Self-sampling for HPV testing in primary cervical screening

Including clinical and health economic aspects

RIINA AARNIO
Abstract

Persistent infection with high-risk human papillomavirus (HPV) is a prerequisite for the development of cervical cancer. HPV testing has higher sensitivity for high-grade cervical intraepithelial neoplasia (CIN2+) than cytology, resulting in more effective screening. As HPV testing also offers an opportunity for self-sampling, it could serve as an even more effective and cost-effective method of cervical screening.

First, we compared repeated self-sampling for HPV testing with Pap smear cytology in detection of CIN2+ in primary cervical screening for women aged 30–49 years (n=36 390). We found a more than twofold higher detection rate of CIN2+ and a fourfold higher detection rate of CIN2 with self-sampling compared with cytology. However, no difference was seen between the arms in the detection rate of CIN3+. It thus seems that CIN is detected at an earlier stage with self-sampling than with cytology, but the impact of this needs to be further explored.

Second, as management of HPV-positive women with normal cytology results is a challenge, we wanted to evaluate the proportion of cases of histological CIN2+ in these women. In this prospective study we performed LEEP and found that 15% (6/40) of the women had undetected CIN2+. These findings can be used in counseling women about the risk of cervical cancer and helping clinicians in decisions on management.

Third, we performed a cost-effectiveness analysis on the same study population as in Study I. Self-sampling for HPV testing resulted in a higher participation rate and more detected cases of CIN2+ at a lower cost and was regarded as more cost-effective than Pap smear cytology in cervical screening. These results can guide policy-makers when planning future screening programs.

Fourth, we compared self-sampling with sampling by medical professionals for HPV testing in detection of CIN2+, using a combination of an FTA card as storage medium and a PCR-based HPV test (hpVIR) in women aged 30–60 years (n=11 951). No difference in the detection rates of histological CIN2+ was found between the arms.

Taken together, self-sampling resulted in a higher participation rate than sampling by medical professionals in cervical screening and that triage with repeated self-sampling resulted in high compliance and detection rate of CIN2+. As repeated self-sampling for HPV testing was also cost-effective, it could serve as an attractive alternative in the development of future cervical screening programs. More research is needed on how to refine the management of HPV-positive women by self-sampling only.

Keywords: HPV, self-sampling, cervical screening, CIN2+, cost-effectiveness

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“Primum non nocere”

To Mikko, Lukas, Ines and Ellen
This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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<tr>
<td>AGC</td>
<td>atypical glandular cells</td>
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<tr>
<td>AIS</td>
<td>adenocarcinoma in situ</td>
</tr>
<tr>
<td>ASCUS</td>
<td>atypical squamous cells of undetermined significance</td>
</tr>
<tr>
<td>ASC-H</td>
<td>atypical squamous cells, cannot exclude high-grade lesion</td>
</tr>
<tr>
<td>CEA</td>
<td>cost-effectiveness analysis</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CIN</td>
<td>cervical intraepithelial neoplasia</td>
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<tr>
<td>CIN1</td>
<td>cervical intraepithelial neoplasia grade 1</td>
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<tr>
<td>CIN2</td>
<td>cervical intraepithelial neoplasia grade 2</td>
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<tr>
<td>CIN2+</td>
<td>cervical intraepithelial neoplasia grade 2 or more</td>
</tr>
<tr>
<td>CIN3</td>
<td>cervical intraepithelial neoplasia grade 3</td>
</tr>
<tr>
<td>CIN3+</td>
<td>cervical intraepithelial neoplasia grade 3 or more</td>
</tr>
<tr>
<td>CIS</td>
<td>carcinoma in situ</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>ECC</td>
<td>endocervical curettage</td>
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<tr>
<td>FDA</td>
<td>(U.S.) Food and Drug Administration</td>
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<td>FTA</td>
<td>Flinders Technology Associates</td>
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<td>HC</td>
<td>hybrid capture</td>
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<td>HPV</td>
<td>human papillomavirus</td>
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<tr>
<td>ICER</td>
<td>incremental cost-effectiveness ratio</td>
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<tr>
<td>LBC</td>
<td>liquid-based cytology</td>
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<tr>
<td>LEEP</td>
<td>loop electrosurgical excision procedure</td>
</tr>
<tr>
<td>LLETZ</td>
<td>large loop excision of the transformation zone</td>
</tr>
<tr>
<td>NILM</td>
<td>negative for intraepithelial lesion or malignancy</td>
</tr>
<tr>
<td>Pap</td>
<td>Papanicolaou</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PPV</td>
<td>positive predictive value</td>
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<tr>
<td>RCT</td>
<td>randomized controlled trial</td>
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<tr>
<td>SCJ</td>
<td>squamocolumnar junction</td>
</tr>
<tr>
<td>SIL</td>
<td>squamous intraepithelial lesion</td>
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<tr>
<td>SNOMED</td>
<td>Systematized Nomenclature of Medicine</td>
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<tr>
<td>TZ</td>
<td>transformation zone</td>
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<tr>
<td>VF</td>
<td>vaginal fluid</td>
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<tr>
<td>VLP</td>
<td>virus-like particle</td>
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Introduction

Cervical cancer

Cervical cancer is the fourth most common cancer in women worldwide, with over 550,000 new cases and 311,000 related deaths in 2018 (1). Cervical cancer has significant differences in properties compared with many other cancers. First, the majority of cases appear at younger ages (47% in women aged <50 years), a period of life when many women are actively involved in their careers and caring for their families and this results also to most years of life expectancy lost (estimated at 29 years) in women. Second, cervical cancer has well-defined precancerous stages which can be diagnosed by the means of cytology and histopathology and subsequently treated by a relatively simple procedure, and development to cancer can thus be prevented (2). This fact resulted in initiation of cytology-based screening programs in many countries since late 1960s. Third, since 20 years it is also known that persistent infection with high-risk human papillomavirus (HPV) is a prerequisite for the development of cervical cancer (3, 4). This has led to further improvement of the screening programs and also the introduction of prophylactic HPV vaccination. By these means could cervical cancer actually be avertable today.

HPV infection

HPV infection is a necessary although not sufficient cause of cervical cancer. Risk factors of cervical HPV infection include young age at sexual debut, a high number of recent or lifetime sexual partners, low socioeconomic status, multiparity, oral contraceptive use, smoking, malnutrition, immune suppression, as well as certain genetic polymorphisms in the human leukocyte antigen system, while male circumcision and condom use are considered to reduce the risk (5).

HPV infection is the world’s most common sexually transmitted disease, but cervical cancer is a rare complication of it (6). The estimated worldwide prevalence of HPV is about 11%, being highest in East Africa and the Caribbean (>30%) and lowest in South Asia and North America (5–7%) (7). Prevalence is also higher at younger ages after sexual debut, being >20% in women aged <25 years and then steeply declining to about 5% in women aged around 50 years in developed countries, but with slightly rising prevalence after that
(8). HPV is easily transmitted in both genders, mostly by mucosal contact, and about 75% of sexually active individuals acquire the infection during their lifetime. HPV infections are asymptomatic and most of them clear within two years, but some infections may become latent or undetectable (9, 10). Persistence of HPV is consistently and strongly associated with the risk of developing high-grade cervical intraepithelial neoplasia (CIN2+) (11), which in turn brings an elevated risk of progression to cervical cancer (12). However, the carcinogenic process of cancer to develop from incident HPV infection usually takes time – approximately 5–10 years at a minimum and 20–25 years on average. HPV also causes cancers of the oropharynx, anus, vulva, vagina and penis and is today estimated to cause about 5% of all cancers globally (13).

HPV

HPV is a double-stranded DNA virus with a capsid which is 50–55 nm in diameter, having icosahedral symmetry (14). The viral genome is circular and approximately 7900 base-pairs long, including the following regions: long control region, early region and late region. While the long control region regulates viral gene expression and replication, the early region encodes proteins E1, E2, E4, E5 and oncoproteins E6 and E7, required for viral gene expression, replication and survival, and the late region encodes the capsid proteins L1 and L2.

HPV life cycle

HPV infects by first entering the basal layer of the epithelium through a microwound. The viral genome is maintained in these cells when genes E1 and E2 replicate episomes. Through expression of E6 and E7 the cells proliferate and move outwards towards the epithelial surface. In the mid layers the cells express all the early genes and the genome is amplified. In the upper layers L1 and L2 proteins are made, allowing the packaging of the amplified
viral genomes. It is likely to be a function of E4 that new virus particles are then released from the epithelial surface in a productive infection. If this viral gene expression is deregulated it can lead to high-grade intraepithelial neoplasia and if the viral genome is integrated into the host cell chromosome, it can then lead to the development of cancer (15).

![Figure 2](image)


**Prevention**

**Primary prevention**

Prevention of a disease in individuals without the disease is called primary prevention. In cervical cancer the issues are, for example, education and prophylactic HPV vaccination of the population.

**Secondary prevention**

Prevention of a disease by interrupting its progression through identification of early stages and then eliminating them is called secondary prevention. In cervical cancer this is done by screening the population and further diagnosing precancerous lesions in screening-positive individuals, and treating them.

**HPV vaccination**

In vaccine development the L1 gene is recombinantly expressed and then self-assembled to virus-like particles (VLPs) containing no viral genome. These VLPs constitute prophylactic vaccines which induce high levels of neutralizing antibodies in hosts.
HPV vaccines

The first-generation prophylactic HPV vaccines were registered in 2006, being a bivalent vaccine against high-risk HPV types 16 and 18, and a quadrivalent vaccine including activity against even low-risk HPV types 6 and 11, which cause genital warts. These vaccines have shown very high efficacy against type-specific infections and precancerous cervical lesions when vaccinating HPV-negative young women (aged 15–26 years) (16). The bivalent vaccine has also shown significant cross-protective effect against other high-risk types, especially HPV31 and HPV45 (17, 18). Safety has been confirmed in randomized controlled trials (RCTs) with over 73 000 participants that showed no difference between intervention and control arms as regards mild or severe systemic side effects (16), and by active surveillance after over 270 million vaccine doses given globally (18) during the last decade that have not shown any serious or unexpected side effects (19).

A national vaccination program was started in 2007 in Australia, and today at least 82 countries have introduced vaccination programs, but with different strategies (19). In Sweden vaccination is school-based and was started in 2012 for girls aged 11 years. In 2014, a next-generation nonavalent HPV vaccine against the same HPV types as the quadrivalent vaccine, and with additional five high-risk types (31, 33, 45, 52 and 58) was registered. While the first-generation vaccines are against the high-risk types that are responsible for about 70% of all cervical cancer cases, the next-generation vaccine is against
the high-risk types that are responsible for about 90% of all cervical cancer cases. As the nonavalent vaccine prevents infection and precancerous lesions related to virus types with similar efficacy as the quadrivalent vaccine, and shows a non-inferior antibody response (20), it has been estimated being cost-saving (21), and many countries are now switching to this alternative. Gender-neutral vaccination, by herd effect, can give comparable protective effectiveness even with low or moderate vaccination coverage (22); hence this is already implemented in many countries and is planned in Sweden next autumn.

Screening

The main principles of screening have been described by Wilson (23), including facts that the disease should be important, have a recognizable latent stage and the natural course should be understood. Secondly, there should be a screening test that is suitable, acceptable, accurate, reliable, sensitive and specific. Thirdly, the treatment should be effective and acceptable and there should be a policy as to who should be treated. Fourthly, the diagnosis and treatment should be cost-effective.

All these principles are partly fulfilled in cervical screening, and organized screening with cytology has resulted in a major reduction in both the incidence of cervical cancer and related mortality in developed countries (24). In Sweden, for instance, the incidence has been reduced to about a half, with 540 new cases and 153 related deaths in 2018 (25). Still, the disease has not disappeared despite screening for the last five decades, and the cancer incidence has stagnated at a reduced level (26). National audits have shown that 45–64% of all cancer cases are diagnosed among non-attenders, and these cancers were also diagnosed at a more advanced stage (27, 28). In a population perspective, non-adherence to screening invitations has been identified as the most important risk factor of incident cervical cancer. This means that it is crucial to make efforts to reach as high rate of participation in screening as possible. In Sweden coverage of screening has stagnated at a level of about 75% of women aged 23–70 years (29).

