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Cervical cancer prevention

Studies on possible improvements

Björn Strander

Doctoral Thesis

Göteborg 2008

The cover illustration is perhaps the first published colposcopic picture reproduced.
Drawing in Hinselmann, Hans. Zur kenntnis der präcancerösen veränderungen der portio.
Zentralbl Gynäkol. 1927;51:901-902

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What is past is prologue

W. Shakespeare. The Tempest Act II scene I

Contents

Abstract

Cervical cancer prevention – Studies on possible improvements
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Aims: The aim of this study is to target and assess possible improvements for women attending cervical cancer screening programs.

Methods: In a randomized study the use of ThinPrep liquid based cytology (LBC) was tested against conventional cytology. 13 484 samples, taken within the screening program at five screening units in the Göteborg area, were evaluated. Main outcome was cervical intraepithelial lesion grade two or more (CIN2+) in histopathology at follow up. A scoring system for colposcopy was constructed containing five parameters and 3 ordered values for each. It was tested in 297 women with abnormal cytology. The relationsship between the scoring units and CIN was modelled with logistic regression. To assess the risk after treatment of CIN3 a cohort of 132 493 women with this diagnose was followed up in the Swedish cancer registry for development of vaginal or cervical cancer. Standard incidence ratios (SIR) and absolute risk difference were calculated with the entire female population as reference and age, time-period of treatment and time since diagnosis were included in a multivariable log-linear regression model. To study the predictive ability of HPV-testing after surgery for CIN2+ a case control study nested in a cohort of women treated for CIN2-3 was performed. Cases were 189 women with recurrence of CIN2+ more than two years after treatment. Exposure was presence of HPV in at least one of two archival smears within two years post treatment.

Results: In the LBC-arm >40% more high grade lesions were found and 30% more women needed follow up. The inadequacy rate of smears fell 60%. The scoring system for colposcopy showed very good ability to find and exclude CIN2+ at certain cut off points and the area under the ROC curve for the system was 0.87. Women treated for CIN3 had 2.5 times increased risk for vaginal or cervical cancer. Risk did not decrease substantially after 25 years and was accentuated when treatment was made in women older than 50. Risk also increased with time-period of treatment. Among women treated for CIN2-3 the odds ratio for recurrence was 2.5 when testing positive for HPV 6 – 12 months post surgery and the sensitivity of the test was 24%.

Conclusions: LBC and a new colposcopic scoring system can improve detection of CIN2+. Women once treated for CIN3 constitute a high risk group that needs to be followed up for a long time, More studies are needed to find the best strategies for such follow up.

Keywords: Cervical intraepithelial neoplasia, cytology, colposcopy, HPV, cervical neoplasm, epidemiology, treatment, follow up
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List of papers

The thesis is based on the following papers, which will be referred to in the text by their roman numerals

I Strander B, Andersson-Ellström A, Milsom I, Rådberg T, Ryd W. Liquid-based cytology versus conventional Papanicolaou smear in an organized screening program : a prospective randomized study. Cancer. Oct 25 2007;111(5):285-291

II Strander B, Andersson-Ellström A, Franzen S, Milsom I, Rådberg T. The performance of a new scoring system for colposcopy in detecting high-grade dysplasia in the uterine cervix. Acta Obstet Gynecol Scand. Oct 2005;84(10):1013-1017.

III Strander B, Andersson-Ellström A, Milsom I, Sparen P. Long term risk of invasive cancer after treatment for cervical intraepithelial neoplasia grade 3: population based cohort study. BMJ. October 24, 2007 2007:bmj.39363. 471806.BE.

IV Strander B, Ryd W, Wallin KL, Wärleby B, Zheng B, Milsom I, Gharizadeh B, Pourmand N, Andersson-Ellström A. Does HPV-status 6-12 months after treatment of high grade dysplasia in the uterine cervix predict long term recurrence? Eur J Cancer. Jul 3 2007;43(12):1849 -1855.

List of abbreviations

AC	adenocarcinoma
AIS	adenocarcinoma in situ
ALTS	ASCUS-LSIL Triage Study
ASC-H	atypical squamous cells-favouring high grade lesion
ASCUS	atypical squamous cells of uncertain significance
CI	confidence interval
CIN	cervical intraepithelial lesion
CIS	carcinoma in situ
FIGO	International Federation of Gynecology and Obstetrics
HGL	high grade lesion
HIV	human immunodeficiency virus
HLA	Human leukocyte antigen
HPV	human papilloma virus
hrHPV	high risk human papilloma virus
HSIL	high grade squamous intraepithelial lesion
HTA	health technology assessment
IFCPC	International Federation for Cervical Pathology and Colposcopy
LBC	liquid based cytology
LEEP	loop electrosurgical excision procedure
LLETZ	large loop excision of the transformational zone
LR	likelihood ratio
LSIL	low grade squamous intraepithelial lesion
mRNA	messenger ribonucleic acid
NPV	negative predictive value
OR	odds ratio
PAP-smear	cytological vaginal smear (from Papaniculaou)
PCR	polymerase chain reaction
PPV	positive predictive value
RCI	Reid's colposcopic index
ROC	receiver operating characteristic
RR	relative risk
SCC	squamous cervical carcinoma
SIR	standardized incidence ratio
WHO	World Health Organisation
VLP	virus like particle

Background

Cervical cancer is the only cancer and one of the few common diseases that has become an uncommon disease in Sweden due to targeted interventions. In that respect it is comparable with some infectious diseases that have been successfully eradicated or radically diminished by vaccination (polio, measles, rubella). But unlike these infectious diseases prevention against cervical cancer until the present day has depended on women's continuous awareness of the necessity to protect themselves throughout life and attend screening. With the recognition of cervical cancer being caused by an infection and the development of vaccines against some of the infectious agents, a new era has been entered in cervical cancer prevention. However the basically surgical technique that so far has been so successful in reducing incidence and mortality of cervical cancer will probably not be outdated for several decades as the present vaccines have limited protection. This is particularly so for the generations of women who have entered sexual activity. This study targets some aspects of the existing screening program and seeks and evaluates possible improvements.

