Cervical cancer studies on prevention and treatment

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Cervical cancer

studies on prevention and treatment

Lotten Darlin

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Cervical cancer is the fourth most common reason for cancer death amongst women worldwide, with 500 000 new cases every year. Cervical screening programs can reduce the incidence by 50%. In Sweden there are approximately 400 new cases of cervical cancer yearly, despite the existence of a screening program. Cervical cancer and dysplastic lesions in the cervix are caused by infection from the Human Papilloma Virus, mainly HPV 16 and 18. An alternative to cytological screening is screening for HPV DNA. The aim of this thesis was to 1. Analyze the screening history of women with cervical cancer. 2. To find new techniques to screen women for cervical cancer. 3. To find out why some women do not attend screening. 4. To investigate the sentinel node technique in cervical cancer treatment.

Of the population of cervical cancer patients diagnosed in the years 2009-2010, one third of the women were over 60 years of age, and one fourth were over 65. The women that followed the screening program had a better prognosis compared to the women above screening age (Hazard Ratio (HR) =5.3 95% CI 2.4-12.0, p<0.001). The vaginal self-sampling device that we validated showed good agreement with HPV analysis of liquid-based cytology (LBC) (kappa value 0.67 95% CI 0.53-0.81).When 1000 women who had not taken a cytology sample for over nine years were offered self-sampling at home, 15% responded. The most common reason for non-attending was "uncomfortable with vaginal examination". The sentinel node concept is safe when bilateral and the tumor is 2 cm or less.

Conclusions: 1. Since so many women develop their cervical cancer after screening age, it would be advisable with an exit test. This exit test should ideally be an HPV test. 2. To reduce the non-attendance rate for cervical cancer screening, vaginal self-sampling for HPV-DNA is a possible method to use. 3. The final conclusion is that the sentinel node concept should be further investigated to make it possible to offer a sharp sentinel node for women with cervical cancer.

Keywords: Cervical cancer, human papilloma virus, HPV, screening, HPV test, self-sampling, sentinel node

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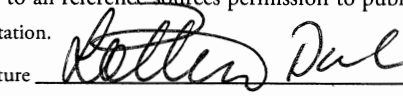
Signature  Date 28/10 2013

Cervical cancer

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Stor är människans strävan,
Stora de mål hon satt –
Men mycket större är människan själv
Med rötter i alltets natt.
Karin Boye

Jag står här och vrålar till kommande sekler:
Jag brinner!
Vladimir Majakovskij

Contents

Abstract

Cervical cancer is the fourth most common reason for cancer death amongst women worldwide, with 500 000 new cases every year. Most of these women live in developing countries. Cervical screening programs can reduce the incidence by 50%, but are not common in the developing world. In Sweden there are approximately 400 new cases of cervical cancer yearly, despite the existence of a screening program.

Cervical cancer and dysplastic lesions in the cervix are caused by infection by the Human Papilloma Virus, mainly HPV 16 and 18. An alternative to cytological screening is screening for HPV DNA.

The aim of this thesis was to analyze the screening history of women with cervical cancer, to find new techniques to screen women for cervical cancer, and to find out why some women do not attend screening. Furthermore, standard treatment for early cervical cancer is surgery including lymph node mapping, and patients have adverse effects from lymphadenectomies, so if a sentinel node procedure is accurate, it would be of value for the women.

Of the population of cervical cancer patients diagnosed in the years 2009-2010, one third of the women were over 60 years of age, and one fourth were over 65. The women that followed the screening program had a better prognosis compared to women above the screening age (Hazard Ratio (HR) =5.3 95% CI 2.4-12.0, $p<0.001$). All the women whose cancer was identified via the screening program were alive at the median follow-up (36 months). This contrasted with the women that had symptoms, where 68 of 98 patients were alive at the median follow-up ($p<0.0001$).The vaginal self-sampling device that we validated showed good agreement with HPV analysis of liquid-based cytology (LBC) (kappa value 0.67 95% CI 0.53-0.81).

When 1000 women who had not taken a cytology sample for over nine years were offered self-sampling at home, 15% responded. In contrast, when 500 women were offered flexible and free open clinic appointments, 4.2% came. The most common reason for non-attending was “uncomfortable with vaginal examination”.

The sentinel node concept is safe when a sentinel node is identified on both sides, and the tumor is 2 cm or less. The negative predictive value was then 100%.

The conclusions are that since so many women develop their cervical cancer after screening age, it would be advisable with an exit test. This exit test should ideally be an HPV test. In the case of hr-HPV positivity, the women could be offered a reflex

cytology test. To reduce the non-attendance rate for cervical cancer screening, vaginal self-sampling for HPV-DNA is a possible method to use. Since so many women seem to be non-attendant due to being uncomfortable with vaginal examination, a self-test could be an attractive alternative.

The final conclusion is that the sentinel node concept should be further investigated to make it possible to offer a sharp sentinel node for women with cervical cancer.

Thesis at a glance

Paper	Aim	Results	Conclusion
Paper 1: *Women above screening age, diagnosed with cervical cancer, show worse prognosis than women of recommended screening age*	To analyze screening-history of women in the southern Region in Sweden with cervical cancer 2009-2010.	All patients diagnosed through the cervical screening program were still alive at median follow-up time (36 months), showing a significantly better overall survival compared to those aged 65 or younger whose disease was discovered due to symptoms (68/98; p<0.001). Significantly worse prognosis for women above screening age compared to those who had a recommended cervical smear (Hazard Ratio (HR) =5.3 95% CI 2.4-12.0, p<0.001).	• Cervical cytology screening has reduced sensitivity in elderly women, then a possible way to improve the screening sensitivity, could be to use the hr-HPV-test, with reflex-cytology. • Women above 65 years of age could benefit from a prolonged HPV-test screening-program.
Paper 2: *Vaginal self-sampling without preservative for human papillomavirus testing* *shows good sensitivity*	To evaluate if self-sampling with new device, without preservatives was adequate for HPV-testing.	The agreement regarding hr-HPV positivity was good between the results from the SS and the LBC tested samples (kappa value 0.67 (95% CI 0.53–0.81)), and moderate when any HPV presence was tested in the two groups (kappa value 0.55 (95% CI 0.37–0.73)). There was no significant difference between the false negative number of patients with the SS or LBC method finding HSIL with cytology or histopathology.	• The self-sampling device validated by us, detects hr-HPV-infections with similar sensitivity as a cervical smear taken by a gynecologist. The self-sampling method costs only 2 euro and is well tolerated The kit is suitable for regular mail transport.
Paper 3: *Comparison of use of vaginal HPV self-sampling and offering flexible appointments as strategies to reach long-term non-attending women in organized cervical screening*	To compare responses for self-sampling or flexible non-fee appointment, among long-term non-attending women. To explore main reasons for previous non-attendance.	The response rate was higher in the self-sampling group (15%) compared with the group invited to the outpatient clinic (4.2%) (p > 0.0001). There were no other significant differences between the two groups. Both in the self-sampling and the outpatient group the most common reason given for nonattendance was “Uncomfortable with vaginal examination” 37% and 43%, respectively.	• Vaginal HPV self-sampling seems to be more effective for increasing the coverage of screening, than the control-group with flexible appointments. • The most common reason for non-attendance in the screening-program was “uncomfortable with vaginal examination”.
Paper 4: *The sentinel node concept in early cervical cancer performs well in tumors smaller than 2 cm*	To evaluate the sentinel node (SLN) concept for lymphatic mapping in early stage cervical cancer.	The overall detection rate of at least one SLN was 90% (94/105 patients) and 94% (61/65 patients) in patients with tumor equal or smaller than 2 cm. The negative predictive value for patients with cervical cancers diameter equal to 2 cm or less was 100%.	• The SLN-technique seems to be an accurate method for identifying lymph-node- metastases, in cervical cancer patients with tumors of 2 cm or smaller. • In case of unilateral SLN. A complete lymphadenectomy should be performed on the radio negative side.

List of abbreviations

ASCUS= Atypical Squamous Cells of Unknown Significance

CI= Confidence Interval

CIN= Cervical Intraepithelial Neoplasia

CIS= Cancer in Situ

Ct-value= cycle threshold-value

CT=Computerized Tomography

ds-DNA-virus= double stranded Deoxyribonucleic acid virus

DNA= Deoxyribonucleic acid

EBRT= External Beam Radiotherapy

FIGO=International Federation of Gynecology and Obstetrics

H&E= Hematoxylin & Eosin

HIV= Human Immunodeficiency Virus

HNSCC= Head and Neck Squamous Cell Carcinoma

HPV= Human Papilloma Virus

HR= Hazard Ratio

hr-HPV= high-risk Human Papilloma Virus

HSIL= High-grade Squamous Intraepithelial Lesion

IARC= International Agency for Research of Cancer

lr-HPV= low-risk Human Papilloma Virus

LBC= Liquid-based Cytology

LSIL= Low-grade Squamous Intraepithelial Lesion

LSG= Lymphoscintigram

MBq= Mega Bequerel

MRI= Magnetic Resonance Imaging

NASBA= Nucleic Acid Sequence-based Amplification

p53=protein 53 or tumor protein 53

PAP= Papanicolau

PCR= Poly Chain Reaction

PET-SCAN=Positron Emission Tomography scanning

pRb=Retinoblastoma protein

RT= Radiotherapy

RT-PCR= Reverse transcript Poly Chain Reaction

SCC= Squamous Cell Carcinoma

SD= Standard Deviation

SLN= Sentinel Node

SPECT-CT= Single-photon emission computed tomography

SS= Self-sampling

Tc-99= Technetium 99

UK= United Kingdom

US= United States

Original Studies; List of publications

The thesis is based on the following studies referred to in the text by the following numbers:

I. Women above screening age, diagnosed with cervical cancer, have worse prognosis than women of recommended screening age. Lotten Darlin, Christer Borgfeldt; Emelie Widén, Päivi Kannisto

II. Vaginal self-sampling without preservative for human papilloma virus testing shows good sensitivity Lotten Darlin, Christer Borgfeldt, Ola Forslund, Emir Hénic, Joakim Dillner, Päivi Kannisto. Journal of Clinical Virology 56 (2013) 52-56

III. Comparison of use of vaginal HPV self-sampling and offering flexible appointments as strategies to reach long-term non-attending women in organized cervical screening. Lotten Darlin, Christer Borgfeldt, Ola Forslund, Emir Hénic, Joakim Dillner, Päivi Kannisto. Journal of Clinical Virology 58 (2013) 155-160

IV. The sentinel node concept in early cervical cancer performs well in tumors smaller than 2 cm. Lotten Darlin, Jan Persson, Thomas Bossmar, Bengt Lindahl, Päivi Kannisto, Anna Måsbäck, Christer Borgfeldt Gynecologic Oncology 117 (2010) 266-269

Introduction

Cervical cancer is the fourth most common cause of cancer death amongst women worldwide (Ferlay et al., 2010). Every year over 500,000 new cases (9% of all female cancers) are diagnosed. Cervical cancer accounts for 275100 individuals (8%) of the total cancer deaths amongst females in 2008. Most of these cases (85%) and deaths occurred in developing countries (fig 1). With the introduction of screening programs in developed countries, the detection of dysplastic lesions and early diagnosis of cervical cancer became more frequent, and thus the incidence of cervical cancer declined (Bergstrom et al., 1999). Simultaneously, deaths from cervical cancer in developed countries became rarer. Looking at the future, HPV vaccination programs could markedly decrease the pain and suffering caused by cervical cancer.

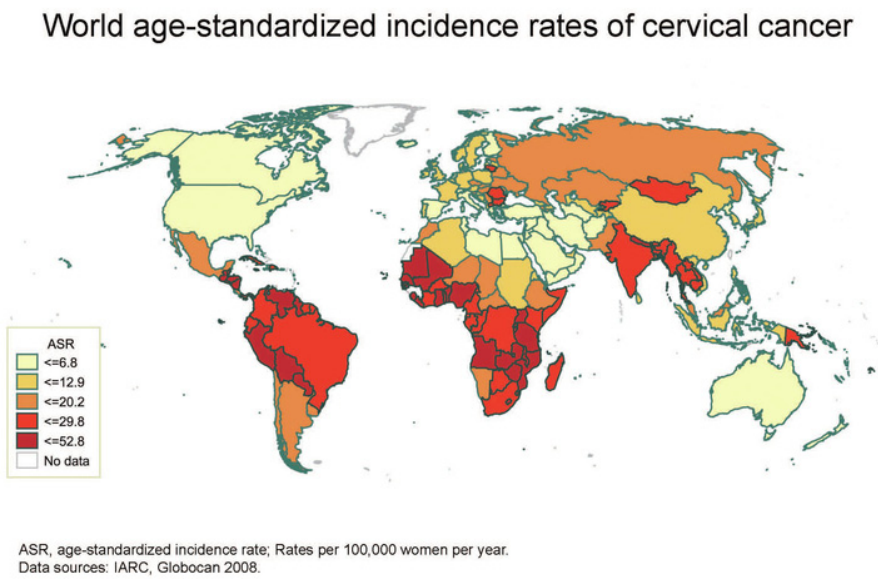


Fig 1. Cervical cancer incidence
World map, to show how the incidence varies in different countries, source GLOBOCAN

Approximately 400 women are diagnosed with cervical cancer in Sweden every year (Cancer Incidence Sweden 2011). The cervical screening program was introduced in Sweden in the late sixties in order to detect and treat dysplastic lesions preceding cervical cancer (Mahlck et al., 1994) (Andrae et al., 2008) (fig 2). Not all invited women attend the recommended cervical screening program. Besides, there is a lower and upper age-limit for the screening, which varies to some extent in the 20 counties in Sweden. In Skane, in the South Sweden region, the women are invited to screening tests every third year between the ages of 23 and 50, and every fifth year between the ages of 51 to 65. In other parts of Sweden the program ends at the age of 60. Since cervical cancer can be prevented by the screening procedure, the challenge is to reach the non-attending women.