Continuous quality control of screening programs has led to identification of some problems. Screening by means of cytology has been unable to prevent cervical adenocarcinoma (30) and the overall low sensitivity of cytology is a well-known drawback. Regular sampling at 2–5 year intervals has, however, partly compensated for this weakness. Nevertheless, there has been an increasing incidence of cervical cancer in Sweden in recent years, mostly among women participating in screening with normal results in cytology (31), indicating a problem with the sensitivity of the technique. The evidence that HPV testing is more effective in reducing cervical cancer incidence compared with cervical cytology (32) has led to implementation of HPV-based primary
screening programs, a step that has not diminished the challenges in organizing existing screening programs.

Developing countries are still meeting major problems in establishing screening programs. The coverage of cervical screening in developing countries is on average only about 20% and the elderly and poor women, with the highest risk of cervical cancer, are least likely to be screened (33). There is an urgent need for educated staff to take care of all aspects of a screening program, not forgetting the education of a population, and cultural barriers. It is estimated that over 80% of cervical cancer cases occur in developing countries, where it accounts for 13% of all cancers in women (34). This important point should not be forgotten in research and when developing screening programs and strategies for the diagnosis and treatment of precancerous lesions.


Consequences of HPV vaccination

Even though HPV vaccination is known to reduce cervical cancer incidence, in near future there will still be a need for an appropriate screening program that must be adapted to a vaccinated population. Although an existing vaccination program will reduce the incidence, there are several issues remaining for attention. The currently used vaccines do not cover all the high-risk HPV types, population-level vaccination coverage is not 100% and the population with sexual debut before the era of vaccination will still exist for several decades.
The effect of vaccination on screening participation has been different in different populations. In Sweden, the vaccinated population has showed higher attendance to screening (35). Concerns have been raised about HPV ‘type replacement’, i.e. that non-vaccination HPV types would emerge after vaccination, but this has not been seen in countries with the longest vaccination programs (36), probably because of HPV’s stable DNA, with a slow evolution rate. However, the consequences of vaccination must still be closely followed. While vaccination eradicates the major high-risk HPV infections, the consequence in screening will be that a positive HPV-test result will be less predictive of CIN3+, because of fewer high-risk HPVs detected (37). This might allow longer screening intervals, and eventual additional triage strategies such as genotyping or assays of other biomarkers could be considered. Also, as HPV types 16 and 18 give rise to cervical cancer at younger ages than the other high-risk types (38), screening in a vaccinated population could be started later.

Cytology

The traditional cytological method in cervical screening has been Papanicolaou-stained cytology (Pap smear) on a glass slide (39). Cytological analysis has a high specificity (about 95%) but has been reported to have a problem with low sensitivity (about 50%) in detecting CIN (40). One crucial fact is that cytology is based on subjective analysis, with moderate interobserver, intralaboratory and interlaboratory variations (41). Efforts in education in morphological assessment of cytological samples, quality assurance of the laboratories and investments in new technologies such as liquid-based cytology (LBC) have been made during the last 50 years to increase the accuracy of cytology. However, the sensitivity is still not higher than approximately 70% at best (41, 42) and LBC is neither regarded as more sensitive nor more specific for detection of CIN2+ compared with conventional Pap smears (43). However, LBC has the advantage that HPV testing can be performed on the same sample, which can be adopted in different triage strategies.

Another, however minor, concern is the rate of false-positive test results, for example, in connection with immature squamous metaplasia, parakeratosis or inflammatory atypia. In settings with HPV testing as primary screening, the observer’s knowledge of the woman’s HPV status might increase the false-positive rate (44). To improve the sensitivity and reduce the rate of false-positive results in cytological screening, continued quality control of cytological laboratories is essential, not least because of steeply falling throughput rates when primary HPV screening is introduced.
Classification of cytology and histology

During the last few decades the terminology has been switching from the three-tier CIN1–3 (Richart) system to the two-tier Bethesda classification system (45) described in 1989, with the following cytological categories: negative for intraepithelial lesion or malignancy (NILM); atypical squamous cells of undetermined significance (ASCUS); atypical squamous cells, cannot exclude high-grade lesion (ASC-H); low-grade squamous intraepithelial lesion (LSIL); high-grade squamous intraepithelial lesion (HSIL); squamous cell carcinoma; atypical glandular cells (AGC); adenocarcinoma in situ (AIS); or adenocarcinoma (46).

The advantage of the Bethesda system is that it is based on the existence of two different forms of HPV infection, with productive infection leading to low-grade SIL and transforming infection leading to high-grade SIL. Current clinical management is mostly based on the two-tier system. This system was created to provide effective communication from laboratory to clinic, to facilitate cytology-histology correlation and to provide more reproducible results. It also gave rise to the concept of ASCUS (47). The Bethesda system is further applied in histological nomenclature. Here, even the CIN grade is often included in the results. However, during the studies included in this thesis the Swedish modification of CIN1–3 classification was used.
Colposcopy
Colposcopy is the standard method to investigate the cervix in women with atypical Pap smears and was first described by Hans Hinselmann in 1925. The purpose of colposcopic examination is to identify diseased tissue for targeted punch biopsy sampling for histological diagnosis. The procedure provides illuminated magnification of the cervix and various solutions (3–5% acetic acid and Lugol’s iodine) are applied for the evaluation.

Transformation Zone (TZ)
To describe and interpret colposcopic findings, colposcopists are recommended to use the terminology of the International Federation of Cervical Pathology and Colposcopy (48). Even this evaluation is subjective, with low reproducibility (49), and different scoring systems, for example the Reid index (50), have been developed for standardization. The latest modification, including even the lesion size in scoring, is the Swede score system (Table 1). The specificity of a score of ≥8 has been reported to be 90–95% for CIN2+, while a score of ≤3–4 speaks against CIN2+ (51, 52). This can be interpreted as recommending ‘see and treat’ management in cases of high scores and possibly refraining from biopsies in cases of low scores. Archiving electronic images taken during colposcopy in patient files is recommended.

Table 1. The Swede score system

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

A crucial assessment is defining the location of the squamocolumnar junction (SCJ) and the type of the TZ. The cervix is covered by both stratified non-keratinizing squamous cells and a single-cell layer of columnar epithelium. These two types of epithelium meet at the SCJ. The buffering action of the mucus covering the columnar cells is interfered when everted columnar epithelium is exposed to the acidic vaginal environment. This leads to the destruction and replacement of the columnar epithelium by newly formed metaplastic squamous epithelium. The metaplastic process mostly starts at the
original SCJ and proceeds centripetally towards the external os through the reproductive period to perimenopause. Thus, a new SCJ is formed between the newly formed metaplastic squamous epithelium and the columnar epithelium remaining everted onto the ectocervix and the TZ is the area between the original and the new SCJ.

This immature metaplastic squamous epithelium in the TZ is sensitive to persistent HPV infections and this is the site for transforming to atypical cells and therefore important to assess. In TZ types 1 and 2 the TZ is fully visible. In TZ type 3 the deeper limit is not visible and this is a common finding in the postmenopausal period when the TZ often retracts into the endocervix (53) (Figure 6).

**Figure 6.** Transformation zone (TZ) type 1 (A), TZ type 2 (B) and TZ type 3 (C). Black line: new squamocolumnar junction. Images by the author.

Evaluation of TZ type 3 is a challenge. Performance of biopsy sampling lacking the TZ is insufficient (54). It is also more difficult to obtain adequate amounts of tissue from the endocervix by cytological sampling or curettage for histology, as the sensitivity of endocervical cytobrush sampling in detecting lesions varies between 44–93%, and is even lower with endocervical curettage (ECC) for histological samples (55, 56); thus use of routine ECC is not encouraged (57). Nevertheless, the sensitivity of colposcopy and biopsy in detection of CIN2+ in women with abnormal cytology is around 70% (58, 59), and more than one biopsy is recommended to improve the accuracy (57, 59). Moreover, both the implementation of more sensitive HPV primary screening and prophylactic HPV vaccination might have an impact on the accuracy of colposcopy.

**Treatment of cervical precancerous lesions**

The procedures for treatment can be divided into ablative (cryotherapy, cold coagulation, radical diathermy and laser ablation) and excisional (cold knife
conization, laser conization, needle excision of the TZ and the loop electro-
surgical excision procedure (LEEP), called large loop excision of the transfor-
mation zone (LLETZ) in the UK. The excisional treatments have been devel-
oped from cold knife conization under general anesthesia to less invasive
procedures such as LEEP under local anesthesia, which is today the most
common procedure. The different treatments are similarly effective (60) but
still have adverse side effects such as infection or bleeding in the short term
and increased risk of late miscarriage and preterm labor (61), or cervical
stenosis (62) in the long term. These risks are higher in connection with more
radical excision techniques (cold knife and laser conization), and increase with
increased cone depth and when treatments are repeated.

The optimal management of women with histological CIN1 is surveillance,
since at least 70% of these lesions will resolve spontaneously and only very
few will progress (63). On the other hand, the recommended management of
histological CIN3 is excisional treatment because of the high risk of progres-
sion to cancer. Excisional treatment of CIN reduces the risk of invasive cervi-
cal cancer by 95% (64). However, it is well known that even in this group
management is mostly overtreatment, since only about 30% of women with
CIN3 develop invasive cancer in 30 years without treatment (65). When it
comes to histological CIN2, Swedish guidelines recommend excisional treat-
ment in women aged ≥25 years. However, the risk of progression to cancer in
this group is lower than in cases of CIN3, being only about 0.5% in two years
(66). During the same time period, the regression rate of CIN2 is 50% and the
progression rate is 18% in an overall population, but in women aged <30 years
the rates are 60% and 11% respectively (66). Knowing this, expectant man-
agement with follow-up in cases of CIN2 can be considered even in ≥25 years
old women in childbearing age. Diagnosis of CIN2 is also morphologically
equivocal, with lower reproducibility than CIN3 (67) (Figure 7). In unclear
cases immunohistochemical staining with labeled antibodies against p16ink4a
and Ki-67 can be used for more exact diagnosis (68). In addition, HPV16-
positive cases with CIN2 have shown a higher rate of persistency or progres-
sion (69). Moreover, DNA methylation panel as a biomarker has shown
promising results in predicting progression of CIN2 (70).
Prophylactic treatment of a precancerous lesion by a relatively simple procedure (LEEP) to avoid progression to cancer is acceptable in most cases. We cannot, however, predict which lesions would eventually become malignant if not treated, but we must keep in mind that any kind of invasive treatment is associated with adverse effects, fear, inconvenience and costs. Management should be based on careful selection of cases depending mainly on the grade of CIN, the type of the transformation zone and the age of the woman concerned.

HPV testing

The advantage of all molecular approaches in testing, such as HPV testing, is that the analysis is more objective, showing reduced variability and being less dependent on personnel compared with cytology. The first clinically validated commercial tests were the hybrid capture (HC)-based FDA (Food and Drug Administration)-approved HC2® test (QIAGEN, Gaithersburg, USA), and the polymerase chain reaction (PCR)-based GP5+/6+ PCR enzyme immunoassay, as they have shown good clinical performance in large randomized controlled trials (71-74). To have good clinical sensitivity and specificity for detection of CIN2+, a candidate test for screening purposes should be validated against either of these, according to guidelines (75, 76), and a more recent protocol for clinical validation of HPV tests, including genotyping, has been developed (VALGENT, Validation of HPV genotyping tests) (77).
HPV testing has a sensitivity for CIN2+ of about 95%, which has resulted in at least a 50% rise in detection of CIN2+ lesions and a significant reduction in the incidence of CIN3+ and invasive cervical cancer compared with cytological screening (78). As a consequence of the nature of HPV infection, which frequently clears, a disadvantage of HPV testing is its specificity, which is on average 6% lower compared with cytology (2), resulting in more screening-positive women needing follow-up. Also, the increase in detection of CIN2 has been reported to be higher than the increase of CIN3+ (73, 79, 80), raising concerns of overdiagnosis of self-clearing lesions. Swedish long-term follow-up of primary HPV screening showed, however, the same cumulative incidence of CIN2+ in both HPV and cytology arms, implying that the improved sensitivity of HPV screening results in earlier diagnosis of CIN2+ rather than overdiagnosis (81). Other HPV tests such as the APTIMA mRNA assay and the HC2 test at a higher viral-load cut-off point have shown higher specificity, with a small loss in sensitivity (82, 83).