A summary of cervical cancer epidemiology

The dominating risk factors for acquiring cervical cancer are lack of screening and infection with high risk human papillomavirus (hrHPV), particularly HPV16. The strength of association with hrHPV is the strongest observed for any carcinogen. In case control or cohort studies odds ratios/relative risks are in the magnitude >50[6, 7]. The exposure of inadequate screening has typically RR/OR of 3 – 10 depending on populations, available data and thresholds[8-14]. All other risk factors found have considerable less influence. Several classical risk factors associated with sexual activity such as the number of sexual partners or the number of partners former partners have been shown to be strongly associated with the risk of acquiring hrHPV infection but their role as independent risk factors for cancer is uncertain[15]. An increased risk with lower social class is consistently found in studies and this disparity is higher in the US than in Europe[16]. In a recent Norwegian study[17] the relative risk for acquiring cervical cancer was 0.38 (95% CI 0.17 – 0.85) for women with a long education compared with women with a short education. In the latest study done in Sweden 1986 Vågerö and Persson[18] found a statistically significant relative risk of 1.22 for blue collar workers, but risk differences of 100% are common

in the literature. The risk difference between social groups can be explained with lifestyle factors and differences in screening participation.

As most people in the western world will be exposed, acquire and clear hrHPV-infection during a lifetime additional cofactors are of great interest. The reasons why a small minority of women develop cancer after HPV-exposure and the majority do not are still very unclear. Some life style factors seem to be of limited importance in studies adjusting for HPV or surrogate markers like sexual activity. There is a relative risk (RR) of 1.60 for smokers compared with never smokers related to squamous cell carcinoma, but smoking does not seem to influence the development of adenocarcinomas[19, 20]. Use of oral contraceptives has a limited dose-response relationship with cervical cancer and more than 10 years of use doubles the risk[19]. The risk decreases after cessation but is still elevated after 8 years[21]. Even injectable progesterone-only contraception seems to be associated with a small increased risk. Co-infections with herpes simplex virus type 2 (OR=2.2 for squamous carcinoma (SCC) and 3.4 for adenocarcinoma (AC)) and Chlamydia trachomatis (OR=1.8 for SCC but OR 1.0 for AC) are associated with increased risk[22]. Of less importance for women in the western world, but a relative substantial risk factor in the third world is high parity[23]. For each term pregnancy the risk for cervical cancer increases significantly with 10% (RR = 1.10)[24]. The effect of nutritients have been discussed for some time and current evidence is that antioxidant nutritients containing e.g. vitamins C and E, folates and beta carotene probably have a protective effect[25, 26]. Immunosupression by HIV or transplantation medication increase risk highlighting the importance of the immune system in clearance/persistence/progression of HPV and cervical lesions. However the increase is modest and progression to cervical cancer does not seem to be linked to CD4+ levels or altered by antiretroviral medication[27].

Some constitutional/genetic risk factors have been proposed. Human Leukocyte Antigen (HLA)-genes code for proteins that are involved in antigen presentations to T-cells. Swedish researchers have found an almost fourfold increased risk, corresponding to an attributable proportion of 30% in a population based nested case control study, for women with HLA class II haplotypes DR15 and DQ6[28]. Some fairly uncommon haplotypes have been associated with limited protection or up to sevenfold increased risk[6]. Very recently certain combinations of class I and II have been found to increase the risk of cervical cancer[29].

There are also some important and established risk factors regarding viral findings that are associated with cancer. The most important factor is that the risk profile differs between HPV types and HPV 16 is attributed the highest risk. Thereafter are found in falling order HPV 18, 52, and 45. HPV 31 actually has the highest odds ratios for cancer (OR=573) in the largest pooled meta-analysis of case control studies but confidence intervals were quite wide[15, 30]. Attributable risk is dependent both on

intrinsic virulence and prevalence of infection. Further, prevalence is dependent on incidence and virulence as virulent HPV-types need longer time for clearance. Persistence of infection[31-34] is today considered a necessary cause, although the absolute risk in a general population associated with persistence seems to be fairly high. A study by Kjaer et al demonstrated a cumulated 10-year risk of CIN3+ after two positiv HPV-tests 2 years apart of 20%[35]. In the SwedeScreen study 17 out of 72 women (24%) with persistent hrHPV-posistivity and negative at base line cytology and a first colposcopic evaluation who underwent close follow up developed CIN2+[36]. High viral load[31, 37, 38] has been shown to increase risk for CIN2+ as well as certain variants of HPV 16 and 18[39]. The risk associated with infections with multiple HPV-types is uncertain. There are studies showing association with persistence and even progression[40].