The aim of this thesis is to evaluate present and newly introduced types of prevention and treatment strategies for cervical cancer.

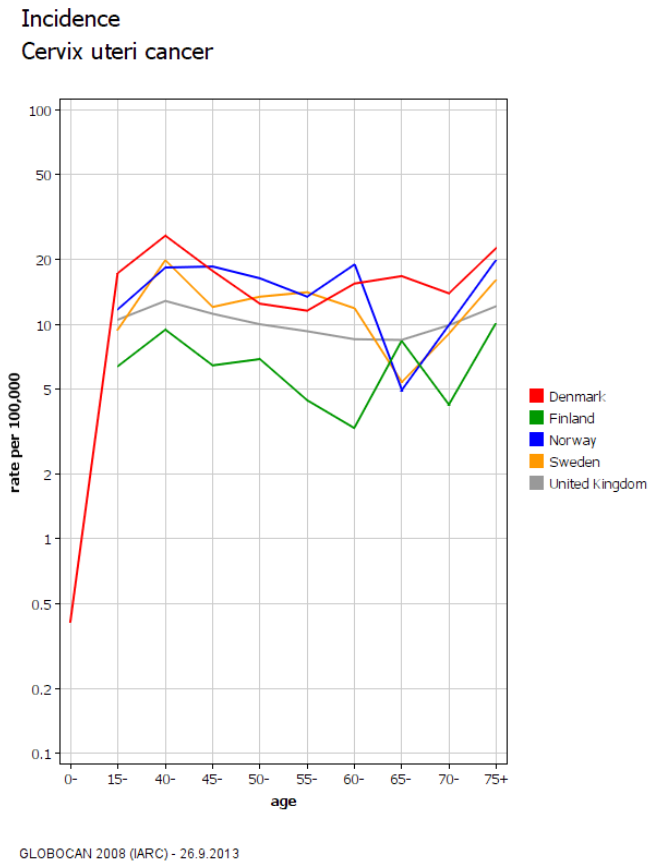


Fig 2. Cervical cancer incidence
Data for Sweden, Norway, Finland, Denmark and United Kingdom. Statistics from GLOBOCAN

Cervical cancer

Background, epidemiology

Cervical cancer is a major health problem amongst women around the world. As mentioned above, it is mainly women from the developing countries that are diagnosed with cervical cancers, with incidence rates of more than half a million individuals yearly. The fact that cervical cancer is a preventable disease makes it important to put effort into reducing the incidence and mortality rates. In many of the developed countries, the strategies used are to screen women and now also to vaccinate against the HPV virus. Since the introduction of Papanicolau smear-based screening in the fifties, the mortality has declined by 70% in the developed countries, and the incidence in the Nordic countries is now about half the incidence in the sixties (fig. 3). However, it is important that worldwide measures are taken against cervical cancer.

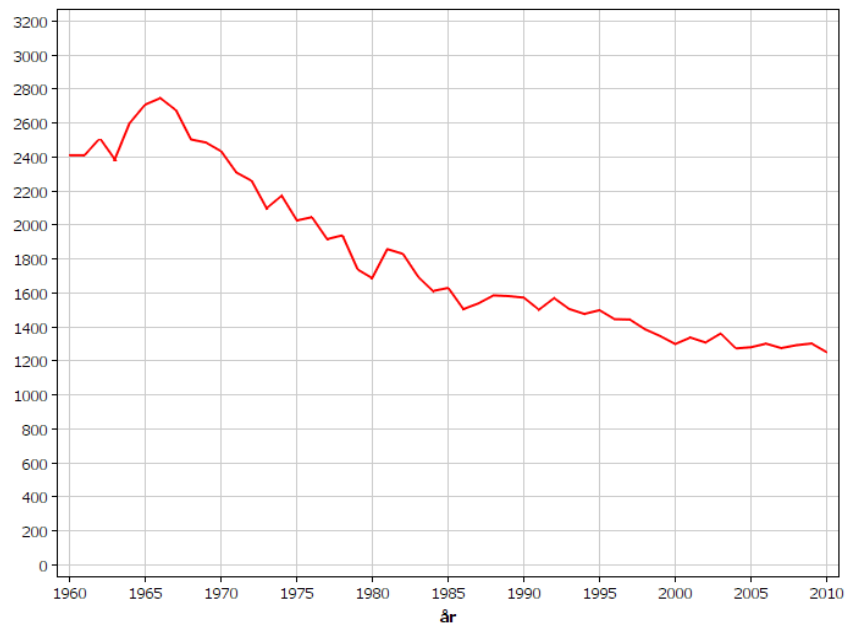


Fig 3.
The incidence of cervical cancer in the Nordic countries. Statistics from NORDCAN.

Etiology

About thirty years ago, the HPV high-risk viruses were identified as a causal agent for developing cervical cancer (Durst et al., 1983). The virus infects the metaplastic epithelium of the cervical transformation zone. The persistent infection makes the epithelium develop pre-cancerous changes and finally the invasion of the basal membrane completes the process of developing cervical cancer. HPV can be detected in at least 99.7% of the squamous and adenocarcinomas of the cervix uteri (Bosch, 2011, Walboomers et al., 1999). The high-grade precursors (CIN 3 and CIS) contain the same 10-15 genetically related HPV viruses (Bosch et al., 2008, Bosch and Munoz, 2002). Factors other than HPV infections are thought to be associated with at least CIN3/CIS. These data have been gathered from epidemiologic studies. Factors associated with CIN 3 lesions are prolonged use of oral contraceptives (Moodley et al., 2003), cigarette smoking(International Collaboration of Epidemiological Studies of Cervical et al., 2006), high parity (Delvenne et al., 2007), and infections with Chlamydia trachomatis (Golijow et al., 2005).

Histology

The epithelial tumors are divided into two main types, i.e. squamous cell carcinoma and adenocarcinoma. Rarer types are: adeno-squamous cancer, adeno-cystical cancer, adenoid basal carcinoma, neuroendocrine tumors (amongst them small cell cancer) and undifferentiated cancer. Other mainly non-epithelial tumors include: mesenchymal tumors, mixed epithelial-mesenchymal tumors, melanoma, germline-tumors, malignant lymphomas, leukemias and various metastases. The most frequent histology is the squamous carcinoma, which accounts for approximately 70% of the cancers, followed by the adenocarcinoma (25%). The screening programs mainly detect the squamous precursors of cancers, and thus they are detected and treated. An apparent relative increase in adenocarcinoma is therefore seen (Bergstrom et al., 1999).

Symptoms and diagnosis

Different reasons bring the woman to the doctor. Post-coital bleeding or inter menstrual bleeding can lead to a clinical examination that could reveal a cervical tumor. Some patients come for cervical screening without symptoms, whereas others present with heavy irregular bleeding or late symptoms such as postrenal failure.

Diagnostics and prognosis

When cervical cancer is suspected, it is important to take biopsies for pathologic diagnosis. All new cases are staged according to FIGO (table1). This is a clinical classification, and does not include lymph node status (Monk and Herzog, 2007). Staging is made by clinical examination, cystoscopy and if needed, rectoscopy. For a more precise identification and localization of cervical tumors, an MRI and vaginal ultrasound may be used (Epstein et al., 2013). MRI should preferably be done before

a conization, since that procedure can disturb the imaging (Charles-Edwards et al., 2011). The MRI can show the margins towards the bladder and rectum, and to what extend there is parametrial infiltration (Hricak et al., 2007). Imaging such as Computerized Tomography (CT) might be helpful for evaluating the status of the parenchymatous organs and para-aortic area. Positron tomography has higher sensitivity and specificity in detecting metastases than MRI or CT (Follen et al., 2003). However, metastases under 4 mm cannot be visualized with a PET scan.(Chung et al., 2009). The prognosis depends largely on stage at diagnosis, and availability of diagnostics and modern treatment methods (Table 2). Prognostic factors for relapse and survival have been identified in several studies. Another important prognostic factor is whether the lymph nodes are positive or not. When 11,775 patient records were analyzed, 7,458 had no data on the lymph nodes, and the five-year survival was 59.8 %. The patients with negative lymph nodes had a five-year survival of 92.1 % (n=3364). As a contrast, the patients with positive lymph nodes had a five-year survival of 64.1 % (n=953)(Quinn et al., 2006). Other important factors are: tumor volume, parametrial spread, and depth of invasion.

Table 1 FIGO staging for carcinoma of the cervix uteri

Revised 2009(Pecorelli, 2009, Pecorelli et al., 2009)

Stage I	The carcinoma is strictly confined to the cervix (extension to the corpus would be disregarded)
IA	Invasive carcinoma which can be diagnosed only by microscopy, with deepest invasion ≤ 5mm and largest extensions ≤7 mm
IA1	Measured stromal invasion of ≤3.0 mm in depth and extension of ≤7 mm
IA2	Measured stromal invasion of > 3.0 mm and not >5.0 mm with an extension of not >7.0 mm
IB	Clinically visible lesions limited to the cervix uteri or pre-clinical cancers greater than stage 1A
IB1	Clinical visible lesions ≤4.0 cm in greatest dimension
IB2	Clinical visible lesions >4.0 cm in greatest dimensions
Stage II	Cervical carcinoma invades beyond the uterus, but not to the pelvic wall or to the lower third of the vagina
IIA	Without parametrial invasion
IIA1	Clinical visible lesions ≤4.0 cm in greatest dimension
IIA2	Clinical visual lesions > 4.0 cm in greatest dimension
IIB	With obvious parametrical invasion
Stage III	The tumor extends to the pelvic wall and/or involves the lower third of the vagina and/or causes hydronephrosis or a non-functioning kidney
IIIA	The tumor involves the lower third of the vagina, with no extensions to the pelvic wall
IIIB	Extensions to the pelvic wall and/or hydronephrosis or a non-functioning kidney
Stage IV	The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum. A bullous edema, as such does not permit a case to be allotted to stage IV
IVA	Spread of the growth to adjacent organs
IVB	Spread to distant organs

Table 2: Survival data

11,639 Patients treated 1999-2001for cervical cancer and eligible for survival analysis. The patients have been reported in Volume 26 of the Annual FIGO Report from different parts of the World (Quinn et al., 2006).

Stage	Patients (n)	Mean age (yrs)	Overall survival (%) at five years
Ia1	829	44.5	97.5
Ia2	275	45.4	94.8
Ib1	3020	48.6	89.1
Ib2	1090	46.8	75.7
IIa	1007	54.4	73.4
IIb	2510	53.5	65.8
IIIa	211	60.3	39.7
IIIb	2028	56.6	41.5
IVa	326	59.5	22.0
IVb	343	56.8	9.3

Treatment

Cervical cancer treatment is based on surgery and radiotherapy. Although both adenocarcinomas and squamous cancers are sensitive to chemotherapy, it is mainly used as a concomitant treatment to radiotherapy. Ongoing studies on neoadjuvant treatment and surgery are promising in locally advanced disease and could be an alternative for primary chemoradiation (Angioli et al., 2012).

Surgery

Treatment is dependent on the stage. In Sweden there are no national treatment guidelines, but generally surgery with radical hysterectomy and pelvic lymphadenectomy is done in FIGO stage 1A2 to 1B1 and in certain 2A cases. The FIGO staging is a clinical determination, and is as easy to perform in developing countries as in developed countries (Monk and Herzog, 2007). The earliest cancers can be treated with large conization or trachelectomy, offering the possibility of preserving fertility.(Sevin, 1999, Lanowska et al., 2011, Monk et al., 2007b, Randall-Whitis and Monk, 2007) In early cervical cancer, identification of tumor spread to regional lymph nodes is mandatory to schedule patients for adequate treatment and to provide prognostic information. A complete pelvic lymphadenectomy is usually performed.