Another important finding when including HPV testing in primary screening with cytology triage is the high negative predictive value for CIN2+ (84-86), this effect being maintained even in primary screening with HPV testing alone (87). This effect is also long-lasting, and screening intervals of six years when using HPV testing are regarded as safe and effective, resulting in higher cost-effectiveness.

**Triage in HPV primary screening**

Because of the relatively low specificity in HPV-based primary screening there is a need for a triage strategy to find out which women are at most risk of cancer development and thus need referral to colposcopy. Currently there is no perfect triage and different algorithms are under research and development. The RCTs presented so far (73, 79, 88, 89) have involved cytology triage and this is therefore considered as a validated option. Consequently, in current European guidelines, cytology is recommended for triage of HPV-positive women (90). However, since the sensitivity of cytology is lower than for the HPV test, HPV-positive women may have CIN2+ despite normal cytology. These women still represent a group with a slightly higher risk of CIN2+ (91, 92) and it is a challenge in developing appropriate follow-up algorithms. One alternative is to use partial genotyping (HPV16/18 or other high-risk type) (93, 94) with different follow-up intervals, and this algorithm was recently implemented in Swedish guidelines.

Other possible triage methods can be categorized into cytological or molecular. Cytological methods include p16\(^{ink4a}/Ki-67\) dual immunostaining (95, 96) and automated cytological evaluation (97). For these methods a cytological sample collected by professionals is needed and self-sampling cannot be adapted. Molecular methods involving HPV testing with extended geno-
typing (98), type-specific HPV viral load (99), viral and/or host gene methylation (particularly high in HPV16 infection, cervical cancer and advanced CIN3) (100, 101), and altered microRNA expression (affecting tumor suppressors or oncogenes) (102) can also be adapted in connection with self-samples. Combinations of different methods including cytology can also be used, e.g. combining extended genotyping with cytology (103), and specific risk-score algorithms have been developed (104). All these methods, however, are still under evaluation and not yet in current clinical use.

As HPV-negative women are at a very low risk of CIN2+ (105), repeated self-sampling for HPV testing to identify persistent infections could provide the highest protection against CIN2+. An alternative procedure for triage is simply repeating the HPV test after a couple of months. By way of this self-sampling strategy about 40% of women that are HPV-positive in their primary screening test have been found to clear the infection after 4–6 months (106), resulting in higher specificity for the detection of CIN2+ after the second sample.

HPV genotypes

Today over 200 different HPV types have been isolated and registered in The International Human Papillomavirus Reference Center. Of all the detected HPV genotypes about 40 are able to infect the genital tract. These can be divided into low-risk and high-risk types, where the low-risk types (e.g. 6 and 11) can cause genital warts (condylomata) while the high-risk types are associated with cervical cancer. In 2012 the International Agency for Research on Cancer defined 12 HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 as high-risk types (107). All these types are included in the alpha-papillomavirus family and the types with highest risk are types 16, 31, 33, 35, 52, 58 from the alpha9 family and type 18 from the alpha7 family; they are all included in nonavalent vaccination. Moreover, type 68 is defined as probably carcinogenic and types 26, 53, 66, 67, 70 and 73 as possibly carcinogenic, with less evidence, and these last six types are usually not included in available HPV tests.

Different types have different properties concerning prevalence, persistence and progression to CIN (108). HPV16 is described as the most common and persistent type, with a worldwide prevalence of about 3%. In total, 96% of all cervical cancer cases are attributable to one of the 12 high-risk types and type 68. The most carcinogenic type is HPV16, being associated with 60% of all cervical cancer. Moreover, HPV16 and 18 together are associated with 70% of all cervical cancer. Types 18 and 45 are not associated with a particularly high risk of CIN but are consistently related to adenocarcinoma (109) and are therefore particularly important. With this background, genotyping for HPV16/18 is proposed as an alternative triage strategy in primary HPV
screening (94), and many commercial HPV tests report positivity for these types, and the other high-risk types as one group.

The level of type-specific viral load is often measured semi-quantitatively, demanding specific characteristics of an HPV test. In serial measurements it has been found that women with high viral loads have more persistent infections (110). The use of viral load in triage may therefore constitute a reasonable strategy. A high viral load was first reported to be important as regards HPV16 infections (111), but later a similar pattern was seen for the other genotypes in the alpha9 family, showing a clear correlation between viral load and CIN2+ (99). Developing management algorithms, however, is complex; every genotype has a different viral-load level giving the same risk of CIN2+ and more data is needed to establish the optimal titer thresholds for stratification of screening-positive women. Multiple infections were earlier described as being associated with a higher risk of CIN2+, but it rather seems that the risk of CIN2+ in a multiple infection is equal to the risk associated with individual genotype with highest risk (99, 109, 112).

**HPV persistence**

A type-specific persistent infection with high-risk HPV is a prerequisite for the development of cervical cancer (3). Even if a persistent HPV infection is a major risk factor of cervical cancer, very few HPV-positive women will develop the disease (113). Most genital HPV infections are transient, with the highest clearance in young women, and in a college population more than 90% will have cleared the infection within 18 months (114). In a population of all women aged >18 years about two thirds of HPV infections cleared within a year (115). Studies on women aged >60 years have shown just over 60% HPV persistence after 3.5 months (116) and about 55% HPV persistence after 5.5 months (117), indicating continuing clearance even in the elderly.

There is still no consensus of opinion concerning the definition of persistency of an HPV infection. In a Columbian prospective study on HPV persistence, they proposed persistent infection to be defined as infection lasting more than the median duration, for example, 9.5 months for HPV16 in women aged >30 years (118). In a large meta-analysis, Koshiol et al. (11) remarked that even testing intervals of ≤ six months produce strong summative relative risks as regards the association between HPV persistence and CIN2+. It is therefore suggested that repeat HPV testing at six months is a valuable way to identify women at increased risk of cervical precancerous lesions and cancer.

It is the type-specific persistency that increases a woman’s risk of CIN2+ or cervical cancer, but to accurately define an individual woman’s HPV persistency is not possible without knowing the exact HPV type. This is the advantage with an HPV test with extended genotyping, and, for example, in ‘test of cure’ it is useful to detect type-specific persistence, that is associated with a high risk of recurrent CIN2+ (119).
HPV self-sampling

Highly sensitive molecular methods such as HPV testing offer the possibility of self-sampling where cytological analysis cannot be adopted (120). The advantages of self-sampling are several: the majority of women prefer it as a more convenient method (121-123) although some women are concerned about test accuracy and their ability to correctly carry out the procedure. Although complementary strategies such as offering invitations with timed appointments (124), reminder letters (125) and telephone calls (126) result in higher participation in screening (124), population coverage is still about 80% at most.

Self-sampling for HPV testing has been shown to increase screening participation in non-attenders vs. other options (127-134). This strategy has also led to higher detection rates of CIN2+ than cytology-based screening (135) and offering self-sampling for non-attenders is recommended in current Swedish guidelines. In a large meta-analysis reduced sensitivity to detect CIN2+ in self-samples was noted when analyzed by way of signal-based assays, but no reduced sensitivity in the detection of CIN2+ in self-samples was reported when HPV testing was performed using amplification-based methods such as PCR (136, 137). A randomized study concerning a clinically validated PCR-based HPV test in a paired screen-positive design showed similar accuracy in self-samples and clinical samples in detection of CIN2+ and CIN3+ (138). Self-sampling has been carried out for the greatest number of years in the Netherlands, where self-sampling is available on request today and is planned to be introduced as a default option in screening in the near future (139).

It has recently been proposed that all HPV tests used in connection with self-sampling should be validated for their accuracy and show agreement with clinician-collected sampling in a designated protocol (VALHUDES, Validation of HPV assays and collection devices for HPV testing on self-samples and urine samples) (140). Every part of HPV testing of self-samples, including sampling material (vaginal fluid, urine), collection device (swab, brush, lavage, tampon), and storage material (liquid, dry), together with the used validated HPV analysis method should also be accredited. Urine sampling might be regarded as more acceptable in self-sampling than vaginal sampling (141) but has shown lower clinical sensitivity for CIN2+ than vaginal sampling (137, 142, 143). Different collection devices and storage media have shown similar results in accuracy (137), but dry storage material is preferred as regards price, safety aspects and easier mailing, where a flat medium is optimal. It is of importance that the self-sampling kit includes clear information of the sampling procedure, which should be easily understood and acceptable for the women. In the choice of test, the possibility of biobanking the samples should also be taken into account.
Self-sampling can be used in different strategies such as opt-in, direct mailing, door-to-door or community campaigns. In an opt-in strategy women need to confirm acceptance of receiving a self-sampling kit, or they pick up the kit by themselves. This strategy has resulted in reduced participation (144-146) in comparison with direct mailing. Door-to-door offering has resulted in a high rate of participation in a low-resource setting but is not feasible in wide-scale screening (133). Before implementing self-sampling for HPV-testing in primary cervical screening, a careful pilot study should be carried out to assess feasibility, the clinical accuracy of the combination of the considered HPV test together with the sampling device and storage medium, not to forget the costs, logistics, and population compliance. Also, not all women feel comfortable with self-sampling and the possibility of sampling by medical professionals should also remain available.

Health economics

Since one of the principles of screening is that the diagnosis and treatment of the disease should be cost-effective, this is a point that should be validated. Any possible changes in an organized screening program should be carefully evaluated. As primary screening with HPV testing gives major reductions in the number of cancer cases even with longer screening intervals and offers an opportunity for self-sampling, it could be expected to be a cost-effective screening alternative.

The results of several studies around the world with somewhat different settings support the notion that HPV-based screening is cost-effective vs. cytological screening when applied among a population aged >30–35 years at five-year intervals (94, 147-153). Self-sampling results in increased response rates among non-responders vs. other options (127, 131-134, 154) and hence results in increased coverage, which can also result in fewer and earlier-detected cancer cases, followed by cost savings. Self-sampling for HPV testing is one of the most effective and cost-effective interventions to improve participation as regards non-responders in several countries (155-160). When self-sampling is offered to non-responders some concerns about costs in regard to switching have been raised, but the costs are compensated for if high-level coverage is reached (157).

When it comes to primary screening, a modelling study has shown that in women aged ≥35 years with repeated vaginal self-sampling, HPV testing is potentially cost-effective compared with conventional clinician-taken Pap smear cytology even in maintained screening intervals (161). In low- and middle-income countries self-sampling in primary screening could be cost-effective if high-level coverage is achieved (162).
Cost-effectiveness analysis (CEA)

In a world with a continuous inflow of new medical technologies, interventions and treatments with rising costs, healthcare providers are in need of ways to evaluate the obtained benefits in relation to additional resources spent. A cost-effectiveness analysis is designed to allow decision-makers to clearly understand the tradeoffs of costs, harms, and benefits between alternative interventions and to combine those considerations into a single metric, the incremental cost-effectiveness ratio (ICER), which can be used to inform decision-makers (163). The ICER is defined as the ratio of the incremental difference in total cost to the incremental difference in effectiveness when comparing alternatives. ICERs can then be used to compare different interventions to define which one provides greatest value for money. A low ICER can mean that intervention improves health at a small additional cost per unit of health. A negative ICER can mean either that the new intervention is less costly than the existing one or that the new intervention is less effective than the existing one. An intervention is ‘dominated’ if it is higher in cost and less effective than the comparator and is not of good value for money. In a CEA with a societal perspective all costs from formal and informal healthcare sectors and costs from the non-healthcare sector should be taken account, while a CEA with a healthcare perspective only includes formal healthcare-sector costs (costs for the patient and costs for a third-party payer [other than patient/healthcare provider]) (164). A cost-effectiveness analysis is validated by a sensitivity analysis of different effects and costs.
Aims

The overall aim of this work was to increase knowledge about the use of self-sampling for HPV testing in primary cervical screening.