Trends in cervical cancer

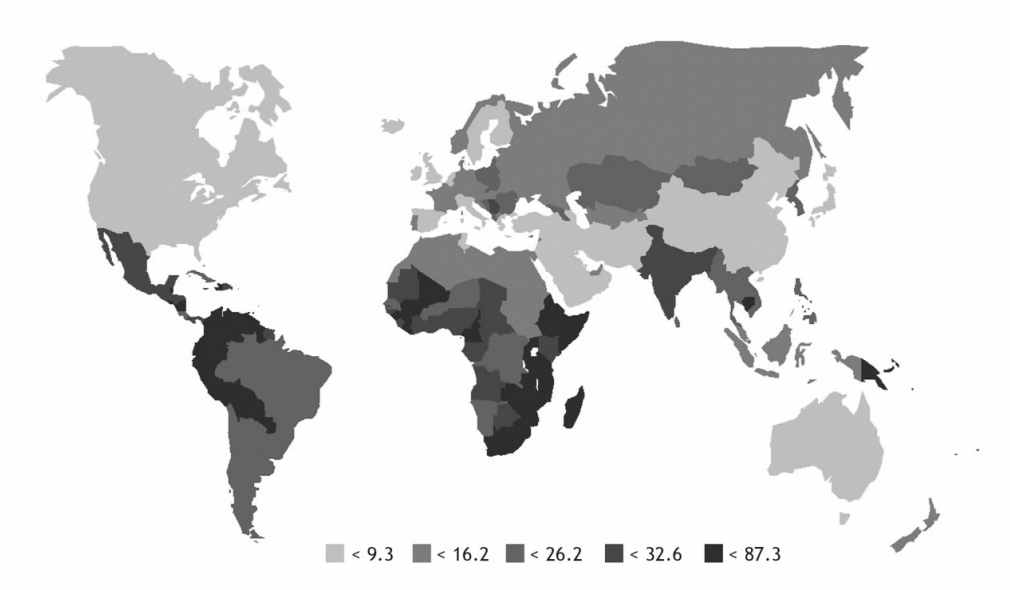

Fig 1. Worldwide age-standardized annual incidence per 100 000 of cervical cancer (from Parkin, Bray 2006[41]).

Cancer of the cervix is the second most common cancer among women in the world[42]. In large parts of the developing world it is the largest single cause of female lost years of life from cancer[43] and the prime overall cause of women years lost in Latin America. The highest incidence rates are found in Latin America and the Caribbean, sub-Saharan Africa and South Asia. Low rates are found in Western

Europe and North America, mainly due to successful screening, but are also found in West Asia, presumably due to low rates of HPV and different sexual behaviour. The lowest rate recorded, 0.4 per 100 000, is from northwest Iran[41].

The Swedish Cancer register was founded in 1958. Until that date cancer registers had been kept at oncological clinics and these registers are still valid sources for knowledge and science[44]. Sweden had introduced the unique personal number system in 1947 consisting of date of birth with 6 digits and at that time another 3 digits showing place of birth, sex and including a serial number. (Modifications have been done later on, e.g. adding a tenth control digit in 1967). A generalized use of personal numbers is not a prerequisite for a cancer register, but has undoubtely facilitated maintaining the high quality of the register.

In the 1950:s cervical cancer was one of the most common female cancers in Sweden.

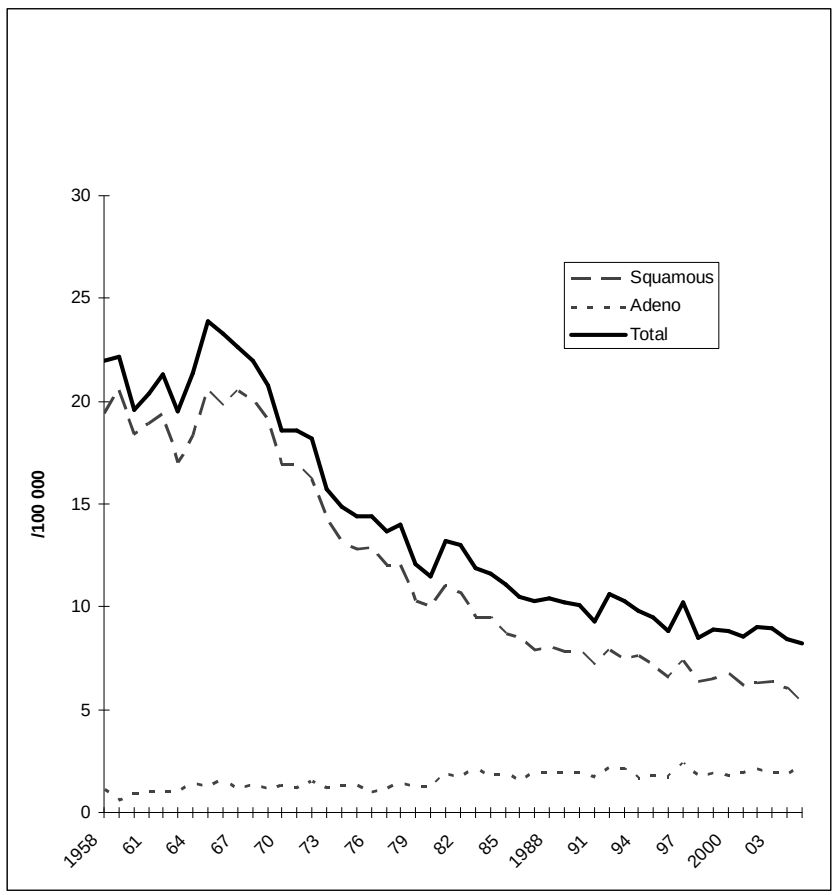

Fig 2. Incidence of cervical cancer in Sweden 1958 -2005, age standardized to the 1970 census. Data from the Epidemiology center (EpC) at the National Board of Health and Welfare[45] .