Cervical cancer surgery is minimally invasive, and for the last five years robot-assisted surgery has been the method of choice in many international centers (Magrina et al., 2008, Magrina and Zanagnolo, 2008, Mettler et al., 2008). Data has shown that laparoscopic robot-assisted operations involve a shorter duration of surgery and less bleeding than traditional laparoscopy (Boggess et al., 2008, Magrina et al., 2008, Magrina and Zanagnolo, 2008). However, a complete pelvic lymphadenectomy is associated with short- and long-term morbidity such as lymphedema, lymphocele and pelvic nerve impairment (Musch et al., 2008),even also when the operative access is minimally invasive.

The sentinel node (SLN) concept has been proven safe in early carcinoma of the breast (Veronesi et al., 2007), (Rob et al., 2005, Giuliano et al., 1994), avoiding total axillary dissection. For the same reasons, the SLN technique is now commonly used in the evaluation of certain malignant melanomas (Callejo Peixoto and Meneses e Sousa, 2005, Reintgen et al., 2004). Moreover, several reports confirm that the SLN concept is also safe for lymphatic mapping in squamous cell carcinoma of the vulva (de Hullu et al., 2000) .

In cervical cancer the SLN technique can be used for smaller tumors with acceptable security (Roy et al., 2011). The sharp sentinel node is not yet generally accepted, but is still a subject of debate and study.

A new classification of radical hysterectomy has been made. The classification is more flexible and is also suitable for minimally invasive techniques, in contrast to the former classification according to Piver (Querleu and Morrow, 2008). When pathology margins are narrow and/or lymph nodes are affected, adjuvant chemoradiation is given. The advanced cancers are treated with primary chemoradiation.

Radiotherapy

Radiotherapy can be given with different intentions. ***Primary, radical curative***: Radiotherapy is the treatment of choice in locally advanced stages 1B2 to IVA. The treatment is a combination of external beam radiotherapy (EBRT), brachytherapy and concomitant cisplatin. The RT standard has been developed through common practice and some studies (Monk et al., 2007a). However, the use of concomitant cisplatinum has been evaluated by several randomized prospective studies, and reduces the risk of death by 30% to 50% (Rose, 2000). The dose and regimen of brachytherapy is summarized by the Nordic Society of Gynecologic Oncology (NSGO; Nordic Society for Gynecological Oncology Advisory Board of Radiotherapy (2001). Guidelines for Irradiation of Advanced Cervical Cancer www.nsgo.org) ***Postoperative, adjuvant radiotherapy***: This is given when the tumor is postoperatively shown to be larger than 4 cm, or to have narrow histological margins (<8 mm) or lymph node metastases. The dose of the external beam radiotherapy (EBRT) is at least 45 Gy to the pelvis. This reduces the risk of recurrence (Rotman et al., 2006), and the addition of weekly cisplatinum further lowers the risk of recurrence (Peters et al., 2000).

Molecular target therapies

There are currently a number of biological agents for modulating different signaling pathways undergoing phase 2 studies. However, none of them are in clinical practice, since no phase 3 studies have been carried out. There are different target mechanisms: inhibiting angiogenesis, targeting epidermal growth factor receptors, histone deacetylases, cyclooxogenase 2 (COX-2) or mammalian target of rapamycin (m-TOR)(Zagouri et al., 2012). There are promising results for bevacizumab, indicating that targeting the VEGF pathway might be an interesting therapy pathway (Monk et al., 2009, Schefter et al., 2012).

Prevention against cervical cancer and HPV infection

Primary prevention:

HPV vaccination:

The vaccines against HPV contain virus-like particles of the major capsid protein (L1) of different virus types. The vaccines contain only viral capsid proteins, without viral nucleic acid, and thus no virus replication occurs. Currently, two different vaccines are available: one is Gardasil®, which has virus-like particles from HPV 6 and 11 (low-risk types, causing kondyloma) and HPV 16 and 18; the other vaccine is Cervarix®, which has virus-like particles from HPV 16 and 18. Both types of vaccine contain an adjuvant to make the immune-response stronger. Both vaccines are safe and well tolerated (Lu et al., 2011), and may prevent at least 70% of all cases of invasive cervical carcinoma (as 70% of the cervical carcinomas are infected with HPV 16 and 18) (de Sanjose et al., 2010).

Condom use:

Constant condom use during heterosexual intercourse has been shown to reduce HPV transmission by approximately 70% (Winer et al., 2006). However, the HPV virus is present not only in the vagina and on the penis, but also on the scrotum, labia and perianal area.

Male circumcision:

Male circumcision is carried out due to cultural and religious beliefs. In Uganda and South Africa, randomized controlled studies of male circumcision show it offers protection against HPV infection and other sexually transmitted diseases (Auvert et al., 2009, Tobian et al., 2009). The studies show decreased HPV infection in men. A study has also shown a partial effect on transmission of HPV infection to women (Wawer et

al., 2011). However, it is important to note that the effect is only partial; circumcised men should continue having safe sex.

Topical microbicides:

There is some evidence that topical microbicides can be useful in blocking the spread of HPV. A gelling agent (carrageenan) has been used in trials, and shows good inhibition of the spread of HPV infection (Buck et al., 2006). Initially it was tested against HIV, but is much more effective against HPV transmission (Roberts et al., 2007). It would be very interesting if carrageenan could be proved to clear up HPV infections.

Secondary prevention:

Cervical cell screening:

Cervical screening works by detection, surveillance and treatment of pre-cancerous changes, which carry a risk of progression to cervical cancer if left untreated. Organized screening programs for detection of cervical dysplasia to prevent cervical cancer have been shown both to reduce the number of new cancer cases and enhance cancer survival (Andrae et al., 2008, Arbyn et al., 2009). In Sweden, almost half of the cervical cancer cases are diagnosed among women who have not participated in the organized screening program (Stenvall et al., 2007a). Organized screening programs also increase early detection of cervical cancer and reduce the number of women diagnosed at advanced stages of the disease, which leads to less complicated treatment and better survival rates (Andrae et al., 2012). Historically, the cervical screening was done by an exfoliative method devised by Papanicolau, called the Pap smear. Lately, many organized programs have switched to liquid-based cytology including HPV reflex testing (Albrow et al., 2012, Froberg et al., 2008, Sarode et al., 2003) The HPV reflex testing of ASCUS avoids unnecessary investigation of HPV-negative women (Arbyn et al., 2006). The algorithm for cervical screening in southern Sweden has integrated the HPV reflex test (fig 4)

HPV DNA testing:

Initially, HPV testing was introduced in screening programs as a triage-method of ASCUS or CIN 1, but also as an evaluating treatment after CIN2-3/CIS and AIS (C-ARG, Cervixcancerprevention Riktlinjer för utredning, behandling och uppföljning av cervikal intraepitelial neoplasi (CIN), 2010 Arbets- och Referensgruppen för Cervixcancerprevention).

Visual Inspection VIA:

In large areas of the world, the screening system is based upon visual inspection. The examiner uses acetic acid on the cervix, and if acetic white areas, the test is positive. A large study in South Africa compared VIA and the HPV DNA test. Both groups were

treated if positive. The end-point was CIN 2+ and cervical cancer rate at six and 12 months, compared to not treated. The level of CIN 2+ and cervical cancer in the two groups with treatment was lower than the expectancy group, and the study claimed that both ways to diagnose and treat cervical lesions were safe and resulted in a lower prevalence of cervical cancer (Denny et al., 2005)(Shastri et al ASCO abstract 2013).

HPV testing versus Liquid-based Cytology

The hr-HPV test has higher sensitivity, especially in women above 50, for finding cervical dysplasia (Gyllensten et al., 2012). The somewhat lower specificity of the hr-HPV test compared with liquid-based cytology may be compensated by analyzing the hr-HPV-positive samples also with liquid-based cytology. Incorporating screening with the modern hr-HPV test and triage of hr-HPV-positive women with cytology provides a good balance between maximizing sensitivity and specificity by limiting the number of referrals for colposcopy and conization (Cox et al., 2013) especially in postmenopausal women (Kinney et al., 2011, Gyllensten et al., 2012, Gyllensten et al., 2010)

Non-attendees to cervical screening

Almost half of the cervical cancer cases in Sweden develop in women who do not attend organized screening or in women who have passed the organized screening program age (Stenvall et al., 2007a). Several strategies have been used to reach the non-attending women in the organized screening program, with varying success rates. The efficiency of a cervical screening program depends mainly on the population coverage of the program, which is dependent on the acceptance of the invitations (Andrae et al., 2012, Stenvall et al., 2007b). Efforts have been made to increase the compliance rate in organized community-based screening programs (Eaker et al., 2001, Oscarsson et al., 2007). One way to increase the number of cervical smears is to use telephone invitations and interviews, reassuring the subjects that they will receive friendly treatment and a suitable appointment time, but such management more than triples the screening costs (Oscarsson et al., 2008a, Oscarsson et al., 2008b). Role models, both personal and in the media, seem to be important for attendance, in addition to information and communication about cervical cancer and cervical screening programs (Knops-Dullens et al., 2007). Another important factor influencing attendance is that many women underestimate the time that has elapsed since their last screening test (Eaker et al., 2001).

Dysplastic lesions in the cervix uterii

The squamous cells with intraepithelial neoplasia are divided into groups, depending on the severity of the cell changes (Table 3). Histologic verification of cytologic anomaly is the golden standard. Usually CIN 2 or worse is the threshold for treatment (Wright et al., 2007b, Wright et al., 2007a). Treatment should be excision by laser, cold knife or LEEP, so that a proper histological analysis of the borders can be made.

Moat protocols recommend treatment of CIN 2, even though 40% of CIN 2 lesions regress spontaneously within two years (Castle et al., 2009, Ostor, 1993). This could lead to overtreatment, with a risk of pre-term delivery in eventual later pregnancies (Sadler et al., 2004).

The greatest problem in cervical screening is the bulk of ASCUS and CIN1. They could possibly be underlying CIN3 or worse, but an HPV reflex test could further classify these women (Group, 2003a, Group, 2003b). Imiquimod has been used to treat CIN2-3, and showed histologic regression in 73% of the imiquimod-group, whereas the placebo-group showed 39%. The human papilloma virus clearance rates were 60% in the imiquimod-group compared to 14% in the placebo-group (Grimm et al., 2012).

Table 3 Intraepithelial dysplasia

Comparison of different terminologies for intraepithelial dysplasia. Adapted from Textbook of Gynecological Oncology Taylor et al. s 83

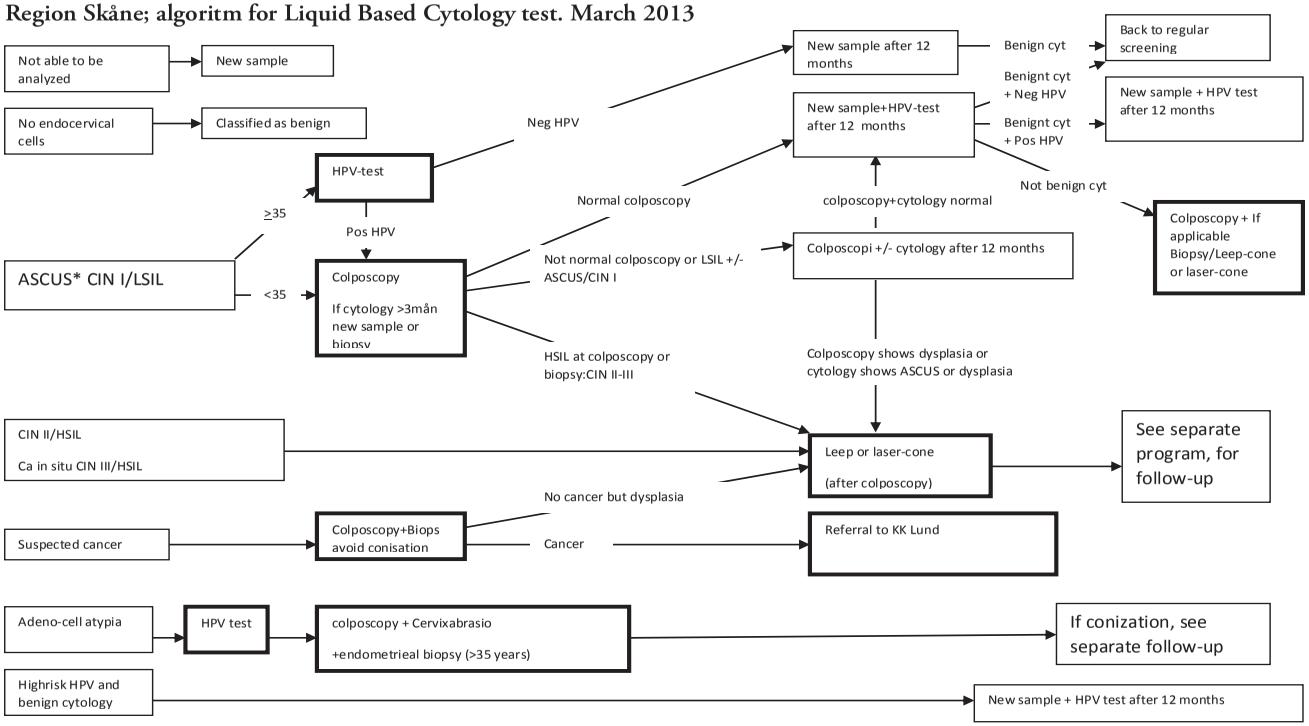
Cytology		Histology	Significance
UK	US		
Borderline	ASCUS	Mixed	Uncertain
Mild dyskaryosis	LSIL	CIN 1	Acute HPV infection
Moderate dyskaryosis	HSIL	CIN 2	Mixture of acute infection, persistent infection with low oncogenic potential subtypes and premalignant lesions
Severe dyskaryosis	HSIL	CIN 3/CIS	Premalignant/ carcinoma in situ

High grade CIN is premalignant, but usually there is a long latency until the invasive disease occurs. A retrospective analysis of an early unethical study of untreated CIN3 found that invasive malignancy without treatment was about 31% over 30 years (McCredie et al., 2008). However, 50% of these women fell ill within 24 months. Another study showed different rates of progression to cancer (Table 4)(Ostor, 1993).