The specific aims of the studies were:

I  To compare repeated self-sampling for HPV testing with Pap smear cytology in detection of CIN2+ in primary cervical screening.

II To evaluate the proportion of cases of histological CIN2+ after LEEP in women with persistent HPV infection and normal Pap smear results.

III First, to compare the cost-effectiveness of repeated self-sampling for HPV testing with Pap smear cytology in primary cervical screening. Second, to estimate the cost of treatment and follow-up of histological CIN2+ in connection with these screening strategies.

IV To compare self-sampling and sampling by medical professionals for HPV testing in detection of CIN2+ and CIN3+ when using a combination of an FTA card as storage medium and a PCR-based HPV test.
Material and methods

Study population

Studies I and III

During 2013–2015 a total of 36,390 women aged 30–49 years (at the date of invitation) scheduled for regular screening invitation in Uppsala County, Sweden, were included. We excluded women with previous hysterectomy, current pregnancy or clinical test results (Pap smear cytology, HPV test or histology) relating to cervical cancer registered within one year before the date of invitation. The follow-up period was 18 months from the date of invitation.

Study II

From April 2013 until March 2016 we prospectively recruited 91 women aged over 40 years with persistent HPV infection without any abnormalities in cytology at the gynecological out-patient clinic, Uppsala University Hospital. We excluded women who had plans for future pregnancies, who could not understand the information in Swedish, and where LEEP was regarded as being technically difficult to perform.

Study IV

During March and April 2016 a total of 11,951 women aged 30–60 years (at the date of invitation) scheduled for a regular screening invitation in Uppsala County were included. After sampling, women with clinical test results (Pap smear cytology, HPV test or histology) relating to cervical cancer registered within one year prior to the start of the study period were excluded from the analysis. The women whose first HPV samples arrived at the HPV laboratory during 2016 were included in the analysis. The follow-up period was 18 months from the start of the study period.

Ethics

All clinical data were coded and analyzed anonymously. In Studies I, II and IV participants received oral and/or written information, and consent was
given. All study designs were approved by the Regional Ethics Committee, Uppsala, Sweden (Dnr 2012/099 for Studies I and III, Dnr 2012/460 for Study II, Dnr 2016/008 and Dnr 2019/929 for Study IV).

Methods

During the study period (Studies I–IV), the regular screening program in Uppsala County was 3-yearly Pap smears for women aged 23–49 years and 5–yearly HPV tests for women aged 50–60 years. Women not attending screening were recalled the following year.

Study I

By means of a computer-based allocation process the women were randomized in two groups, one to perform self-sampling of vaginal fluid (VF) for HPV testing (n=17,997, HPV arm) and the other group to undergo screening by Pap smear cytology (n=18,393, control arm).

HPV arm

Women in the HPV arm were sent an invitation including information on how to perform the sampling at home, a sampling brush, an FTA (Flinders Technology Associates) card and a preaddressed return envelope. The FTA card was returned by regular mail to the HPV lab at Uppsala University for HPV testing. A reminder was sent to women who did not return their self-sample within three weeks. Women who were HPV-positive in their first self-sample were informed of the test result and told that they could contact a gynecologist if they had any questions or symptoms. These women were sent a new kit in 3–6 months to repeat the self-sampling. Women who were HPV-positive in two consecutive self-sampling tests were referred to colposcopy. HPV-negative women in the first or second HPV test were referred back to the regular screening program. Women who chose not to participate in the study were returned to the regular screening for Pap smear sampling.

Control arm

Women in the control arm were managed according to the regular screening program in Uppsala County during the study period where a midwife performed cervical sampling for Pap smear cytology. Women with CIN2+ based on cytology were referred to colposcopy within a month, while women with CIN1/ASCUS based on cytology were offered follow-up with HPV test and Pap smear cytology after three months, according to the clinical routine. All HPV-positive women and women with CIN2+ in follow-up cytology were referred to colposcopy and eventual biopsies. HPV-negative women without
CIN2+ in follow-up cytology were referred back to the regular screening program (Figure 8).

**Self-sampling**

The method for self-sampling of VF has been described previously (165). The women were instructed to perform self-sampling of VF using a Viba-brush® (Rovers Medical Devices, Oss, The Netherlands) and to apply the VF sample to the indicating FTA elute micro card (GE Healthcare, Cardiff, UK, art. no WB129308) (Figure 9). Together with the sampling kit women received instructions on how to perform the collection of VF and a link to a dedicated homepage at Uppsala University Hospital with animation of the self-sampling procedure. Briefly, the women were asked to place the brush approximately 5–10 cm into the vagina and gently rotate it once. Then they were instructed to remove the brush and apply the vaginal sample to the FTA card by placing the brush in the middle of the application area and rolling it one full circle across that area and letting it air-dry for a few minutes. They were then required to close the lid, place the card in the envelope and send it by regular mail to the Department of Immunology, Genetics and Pathology at Uppsala University (HPV laboratory) for HPV testing.
**Sample processing**

At the HPV laboratory, the FTA cards were processed using an automated laboratory system (easyPunch STARlet; Hamilton Robotics, Bonaduz, Switzerland). A robot arm picks up each card, takes a photograph of the sampling area, and using machine-learning software for calculation of which parts of the card contain the highest concentrations of cells, and thereafter punches four circular pieces of 3 mm diameter. All four pieces are collected into a single well in a 96-well microtiter plate and DNA extracted as described earlier (166).

**HPV testing**

HPV testing was performed using the real-time PCR-based assay hpVIR (167, 168). Clinical validation of this test was performed after Paper I but during this thesis work (169). This test detects and quantifies a human single-copy gene (housekeeping gene), HMBS (Homo sapiens hydroxymethylbilane synthase; GenBank accession no. M95623.1) as a control to ensure that the sample contains enough cellular material for the test to be informative. The test detects and quantifies the following HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. The results are presented as individual types, except for HPV18/45, detected as a group, and HPV33/52/58, also detected as a group. The limit of detection of HPV is 10 HPV copies per PCR. In order for a sample to contain enough material for the HPV test to be informative, a threshold of 10 copies of the nuclear single-copy gene per PCR is used (167).

**Colposcopy, cytology and histology**

At the colposcopy visit a Pap smear was collected in cases with no previous cytology results during the study. The colposcopic evaluation included identification of the squamocolumnar junction and TZ with application of 5% acetic acid and iodine solution. Directed biopsy samples were obtained from all the identified abnormal areas and a random biopsy sample was taken in women with normal results in colposcopy. In cases of TZ type 3 with an invisible squamocolumnar junction, an additional sample for endocervical cytology was taken. All cytology and histology was performed at the Clinic of Pathology and Cytology, Uppsala University Hospital. Classification was carried out according to the Swedish modification of SNOMED (Systematized Nomenclature of Medicine; College of American Pathologists, Skokie, IL, USA), describing the findings in cases of CIN, and the highest histological grade found in each patient was used for interpretation of the results.

**Statistics**

The data were first analyzed using a *per-protocol approach*, including only women assigned to the two arms who complied with the protocol. The cumulative prevalence of CIN2+ based on histology per 1000 women screened, as
well as per 1000 woman-years screened was calculated in the two study arms. The data was also analyzed using an intention-to-treat approach, also including women in the HPV arm who were HPV-positive in their self-sample screening test, but who on their own initiative had a clinical examination performed before receiving their second kit for self-sampling. All statistical calculations were performed using R (R Core Team, 2014). Two-sided Wilcoxon’s tests were used in comparison of the participants’ ages and days to diagnosis between the arms. Binomial tests were used in comparison of participation, prevalence and positive predictive value (PPV) between the arms. P-values were corrected for multiple testing using Bonferroni correction and P-values <0.05 were considered significant, unless stated otherwise.

Study II
Eligible women attended a gynecological examination including an HPV test, a Pap smear, endocervical cytology and colposcopy with biopsies and a diagnostic LEEP. All postmenopausal women were treated with local estradiol for at least two weeks before the visit to optimize the vaginal mucosa and minimize the possible risk of postoperative cervical stenosis. All women that were HPV-positive at the study visit underwent follow-up 6–12 months after LEEP, with a Pap smear and an HPV test. Women that did not participate in the study were followed up with annual Pap smears and HPV tests.

Colposcopy, cytology and histology
As described for Study I. All biopsy and LEEP samples were subjected to histological examination.

HPV testing
Samples for HPV testing were collected with a cytobrush and applied to an indicating FTA elute micro-card and processed and analyzed as described in Study I.

Study III
For health-economic evaluation, clinical data used in Study I were retrieved from a database at the Department of Pathology and Cytology, Uppsala University Hospital. All events from invitation until diagnosis and treatment were noted for each patient in both study arms. The treatment records, including further preoperative assessment and follow-up after treatment in cases of CIN2+, were manually checked in the patient files up to 31 December 2018. All events were included after LEEP until the ‘test of cure’ was accepted (HPV-negative and Pap smear cytology <CIN2), or after surgical treatment of cancer, until the first postoperative visit. Follow-up and possible treatments in cases of CIN1 were not included in this analysis.
Treatment of precancerous lesions and cancers

Women with histological CIN2+ were treated according to current clinical recommendations. In the Pap smear arm, about one fifth of the women with CIN2+ were treated at a regional hospital (Enköping hospital, Enköping). The rest of the women with CIN2+ and women with cancer were treated at the Department of Gynecology and Obstetrics, Uppsala University Hospital. Precancerous lesions and micro-invasive cancers were treated by LEEP, most of them under local anesthesia but some under general anesthesia (e.g. all women in Enköping). Treated women were invited for a ‘test of cure’ appointment with a midwife or a gynecologist in 4–6 months. At this appointment, the midwives collected a Pap smear and a sample for HPV testing, and in addition, the gynecologist also carried out colposcopy. The cancer cases were discussed at a multidisciplinary meeting after requisite radiological investigation, usually chest and abdominal CT scans and a pelvic MR scan. Surgical treatment consisted of either simple or radical hysterectomy or trachelectomy. Radical surgery included excision of the upper vagina and parametria with bilateral pelvic lymphadenectomy beyond removal of the uterus (hysterectomy) or the cervix (trachelectomy). Surgery was performed either by laparotomy or in most cases by means of minimally invasive techniques, such as laparoscopy or robotic-assisted laparoscopic surgery.

Cost-effectiveness analysis (CEA) and cost estimation

A CEA was performed using a healthcare provider perspective (170). The unit costs for each screening event were retrieved from the HPV laboratory and Uppsala-region financial records. Direct medical costs of inpatient and outpatient healthcare were retrieved from the financial records at Uppsala University Hospital. When needed, costs were adjusted for inflation by using the consumer price index (CPI) (171) and converted to 2019 Euros (€ 1 = 10.5912 SEK). A cost per screened woman was calculated in each study arm. Screening strategies (HPV self-sampling vs. Pap smear) were ranked from the lowest to the most costly. Incremental cost-effectiveness ratios (ICERs) per extra screened woman were calculated by dividing the cost difference (cost) by the difference in number of screened women (effect) between the two screening arms. If a screening arm was more costly and less effective than the comparative one, it was defined as strongly dominated. A sensitivity analysis was performed to account for the uncertainty of screen participation and trends in direct medical costs. Moreover, using the same cost data we estimated the cost of treatment and follow-up of histological CIN2+.
Study IV

By using a computer-based allocation process the women were randomized into two groups, one to perform vaginal self-sampling (n= 5961, SS arm), and the other group to receive an invitation to undergo cervical sampling by medical professionals (n= 5990, SMP arm), with subsequent HPV testing of all samples.

