetsgraderna (CIN). Cellprovet har dock en relativt låg sensitivitet och en fjärdedel av de kvinnor som drabbas av livmoderhalscancer har deltagit i screeningen och haft ett eller flera cellprover som bedömts som normala. En annan svaghet med screeningen är att drygt 20% av svenska kvinnor inte deltar i screeningen och att hälften av de som drabbas av livmoderhalscancer tillhör den gruppen. Låg sensitivitet för cellprovet och lågt deltagande i screeningen bidrar till att vi fortfarande har ungefär 450 nya fall av livmoderhalscancer per år i Sverige.

framför att gå till en barnmorska för att ta ett cellprov. I Uppsala har man sedan några år erbjudit vaginal självprovttagning med HR-HPV-test till kvinnor som inte deltagit i screeningen. I min avhandling har jag studerat och beskrivet resultat ifrån självprovtagningen av kvinnor som ej deltagit i screeningen utifrån deltagandet och säkerheten i provet jämfört med cellprov. Jag har också jämfört självprovtagning av HR-HPV med HR-HPV-prover tagna av läkare och dessutom har jag studerat vaginalt HR-HPV hos kvinnor som tagit självprover dagligen under en kortare tid.


I det andra arbetet studeras också kvinnor som ej har deltagit i screening på över 6 år och som har fått och accepterat erbjudande om självprovttagning för analys av HR-HPV och befunnits vara infekterade med HR-HPV. Kvinnorna studeras med avseende på skillnaden mellan ett och två konsekutivt tagna positiva HR-HPV-prover för att identifiera kvinnor med allvarliga cellförändringar. Resultatet visar att två positiva HR-HPV-prover med 1-3 månader mellan proven ger testet en högre specificitet än vad ett enstaka positivt prov gör.

I det tredje arbetet studeras förekomst av HR-HPV hos 50 kvinnor som tagit vaginalt självprov för HR-HPV och vid samma tillfälle provtagits av läkare med cervikalt HR-HPV-prov. Biopsier från livmoderhalsen har också tagits från samtliga kvinnor och analyserats histologiskt. Resultatet visar att de kvinnor som varit positiva för HR-HPV när läkaren tagit cervikala prover också, utan undantag, var positiva i självprovet, vilket visar att självprovet har en lika hög sensitivitet som prov som tagits av läkare. Några av kvinnorna hade positiva självprover där det cervikala provet var negativt och hos dessa kvinnor påvisades inte några allvarliga cellförändringar.

I det fjärde arbetet studeras daglig förekomst av HR-HPV hos kvinnor under en kortare sammantagen tid, motsvarande ungefär en menstruationscykels längd hos både menstruerande och postmenopausala kvinnor. Kvinnorna tar dagliga prover med självprovtagning. Resultatet visar att hos majoriteten av kvinnor med en HR-HPV-infektion påvisas virus varje dag och hos de som inte är infekterade förekommer inte virus i något prov. Hos ett fåtal kvinnor ligger virusmängden av ett specifikt virus lågt och varierar mellan svagt positivt och negativt prov under provtagningstiden. Sammantaget visar
resultatet att ett enstaka självprov för HR-HPV är ett representativt prov för att bedöma om en kvinna har en aktuell HR-HPV-infektion.

Sammanfattningsvis ger avhandlingen belägg för att självprovtagning för analys av HR-HPV är ett mer sensitivt test för att upptäcka allvarliga cellförändringar som kan orsaka livmoderhalscancer än vad det konventionella cellprovet är. Den visar också att specificiteten ökar om provtagning för analys av HR-HPV upprepas inom 1-3 månader efter att det första provet tagits och att även det andra provet är positivt för HR-HPV. Vidare visar avhandlingen att det vagina självprovet har en lika stor säkerhet som det cervikala läkartagna provet för att upptäcka allvarliga cellförändringar och att ett enstaka självprov i de flesta fall ger en rättvis bild av om en kvinna har en aktuell HR-HPV-infektion. HR-HPV-självprovtagning kommer troligtvis att utgöra en viktig del av screeningen för att minska förekomsten av livmoderhalscancer eftersom metoden är säker, användarvänlig och kostnadseffektiv.
Acknowledgements

I would like to take the opportunity to thank all of you at the Gynaecological Surgery at Akademiska University Hospital in Uppsala, the Department of Women’s and Children’s Health and the Department of Immunology, Genetics and Pathology, Uppsala University, who have contributed to the creation of this thesis. Many thanks are due to all the women who in an altruistic way performed daily vaginal sampling for the sake of research. In Swedish, my mother tongue, I would like to express my special and sincere gratitude to:


**Ingrid Wikström**, överläkare i obstetrik och gynekologi, klinisk utredare i kvinnosjukdomar på Läkemedelsverket och min handledare. Tack för kunngigt och värmande handledarskap från början till slut. Det var tack vare dig som idén med det här forskningsarbetet föddes.

**Erik Wilander**, överläkare och professor i cytotologi och patologi och min handledare. Tack för din outsinliga kunskap i ämnet och ditt otroliga engagemang som har genomsyrat och gett mening till mitt avhandlingsarbete.

På Institutionen för immunologi, genetik och patologi, Uppsala Universitet:

**Inger Gustavsson**, projektledare och medförfattare. Du är passionerad av HPV tester och är en av de mest välvikterade människor jag träffat, i labbet, på forskningsmöten men också när det gäller att hitta restauranger i San Juan, Puerto Rico. Tack för all ovärderlig hjälp!


Andra viktiga personer:


Monica Lindell, molekyläribiolog på klinisk patologi och cytologi, Akademiska sjukhuset och medförfattare. Tack för att du har hjälpt mig skaffa kort och pinnar.

Lars Berglund, statistiker vid Uppsala Research Clinical Center. Tack för mänsklig hjälp med statistiken.

Lars-Åke Berndalen, f d klinikchef och Kerstin Sterner Mälstam, nuvarande vårdenhetschef på Hudkliniken, Gävle sjukhus. Tack för att ni har visat förståelse för mitt forskningsintresse.

Min familj:

Tack pappa Lars-Erik, för att du funnits vid min sida, död som levande. Tack mamma Marianne, för att du som alltid har varit närvarande och engagerad och hjälpt mig med språkliga spörs mål. Tack till mina bröder, Mikael och Jörgen för mänskligt stöd och till Inga, min syster som bidragit med akademiskt förnuft och värmande systerskap genom hela min forskarutbildning.

Tack till min livskamrat Thomas (och min Executive Assistant) för ditt tålmodiga stöd med allt från kloka synpunkter och korrekturläsning till hushållsnära tjänster under dygnets alla timmar.

Tack till mina barn, Gabriel och Rakel som har data-supportat, handskrivit kort och bistått till mitt arbete med föredömlig lojalitet!

The work within this thesis was supported by the Department of Women´s and Children´s Health, Uppsala University, the Department of Obstetrics and Gynaecology, Akademiska University Hospital, the Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, CFUG, Landstinget Gävleborgs FoU-enhet and by grants from Lions Cancerforskningsfond, Akademiska sjukhuset, Uppsala and from Stiftelsen Sigurd och Elsa Goljes minne.


47. CARG, Cervixcancerprevention Riktlinjer för utredning, behandling och uppföljning av cervikal intraepitelial neoplasii (CIN), in 63. 2010, Arbets- och Referensgruppen för Cervixcancerprevention.
62. Brink, A.A., et al., High concordance of results of testing for human papillomavirus in cervicovaginal samples collected by two methods,


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.