After an initial rise in incidence in the 1960:s, that can be attributed to the detection of prevalent cases when screening started, the trend has been a steady decline that is still noticeable. The decrease is entirely attributed to a strong decreasing trend in squamous cervical carcinoma (SCC) while the incidence in adenocarcinomas increased between 1958 and 1981 and has been fairly stable since 1981, confirming and extending observations made by Bergström and co-workers[46]. Adenocarcinomas constituted 4% of all cervical cancers 1958 – 62 but were 23% during the period 2001 – 2005.

When studying the incidence of SCC, from cancer registry data 1964 - 98 using an age-period-cohort model Bray and co-workers confirmed a very strong effect of period in Sweden which is attributed to screening[5]. In the model the effect of age was adjusted for in calculating effects of Period and Birth cohort. Like Norway, but in contrast with most European countries studied, including Denmark and Finland, there is no increasing trend for the cohorts born after 1950 and that is also attributed to successful screening programs.

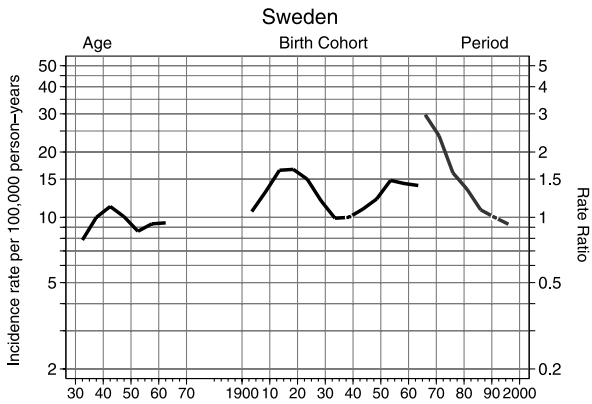

Fig 3. Incidence of squamous cervical carcinoma in Sweden in a model were Period and Birth cohort were adjusted for age. (From Bray et al 2005)[5]

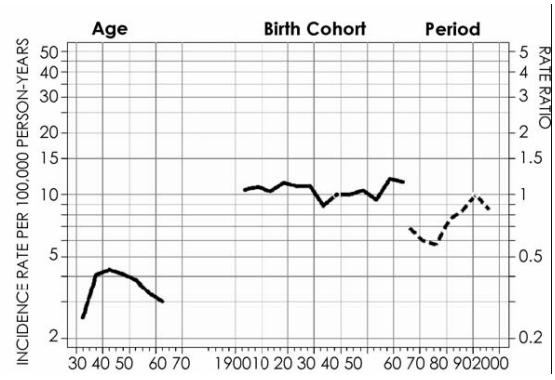

Fig 4. Incidence of adenocarcinoma in Sweden in a model were Period and Birth cohort were adjusted for Age (From Parkin, Bray 2005)[41] .

Incidence rates of adenocarcinomas have also been studied with the same model and compared between 13 European countries[4]. The authors stress that data should be interpreted with some caution as classification may have changed with time. In contrast with previous studies[46, 47] a slight decreasing trend among women >30

years of age in Sweden is shown, that was attributable to screening. Over the 13 countries studied an increasing trend was noted for birth cohorts born after 1940 which is interpreted as an increase of risk factors, mainly exposure to HPV that more (Sweden, Norway) or less (Finland) successfully are compensated by screening. A decrease in mortality from adenocarcinoma without a decrease in incidence has previously been reported from Finland [48].

Contrary to screening for cancer in other organs, incidence rates are valid markers for the success of cervical screening as primarily precancerous lesions are found and removed from the population, thus protecting from the disease. After a rise from 4 to 8/100 000 during the 1950:s[46] there has also been a steady and still ongoing decreasing trend in mortality as shown in fig 5.

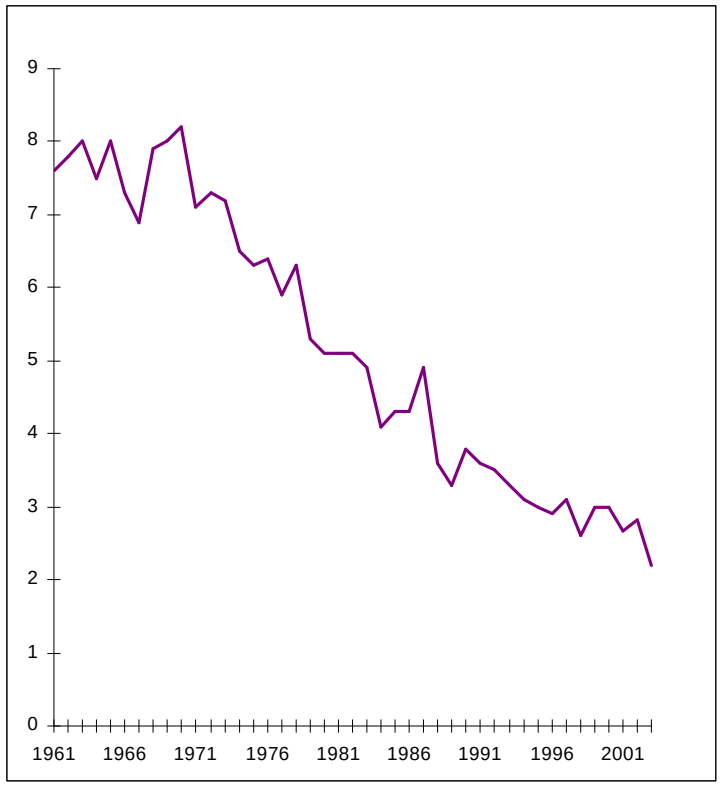

Fig 5. Cervical cancer mortality in Sweden 1961 -2003. Standardized to the census 1970 (FoB). Data from Statistics Sweden and Epc.