Self-sampling (SS arm)

Women in the SS arm were sent an invitation together with a kit including a Rovers® Viba-brush, an FTA card, a postage-paid return envelope and information on how to perform the sampling, described in Study I. Women that were HPV-positive in their first self-sample were informed that they would be sent an additional kit to repeat the self-sampling about six months after the first sample was collected, but that they could contact a midwife or a gynecologist if they had questions or symptoms. Women that were HPV-positive in two consecutive samples were referred to colposcopy. Women that were HPV-negative in their first or second sample were referred back to regular screening.

Sampling by medical professionals (SMP arm)

Women in the SMP arm were sent an FTA card together with an invitation to book an appointment at a local midwife clinic for cervical sampling with a cytobrush. After sampling, the FTA card was sent to the HPV laboratory for HPV testing. Women that were HPV-positive in their first sample were informed that they would be sent an additional FTA card with an invitation to book an appointment at the midwife clinic for repeated sampling about six months after the first sample was collected, but that they could contact a midwife or a gynecologist earlier if they had questions or symptoms. Women that were HPV-positive in two consecutive samples were referred to colposcopy. Women that were HPV-negative in their first or second sample were referred back to regular screening.

HPV testing

As described for Study I.

Colposcopy, cytology and histology

As described for Study I.

Statistical analysis

The data were analyzed by using both a per-protocol approach and an intention-to-treat approach. The primary outcome was the prevalence of detected CIN2+ and CIN3+ per 1000 screened women. Statistical calculations were performed by using R (version 3.5.3) and IBM SPSS (version 26) software.
Fisher’s exact test was used to compare proportions between the two independent study groups with respect to nominal variables (sampling method, participation and diagnostic outcomes), but the binomial test was used in comparison of PPVs. P-values <0.05 were considered to indicate statistical significance.
Results

Study I

The number of women included and excluded at each stage of the study and the number of CIN2+ detected is shown in Figure 10. The HPV arm included 17,046 eligible women that were sent a sampling kit, and among these 7,997 performed self-sampling for HPV testing. The control arm included 16,364 eligible women who were invited to Pap smear cytology, out of which 6,364 were sampled. The mean age of the participants was similar in the two study arms. The participation rate in the HPV arm was 47% as compared with 39% in the cytology arm (p<0.01).

In the HPV arm, 6.3% of the women (554/7997) were HPV-positive in the primary screening test. A high proportion of these women (90%, 501/554) performed repeat self-sampling for HPV testing, on average 4.4 months after the first sampling. Of those positive in the first test the second HPV test was positive in 71% (355/501) of the women. Following the per-protocol approach, 162 women received a CIN2+ diagnosis, and of these women, 48% (77/162) had CIN3+ and 52% (85/162) had CIN2.

Among the women that were HPV-positive in their first self-sample, 53 did not perform the second self-sampling and 37 of them requested clinical follow-up before receiving the second kit. Among these women, 13 received a CIN2+ diagnosis (four had CIN3+ and nine had CIN2). These were included in the intention-to-treat calculation. In total, 175 women had CIN2+, 94 women had CIN2 and 81 women had CIN3+ (including six cancers) in the HPV arm.

In the control arm, 3.5% (222/6364) had an abnormal cytology result (≥ASCUS) in their Pap smear screening and 85% (188/222) of these women participated in clinical follow-up. Among these women, 69 received a CIN2+ diagnosis and of these women, 75% (52/69) had CIN3+ (including five cancers) and 25% (17/69) had CIN2.
The cumulative prevalence of CIN2+ per 1000 women screened was 21.9 (175/7997 × 1000) (95% CI 18.68–25.09) in the HPV arm (per-protocol and intention-to-treat) compared with 10.8 (69/6364 × 1000) (95% CI 7.77–12.70) in the control arm (Figure 11B) (p<0.01). The cumulative prevalence of CIN2+ was statistically significantly higher in the HPV arm than in the control arm even when divided into age groups of 30–39 and 40–49 years.

The two screening strategies identified about the same number of cases of CIN3+ per 1000 women screened (HPV arm: 10.1 (81/7997 × 1000) (95% CI 7.9–12.3), control arm: 8.2 (52/6364 × 1000) (95% CI 6.0–10.4)) (p=0.03). However, in the HPV arm four times as many CIN2 lesions per 1000 women screened were identified as in the control arm (HPV arm: 11.7 (94/7997 × 1000) (95% CI 9.3–14.1), control arm: 2.7 (17/6364 × 1000) (95% CI 1.4–4.0)) (p<0.01) (Figure 11A).
The PPV (per-protocol) for detection of CIN2+ was 0.46 (95% CI 0.41–0.51, n=162 of 355) in the HPV arm and 0.37 (95% CI 0.30–0.44, n=69 of 188) in the cytology arm (p<0.01).

Study II

In the 40 women who underwent examination including LEEP, the mean duration of known HPV persistence was 20 months (median 12, range 4–93). The mean age of the women was 58 years (median 59, range 41–77) and 83% (33/40) of the women were postmenopausal. Before the study, 25/40 women had undergone one Pap smear, 13/40 had undergone two Pap smears, 1/40 had undergone three Pap smears and 1/40 had undergone seven Pap smears, with normal results. Although all the Pap smears and endocervical samples obtained at the study visit were normal, 3/40 Pap smears were reported to lack columnar cells. At colposcopy, 28/40 (70%) of the women had TZ type 3. None of the biopsy samples confirmed CIN2+, but six showed CIN1. In five cases a biopsy sample was lacking because it was difficult to obtain for technical reasons. The LEEP samples showed that 20 women had no CIN, 14 women had CIN1, two women had CIN2 and four women had CIN3. Four out of six women with CIN2+ had TZ type 3, but two women with CIN2+ had a fully visible normal TZ. Five out of six CIN2+ excisions showed free endocervical margins and 34/40 histological samples included the whole TZ.

HPV analysis of the samples obtained at the study visit revealed that 21/40 women had cleared their HPV infection, while 19/40 women still had a persistent HPV infection. The most common HPV types that persisted were...
HPV16 (n=5) and the HPV33/52/58 group (n=5) and all the women with CIN2+ were positive for different HPV types. The known mean duration of HPV persistence among the women with CIN2+ was 13 months (range 7–20) and the known mean duration of HPV persistence among the women without CIN was 26 months (range 7–93). None of the 21 women who were HPV-negative at the study visit showed CIN2+ in histology.

At follow-up 6–12 months after LEEP, all six women with CIN2+ had become HPV-negative and had normal cytology. Among eight HPV-positive women with no dysplasia in the LEEP sample, six women still had a persistent HPV infection at follow-up (Figure 12).

**Study III**

**CEA on primary screening including clinical follow-up**

The total cost of primary screening was higher in the Pap smear arm (€ 781 139 for 6364 women participating) than in the HPV self-sampling arm (€ 228 642 for 7997 women participating), and the Pap smear arm was thus strongly dominated (more expensive and less effective) (Table 2). The HPV self-sampling arm also revealed the detection of more histological CIN2+ at a lower cost in comparison with the Pap smear arm and is thus a cost-saving alternative (Clinical follow-up, Table 2). Sensitivity analysis of participation
rate, screening-test cost (Pap smear analysis and HPV test analysis) and self-sampling kit cost did not affect the results (Table 3).

Table 2. Resources required per screened woman by intervention arm, with associated costs in 2019 (€ 1 = 10.5912 SEK).

<table>
<thead>
<tr>
<th></th>
<th>Pap smear</th>
<th>HPV self-sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=18 393)</td>
<td>(n=17 997)</td>
</tr>
<tr>
<td><strong>Unit Cost (€)</strong></td>
<td><strong>Costs (€)</strong></td>
<td><strong>Units</strong></td>
</tr>
<tr>
<td>Primary screening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pap smear cytological analysis</td>
<td>25</td>
<td>6 364</td>
</tr>
<tr>
<td>Midwife appointment for sampling</td>
<td>98</td>
<td>6 364</td>
</tr>
<tr>
<td>First HPV self-sampling</td>
<td>27</td>
<td>7 997</td>
</tr>
<tr>
<td>2nd HPV self-sampling</td>
<td>27</td>
<td>501</td>
</tr>
<tr>
<td><strong>Total cost of primary screening</strong></td>
<td>781 139</td>
<td>228 642</td>
</tr>
<tr>
<td>Screened women</td>
<td>6 364</td>
<td>7 997</td>
</tr>
<tr>
<td>Abnormal cytology in need of clinical follow-up</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>2nd HPV-positive in need of clinical follow-up</td>
<td>355</td>
<td></td>
</tr>
<tr>
<td>Incremental effect (screened women)</td>
<td>1 633</td>
<td></td>
</tr>
<tr>
<td>Cost per screened woman</td>
<td>123</td>
<td>29</td>
</tr>
<tr>
<td>ICER per extra screened woman</td>
<td>-338</td>
<td></td>
</tr>
<tr>
<td>Clinical follow-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pap smear cytological analysis</td>
<td>25</td>
<td>238</td>
</tr>
<tr>
<td>HPV analysis*</td>
<td>38</td>
<td>173</td>
</tr>
<tr>
<td>Midwife appointment for sampling</td>
<td>98</td>
<td>166</td>
</tr>
<tr>
<td>Colposcopy appointment</td>
<td>470</td>
<td>114</td>
</tr>
<tr>
<td>Biopsy histological analysis</td>
<td>147</td>
<td>114</td>
</tr>
<tr>
<td><strong>Total cost of clinical follow-up</strong></td>
<td>99 130</td>
<td>247 269</td>
</tr>
<tr>
<td><strong>Total cost of primary screening + clinical follow-up</strong></td>
<td>881 902</td>
<td>476 715</td>
</tr>
<tr>
<td>CIN2+</td>
<td>68</td>
<td>175</td>
</tr>
<tr>
<td>Incremental effect (CIN2+)</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Cost per woman with CIN2+</td>
<td>12 964</td>
<td>2 724</td>
</tr>
<tr>
<td>ICER per extra detected CIN2+</td>
<td>-3 787</td>
<td></td>
</tr>
</tbody>
</table>

*Total cost of HPV test including HPV kit and analysis performed at the HPV laboratory, Uppsala University and transfer of the results to the database at the Department of Cytology and Pathology, Uppsala University Hospital.
Table 3. Sensitivity analysis (ICER per extra detected women with CIN2+).

<table>
<thead>
<tr>
<th>Efficacy parameters</th>
<th>Unit Cost (€)</th>
<th>Units</th>
<th>Costs (€)</th>
<th>Units</th>
<th>Costs (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participation rate +25% Pap smear</td>
<td>7 955</td>
<td>5 451</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participation rate +25% HPV</td>
<td>-3 124</td>
<td>9 996</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participation rate -25% Pap smear</td>
<td>4 773</td>
<td>-111</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participation rate -25% HPV</td>
<td>-4 129</td>
<td>5 998</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Screening cost variation             |               |        |           |        |           |
| HPV testing +25%                     | 34            | -3 092 |           |        |           |
| HPV testing -25%                     | 20            | -4 160 |           |        |           |
| Pap smear +25%                       | 32            | -3 990 |           |        |           |
| Pap smear -25%                       | 19            | -3 262 |           |        |           |
| Midwife appointment +25%             | 122           | -5 076 |           |        |           |
| Midwife appointment -25%             | 73            | -2 176 |           |        |           |
| Midwife appointment -50%             | 49            | -727   |           |        |           |
| Midwife appointment -75%             | 24            | 723    |           |        |           |
| Midwife appointment -85%             | 20            | 1 013  |           |        |           |

Cost estimation of treatment and follow-up of CIN2+
In the Pap smear arm, 68 women had CIN2+, i.e. five women with invasive cancer, 17 women with CIN2 and 46 with CIN3 or AIS (Table 4). In the HPV self-sampling arm, 175 women had CIN2+, i.e. nine with invasive cancer, 94 with CIN2 and 72 with CIN3 or AIS. As a result of different data-retrieval procedures, numbers differ somewhat from those in Study I. The total cost of treatment of histological CIN2+, was € 444 125 for 192 treatments in the HPV self-sampling arm and € 235 211 for 70 treatments in the Pap smear arm. Cost per treated woman was 45% higher in the Pap smear arm (€ 3675) compared with the HPV self-sampling arm (€ 2538) (Table 4).
Table 4. Resources required for treatment including further preoperative assessment of CIN2+ among women by intervention group, with associated costs in 2019 (€ 1 = 10.5912 SEK).