Table 4 natural history of CIN

	Regress	Persist	Progress to CIN3	Progress to invasive cancer
CIN1	57%	32%	11%	1%
CIN2	43%	35%	22%	5%
CIN3/CIS	32%	<56%		>12%

Region Skåne; algoritm for Liquid Based Cytology test. March 2013



Women with benign cytology and highrisk HPV shall be offered a new cytology-sampling and HPV-test after 12 months at referral gynecological out patient clinic.
*ASCUS=Atypical Squamous Cells of Undetermined Significance

Human Papilloma Viruses

The family Papillomaviridae has 200 types of papillomaviruses, and as many as 170 types can affect humans (de Villiers, 2013). They are divided into five different genera, whose members share similar tissue tropism (Bernard et al., 2010). Approximately 35 HPV types can infect the human anogenital region. The high-risk-HPV types, however can cause cancer if the infection becomes persistent (Rositch et al., 2013). The viruses that cause cervical cancer come from the genus alpha-papilloma viruses (Bernard et al., 2010). Thirteen anogenital HPV types are classified as hr-HPV; 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The probable high-risk types are: 26, 53, 66, 73 and 82 (Bouvard et al., 2009). HPV is viewed as a necessity for developing cervical cancer (Munoz, 2000). The incubation time is from 3-4 weeks up to several years. However, most infections do not cause any symptoms (Stanley, 2010b, Stanley, 2010a). The most carcinogenic genotypes are HPV 16 and HPV 18 (Bosch et al., 2008). HPV 16 and HPV 18 account for 70% of all cervical cancers and 50% of CIN3 (CIS). HPV accounts for 83% of all anal cancers, and 25% of oropharyngeal cancers (De Vuyst et al., 2009, Kreimer et al., 2005). However, there seems to be an increase in oropharyngeal squamous cell carcinoma, despite a decrease in smoking (Panwar et al., 2013). A total of 70% of vaginal cancers and 40% of vulvar cancers are caused by HPV infection(De Vuyst et al., 2009).

Some of the HPV types are considered low-risk infections, which can generally be resolved by the immune system of the host (Lacey et al., 2006).

Prevalence, Worldwide and in Sweden

Of the 610 000 cancers worldwide caused by HPV infection, the greatest bulk of new cases are cervical cancers (86.9%)(Forman et al., 2012). To determine the prevalence of HPV amongst the population, a meta-analysis has been made that shows an HPV prevalence of around 11-12% in cervical samples (Bruni et al., 2010). Different countries and regions have different patterns of HPV prevalence. Women undergoing routine cervical testing in the UK were tested for HPV virus prevalence (fig 5), where the peak in prevalence is in the early twenties. When age-related cervical cancer prevalence is investigated, it is notable that the peak in incidence is around 35 years of age (fig 6); that is, 10-15 years later than the peak of HPV prevalence. A study on the cumulative risk of cervical cancer, depending on HPV positivity shows a low risk of cervical cancer when no HPV 16 or HPV 18 is present at the start of the control (fig 7).

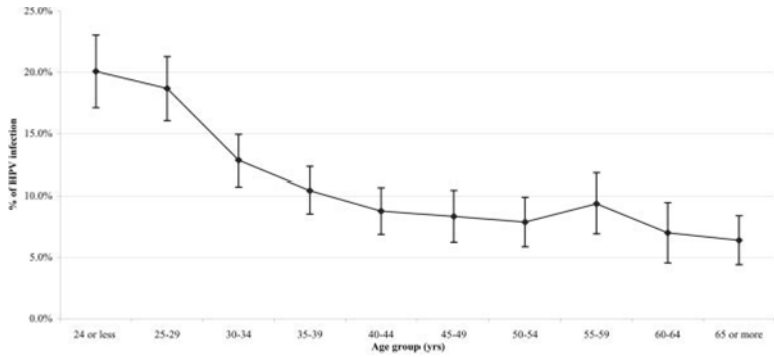


Fig 5 HPV prevalence according to age
Prevalence of HPV infection.(Petignat et al., 2005). Reprinted with permission

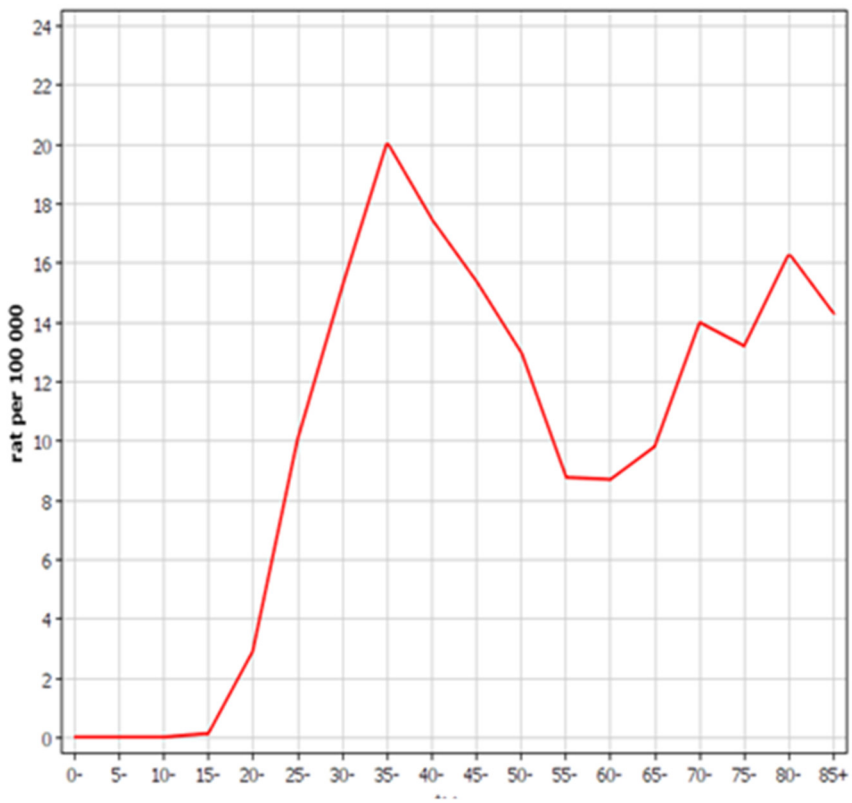


Fig 6 Age-specific cervical cancer in the Nordic countries. Statistics from NORDCAN

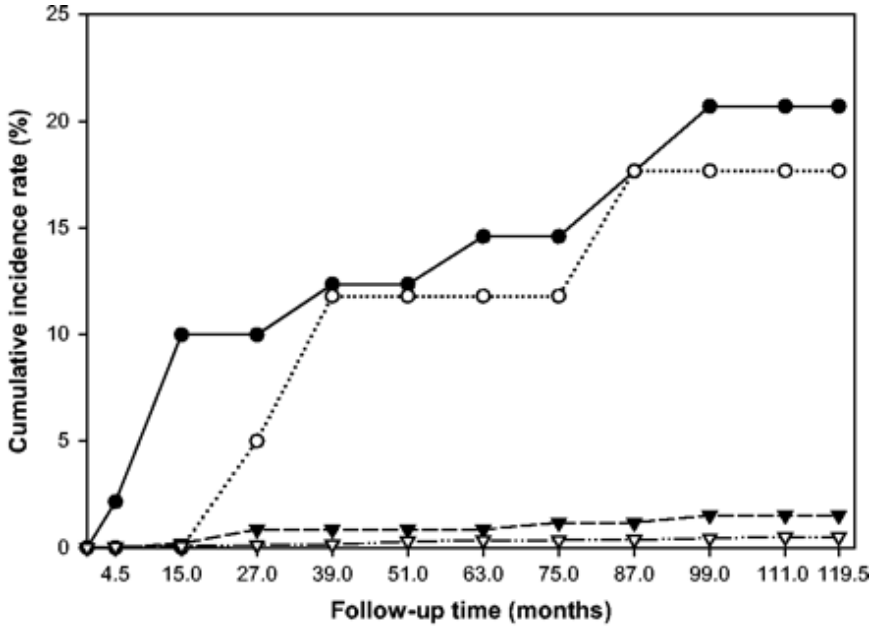


Fig 7 Cumulative incidence of CIS + Invasive cancer over a 10-year period.

Filled circles HPV16+, White circles HPV 18 +, Filled triangles other hr-HPV+ and white triangles no hr-HPV. Reprinted with permission (Khan et al., 2005)

HPV and carcinogenesis

The HPV virus is a double-stranded DNA-virus, where the DNA-structure is wrapped in a protein shell of two capsid proteins, namely L1 and L2. The other six proteins are E1, E2, E4, E5, E6 and E7. These proteins are necessary for virus replication, genome amplification, proliferation and oncogenesis (Munoz et al., 2006).The infection starts with the introduction of the virus to the basal cell, induced by inflammation, a cut or a tear (Schiller et al., 2010). The virus needs cell proliferation for its own replication (Pyeon et al., 2009). Different HPV proteins are expressed during the maturation of the cells until the normal squamous cells are desquamated. The oncoproteins E6 and E7 are expressed just suprabasally in the epithelium, and stimulate the proliferation of infected basal cells (Doorbar et al., 2012).

Large prospective studies have shown that the carcinogenicity of the HPV virus is not related to the actual persistence. When women in Costa Rica with different HPV infections were followed, it was found that nearly 20 % of the women with HPV 16 developed CIS/cervical cancer. It was clear that the HPV 16, and other carcinogenic HPV types, codes for an E5 protein, which is involved in the host's lack of immune-response(Ashrafi et al., 2006).

The two major oncogenes, E6 and E7, are integrated in the cells genome, and they become up-regulated and produce the viral proteins E6 and E7(Doorbar et al., 2012).

They target two major cellular regulators. The first is pRB (retinoblastoma protein), which causes uncontrolled cell-proliferation when E7 is bound to it. The second is p53, which is bound to E6, and this complex causes loss of DNA repair and prevents the cell from undergoing apoptosis (Moody and Laimins, 2010). The mature virus is only produced in the most superficial layers of the epithelium (fig 8).

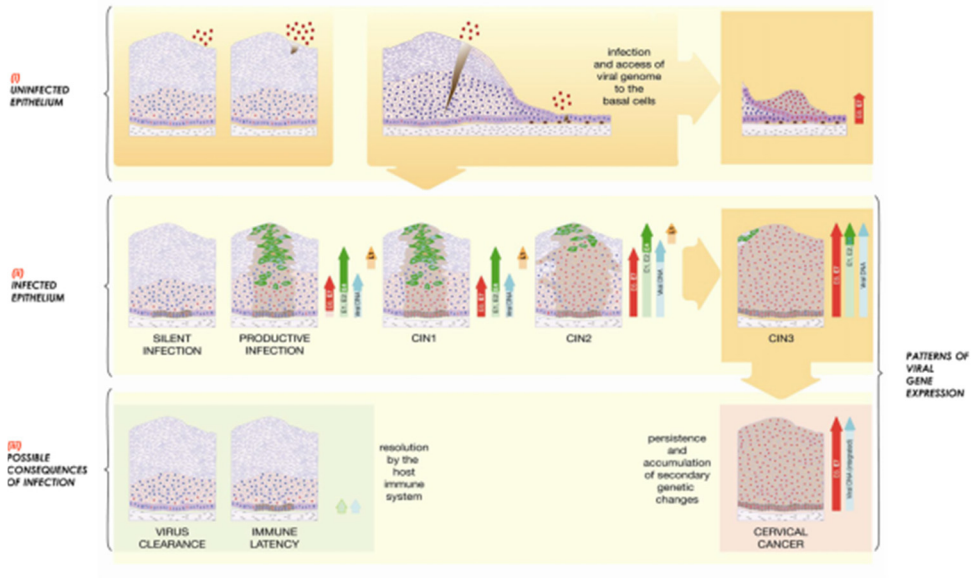


Fig 8 High risk HPV infection and possible consequences.