A brief history of cervical cancer screening in Sweden

Olle Kjellgren was perhaps the first Swede to write about PAP-smears. In a report from a study-trip in the US, published in Svenska Läkartidningen (the Swedish Medical Journal) 1951[49], he stated "A condition of great importance is that the cytological smear turns positive at a very early stage of cervical cancer when the portio is normal at palpation and inspection". Pilot projects started very soon thereafter, among other places at hospitals in Sabbatsberg and Göteborg[50], and five years later the first Swedish cytological laboratory was established in Malmö[51]. The PAP-smear had been introduced in Sweden. Initially cytology was used for "uterine cancer"[50] but soon focus was more specifically on cervical cancer. Smears were used as a complement in cancer diagnostics and finding recurrences after radiation therapy. But included in cancer diagnosis was also stadium 0 or carcinoma in situ, a diagnosis that was very rare before the breakthrough of cytology. From being a tool in gynecologic oncology, cytological smears became above all a method for finding pre-cancerous lesions and making prevention possible. The very rapid spread of the method among gynecologists and laboratory doctors during the 1960:s may partially be explained in that this was in the period before the "seven kronor reform" and that the economical reward for the good doctor was often quite decent.

Already in the middle of the fifties visionaries started talking about screening of large populations but were aware that resources were too scarce[50, 52]. During the powerful expansion of health care in the 1960:s the impossible became possible. After a few years of preparations the National Royal Board of Medicine (Medicinalstyrelsen), forerunner of the present Board of Health and Welfare, 1967 established recommendations on screening for all women aged 30 – 49[53]. Although pilot projects with comprehensive gynecological and breast screening had started[54] the board recommended that examinations should be limited to the cervix as this rightly was considered more fruitful than examining breasts, uterine cavities and ovaries. This limitation opened the door for midwives replacing doctors as smear takers, a part of the recommendations that was quite controversial[55-57]. Even the decision to use a spatula as an instrument for smeartaking instead of cyto-pipetting stirred a debate[58]. Screening was to be be organized by the counties. Lines of decisions were short and the level of ambition was high. Östergötland was first out and the Swedish population based screening started there in 1964. By 1970 16 counties had launched screening programs and the rest joined in line during the 1970:s with Göteborg as the last in 1977. Actually the counties of Blekinge (1975) and Kristianstad abandoned their screening programs for some years and it was not until 1983 the entire Swedish female population was covered simultaneously[59].

The comprehensive Swedish population registers, based on the unique person numbers were used for invitation in combination with modern computer technology. Midwives were trained and had strong support from their professional organisation in this new task. In the city of Lund the curriculum included smeartaking under supervision of doctors for ten hours and a computerized system was developed where also colposcopic findings could optically be registered from a mould filled in by hand[2].

The success of the programs could be described at an early stage and in 1974 a reduction in incidence to 1/6 was reported for participants compared with non-participants[60], a downstaging of invasive cancers among participants 1976[61], noticeable even for those who presented with symptoms, regional coverage of 86% in 1977[62], and a decrease in mortality was reported regionally 1982[63] and nationally 1984[64].

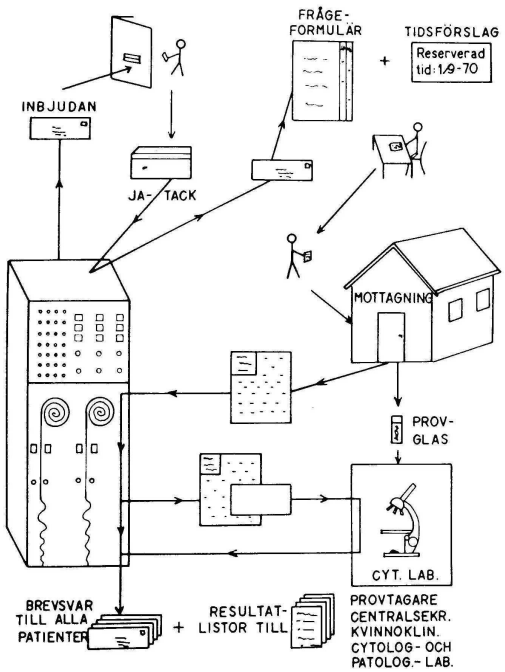

Fig 6. Flow-chart for computerized handling of invitations, letters and reports in Malmöhus 1971 for "women without former or present cytological abnormalities"[2] .

Computer technology allowed compilation of huge amounts of data[65] and a national register was in effect 1967 – 1977 at the Radium hospital in Stockholm. Data from this database had decisive importance for the IARC/WHO international recommendations on screening intervals and age groups to target[9].

In the beginning there were high thresholds for treatment, most often based on cytology alone. Normally two consecutive "malignant" smears were required for treatment. And the threshold in cytology also seems to have been high. From Danderyd, where 15 000 smears were processed 1966, 97.7% of all smears were reported as normal[66]. Treatment during the 1950:s was hysterectomy, although the "conservative" method conization gained ground the proceeding decade, first as a diagnostic method but later as therapeutic[67, 68], although hysterectomy and even radiotherapy were sometimes considered in older women[69].