| Study IV                          | The number of women included and excluded at each stage of the study and the number of CIN2+ detected is shown in Figure 13. In the SS arm 5767 women received the invitation (no returning mail) and in the SMP arm 5844 women received the invitation. The participation rate was 44% in the SS arm, |

<table>
<thead>
<tr>
<th></th>
<th>Pap smear</th>
<th>HPV self-sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unit Cost (€)</td>
<td>Units</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>775</td>
<td>2</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>775</td>
<td>3</td>
</tr>
<tr>
<td>CIN2</td>
<td>775</td>
<td>17</td>
</tr>
<tr>
<td>CIN3/AIS</td>
<td>775</td>
<td>46</td>
</tr>
<tr>
<td><strong>CIN2+</strong></td>
<td><strong>68</strong></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excision under local anesthesia</td>
<td>753</td>
<td>40</td>
</tr>
<tr>
<td>Excision under general anesthesia</td>
<td>1 767</td>
<td>23</td>
</tr>
<tr>
<td>Hysterectomy (mini-invasive)</td>
<td>5 825</td>
<td>3</td>
</tr>
<tr>
<td>Hysterectomy/trachelectomy (radical)</td>
<td>11 973</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total number of treatments</strong></td>
<td><strong>70</strong></td>
<td></td>
</tr>
<tr>
<td>Inpatient care (mean)</td>
<td>968</td>
<td>24</td>
</tr>
<tr>
<td><strong>Radiology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal CT scan</td>
<td>145</td>
<td>5</td>
</tr>
<tr>
<td>Thorax CT scan</td>
<td>131</td>
<td>5</td>
</tr>
<tr>
<td>Pelvic MR scan</td>
<td>339</td>
<td>4</td>
</tr>
<tr>
<td>Multidisciplinary meeting (primary)</td>
<td>2 647</td>
<td>6</td>
</tr>
<tr>
<td>Multidisciplinary meeting (repeated)</td>
<td>1 511</td>
<td>3</td>
</tr>
<tr>
<td><strong>Treatment cost</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total cost</strong></td>
<td><strong>235 211</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Treated women</strong></td>
<td>64</td>
<td></td>
</tr>
<tr>
<td><strong>Cost per treated woman</strong></td>
<td>3 675</td>
<td></td>
</tr>
</tbody>
</table>
compared with 27% in the SMP arm (p<0.001). After exclusion, 2466 women were eligible in the SS arm and 1519 women were eligible in the SMP arm. The mean age of participating and eligible women was 42.4 years in the SS arm and 41.5 years in the SMP arm, with a difference between the arms (p=0.001).

Figure 13. Study design with number of women included and excluded at different steps in the self-sampling arm and the sampling by medical professionals arm.

The prevalence of HPV in the first test was 6.8% (167/2466) in the SS arm and 7.8% (118/1519) in the SMP arm (p=0.26) (Table 5). Compliance of HPV-positive women for second sampling was high, 88.6% (148/167) in the SS arm and 89.8% (106/118) in the SMP arm (p=0.85). The second sample was collected on average 7.2 months after the first in the SS arm and 7.7 months after the first in the SMP arm. Of those positive in the first test the second HPV test was positive in 67.6% (100/148) in the SS arm and in 61.3% (65/106) in the SMP arm (p=0.37). The screening positive rate after the second HPV test was 4.1% (100/2466) in the SS arm and 4.3% (65/1519) in the SMP arm (p=0.74). When the study population was divided into age groups, there was no significant difference in prevalence but there was a trend in the youngest group (30–39 years) where the HPV prevalence tended to be lower in the SS arm (7.6%) than in the SMP arm (10.3%) (p=0.06) in the first HPV test.
Table 5. HPV prevalence in the study arms and different age groups (per-protocol).

<table>
<thead>
<tr>
<th></th>
<th>Self-sampling n (%)</th>
<th>Professional sampling n (%)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1st HPV test</strong>, all ages</td>
<td>167 (6.8)</td>
<td>118 (7.8)</td>
<td>0.26</td>
</tr>
<tr>
<td>30-39 years</td>
<td>74 (7.6)</td>
<td>68 (10.3)</td>
<td>0.06</td>
</tr>
<tr>
<td>40-49 years</td>
<td>65 (6.3)</td>
<td>37 (5.5)</td>
<td>0.53</td>
</tr>
<tr>
<td>50-60 years</td>
<td>28 (6.1)</td>
<td>13 (6.9)</td>
<td>0.72</td>
</tr>
<tr>
<td><strong>2nd HPV test</strong>, all ages</td>
<td>100 (4.1)</td>
<td>65 (4.3)</td>
<td>0.74</td>
</tr>
<tr>
<td>30-39 years</td>
<td>42 (4.3)</td>
<td>39 (5.9)</td>
<td>0.16</td>
</tr>
<tr>
<td>40-49 years</td>
<td>40 (3.9)</td>
<td>20 (3.0)</td>
<td>0.35</td>
</tr>
<tr>
<td>50-60 years</td>
<td>18 (3.9)</td>
<td>6 (3.2)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

The prevalence of different HPV types in the first HPV test was similar in the two study arms. HPV16 was the most common type, followed by HPV33/52/58 in both study arms. HPV51 was the only type more common in the SMP arm than in the SS arm, but in the second HPV test this difference had disappeared and most HPV51 infections turned out to be transient.

In the SS arm, 43 women with a positive second HPV test result received a CIN2+ diagnosis and 35 received a CIN3+ diagnosis (per-protocol approach). Among the women that were HPV-positive in their first self-sample, 19 did not perform the second self-sampling, but 10 of them instead requested an earlier clinical follow-up. Among these women, five received a CIN3 diagnosis and were included in the intention-to-treat calculation. In total, 48 women had CIN2+ and 40 women had CIN3+ in the SS arm. In the SMP arm, 32 women with a positive second HPV test result received a CIN2+ diagnosis and 23 received a CIN3+ diagnosis (per-protocol approach). Among the women that were HPV-positive in their first self-sample, 12 did not obtain a second sample by midwife but five of them instead requested an earlier clinical follow-up. Among these women, three received a CIN3 diagnosis and one a CIN2 diagnosis and were included in the intention-to-treat calculation. In total, 35 women had a CIN2+ and 25 women had a CIN3+ in the SMP arm.

The prevalence of CIN2+ per 1000 screened women was 17 (43/2466 × 1000) (95%CI 13–24) in the SS arm and 21 (32/1519 × 1000) (95%CI 15–30) in the SMP arm (p=0.47). The prevalence of CIN3+ per 1000 screened women was 14 (35/2466 × 1000) (95%CI 10–20) in the SS arm and 15 (23/1519 × 1000) (95%CI 10–23) in the SMP arm (p=0.79) in per-protocol approach. Including women from the intention-to-treat approach did not influence the prevalence markedly. Neither was there a significant difference in prevalence when dividing the women into age groups, but in the youngest age group (30–39 years; per-protocol approach), a tendency towards a slightly lower prevalence of CIN2+ and CIN3+ was observed in the SS arm compared with the SMP arm (Table 6).
Table 6. Detection of CIN2+ and CIN3+ in the study arms and different age groups (per-protocol approach).

<table>
<thead>
<tr>
<th>Prevalence per 1000 women screened [95% CI]</th>
<th>Self-sampling (n=2466)</th>
<th>Professional sampling (n=1519)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CIN2+, all ages</strong></td>
<td>17 [13-24]</td>
<td>21 [15-30]</td>
<td>0.47</td>
</tr>
<tr>
<td>30-39 years (n=976)</td>
<td>17 [11-28]</td>
<td>30 [19-47]</td>
<td>0.09</td>
</tr>
<tr>
<td>40-49 years (n=1030)</td>
<td>19 [12-30]</td>
<td>15 [8-28]</td>
<td>0.57</td>
</tr>
<tr>
<td>50-60 years (n=460)</td>
<td>13 [5-30]</td>
<td>11 [2-42]</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>CIN3+, all ages</strong></td>
<td>14 [10-20]</td>
<td>15 [10-23]</td>
<td>0.79</td>
</tr>
<tr>
<td>30-39 years (n=976)</td>
<td>14 [8-25]</td>
<td>27 [17-44]</td>
<td>0.07</td>
</tr>
<tr>
<td>40-49 years (n=1030)</td>
<td>15 [8-24]</td>
<td>6 [2-16]</td>
<td>0.16</td>
</tr>
<tr>
<td>50-60 years (n=460)</td>
<td>13 [5-30]</td>
<td>5 [0-34]</td>
<td>0.68</td>
</tr>
</tbody>
</table>

The PPV in connection with detection of CIN2+ (per-protocol approach) was 0.43 (95% CI 0.33–0.53, n= 43 of 100) in the SS arm and 0.49 (95% CI 0.37–0.62, n= 32 of 65) in the SMP arm (p=0.52).

In all women with histological CIN2+, the Pap smears collected at the study visit were abnormal (≥ASCUS) in 38/48 (79.2%) in the SS arm and in 18/35 (51.4%) in the SMP arm. In all women with histological CIN3+, the Pap smears were abnormal (≥ASCUS) in 31/40 (77.5%) in the SS arm and in 16/25 (64.0%) in the SMP arm. In the overall study population, the sensitivity of Pap smear was 67.5% in the detection of histological CIN2+ and 72.3% in the detection of histological CIN3+. 
Discussion

Low population coverage of screening has been identified as an important risk factor of incident cervical cancer. Cases detected outside screening programs are usually more advanced, with worse prognosis, and participation in screening is thus of utmost importance. Earlier studies have shown a higher participation rate among non-attenders when offered self-sampling. The same pattern was seen in Study I, where the participation rate among a primary screening population was significantly higher in the HPV arm (self-sampling) compared with the cytology arm (sampling by medical professionals). This was also seen in Study IV, where the participation rate was higher for self-sampling than sampling by medical professionals. In the cost-effectiveness analysis (Study III) the improvement in effect (defined as women participating) was an additional factor why cytology was ‘dominated’ by self-sampling for HPV testing. However, the participation rate remained under 50% in Studies I and IV, while the participation rate in screening in Uppsala County was over 60% during the same time period. This difference can be explained in two ways. First, there is usually lower participation in studies, and this is probably the major reason for the difference we saw. Second, a shorter time for follow-up than in regular screening might also explain a significant part of the difference.

As HPV testing is a more sensitive but less specific screening strategy than cytology, it results in more screening-positive women needing follow-up. Additional triage is required to reduce the number of women needing inconvenient, resource-demanding colposcopy by a specialist. An optimal triage strategy would be a more specific method that does not lose too much in sensitivity. In HPV-based cervical screening, the most validated triage method today is cytology, maybe combined with partial 16/18 genotyping. However, the lower sensitivity of the cytological method results in a group of HPV-positive women with normal cytology who still have a slightly higher risk of CIN2+. An alternative triage method is repeated self-sampling for HPV testing after a couple of months, as used in Studies I, III and IV. By this means, in Study I, only 71% of the initially HPV-positive women had a persistent infection, resulting in a screening-positive rate needing clinical follow-up of 4.4% after triage by repeated self-sampling. In Study IV, self-sampling and sampling by medical professionals resulted in 60% and 55% HPV persistency, respectively, and 4.1% and 4.3% screen-positive rates needing clinical follow-
up, respectively, using this strategy. The screen-positive rates needing clinical follow-up in cytological screening were 2.7–9.5% in Sweden in 2014.

It would be advantageous to carry out triage on the initial screening sample, since no women would be lost to follow-up by having second sampling. This strategy is used for LBC samples that can be analyzed for both HPV and cytology. The need for a second sample when considering repeated sampling should be carefully monitored, since there is an obvious risk of loss to follow-up. An overview of eight RCTs showed that compliance with repeated clinician sampling after 6–12 months among HPV-positive/cytology-negative women was 55–73%, while compliance after direct referral to colposcopy was around 90% (172). This might also reflect the impact of the time interval between interventions. However, a meta-analysis of RCTs on non-attenders to screening showed over 80% compliance to direct follow-up (either by cytology or colposcopy) after an HPV-positive self-sample.