(i)Shows infection with HPV virus (ii) Shows silent infection, where the viral genome is retained in the basal layers without apparent disease. This could lead to productive infection, where virus particles are released (CIN1),or deregulated viral gene expression and high grade neoplasia (CIN2/Cin3/CIS) Persistent infection with high grade neoplasia, is associated with increased risk of integration of the viral genome into host chromosomes, thereby leading to cancer. (iii) In most cases, HPV infections are cleared by the host's immune system (cell-mediated response). However, if the genetic changes in the host cell lead to overexpression of the oncoproteins E6 and E7, the apoptosis is disturbed, and this is de-regulated in cervical cancer. Reprinted with permission of Elsevier.(Doorbar et al., 2012)

HPV and non-cervical diseases

HPV infections near a transformation zone increase the risk of carcinogenesis. It is in the transformation zones of the cervix, anus or oropharynx, that the oncogene HPV viruses are most prone to causing cancer (Giuliano et al., 2008). In vulvar squamous carcinoma, HPV can be detected in 71% of biopsy samples, but for vulvar cancer, independent of histology, approximately 40% of the samples are HPV-positive (De Vuyst et al., 2009).The correlation between HPV and anal cancer is also strong, since 88% of anal cancers are HPV-positive. Of these cases, 73% were infected with HPV 16. Penile cancer is rare; about 26300 new cases worldwide per year. About half of the cases are HPV-prevalent; usually HPV 16 or HPV 18 (Miralles-Guri et al., 2009).

Head and neck squamous cell carcinoma (HNSCC) incidence rates are increasing worldwide. The causes are multifactorial, but the most common risk factors include alcohol and tobacco use. Another risk factor is HPV 16 infection. Apparently HPV-DNA has been found in 35.6% of oropharynx cancers, 24% of larynx cancers and 23.5 in oral cavity cancers (Kreimer et al., 2005).

Some authors have suggested HPV infections as a possible co-factor in esophagus-carcinoma, but data are very inconsistent (Gillison and Shah, 2003). The fact is that HPV has been identified from the human esophagus (Jarrett et al., 1978, Syrjanen et al., 1982).

Certain types of skin cancers are connected to HPV infections, namely squamous cell carcinoma (SCC) and its precursor lesions (Aldabagh et al., 2013). There is evidence that HPV plays a role in the development of epidermodysplasia verruciformis (EV) (Majewski and Jablonska, 1997), but these HPV virus types belong to other families than the alphaviridae. There have been indications that immunosuppression can lead to skin cancers with possible HPV linkage (Bouwes Bavinck et al., 2001), but further investigations must be carried out.

HPV analysis

The HPV detection assays that are used today are mainly based upon detection of viral nucleic acids, and mostly viral DNA. They can further be divided into target amplification methods and signal amplification methods.

Target amplification:

The most common method to amplify Viral DNA is PCR. The process requires polymerase, which can synthetize DNA from the target sequence, primers that can bind to specific targets, and the four deoxynucleic acids in adequate amounts. This makes it possible to amplify the potential DNA sequences to detectable levels. It is also possible to use RNA in the PCR, then the RNA is converted to cDNA by a reverse transcriptase prior to the actual PCR (RT-PCR). An isothermal amplification method has also been developed, which is particularly well suited for m-RNA-amplification. That method is called NASBA (nucleic acid sequence-based amplification)(Smits et al., 1995).

Signal amplification:

Signal-amplification methods, such as Hybrid Capture 2, are based on an initial hybridization, where the signal from the hybridization is amplified and visualized (Snijders et al., 2010).

Consensus-primers with targets in several HPV types are used, almost exclusively directed against L1 or E1 sequences of the HPV genome. Commonly used PCR assays employ the MY09/11 and GP5/6-primers (Snijders et al., 2010).

The reading system for detecting the PCR product is essential. Most systems use some kind of labeled PCR product, which ultimately can be visualized through colorimetric or fluorescent staining procedures.

Reverse hybridization techniques have been introduced for genotyping. For genotyping of HPV, such an approach is used in reversed hybridization techniques with probes of strips, filters, microarrays and microsphere (Luminex, Austin; TX, USA) beads being employed.(Snijders et al., 2010)

Present studies

When the work on this thesis started, the first study (paper IV) investigated a surgical method using the sentinel node technique. The following work then concentrated upon reasons for falling ill with cervical cancer, and ways to prevent this. The history of screening results was analyzed in cervical cancer patients. It was clear that screening was less frequent in women who had the cervical cancer diagnosis. The further investigations were about HPV and self-testing. However, the self-tests on the market were expensive and messy. We started brainstorming about simple and cheap devices to collect self-sampling-material. This ended up in the discovery of self-sampling device that is validated in paper II. The actual study about non-attendees then resulted in paper III.

Aim of the studies

Paper I:

To audit the cervical screening program in the South Sweden Region. All women with cervical cancer diagnosed between January 2009 and December 2010 were included in the audit, which analyzed their previous screening history.

Paper II:

To evaluate if vaginal self-sampling without preservatives was adequate for HPV testing.

Paper III:

To compare the responses and the HPV prevalence in responding women among long-term non-attending women with (i) HPV testing of a self-collected vaginal sample, and (ii) cytological screening with a flexible no-fee appointment for sampling at an out-patient clinic.

To explore the main reasons for previous non-attendance.

Paper IV:

To evaluate the sentinel node (SLN) concept in early stage cervical cancer.

Materials

Paper I:

Between January 2009 and December 2010, 165 women were diagnosed with cervical cancer in the south Sweden region. These patients were referred to the gynecologic oncology department, where they were staged by an oncologic gynecologist and a gynecologic oncologist. The women´s cytological tests prior to their cervical cancer diagnosis were analyzed. The study was approved by the Regional Ethical committee DNR nr 2009/345

Paper II:

One hundred and twenty-one women, aged 18-65 (mean 34 years), who had been found to have an abnormal cervical smear in the organized screening program, were invited to the out-patient colposcopy clinic at Lund University Hospital. Tests from 108 women, who had given informed consent, were analyzed. Oral and written instructions on how to make the sampling were given. The study was approved by the Regional Ethical committee. DNR nr 2009/401

Paper III:

From the Swedish population registry, there were 242,678 women aged 32-65 in the county of Skane, who had been resident during the years 2006-2011. Comparison with the Southern Sweden Regional cervical screening registry showed that from the Swedish population registry, there were 242,678 women aged 32-65 in the county of Skane, who had been resident during the years 2006-2011. A comparison with the Southern Sweden Regional cervical screening registry revealed that 28,636/242,678 women (11.8%), had not taken a smear for nine years or more. The screening registry contains information on all smears taken in the region (both organized and spontaneously taken smears). There were 6,523/28,636 long-term non-attendees who were resident in the Lund University Hospital district. The women were randomized using computer-generated random numbers. Among these, 1,500 eligible women were selected by randomization, and were further randomized 2:1 to HPV self-sampling or to flexible no-fee appointments. DNR nr 2009/401

Paper IV:

From March 2005 to April 2009, a total of 105 women presenting with early stage (1a1-2a) cervical cancer were scheduled for the sentinel node procedure in conjunction with a complete pelvic lymphadenectomy at the Department of Obstetrics and Gynecology at Lund University Hospital, Lund, Sweden. For patient characteristics, see (Fig.) 1. The study was approved by the Regional Ethical committee DNR nr 2009/345

Methods

Database searching; Paper I:

The women, who had been diagnosed with their cervical cancer between 2009 and 2010 in the southern Swedish region, had their screening history examined in a database of cervical cytology samples. The screening history was then classified as correct screening, under-screened or not screened. The women beyond screening age could have had a normal screening history or an abnormal screening history. Normal screening history was two screening samples without dysplasia and taken within the correct timespan. The cancer was classified as screening-detected if an abnormal smear leading to diagnosis was recorded 1-6 months prior to diagnosis.

Self-sampling device; Papers II and III:

We present a new cost-effective sample method, which consists of a cotton swab (Selefa Trade, Sweden) and a dry sterile 2 mL plastic container (Cryotube, Nunc A/S, Denmark) (Fig 9). No transport medium is needed, which makes it suitable for regular mail transport. For paper II, both written and oral instructions were given; and for paper III only written instructions on how to take the vaginal self-collected sample (SS) were provided. Briefly, the women were asked to rotate the swab 360 degrees, 3-4 times inside the vagina. They then put the wooden stick into a sterile cryotube, broke off the upper part of the stick and put the tube cap on.

The SS device was then sent to the Microbiology Department in the Hospital of Malmo. This was done by hospital mail for paper II and by regular mail for paper III.

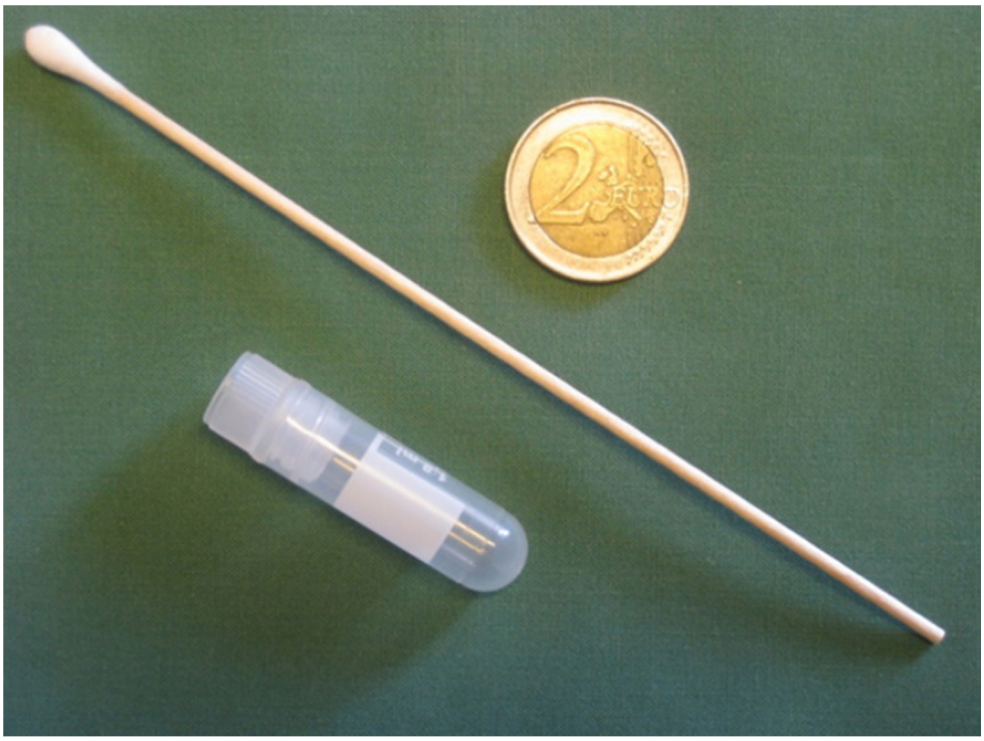


Fig 9 Self-sampling device
This simple and cheap device is validated in paper II.

Liquid-based Cytology (LBC); Paper II:

The LBC was collected by a plastic device called a Rovers Cervex-Brush Combi. This scraped cells from the portio, and these were put into a "Thin Prep preservCyt Solution". An aliquot was sent to the Microbiology Department of the Hospital of Malmö.

Luminex-method HPV analysis; Paper II and paper III:

In the laboratory, the cotton tip from the self-sample was incubated with 0.5 mL saline for ten minutes at room temperature, and then the cotton tip was rinsed in the solution by pipetting. From each sample, 200 µL was then used for automatic DNA extraction by MagnaPure (Roche) and eluted in 100µL. 5 µL was used for HPV DNA amplification by PCR with modified GP5+/6+ (MGP) primers (Soderlund-Strand et al., 2009). After amplification, Luminex-based HPV genotyping was used to identify HPV types. The technique allowed the detection of 38 HPV genotypes of which 18 are high-risk HPV and five are probable high-risk HPV. The potential high-risk HPV types were classified into the high-risk group in our studies. Beta-globin real time PCR was included as a separate test of sample adequacy for PCR, where the threshold (Ct)

values reflect the relative number of cells in the sample. A lower Ct-value represents a relatively higher number of cells. The lowest detection level was about five diploid cells per PCR, yielding a mean threshold Ct-value of 37.2 (CV 2.8%).

Self-sampling versus flexible appointment; Paper III:

The HPV testing group was invited by mail to participate and each person was asked to collect a vaginal sample for HPV analysis (as described under self-sampling device above, and analyzed as described above under the Luminex-method). The letter contained user instructions, a questionnaire, and the actual self-sampling kit. The women returned the self-taken sample to the laboratory in a stamped addressed envelope provided. One month after invitation, a reminder was sent to non-responding women. The reminder included another sampling kit, identical to the one in the first mailing.