In the late 60:s an estimated 5000 conizations were performed yearly[70]. In his thesis Bjerre noted that for 1000 women screened about 60 inpatient days at hospital were needed due to abnormal smears[54]. The increasing number of young women with only precancerous smears made preservation of fertility an issue. Conization was regarded as a success but some colleagues noted with concern that 13 - 17% of the patients had abnormal smears at follow up and later needed re-treatment, often hysterectomy[70, 71]. Complications such as premature deliveries in subsequent pregnancies, cervical stenosis and dysmenorrea were recognized[68] and there was a need for even less mutilating interventions, particularly as removal of less advanced lesions became common practice. Cryo-surgery developed during the 1970:s as a simple, cheap method with few complications[72-74]. Often, but far from always this technique was combined with colposcopic skills and meticulous biopsing we now know is a prerequisite for safe treatment. The method used particularly in west Sweden, combining multiple punch biopsies with diathermy had the advantage of providing some material for histopathology but was quite blunt in the absence of colposcopy. The next treatment technique à la mode was laser, used for vaporisation or conization[75]. One substantial asset with these machines was that training and skill was needed which limited the number of users. However this learning was not often used when loop excision had its breakthrough in the 90:s. Again the youngest, least experienced intern could start his/her surgical career with a seemingly simple intervention.

Colposcopy has had an uneven spread in Sweden. Many doctors were discouraged by lack of consistency in grading of findings. In the earlier years the focus was often on rather unspecific vessel patterns and light sources were often sub-standard. Cervical cancer prevention was primarily based on cytology. Some authorities did not see the need for more specific diagnostic procedures[70] and the belief in cytology was strong with reported figures on sensitivity of up to 99.2%. Results of conizations 1962 – 67 were reported from Karlskrona[71], without colposcopy and actually without calculating the proportion of women unnecessary treated and this might reflect that specificity was not much of an issue at the time. When we do this calculation of specificity 37 years later it is a meagre 68%. Others however have advocated the advantages with colposcopy throughout the years. Rubenstein provides a modern and structured approach already in 1967 with the use of acetic acid[76], and in a clinical overview Ahlgren and co-workers advocated the use of colposcopy[77]. However this was without the use of acetic acid, an approach that seems to have been prevalent at the time. (In paper II we show that the findings most predictive for high grade lesions are results from the use of acetic acid). Other publications in these early years, from other parts of Sweden advocate the use of colposcopy[52, 78-81] and the need to have histopathological verification of the cytological diagnosis before treatment was underlined already in the 1950:s[82]. Nevertheless in some local programs, treatment still is initiated more on repeated minor cytological abnormalities than actual colposcopic and histological findings.

Early attempts were also made to automatize parts of the routine screening in the laboratories or replace conventional cytology with automatic methods[83, 84]. In the 1980:s an imaging interpretation program was quite successful in separating benign smears from abnormal[85].

As reflected in the chapter on Trends in cervical cancer, screening in Sweden has been a success, saving hundreds of lives every year for more than three decades, and merits a place among the great medical conquests in Sweden. Cervical cancer, a disease that in advanced stages struck 40-year old patients who filled the gynecology wards has been reduced to a rare disease that mainly is found in an early treatable stage. In the youngest ages as much as half of the cases are detected in stages where fertility preservation is possible. The screening programs were launched in an era when randomized controlled trials were rarities almost unheard of. However the results have been quite obvious and observational studies have gradually built up evidence to the effect that the basic effectiveness of these programs is not questioned today. In Sweden the pioneers made wise priorities concerning organisation, age intervals and age groups from existing resources and research activity in the field was substantial up to the eighties. In the 1980:s the recommended screening ages were expanded[59] and the protection of the Swedish women has increased throughout the years as more and more women have taken more and more smears.

However after some years the air went out of the systems when the enthusiasts left the scene. Many procedures rolled on in old tracks. Midwives were often left alone to defend the screening organisation, often with minimal feed back, no continuous education and only sporadic commitment from doctors. The effects of course were cut backs, introduction of patient fees and decline in attendance. This development has been paralleled by a shift in the proportion of smears taken outside and inside the organized screening program. In 1985 less than 25% of all smears were taken as organized screening and in 2002 this figure was 55% (data recalculated from Sparén 2006[86]). Data-systems once tailored made for the need of efficient screening organisation have been dismantled, or distortedly transformed and integrated in commercially provided systems and the knowledge of what should be kept track of, why and how have often faded away in organisations without memory. The last five years improvements have been made and lot of activities have commenced but Sweden still has a way to go. The potential for improvements of the screening programs are substantial, and the resources necessary for these changes are relatively small.

The protective chain

The protective chain is the chain of events that constitute a screening program. Every link has its strengths and weaknesses, contributing to success or failures. In Sweden the different parts of the program are administered by different organisations which increase the risk of losing information and underlines the need for coordination and surveillance.

The protective chain is described in fig 7 with the possible pitfalls within and between the links. The parts of the chain that are specifically investigated in this study are represented by ticked boxes.