Studies on repeated self-sampling for HPV testing in primary screening have consequently shown high rates of compliance to second sampling: in Study I, 90%, and in Study IV nearly 90% and 93% (173). These studies support the fact that compliance is fairly good when applying repeated self-sampling. Over 50% of women not performing a second self-sampling are lost to follow-up because of symptoms and they seek an immediate appointment instead. The high rate of compliance to second self-sampling can be explained by the fact that women are aware that they had an abnormal test result and this made them highly motivated to perform second self-sampling. Also, the sampling method was already known and easy to perform at home. So, even if triage on the initial screening sample might be desirable, repeated self-sampling seems to be a feasible possibility, with a high rate of compliance.

Even though specificity is increased after repeated self-sampling for HPV testing we still noticed an approximately threefold increase in the number of colposcopies in the HPV arm compared with the cytology arm (Study I). This suggests a need for additional triage. One possible option could be to use extended genotyping, since there is a great difference in risk of CIN2+ with the different types of high-risk HPV, a strategy that also opens up investigation of type-specific persistency. This could have been an alternative, as the used HPV test (hpVIR) already presents the results as individual HPV types, except for HPV18/45 and HPV33/52/58, which are detected as groups. In addition, as hpVIR even quantifies individual HPV types and groups, viral load could also serve as a possibility for triage (174). Preliminary results on other molecular markers such as methylation have not been promising because of the small amount of DNA on the FTA card. Successful early triage would mean that women at a higher risk of CIN2+ and cancer could be identified and referred directly for colposcopy as early as after the first self-sample, and women at a lower risk could have a longer time interval between the first and second self-sample, allowing a greater chance to clear their HPV infection.
The increased number of colposcopies after introducing repeated self-sampling for HPV testing might be temporary, since a new screening test with better sensitivity detects a larger proportion of prevalent lesions in the screened population. Earlier RCTs on HPV primary screening with cytology triage revealed that a large proportion of the screened women needed referral during the transition period to this approach. In time this problem was resolved and increased referral was limited to the first round of testing. By the second round the referral rate had declined to the same level or an even lower one compared with cytology (74, 175). While concerns have been raised about the increase in number of colposcopies carried out in connection with HPV-based screening, it should not be forgotten that the total number of colposcopies also depends on clinical follow-up guidelines and individual clinical management. The fact that HPV testing has a high negative predictive value for CIN can also add support to decision-making by clinicians, avoiding repeated unnecessary colposcopies.

In Study I, repeated self-sampling for HPV testing resulted in detection of more than twice as many cases of histological CIN2+ compared with Pap smear cytology in primary cervical screening. Even though a higher number of screening-positive women needed clinical follow-up after self-sampling, in the cost-effectiveness analysis of primary screening, including clinical follow-up (Study III), self-sampling for HPV testing was still ‘dominant’, mostly because of significantly fewer screening appointments at healthcare facilities. Also, the cost per women with detected CIN2+ in the HPV self-sampling arm was only about 20% of that in the Pap smear arm. Analysis of CIN2 and CIN3+ separately (Study I) resulted in four times as many cases of CIN2 per 1000 women screened in the HPV arm, but no statistically significant increase in the detection rate of CIN3+ between the arms. The study was powered to show a difference in the detection rate of CIN2+ and not CIN2 and CIN3+ separately, thus meaning that the study probably was under-powered to show a difference in the detection rate of CIN3+.

These results might be interpreted as giving an earlier detection of CIN, but as CIN2 clears more often than CIN3, there is a risk of over-diagnosing and unnecessary treatments. Also, CIN2 is an equivocal diagnosis with a lower reproducibility than CIN3, and therefore some cases of CIN2 might constitute CIN1 and some CIN3. The current routine for increasing the accuracy of histological diagnosis is to use immunostaining with p16ink4a in unclear CIN2 cases, a strategy that could be expanded to all CIN2 cases to reduce the false-positive rate. However, even p16ink4a-positive CIN2 infections clear in most cases, unlike CIN3, where a larger proportion persist or progress to cancer (176). This new screening strategy with repeated self-sampling for HPV testing does not include cytology as in all the former screening strategies, and hence the role of colposcopy becomes even more challenging. Using more sensitive screening might result in discovery of smaller lesions, reducing the proportion of larger lesions detected (177). Histological CIN2 detected after
an abnormal cytology result might indicate a larger lesion in the cervix, while histological CIN2 detected after a positive HPV result alone could be more local. There might therefore be differences in the risk of malignant progression in CIN2 detected by HPV testing compared with CIN2 detected by cytology. Furthermore, as the observers’ knowledge of a woman’s HPV status might increase the false-positive rate in cytology (44), there might also be the same impact as regards histological evaluation. However, in cases with HPV type 16/18 infection, the accuracy of colposcopy is better than in women with other types of HPV (57, 178). Direct referral for colposcopy in cases with HPV 16/18 positivity is recommended in some national guidelines, for example in Australia.

Hence, as many guidelines recommend immediate excisional treatment in all cases with histological HSIL (CIN2 or CIN3) in women aged ≥25 years, the risk of emergent overtreatment with repeated self-sampling for HPV testing needs to be considered. Since the youngest women (aged <25 years) with CIN2 are already monitored conservatively (avoiding immediate treatment), this management could possibly be expanded to somewhat older age categories, in particular to women of reproductive age (179). Here, however, it is crucial to have good adherence to follow-up at the same time as inconvenient and costly unnecessary extra appointments should be avoided.

A screening strategy with repeated self-sampling for HPV testing results in high sensitivity for CIN2+ and at least to some extent separates transient infections from persistent ones, thereby increasing the specificity. About 30–45% of HPV-positive women had cleared their infection at the repeat test (Studies I and IV) and were thus referred back to screening. However, a recent Dutch post-hoc analysis describes a higher risk of CIN3+ five years later for women that were HPV-positive in the first screening round and were then HPV-negative at follow-up (180). These women might thus require different surveillance and this should be emphasized in future studies and considered when updating guidelines on screening intervals.

Concerns regarding the emotional impact of screening by testing for a sexually transmitted HPV infection have been raised and studied after triage because of LSIL cytology and ‘test of cure’, but this impact has not been shown to be more pronounced among women that were HPV-positive compared with those with abnormal cytology (181, 182). However, attitudes towards self-sampling for HPV testing have been positive in screening populations with only some concerns about accuracy. Hence, the importance of sufficient comprehensive information for women testing positive is of utmost importance. After the first self-sampling in Studies I and IV, women with a positive HPV-test result received information on what an HPV infection means, and the fact that they would be offered repeat self-sampling after 4–6 months. They were also advised to contact a gynecologist if they had any questions or symptoms. A few (7% in Study I and 6% in Study IV) of the women in the self-sampling arm requested direct follow-up, some of these
because of symptoms. In sampling by medical professionals in Study IV, slightly fewer (4%) requested direct follow-up, a difference that might be a result of personal oral information or greater confidence in sampling. It is clear, however, that women feel confident and comfortable with repeated self-sampling and that the intervals between the first and second self-sampling (about four months in Study I and about seven months in Study IV) were acceptable to most women.

The emotional impact of HPV-positivity without cytological and/or colposcopic abnormalities should not be neglected. Knowledge of an untreatable infection with an elevated risk of precancerous lesions that might be difficult to detect, often resulting in long-term surveillance, might result in psychological stress, and, further, unnecessary treatments. In Study II, 6/40 (15%) women with persistent HPV infection and no detectable abnormalities in cytology had histological CIN2+ in the LEEP sample. Similar or slightly lower rates have been described in two recent Swedish studies on postmenopausal women who underwent LEEP because of HPV persistency (116, 183), but the cytological results differed somewhat between the studies. All three studies also showed relatively high HPV clearance (30–50% during ≤ one year), even among older women, which is important to remember at follow-up. Long-term follow-up of a large Swedish RCT (Swede screen) among younger women (32–38 years of age) showed that HPV infection among cytology-negative women resulted in either clearance or development of HSIL in six years (184). None of these studies have shown CIN2+ in cases with cleared HPV infection, supporting earlier evidence of the high negative predictive value for CIN2+ of HPV testing.

Concerning the impact of age on the risk of developing CIN, the incidence of CIN2+ was higher in the younger population in the Swede screen study than in older women and an earlier study showed that women aged 30–44 years had a significantly higher risk of CIN3+ than women aged 45–64 years (185). However, in older women the accuracy of cytology and colposcopy is lower and there is a risk that CIN might remain undetected (116, 117). Theoretically, CIN might carry a higher risk of cancer development in older women, since their immune defense is not as good as in younger women. The risk of cancer in connection with a new HPV infection after the age of 40 years is estimated to be low (186), but at the same time there is growing evidence that reactivation of latent HPV infections can be associated with a higher risk of CIN2+ (9). These results highlight the importance of continued follow-up of women as long as HPV infection persists, despite normal cytology, because of the risk of undetected CIN2+. In selected cases can LEEP be an opportunity to detect and treat a lesion simultaneously.

Type-specific genotyping may not be necessary in cases of verified CIN2+ but it could serve as a research opportunity at follow-up of HPV-positive women with normal cytology. The reason why extended genotyping might be important in such cases is that type-specific persistence is defined as a risk
factor of CIN2+. HPV16 is identified as the most prevalent single high-risk genotype in women aged ≥25 years and has been shown to carry the highest risk of CIN3+ in a screening population covering all ages (187). In Study II, however, only one of the six women with CIN2+ were persistently infected with HPV16. Similar results have been shown in three studies on older women, where the majority of cases of CIN2+ were caused by types other than HPV16 (183, 188, 189). This indicates that follow-up of non-HPV16/18 also is of utmost importance, in particular in older women. In addition, HPV18 and 45 have been consistently related to adenocarcinoma and show a lower risk as regards CIN. Persistent positivity of these two genotypes may indicate a lesion in columnar epithelium not easily detectable by colposcopy, and might warrant further investigations deeper in the endocervical canal (cytology/ECC/LEEP), in particular in cases with TZ3.

The optimal management of women with histological CIN1 is surveillance, since at least 70% of these lesions will resolve spontaneously and only a few will progress. However, in persistent cases with additional risk factors such as smoking, LEEP can be considered as a valid option. Even though LEEP performed because of CIN1 is associated with a higher HPV-positive rate at follow-up when compared with treatment of CIN2+. Also, in Study II, LEEP carried out in women with CIN2+ tended to clear the HPV infection earlier than when carried out in women with CIN1. This can probably be explained by the fact that in addition to a real high-risk HPV infection, a histological appearance suggestive of CIN1 can result from a low-risk HPV infection or some other minor disturbance of the cervix. In addition, it does not seem possible to treat a persistent HPV infection without CIN by means of LEEP, since most of these women (6/8) still tested HPV-positive at follow-up (Study II). Sometimes CIN2+ can be excluded with certainty only by LEEP with benign histology. Even if an HPV infection were to persist after treatment, the exclusion of CIN2+ could be reassuring in these cases.

The most commonly identified reason why self-sampling is cost-effective is because of a higher participation rate in screening (191). In the cost-effectiveness analysis (Study III), the major reasons for superiority of HPV self-sampling were that women did not need an appointment with a healthcare provider, together with a higher participation rate. This resulted in profitable cost-effectiveness and HPV self-sampling was regarded as ‘dominant’. Greater test sensitivity and longer intervals between tests further improve cost-effectiveness in the long run. In Study III, only direct medical costs during the study period were included. With current knowledge of the cost-effectiveness of HPV-based screening and the effect of self-sampling on participation, it could be predicted that in the long run cost-effectiveness would be even larger in this population. Also, the inclusion of costs from a societal perspective, for example direct non-medical costs (like transportation) and indirect costs (like
cost of time out of work), further improves the cost-effectiveness of self-sampling (192).