In the flexible appointment arm, women were invited to sampling by a gynecologist at an outpatient clinic. The visit was free of charge, and the invitation included several alternative appointments in the daytime, evenings or on Sundays, so that access to the appointments would be as flexible as possible. An LBC sample for HPV analysis was taken. If the women did not turn up, another invitation with five different options for appointments was sent.

The participating women signed informed consent forms, and were asked to answer a questionnaire covering their reasons for not attending the organized cervical screening program, their sexual history, their parity, smoking habits and educational level.

Women with positive hr-HPV tests, including possible hr-HPV, were invited to the outpatient colposcopy clinic for examination. In the case of positive colposcopical findings, biopsies and an LBC were taken. If a woman with hr-HPV did not attend, efforts were made to contact her by phone.

Surgical methods, SLN detection; Paper IV:

Surgical methods:

In 90 of 105 patients (86%) a robot-assisted laparoscopic approach (da Vinci Surgical system, Intuitive Surgical Inc., Sunnyvale, CA) was used, whereas the remainder underwent either open surgery or a traditional laparoscopic approach.

Radiotracer:

As a radiotracer, we used 1.5 mL (120 MBq) Tc-99 human-albumin nanocolloid (GIPHARMA, Saluggia, Italy). Under direct visualization, a four-quadrant submucosal peritumoral injection of the radiotracer was performed approximately 18 h before onset of surgery by the surgeon or the assisting surgeon. Immediately after the injection, a 15-min dynamic (anterior) Lymfoscintigram (LSG) with a final picture after 45-60 min

was performed. For logistics reasons, seven patients were injected with Tc-99 on the morning of the surgery, but had no LSG.

Detection of tracer:

During surgery, we used a gamma probe (Neo2000® laparoscopic probe, Neoprobe Corporation, Dublin OHIO) to detect the SLN having the LSG chart exposed for additional guidance. With the probe, we systematically scanned the pelvic side walls, the presacral area and the para-aortic area, up to the level of the inferior mesenterical artery. Any lymph node with a radioactivity of at least five times the background count was considered an SLN and was sent separately for pathohistological evaluation (frozen section as well as full final evaluation). We also separately removed enlarged but radio-negative nodes. Then, a complete bilateral pelvic lymphadenectomy was performed starting with the common iliac nodes (boundary 5 cm cranial of the bifurcation of the iliac artery), followed by the external iliac nodes (distal boundary the Cloquets node, lateral boundary the genitofemoral nerve), and the obturator nodes (distal boundary the pubic bone, dorsal boundary the obturator nerve).

If the case of metastatic nodes, the radical hysterectomy was abandoned in favor of radiation treatment with concomitant weekly cisplatinum.

Pathology processing of the SLNs; Paper IV:

The SLNs were divided into at least two pieces for frozen section, and at least one section was stained from each piece with haematoxylin and eosin (H&E), and evaluated microscopically during the operation. Thereafter, the tissue was fixed in 4% phosphate buffered formaldehyde and further processed for permanent sections. If no metastases were found, at least two additional sections were obtained from paraffin-embedded tissue, at distances of 0.2 mm, and stained with H&E. Beginning in December 2007, negative SLN slides were additionally stained by a pan cytokeratin cocktail MNF116 (Dako Canada, ON) immune-peroxidase stain. The remaining non-SLNs were fixed in 4% phosphate buffered formaldehyde. After fixation, each lymph node was cut into 3 mm thick slices and at least one slice per lymph node was histopathologically analyzed after staining with H&E.

Statistical analyses:

Paper I:

Data were controlled for normality with Levene´s test. Parametric data are presented as mean + standard deviation (SD) within parenthesis. The Student's T-test was used for analysis of descriptive parametric data. A Chi-square test or Fisher's exact test was used when appropriate for analysis of grouped data. All comparisons were two-sided and a p-value <0.05 was considered statistically significant. The survival statistics were prepared using the Cox proportional hazard model. Assumptions of proportional hazards were verified graphically. For a graphical presentation of overall survival, the

Kaplan-Meier method was used. Analysis was performed using the IBM Statistical Package for Social Sciences (SPSS) 21.0 (SPSS Inc., Chicago, IL, USA).

Papers II and III:

The tests were based on the binomial distribution and the exact confidence intervals (CI) are given. Kappa values were calculated using standard methods. Kappa statistics indicate how two tests reproduce one another. A kappa value of 1.0 shows a perfect agreement, a value of more than 0.8 is almost perfect, more than 0.6 is good, a value between 0.4-0.6 is considered moderate, and below 0.4 is poor. A value of 0 is considered a random distribution. All comparisons were two-sided, and P values less than 0.05 were considered statistically significant. Fischer´s exact test was used to compare the different groups. Statistical analysis was performed using SPSS (PASW) version 18.0 (SPSS Inc., Chicago, IL, USA) and OmniStat (SBU, Sweden).

Paper IV:

The performances of the diagnostic tests were summarized regarding sensitivity, specificity and negative predictive values with exact confidence intervals (CI) based on the binomial distribution.

Results

Audit of the cervical screening program in the South Sweden Region (Paper I):

The histology of the cervical cancer of the 165 women was squamous cell carcinoma in 71% and adenocarcinoma in 28 % of the cases (table 1). In advanced FIGO stages II-IV the squamous cell carcinoma was more frequent compared to the adenocarcinoma but the difference was not statistically significant (comparison between stages Ia-Ib vs. II-IV) (table 1).

The mean age at diagnosis was 53 years (SD=18, range 22-94); in the adenocarcinoma group the mean age was 46 years (SD 14) and for the squamous carcinoma group it was slightly higher, 55 years (SD 19)(ns). One quarter of the women (n=43) were above 65 years of age at diagnosis, and one third of the women (n=56) were older than 60 years of age (Figure 1). The screening-detected cancer patients (n=30; 18 %) were all in stage I a-b except for one patient in stage II (Figure 2). The pattern of FIGO stages in women of screening age was the same whether they had followed the recommended screening program or not. Women aged over 65 years had significantly more often an advanced stage of disease (FIGO II-IV) (n=36 out of 43; 84 %) compared to those below 65 years of age (n=35 out of 122; 29 %) ($p<0.001$).

The overall survival using univariate Cox regression analysis indicated a significantly worse prognosis for women above screening age compared to those who had a recommended cervical smear (Hazard Ratio (HR)=5.3 95% CI 2.4-12.0, p<0.001).(Figure 3). The women of screening age who had not had a recommended screening test showed a tendency to have a worse prognosis compared to the women with a normal screening history, (HR=2, 95% CI 0.8-4.7). All patients diagnosed through the cervical screening program were still alive (30/30) at the median follow-up time (36 months), and showed a significantly better overall survival compared to those aged 65 or younger whose disease was discovered due to symptoms (68/98; p<0.001).

Vaginal self-sampling for HPV testing (Paper II):

In total, 121 patients were included in the study, but 12 patients were excluded. One additional patient was excluded due to an inadequate self-sampling. We included one woman who had a non-adequate Beta-globin result in the self-sampling (SS) presenting hr-HPV DNA in the LBC-sample. The evaluation of the SS and the LBC-samples included 108 women. The mean Ct-values for the Beta-globin adequacy-test in the SS group and LBC group were 28.7 (range 21.4–39.7, CV 12.4%) and 27.9 (range 22.3–36.9, CV 10.7%), respectively. Positive hr-HPV DNA was found in 56 women with both tests. The SS method detected another nine (65/108 60%; 95% CI 0.50–0.69), and the LBC method detected eight additional positive hr-HPV individuals (64/108 59%; 95% CI 0.49–0.69) resulting in an hr-HPV positivity of 68% (95% CI 0.58–0.76) in the test cohort. Test persons positive to any HPV type were found in 89 of 108 women (82%; 95% CI 0.74–0.89) (Table 1b). The SS detected any HPV in 83/108 (77%; 95% CI 0.68–0.84) and in the LBC samples in 76 of 108 women (70%; 95% CI 0.55–0.74). The agreement regarding hr-HPV positivity was good between the results from the SS and the LBC-tested samples (kappa value 0.67 (95% CI 0.53–0.81)), and moderate when any HPV presence was tested in the two groups (kappa value 0.55 (95% CI 0.37–0.73)). Comparisons between hr-HPV found in the SS and the LBC samples in relation to cytology and histopathology are shown in Tables 2a and 2b. Cytology was available for 57 women (53%) and biopsy-verified histology was available for 47 patients (44%). There was no significant difference between the false negative number of patients in which HSIL was found with cytology or histopathology using the SS or LBC methods. The sensitivity for SS with hr-HPV to find HSIL was 77% (95% CI; 62–91%) with a specificity of 47% (95% CI 35–59%), and the sensitivity for LBC with hr-HPV to find HSIL was 79% (95% CI 66–93%) with a specificity of 50% (95% CI; 38–62%).

Self-sampling versus flexible appointment (Paper III):

Self-collected vaginal samples (SS) from 147 (14.7%) women were returned to the HPV laboratory from the 1000 invited women. The DNA was inadequate in two of the samples, leaving 145 samples with adequate HPV tests. Ten women had high-risk-HPV (hr-HPV) infection (6. 9%). They were offered a gynecological examination, and

seven of them turned up. Six of them had normal cytology, and one woman, who in fact had had a hysterectomy, had LSIL. Thus, no HSIL was found in these women. The following HPV types were detected: HPV 16 (three women), HPV 18 (one woman), HPV 51 (one woman), HPV 52 (one woman), HPV 53 (one woman), HPV 56 (one woman) and finally HPV 58 (2 women). Seven women had low-risk HPV infections. Twenty-one of the 500 invited women came to the outpatient clinic and had a cervical sample taken (4.2%). Two women had hr-HPV (HPV 51 and HPV 53 respectively). The woman with hr-HPV 51 had HSIL in cytology, and the other with hr-HPV 53 had benign/atypia cytology. Four women had a low-risk HPV infections, and normal cytology. In total, from the flexible appointment group the cytology was normal in 19 cases, one case had atypia and one had HSIL. The response rate was higher in the self-sampling group (15%) compared with the group invited to the outpatient clinic (4.2%) ($p > 0.0001$). There were no other significant differences between the two groups. One quarter of the self-test group and half of the outpatient clinic group reported previous negative experiences of gynecological examinations. One quarter of the outpatient clinic group and 14% of the self-test group reported previous negative sexual experiences. The questionnaire allowed the women to give multiple reasons for not attending earlier invitations to cervical screening. Several women gave more than one reason. Both in the self-sampling and the outpatient group the most common reason given for non-attendance was "Uncomfortable with vaginal examination" 37% and 43%, respectively. The other common reasons were "Feel healthy", "Lack of time" and "Experience of unfriendly health workers".

Sentinel node (SLN) concept for lymphatic mapping in early stage cervical cancer (Paper IV):

The median age of the patients in this study was 40 years (range 24–76). The tumor was less or equal to 2 cm in 62% (n=65), and larger than 2 cm in 38% (n=40) as measured preoperatively by visualization, CT-scan or MRI (mean 1.8 cm and median 1.5 cm). Of the women, 60 (57%) had a squamous cell carcinoma, 44 (42%) had an adenocarcinoma and one woman had a tumor with a predominant neuro-endocrine histopathology. The most frequent stage was 1b1 (66%). The vast majority of surgical procedures (86% n=90) were performed using robot-assisted laparoscopy. One patient's procedure was switched from robot-assisted laparoscopy to laparotomy due to robot arm failure. Radical trachelectomy was performed on nine patients (four with the robot), 83 patients had radical hysterectomy and in 13 patients only pelvic lymphadenectomy was performed, since lymph node metastases were diagnosed during surgery. The overall detection rate of at least one SLN was 90% (94/105 patients) and 94% (61/65 patients) in patients with tumors equal to or smaller than 2 cm. Bilateral SLNs were identified in 59% (62/ 105) of the patients. In patients with tumors equal to or smaller than 2 cm, bilateral SLNs were detected in 65% of cases (42/65), whereas in patients with tumors larger than 2 cm, bilateral SLNs were found in only 50% of cases (20/40). No difference in detection rate between squamous cell carcinomas and

adenocarcinomas was observed. The LSG showed "hot" SLNs in 85 out of 97 patients (88%), which was slightly less compared to the detection rate with the gamma probe. The median number of SLN/side was 1 (range 0–4) on both the right and left side. The mean number of SLN/side was 1.4 (SD 1.1) on the right side and 1.2 (SD 1.1) on the left side. The mean number of removed and analyzed pelvic lymph nodes per side was 12.2 (SD 6.4) on the right side and 11.6 (SD 5.6) on the left side. Two women, both with tumors larger than 3 cm, had no identified SLNs either with the probe or with the LSG but one had a bulky metastatic node and the other woman had metastases in 14 out of 27 analyzed lymph nodes.