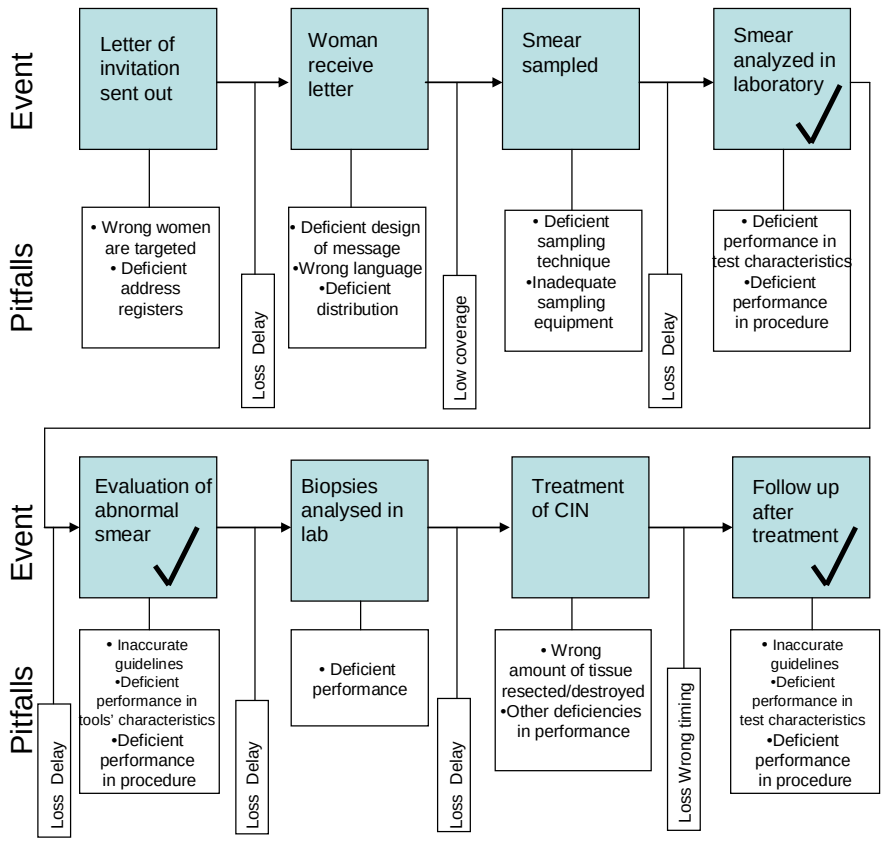

Fig 7. The protective chain of cervical screening and possible pitfalls and areas of deficiencies in and between the links. Ticked boxes represent areas of this investigation.

Recently a study has been made auditing the cervical screening programs in Sweden, made as a case control study including all cases of cervical cancers 1999 – 2001[87]. The presence or absence of a smear taken within the recommended time before diagnose of cancer was assessed. (Only limited data are presented from this important study as it has not been published yet). The cytological background for the past screening round is shown in figure 8.

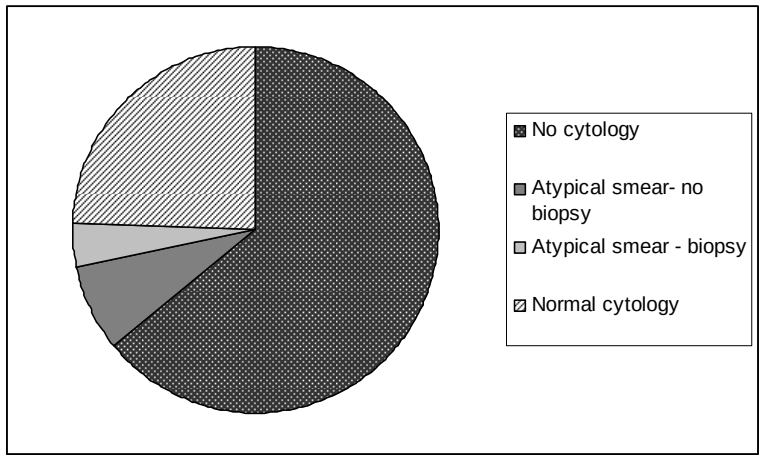

Fig 8. Recent screening background of cancer cases in Sweden. Calculated from Andrae et al 2007[87].

From this study, and other case control studies with similar results[88-91], conclusions can be drawn about how to improve the screening program. Each of the sectors can be attributed to a link in the protective chain.

1) *No cytology* - Coverage should be improved

2) *Normal cytology* - Measures to increase sensitivity in screening should be considered

3) Women with atypical smears may have to be followed up more rigorously. Here the fallacies could be:

a) *Atypical smear – no biopsy registered.* Abnormal cytology without colposcopical/histological evaluation (perhaps repeat cytology was normal)

b1) *Atypical smear – biopsy registered.* Atypical smear, normal colposcopy and biopsy - Colposcopical evaluation incorrect

b2) *Atypical smear – biopsy registered.* Correct evaluation, CIN found in biopsy but unsuccessful treatment of CIN and/or follow up after treatment

With this scheme as a starting point the present study addresses point 2, 3b1 and 3b2.

Invitation

The chain protecting for cervical cancer generally starts with an invitation letter. Studies[92-94] have not surprisingly shown that coverage benefits from women having an invitation letter vs. not having one and some studies have superficially addressed issues about the content of the letter. There are no nation-wide surveys made on how these invitations are made or designed but it is known that communication professionals are rarely used. In general in Sweden laboratories are responsible for invitations and the design of the invitation often is a task where cytotechnicians, pathologists and perhaps gynecologists and midwives are formulating the message but communication experts are seldom involved. In West Sweden it has been a deliberate strategy that the core message should be that participating in screening is not primarily a way to detect cancer (unlike mammography) but to protect one-self from ever getting cervical cancer. This is a positive message that would have a chance to appeal also to the group of women who tend to abstain out of fear from the result[95], emotions linked to general anxiety, feeling of hopelessness and lack of control over health and life[96].