Total costs are important from a societal perspective and many cost-effectiveness studies have been concentrated on life-time costs. From a payer’s perspective (e.g. healthcare), decisions need to be made at a level where costs must be set in a real-life scenario for easier comparison of different alternatives. Here, the intermediate costs of different effects such as cost per women screened or cost per CIN2+ diagnosed/treated are of importance and presented in Study III. A limitation of this study was the difficulty of retrieving reliable cost data from several different places/people involved in healthcare. Strengths include the fact that all the costs were obtained from the same county and applied to a population in a randomized study.

Costs of sampling material and HPV analysis also influence cost-effectiveness. For self-sampling, direct mailing of kits has been associated with a higher participation rate than in an opt-in strategy where women need to confirm their wish to receive a kit. Direct mailing of kits was adopted in Studies I and IV. However, concerns about cost-effectiveness have been raised because of costs associated with unused kits (193). Two studies on young women, carried out to compare these strategies, however, showed that direct mailing of kits is likely to be more cost-effective than an opt-in strategy, but with higher overall costs (158, 194). In the end it is the price of the self-sampling kit that is crucial. A low cost per kit balances the cost of unused kits. In the cost-effectiveness analysis in Study III, the price of an HPV kit was defined as being 30% higher than the original approximated cost in order to compensate for wasted kits, based on a known 70% participation rate in screening. However, the participation rate in the study was just under 50%. As a consequence, the calculated cost of a kit should have been 50% higher than the original price. This would have resulted in a € 25 000 rise in costs in the HPV self-sampling arm (Study III), which would then have resulted in a slightly reduced difference in the results. It must be remembered that the cost of a sampling kit is only about one third of the total cost of self-sampling, including HPV testing in the laboratory.

Even though PCR-based HPV testing is regarded as accurate in self-samples, clinical performance in connection with the detection of CIN2+ should be evaluated for every combination of self-sampling kit and HPV test. When combining FTA cards as storage media with the PCR-based test hpVIR, performance regarding the detection of CIN2+ seems to be similar for self-sampling and sampling by medical professionals, as has been shown in a previous study on women aged 50–60 years (173), and in Study IV on women aged 30–60 years. Before introducing self-sampling as a default option in primary screening, it is important to have a well-organized infrastructure, and after introduction outcomes should be closely monitored at all levels, from a system for invitation to follow-up. Today there are still several problems with liquid-based techniques that need attention, including problems with
biobanking the samples for future retesting and research (195). FTA cards, on the other hand, are easy to apply in a screening setting, since extraction of DNA is fast and automated, and requires only a simple wash with warm water (166) before the PCR-based HPV analysis (169), and FTA cards are stable and easy to biobank.

In addition to screening, self-sampling for HPV testing could be extended to other clinical contexts. As a positive HPV test result after treatment of CIN2+ predicts treatment failure accurately (196), self-sampling for HPV testing might even be used as a ‘test of cure’ (197). It could also be possible to use self-sampling for HPV testing in follow-up of histological CIN1, since a negative HPV test result has a very high negative predictive value for CIN2+, a strategy that could serve as an opportunity to reduce the need of repeated and in many cases unnecessary colposcopies. These and maybe other possibilities could serve as safe, feasible and convenient alternatives for women, which could also result in cost savings.

In recent decades, gynecology as a specialty has developed from a tradition with regular control appointments without symptoms to what nowadays has become a practice of seeking a gynecologist when having symptoms/need help. Today the major reason for symptom-free women to have a gynecological examination is for sampling within a screening program. Most women experience gynecological examination as a very special situation that may violate their integrity and often be experienced as inconvenient, even if most women can cope with it. Also, some women experience a huge barrier to gynecological examinations because of unaddressed fear, earlier trauma or divergent gender identity. As such women are also often identified as possessing a higher risk of cervical cancer, self-sampling is a great opportunity for them to obtain a sample. In cases of HPV positivity as an additional risk factor, even these women may accept gynecological examination or could obtain further help. In some cases general anesthesia may be needed for further sampling.

To conclude, secondary prevention of cervical cancer is still beset with several problems such as organization and coverage of screening programs, sensitivity of cytology, accuracy of colposcopy as well as treatment of cervical lesions in forms of both overtreatment and undertreatment. With a high population coverage using a safe and highly effective prophylactic HPV vaccination, cervical cancer can be preventable. With efforts made towards this ultimate goal, I truly believe that self-sampling for HPV testing will be the best strategy in primary cervical screening in the near future. Self-sampling for HPV testing will above all facilitate women’s participation in screening. With continuous societal pressure to cut costs in healthcare, self-sampling for HPV testing would also serve as a cost-saving alternative to existing cervical screening programs.
Future perspectives

The number of colposcopies was significantly higher in connection with HPV-based screening compared with cytology-based screening according to the results of Study I. Earlier, the results of RCTs have indicated that this rise would be temporary and decrease in subsequent screening rounds. It is also unclear if the same proportion of CIN2 cases detected by HPV testing would have progressed to CIN3+ as those found with cytology. That is why it would be interesting to study this population in follow-up after some 10 years. In the time period when the studies were performed, routine screening in Uppsala County consisted of 3-yearly Pap smears for women aged 23–49 years and 5-yearly HPV tests for women aged 50–60 years. In February 2017 even women aged ≥50 years returned to Pap smear screening. LBC was introduced in 2018 and HPV-based primary screening among women aged ≥30 years was introduced in summer 2019. A follow-up study could thus be based on screening data obtained during the coming 3–4 years, since the screening interval is three years. It would also be interesting to compare the recently implemented HPV/liquid-based screening program with repeated self-sampling for HPV testing, both according to the detection rate of CIN2+ and cost-effectiveness.

Today there is no ultimate strategy concerning women that are HPV-positive to select those that need immediate clinical follow-up and treatment from those that can be observed and retested. The PCR-based HPV test (hpVIR) used in Studies I and IV detects, quantifies and presents the results separately for HPV types 16, 31, 35, 39, 51, 56 and 59, and in groups for HPV types 18/45 and 33/52/58. Both quantification and extended genotyping could serve as opportunities in triage and should be subject to future studies. Types 16, 18, 31 and 33 are associated with the highest risk and types 35, 45, 52 and 58 for next highest risk (99, 108), and this, together with viral load, could be used to guide management.

Management of persistently HPV-positive women with TZ3 and normal cytology is still a challenge. Current Swedish guidelines recommend diagnostic excision in cases with 1–2.5 years of HPV persistency. But, as the related treatments cause inconvenience and complications, and are costly, and the fact that it is not known whether this is optimal, there is an urgent need for further studies. As shown in Study II and by others, histological results should be carefully monitored in order to acquire knowledge and for further development of guidelines, particularly among older women. This includes studies on
more expectant management in cases with HPV types 39, 51, 56 and 59, which are associated with a lower risk of cancer development.

The importance of a high rate of participation in screening and the superiority of screening by self-sampling in comparison with current screening as regards cost-effectiveness are now well known. This should encourage increasing implementation of self-sampling as the new routine in screening programs. Text-message reminders (short-message service [SMS] reminders) have moderately increased participation in cancer screening (198), but this strategy is not studied in cervical screening although it could be an attractive opportunity, complementary to self-sampling, for more fluent cervical screening. A next step would be implementation of a possible future screening program based on repeat self-sampling for HPV testing with careful monitoring. This, combined with better strategies for triage, and SMS reminders, would imply better coverage, health-economic benefits and, finally, fewer cases of cervical cancer.
Conclusions

Study I
Repeated self-sampling for HPV testing showed a more than twofold higher detection rate of CIN2+ compared with Pap smear cytology in primary cervical screening. No difference was seen between the two study arms in the detection rate of CIN3+, but repeated self-sampling for HPV testing resulted in a fourfold higher detection rate of CIN2.

Study II
In women with persistent HPV infection and normal Pap smear results 15% (6/40) were revealed to have histological CIN2+ in LEEP samples.

Study III
Repeated self-sampling for HPV testing is a cost-effective alternative compared with Pap smear cytology in primary cervical screening. In estimation of treatment costs, self-sampling for HPV testing resulted in an approximately one third lower cost per treated woman, including follow-up, than Pap smear cytology.

Study IV
Self-sampling and sampling by medical professionals for HPV testing resulted in similar rates of detection of CIN2+ and CIN3+ when using a combination of an FTA card as storage medium and a PCR-based HPV test.

Humant papillomvirus av högrisktyp (HPV) är den helt dominerande orsaken till livmoderhalscancer. Analys av HPV är en känsligare metod för att upptäcka cellförändringar än cellprover för cytologisk analys. Socialstyrelsen rekommenderar därför idag att screening för livmoderhalscancer primärt bör göras med analys för HPV för kvinnor över 30 år. Utöver fördelen med högre känslighet kan provet för analys av HPV tas av kvinnan själv, vilket inte är möjligt vid cytologisk analys. Själprovtagning hemma fördelas av de flesta kvinnor och har medfört högre deltagande hos kvinnor som tidigare inte deltagit i screeningen.


I den andra delstudien utvärderades hur stor andel av de kvinnor som de är HPV-positiva men med normalt cellprov som har höggradiga cellföränd ringar. Fyrtio HPV-positiva kvinnor genomgick en gynekologisk undersökning som avslutades med en behandling där en del av livmodertappen togs bort (konisering) och analyserades för eventuella cellförändringar av en patholog. Av dessa 40 kvinnor hade 15 % höggradiga cellförändringar som inte hade kunnat upptäckas på annat sätt. Resultatet från den andra delstudien kan påverka hur man kliniskt bedömer kvinnor som är HPV-positiva men har normalt cellprov samt vilken information som ges till dessa kvinnor.

I den tredje delstudien undersöks kostnadseffektiviteten av primärscreening med upprepad självprovtagning för analys av HPV jämfört med
cellprov taget hos barnmorska i samma population som i den första delstudien. Självprovtagning för analys av HPV visade sig vara ett kostnadseffektivt alternativ jämfört med cellprov. Detta berodde bland annat på högre deltagande och upptäckt av fler fall av höggradiga cellförändringar till en lägre kostnad vid primärscreening med självprovtagning för analys av HPV jämfört med cellprov. Även kostnaderna för behandling av höggradiga cellförändringar jämfördes, men detta ingick ej i kostnadseffektivitetsanalysen. Kostnaden per behandlad kvinna med höggradiga cellförändringar var 47 % högre i cellprovgruppen (€ 3675) jämfört med självprovtagningsgruppen (€ 2495). Denna information kan utgöra en del av underlaget för beslut om framtida screeningprogram.

I den fjärde delstudien jämfördes upprepad självprovtagning med provtagning hos barnmorska för analys av HPV beträffande upptäckt av höggradiga cellförändringar vid screening för livmoderhalscancer. Det är viktigt att utvärdera tillförlitligheten i varje kombination av självprovtagningskit och metod för analys av HPV. Under mars och april 2016 randomiserades 11 951 kvinnor i åldrarna 30-60 år till självprovtagning eller provtagning av en barnmorska för analys av HPV. Provet applicerades på FTA-kort och analysen gjordes med en PCR-baserad metod (hpVIR). Upptäckten av höggradiga cellförändringar var likartad oavsett om provet togs av kvinnan själv eller av en barnmorska. Denna studie ger alltså stöd för att den använda kombinationen av självprovtagningskit och HPV-analys skulle kunna användas för primärscreening av livmoderhalscancer.


Kolmannessa osatutkimuksessa arvioitiin kustannustehokkuutta toistetun HPV-kotinäytteenoton ja Papa-testin välillä kohdunkaulansyövän seulonnassa samassa väestössä kuin ensimmäisessä osatutkimuksessa. HPV-kotinäytteenotto oli kustannustehokkaampaa kuin Papa-testin käyttö kohdunkaulansyövän seulonnassa. HPV-kotinäytteenotoryhmässä osallistumisprosentti oli korkeampi, vaikeita solumuutoksia löytyi enemmän ja kustannukset


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References


25. Statistikdatabas för cancer [Internet].


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)

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