Among 18 women with at least one metastatic lymph node, 17 also had a metastatic SLN. One woman with a stage 2a squamous cell carcinoma of 3.5 cm had one metastatic non-SLN on the left side but the bilateral (one on each side) SLNs were without tumor. Five out of the 61 women (8%) with a tumor size of 2 cm or less had lymph node metastases, all identified in SLNs (sensitivity 100%). Another woman with a stage 1b1 squamous carcinoma of 1.5 cm had metastatic SLNs on both sides but also one radio-negative metastatic bulky node. The negative predictive value for patients with cervical cancers having a diameter equal to 2 cm or less was 100%.

The laparoscopic robot-assisted pelvic SLN procedure was performed on 90 patients. In the separate analyses including only the laparoscopic robot-assisted procedures, the detection rate, sensitivity and negative predictive value did not differ from the total material with different surgical methods. The intraoperative frozen section of SLNs identified metastatic disease in 14 out of 18 patients, with metastatic SLNs in the final histology. The remaining four "frozen section negative" SLNs contained micro metastases between 0.1 and 0.5 mm. Metastases were found exclusively in the SLNs in 14 out of 18 patients. Two of these patients had metastases in two of the SLNs.

Discussion

Screening for cervical cancer

In 2011, women in Sweden had an estimated life expectancy of 83.5 years, which means that for one third of their lives they are not screened (SCB, Swedish Official Statistics; 2012). The recommended cervical cancer screening system in Sweden covers women aged up to 65 years, and our results show that more than one quarter of cancer cases cannot be detected by the national screening program. The breast cancer screening program ends at 75 years of age. The major causes of death for women are cardiovascular diseases and malignancies (Causes of death 2012, Socialstyrelsen). However, a large proportion of women over 65 are healthy and have an active sexual life (Beckman et al., 2008). They respond well to curative treatment for dysplasia and cervical cancer if the disease is discovered at an early stage, as shown by the results from

other studies (Andrae et al., 2008). The efficiency of a cervical screening program depends mainly on the population coverage of the program, which is dependent on the acceptance of the invitations (Andrae et al., 2012, Stenvall et al., 2007b). The recommended screening program is ended at a relative young age in many countries, at least partly because of the declining sensitivity of the pap smear in postmenopausal women (Colgan et al., 2002, Leinonen et al., 2009, Gyllensten et al., 2010, Gustafsson et al., 1995). A possible way to improve the screening sensitivity is to use the hr-HPV test with reflex cytology as an "exit test" in the screening program. Another improvement would be to use a prolonged HPV test screening program to reduce the risk for cervical cancer in women above 65 years of age.

Self-sampling as a solution

A suggestion for a solution to nonattendance could be testing for the presence of hr-HPV among vaginal self-samples, as validated in paper II. There has been increasing interest in finding a feasible method for self-sampling vaginal tests to detect hr-HPV DNA since compliance in attending cervical screening programs is not complete. Many women choose not to have a cervical screening test at all, or have the test taken on demand, more frequently than suggested in the organized screening programs. This study shows a new self-sampling method with a similar detection rate of hr-HPV DNA as the one taken by a gynecologist. Our study is in accordance with earlier studies and a large systematic review including a meta-analysis.(Petignat et al., 2007, Gustavsson et al., 2011) Previous papers have shown that vaginal HPV sampling is not dependent on menstrual cycle, recent sexual intercourse or the timing of the sampling, which all contribute to stable requirements for a self-sampled vaginal test.(Harper et al., 2003) The sterile cotton swab used in this test seems able to collect enough vaginal and cervical cells, since the results obtained show a sufficient amount of DNA in more than 99% of the self-collected samples. The smooth and narrow swab is easy to use and does not cause any major discomfort (personal communication). The test individuals did not report any difficulties or problems with the instructions, probably due to the widespread use of tampons for menstrual bleeding. Several other studies have evaluated the acceptability of self-sampling versus physician sampling, showing that women prefer self-sampling.(Harper et al., 2003, Sellors et al., 2000) Compared to other self-sampling methods, where vials are stored or transported in liquid, our method does not need any added liquid, allowing use of regular mail for delivery to the laboratory for a total cost of approximately two Euros. These results make this self-collected vaginal smear sample very convenient and cost-effective for HPV analysis (Andrae et al., 2012, Stenvall et al., 2007b).

The study reported in paper III shows that long-term non-attendees replied three times more often when offered a self-sampling HPV test (15 %) than when they were invited to an outpatient clinic (4.5 %)(p value < 0.0001). Women who had not had a screening test for nine years or more appeared to be very reluctant to reply to any kind of

invitation. When the women responded to a question about why they had not attended the tests, the most common answer was "Uncomfortable with vaginal examination". Other common reasons were "lack of time", "feeling healthy" or "experience of unfriendly health workers".

Who benefits from self-sampling?

The vaginal self-sample can be a suitable and attractive alternative for persons older than the screening population, especially among non-attendees (Tamalet et al., 2010). Also, women who have never been screened have a preference for self-sampling (Gok et al., 2012).

Immune-suppressed individuals have a higher risk of HPV infections not healing and developing cytological pathologies leading to cervical cancer (Paternoster et al., 2008, Ahdieh et al., 2000, Grulich et al., 2007). These high risk women may benefit from more frequent self-sampling HPV tests which are easier and more cost-effective to perform than the normal screening programs.(Serraino et al., 2007).

Why do women not attend cervical screening programs?

In our questionnaire, nearly 40 percent of respondents thought the gynecological examination would be unpleasant. Women with negative feelings towards gynecological examinations may prefer to use the self-sampled test at home instead of being confronted once again with experiences they previously found to be emotionally unpleasant. For these women, an acceptable alternative to no regular cytological screening could be a primary self-sampled HPV test. Women who state that they lack the time to attend screening may also prefer the time-saving self-test. Women giving "feeling healthy" as a reason for not attending will be hard to convince, but information campaigns and the vaccination debate may persuade them to attend the program. In rural areas with a high percentage of immigrants, the compliance rate with the screening program is generally low, indicating that other strategies or programs to recruit these women are needed. In some areas in the city of Malmoe in Southern Sweden, there is a compliance rate of about 50 %(Bjelkekrantz K. Cervixcancerscreening Rapport 2010+2011).

We do not have information about the attitudes of the non-attendees who also did not attend the present study, even though we believe that the responders in this study gave similar answers in the questionnaire as the current non-responders would have done. Other studies have shown high response rates, but the inclusion criteria were different. In those studies, the women had not taken a screening test for five or six years (Giorgi Rossi et al., 2011, Gok et al., 2010, Gyllensten et al., 2011, Sanner et al., 2009, Wikstrom et al., 2011, Virtanen et al., 2011a, Virtanen et al., 2011b), whereas the time interval in our study was rather extended; nine years or longer. The screening register does not always contain information about prior hysterectomy, and a few percent of the invited women did not respond because they knew they did not have a cervix.

HPV prevalence and histology in former non-attendees

In the study of self-sampling, the rate of hr-HPV infection was similar to other studies, even though the number of years since the last smear differed (Gyllensten et al., 2011, Virtanen et al., 2011b, Piana et al., 2011, Szarewski et al., 2011, Wikstrom et al., 2007). The cytological-pathological results revealed no HSIL in this study, which was in accordance with the study by Virtanen from Finland in which there were only nine cases with HSIL out of 125 women (7 %) in the non-attendant group with hr-HPV. The women in our study had previously received screening invitations and reminder letters to attend regular cervical screening. Both our study and other studies indicate that the percentage of HSIL in non-attending women who perform self-sampling remains low, at 0-3%, even when self-sampling is offered as a third intervention. In addition, when women with known hr-HPV were invited several times to clinical examination and follow-up, three of the women did not turn up (30%).

There were no monetary rewards for participating, but the women were neither charged for the ambulatory visits to the clinic, nor for the HPV test. No other rewards were offered. It is possible that some patients in both groups responded because the examination was free, since the normal screening visit costs approximately 15 Euros in southern Sweden. In the hr-HPV-positive women, 70% came for a follow-up. A thorough follow-up is a necessity, and repeated reminders are given (Piana et al., 2011). It is a challenge to persuade these individuals to come for further investigation, even when they know that they have an hr-HPV infection. The organized program in the area where this study was performed has a population coverage of approximately 75%. The organized self-sampling increased the population coverage by 4%. Although this is a small improvement, the increased cervical cancer risks in this group may still motivate the use of this strategy.

The sentinel node procedure

When a woman has her cervical cancer treated it is important that the treatment is safe, and has as low morbidity as possible. In paper IV, a surgical method of carrying out a sentinel node procedure, as an alternative treatment to full lymphadenectomy is investigated. The detection rate of an SLN in this study was 90% which is similar to other published series (Hauspy et al., 2007b, Altgassen et al., 2008). In tumors equal to or smaller than 2 cm the detection rate was higher (94 %), as also shown by Altgassen et al. (Altgassen et al., 2008). In other tumor types such as malignant melanoma, breast cancer, vulvar cancer or penis cancer the SLN concept is reliable when the tumor is not too large (Hauspy et al., 2007a).

From studies on breast and vulvar cancer it is known that bulky metastatic nodes may cease to receive lymphatic flow due to a blockage of the lymphatic channels (Fons et al., 2004). In this study, five patients had bulky, suspiciously metastatic nodes at surgery and metastases were found in all these nodes. In a study of Altgassen et al. there was no data on or any discussion about enlarged or suspicious lymph nodes (Altgassen et al.,

2008, Hauspy et al., 2007b). When the disease is metastatic, the lymphatic flow may bypass a bulky metastatic node and the radiotracer can take another route and identify any possible lymph node as SLN. Pre-operative imaging by MRI and/or CT scan increases the possibility of identifying enlarged bulky nodes. Furthermore, we believe the enhanced visualization with the robotic laparoscopic 3D vision and magnification facilitates the identification of lymph nodes in general and non-SLN tumor suspect nodes in particular, adding extra accuracy to the SLN concept.

Frozen sectioning and micro-metastases

In patients with tumors sized 2 centimeters or less the sensitivity for the SLN concept was 100 % as all five women with lymph node metastases were identified. Four SLNs were negative in the frozen section but micro metastases less than 0.5 mm were found at serial sectioning and staining with H&E. The false negative SLNs in frozen section indicate the importance of further formalin fixation and serial sectioning of the SLNs. In our study, intraoperative assessment of SLNs allowed immediate detection of metastases to determine whether radical hysterectomy or chemo-radiation should be performed. Serial sectioning to evaluate the SLNs has demonstrated an increased detection rate of metastases in up to 10–15 % more than the ordinary lymph nodes. It has been shown in breast cancer patients that 10 % had occult lymph node metastases, 16 % in the SLNs and 4% in other lymph nodes (Weaver, 2006, Weaver et al., 2006). However, the clinical significance of a micro-metastasis (0.2-2.0 mm) or even smaller tumor cell conglomerates (<0.2 mm) has not yet been determined but those patients may have increased risk of loco-regional recurrences. In cervical cancer a local regional recurrence worsens the prognosis significantly and leads to major surgery, often combined with chemo-radiation in cases where the recurrent tumor may be curable. The search for micro-metastases by serial sectioning of all lymph nodes is time consuming whereas serial sectioning on targeted SLNs only is less labor intensive and may result in high metastatic yield.

Finding lymph nodes in unusual locations:

The SLN concept with a gamma probe and a pre-operative LSG may improve the chance of finding metastasis in unusual locations such as the presacral area, the higher common iliac region and the lower para-aortic areas, where up to 10% of the metastatic nodes are found (Rob et al., 2005). On the other hand, radioactive lymph nodes may be difficult to find close to the cervix, due to background radioactivity from the injection in the cervix. However, if the parametria contain metastatic lymph nodes the nodes are removed and analyzed en bloc with the cervical specimen in radical hysterectomy or radical trachelectomy. The negative predictive value for an SLN free of disease in this study was 99%, which indicates a low probability of failure. If the patients are categorized according to tumor size preoperatively, the negative predictive value for tumors equal to 2 centimeters or smaller was 100% in this study. Thus, the concept for tumors equal to 2 centimeters or less is safe. In cervical tumors of 2

centimeters or less, a similar high negative predictive value 99.1 % has been shown in a large multicenter study by Altgassen et al. (Altgassen et al., 2008). On the other hand, in our study the negative predictive value for patients with tumors larger than 2 centimeters was 95 %.

Visualizing suspect nodes:

Pre-operative LSG may enhance the possibility of detecting SLNs in the presacral and the common iliac artery or lower para-aortic region. However, in our material the detection rate in the pre-operative LSG was lower, compared with the per-operative gamma probe, corresponding with other reports (Vieira et al., 2009). However, SPECT-CT with three dimensional images may improve pre-operative imaging and make the detection easier and more precise (Ibusuki et al., 2009, Vermeeren et al., 2009) .

Studies with other new techniques such as CT-PET have shown high specificity in predicting metastatic lymph nodes, but limited sensitivity. CT-PET may be used as part of the preoperative investigation of cervical cancer patients but cannot replace lymphatic surgery, as CT-PET presently is unable to identify metastases smaller than 4 millimeters (Chung et al., 2009).

Final remarks on the SLN technique:

The results from the present and other studies indicate a role for the SLN concept with patients with cervical tumors of 2 centimeters or less, to a low false negative rate. If there is no identifiable SLN on either of the pelvic walls of the patient, a complete lymphadenectomy should be performed on this side. For reasons discussed earlier it is important that bulky nodes are removed. For early cancer of 2 centimeters or less without bulky nodes and a detectable SLN on each pelvic side there is reason to recommend a sharp SLN protocol instead of a complete pelvic lymph node extraction. The recommendation would include a follow-up protocol with an observational prospective multi-center study, including Quality of Life analyses to find negative side effects of the complete pelvic gland extraction compared to the sharp SLN concept, but should also be initiated to further establish the safety of omitting complete lymphadenectomy in patients with no metastases in sentinel nodes.

General conclusions

- Women above 65 years of age may benefit from a prolonged HPV test screening program.
- The self-sampling device validated by us detects hr-HPV infections with similar sensitivity to a cervical smear taken by a gynecologist.
- Vaginal HPV self-sampling seems to be more effective for increasing the coverage of screening than the control-group with flexible appointments for regular cervical smear performed by a gynecologist or midwife.
- The most common reason for non-attendance in the screening-program was "uncomfortable with vaginal examination".
- The SLN technique seems to be an accurate method for identifying lymph-node metastases in cervical cancer patients with tumors of 2 cm or smaller.

Future perspectives

It is important to see what effect the HPV vaccination has on the cervical cancer burden. The virus comes in many variants and although the vaccines have some cross-protection, there are still some variants that are not covered by vaccination. Will the screening program continue as previously? No, I think there will be a shift towards HPV testing and probably self-sampling. We must keep track of the development so we do not lose a generation in the screening. The women that have not received the vaccination must continue screening. Even the women that have received their vaccination must continue to be screened; however maybe this process will be different. The self-sampling hr-HPV test may be used as a follow-up after vaccination.

How about the population in developing parts of the world? It is of the utmost importance to expand the vaccination program to areas where cervical cancer is most frequent, and the possibilities to diagnose and treat the disease are limited. The simple idea of self-sampling could be used in large populations, and since there is no need for preservative, the method is easy. A problem could be anatomic knowledge about the actual female body. When we conducted the validation in Sweden, we used an information sheet containing pictures of the way to sample. How do we do this in

communities where people have limited knowledge about their own bodies? Maybe the sampling could be combined with family-planning advice, or maternity examinations; then it would improve female health in many ways.

It would be interesting to use self-sampling to investigate the viral pattern in certain groups, e.g. immune-compromised patients.

The sentinel node concept is now developing further with the IsoCyanoGreen method. This may make it easier to identify the sentinel nodes. Use of sharp sentinel nodes is an area that needs improvement, and studies are now ongoing.

We are planning further studies on HPV typing in cervical cancer tumors, and also with nodal viral-DNA. We have plans to conduct further investigation into patients suffering relapses, and see if their nodes were HPV-positive when the first surgery took place. If this was the case, it may in the future be used as a prognostic factor.

Sammanfattning på svenska

Livmoderhalscancer är den näst vanligaste cancern för kvinnor i världen, över 500 000 kvinnor insjuknar varje år. Varje år dör dessutom 275 000 kvinnor av denna sjukdom. De flesta av de som insjuknar och dör bor i utvecklingsländer. I Sverige drabbas ca 400 kvinnor varje år.

På sextiotalet i Sverige började vi screena för denna cancertyp i Sverige. Detta genom hälsokontroller, där celler från livmoderhalsen skrapades av vid en gynekologisk undersökning. Dessa celler undersöktes sedan i mikroskop, för att se om cellförändringar förelåg. Cellförändringar kan finnas i olika grader, och behandlas genom att ta bort en bit av livmoderhalsen. Denna behandling kan då stoppa utvecklingen till livmoderhalscancer. Sedan screeningen började i Sverige har antalet livmoderhalscancer minskat med hälften.

Det är ett problem att många kvinnor inte kommer till sina cellprovsundersökningar. Ca 20 % kommer inte till undersökningarna. Det har visat sig att en stor del av de kvinnor som får livmoderhalscancer tillhör denna grupp, och olika sätt att få dessa kvinnor till undersökningar har provats.

Vi vet idag att livmoderhalscancer orsakas av ett virus, Humant Papilloma Virus. Detta virus finns i ca 200 varianter. Alla orsakar inte cancer, en del är lågriskvirus, och orsakar exempelvis vårtor. Viruset är vanligt förekommande, stora undersökningar på friska kvinnor visar att ungefär 12 % är positiva för viruset. De allra flesta får infektionen i unga år, då de börjar sitt sexuellt aktiva liv, och träffar flera olika partners. De allra flesta infektioner läker ut, medan några blir kvarstående, persisterande.

Det är dessa kvinnor med en persisterande HPV-infektion som löper högst risk för att få livmoderhalscancer.

De vanliga cellproven har låg sensitivitet, till skillnad från HPV-provtagning, som har högre sensitivitet. Ett problem är dock att många unga kvinnor har en HPV-infektion, som de sedan läker ut. Det kan då bli många falskt positiva prov. Bland äldre kvinnor är andelen med HPV-infektion lägre, då de flesta har läkt ut eventuella infektioner, och många lever i monogama relationer. Man ser dock en topp av HPV-infektioner i 40-50 års ålder igen, kanske beroende på nya relationer eller att viruset reaktiveras i kroppen. Klart är att de, som har försvagat immunförsvar har högre risk att få en persisterande infektion, och livmoderhalscancer.

I våra studier har vi inriktat oss på att hitta orsaker till att kvinnor inte går på sin screening, och försökt hitta nya sätt att få kvinnor att genomgå undersökning. Vi har även tittat på hur kvinnor med livmoderhalscancer i södra sjukvårdsregionen har följt sina cellprovskontroller, samt undersökt ett nytt sätt att behandla livmoderhalscancer.

I det första arbetet har jag analyserat de kvinnor, som 2009 och 2010 har insjuknat i livmoderhalscancer, och retrospektivt kontrollerat hur de har följt sina screeningkontroller. Jag har tittat på två kontroller bakåt, och om de är inom rätt tid, klassificeras kvinnan som att ha följt screeningen. Om ett screeningprov fanns inom 1-6 mån från diagnosen, och det var det som ledde till diagnosen livmoderhalscancer, räknas kvinnans cancer som screeningupptäckt. Annars räknas cancern som symtomupptäckt. Cellprovtagningen slutar vid 65 år ålder i Skåne, och vid 60 i resten av södra sjukvårdsregionen. Det visar sig att en tredjedel av alla cancerfall upptäckt efter 60 år, och en fjärdedel efter 65 år. Detta gör att vi behöver ett slut-test innan kvinnan slutar bli screenad. Vårt förslag är ett HPV-test, som om det är negativt gör att risken för senare insjuknande i livmoderhalscancer vore mycket låg. Om HPV-provet är positivt kan man göra ett reflex-cytologitest; d.v.s. ta ett vanligt cellprov automatiskt på de HPV–positiva. Ingen skulle släppas från screeningen förrän de har ett negativt HPV-test. Vi visar att dödligheten för de icke-screeningupptäckta cancrarna är mycket högre, än för de screeningupptäckta, där vid uppföljningen efter ca 3 år, ingen hade dött.

I det andra arbetet har vi testat en ny metod att ta självtest inför HPV-test. Vi ville göra en självteststudie, men de självprovtagnings-kit som fanns att tillgå var dyra eller omständliga. Därför hittade vi andra material att samla provmaterial, och undersökte sedan tillförlitligheten i det materialet. Vi lät kvinnor på en mottagning ta ett självtest innan läkarundersökningen, och tog sedan ett vanligt läkar-taget cellprov för HPV-analys. Det visade sig att vårt självtagnings-set var jämförbart med ett läkar-taget prov. Kvinnorna tyckte att det var enkelt att ta testet.

I det tredje arbetet identifierade vi 1500 kvinnor i Lunda-trakten, som inte tagit cellprov på 9 år eller mer. Till 1000 av dem skickade vi självprovtagning-kit, samt ett frågeformulär där vi efterfrågade anledningarna till varför de ej tagit cellprov på så länge. Till 500 kvinnor skickade vi erbjudande om kostnadsfria mottagningsbesök på flexibla tider; kvällstid och på helger. Besöken var av typen drop-in, så de kunde bara dyka upp. De kvinnorna fick samma frågeformulär om varför de ej varit på sina cellprovtagningar.

15 % av kvinnorna skickade in sina självprovtagnings-kit, jämfört med 4.2% av kvinnorna, som kom på den öppna mottagningen. Kvinnorna i de två olika grupperna skilde sig inte åt med avseende på olika karakteristika som rökning, civilstånd, utbildning osv.

Det vanligaste svaret på tidigare uteblivna cell-provtagningar var ”obehag vid vaginal undersökning”. Vår studie visar att självprovtagning för HPV kan vara ett sätt att nå kvinnor, som annars inte kommer på sina cellprovtagningar. Man kommer då undan det faktum att många inte vill göra vaginal-undersökningar.

Det här självprovtagnings-kitet är ju att attraktivt alternativ i utvecklingsländer, då provet är billigt, inte behöver konserveringsmedel o är lätt-fraktat. Det är dock viktigt med basal kunskap om kvinnokroppen, för att kunna ta testet.

Det fjärde arbetet handlar om kvinnor, som redan har fått sin livmoderhalscancer-diagnos. De tidiga cancrarna behandlas genom kirurgi, där man opererar bort livmodern, och en del av stödjevävnaden runt livmoderhalsen. Man tar även bort lymf-körtlar på insidan av bäckenet och eventuellt längs den stora kroppspulsådern. Detta för att analysera om cancern har spridit sig till lymfkörtlarna. Man ska då ge strålbehandling och cellgiftsbehandling efter operationen. Lymfkörtel-borttagningen kan innebära problem med svullna vävnader, infektioner, lymfvätske-ansamlingar och obehag. Vi ville därför utvärdera ett sätt att selektivt ta bort lymfkörtlar. Idén är att det finns speciella lymfkörtlar, som är de första som nås från lymfbanorna från tumören. De kallas portvakts-körtlar. Den tekniken med att identifiera portvakts-körtlarna är redan känd från bröstcancer-kirurgin och behandlingen av malignt melanom.

Vi sprutade in en svagt radioaktivt märkt vätska runt livmoderhalstumören innan operationen. Underoperationen avlyssnades strålning med hjälp av en gamma-detektor. De körtlar som hade över fem gånger bakgrundsstrålningen identifierades som sentinel nodes portvaktskörtlar, och skickades för fryssnitts undersökning under tiden operationen fortsatte. Vi tog därefter bort alla lymfkörtlar på patienten, precis som den traditionella metoden säger. Detta för att patienterna skulle vara sina egna kontroller. Det visade sig att det är säkert att ta bort bara portvaktskörteln/körtlarna, bara det fanns någon på varje kroppshalva. Om inte ska en fullständig lymfkörtelutrymning göras. Vi fann en gräns på 2 cm i tumörstorlek, om tumören var större än så, är tekniken inte säker. Detta troligen pga. att lymfbanor har blivit förstörda av den allt större tumören, så resultatet blir då inte säkert.

Det är ännu inte fullt i kliniskt bruk med sentinel-node-konceptet för behandling av livmoderhalscancrar, men förhoppningsvis kan snart alla kvinnor med cancrar under 2 cm erbjudas detta.

Sammanfattningsvis ger avhandlingen belägg för att kvinnor borde erbjudas ett utträdesprov ur screeningprogrammet, då en tredjedel av kvinnorna får sin cancer efter 60 års ålder. Exit-testet kan lämpligen vara ett HPV-test, där man kan gå vidare med vanlig cellprovtagning vid behov. Det kan vara bra med ett självprovtagningstest, som kvinnorna kan ta hemma. Det är klart att överlevnaden är bättre hos de screening-upptäckta cancrarna. Sämst går det för kvinnor, över screeningåldern.

Vårt självtagningstest för HPV är lika bra som ett läkar-taget HPV-test. När kvinnor, som ej tagit cellprov på 9 år eller mer, är det 15 %, som svarar och skickar in ett självtest. Den vanligaste orsaken till att man inte varit på cellprov är att man ”tycker det är obehagligt med vaginala undersökningar”.

Kvinnor med livmoderhalscancer under 2 cm kan erbjudas tekniken med att bara ta bort portvakts-körtlar och behöver inte göra en fullständig utrymning av lymfkörtlarna.

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