The invitation letter should not be sent to women who recently have taken a smear according to the most recent recommendations on cervical screening from the National Board of Health and Welfare[97]. Some minor confusion among the public can be prevented and foremost resources can be saved. The existing data programs in the laboratories can sort out women who have smears registered in the same laboratory but in the larger cities several laboratories might be involved in the same geographical area which makes things more complicated.

Invitations must be distributed to the correct addresses. In other countries this really has posed a problem. In the early 90's it was reported that 30 % to 60 % of invitations in the London and Manchester areas were sent to the wrong addresses[98]. Even in Sweden incomplete and not up to date copies of existing population registers cause problems. An ill defined catchment area for screening units is another reason for invitations that fail to reach their targets. Although the National Board of Health and Welfare has set standards for the maximum number of invitations that do not reach target[97] this is generally not audited. This measurement is not included in the quality assurance standards neither the ones set for laboratories by the profession (KVAST - Kvalitets och standardiseringskommittén inom Svensk Förening för Patologi och Klinisk Cytologi) nor for accreditation of laboratories. (SWEDAC- Swedish Board for Accreditation and Conformity Assessment).

Attendance and coverage

Attendance is the proportion of women who respond to an invitation and actually take a smear. More precisely attendance can be defined in several ways and there is no national or international standard set for this term. In a national report the term is

defined as any smear that is taken within a year from the date of invitation and data are presented with Kaplan-Meier curves[86]. In a report from West Sweden attendance is defined as the proportion of women who take a smear within 3 months after date of invitation.[99]. This report shows substantial variations in attendance between communities in West Sweden ranging from 30 – 77%.

Coverage is the term for the proportion of women within a catchment area who take at least one smear within a defined time regardless of relation to an invitation. This could appear to be a more clear cut term but it too lacks a commonly accepted definition which makes comparisons between areas difficult or impossible. In international literature figures of coverage can evolve from surveys and interviews[100] and only a few countries can report national figures[86, 101, 102 2007, 103, 104].

In countries and areas with screening programs coverage seems to be the weakest link. In several studies not having a recent smear, sometimes specified as non-participation in existing screening program, is found to be a major background factor of invasive cancer although few studies make direct comparisons with other risk factors in screening history[105, 106]. Such risk factors are having an atypical smear and having a history of earlier treatment for CIN. However none of the other risk factors have been reported to constitute the same amount of risk as abstaining from screening. This is confirmed by the recent audit by Andrae and coworkers that do compare these risk factors[87] as well as a recent Dutch study[107].

Predictors of attendance

A number of important background factors for attendance have been known for years and largely been confirmed in recent research, although this kind of data should be looked upon with some caution. Health care systems vary greatly and so does socioeconomic and cultural factors between different settings. In rural India for example more affluent women had a lower participation in cervical cancer screening that was organized in public institutions compared with poorer women[108], although in this as well as another Indian study attendance was correlated with literacy[109]. In recent research in developed countries classical predictors for screening attendance are found: Higher income, higher educational level[96, 110-115] and being non-migrant[112, 116]. Age and urban/rural residence present conflicting results [113, 117-119] most probably due to heterogeneity among the populations studied.

Interventions that affect attendance and coverage

Several randomized trials have been made evaluating factors related to participation in screening. Segnan et al studied the response rate after sending 16 454 invitations. Half of them concerned cervical screening and the other half breast screening. Four arms differentiated between varied forms of appointments, senders and messages.

When an open appointment (necessitating the booking of an appointment) was compared with sending a prefixed time there was a significant 39% reduction of attendance. The results were similar in breast cancer screening. A more detailed description of the disease and the procedure did not alter participation[120]. Other studies have also found that making a fixed appointment increases participation[93, 119, 121].

A Swedish trial[122] tested three interventions in a randomized trial. Modifying the invitation letter did not alter attendance, but sending a reminder letter increased attendance 9% and a phone reminder by as much as 34%. The results were in agreement with a US study[123] that also stressed the need for registrations of hysterectomies in order to avoid unnecessary reminders. British researchers however had less success in getting women without smears for more than 15 years to participate using similar measures[124]. It is perhaps noteworthy that having reminders signed by the screening organizer in this study seemed to increase attendance somewhat more than a letter signed by a celebrity(!).

A huge study in New Zeeland[125] (n = 90 000) found a 50% increase in attendance with a reminder compared with no reminder, a higher response from older women on reminders compared with younger and concluded also that reminders rather should be sent after and not before Christmas (!). Several other studies have confirmed the benefit of reminders[126-128].

A Cochrane review was published 2001[129]. The authors made few conclusions, mainly due to heterogeneity between studies that limited the possibility of pooling data. Actually the only clear conclusion made was that invitations and educational material increases uptake in screening. However the authors succeeded in pooling the results from three studies comparing fixed and open appointments and found a relative risk for attendance of 1.49 (95 % CI: 1.27 – 1.75) in favour of fixed appointment (Fig 9). This review stressed the concept of "informed uptake" of screening, recognizing that many women are not fully aware of the implications of participating and that cervical screening may have unwanted side-effects.

The importance of reimbursement for the service, given to screening units has been studied in England. The payment for coverage in the area of 80% or more was quadrupled compared with 50 – 79% coverage. This measure was considered a key factor in increasing screening coverage from less than 40% to over 80% in the 1990:s[130]. In Sweden the models for financing vary greatly but how this affects coverage has not been studied.

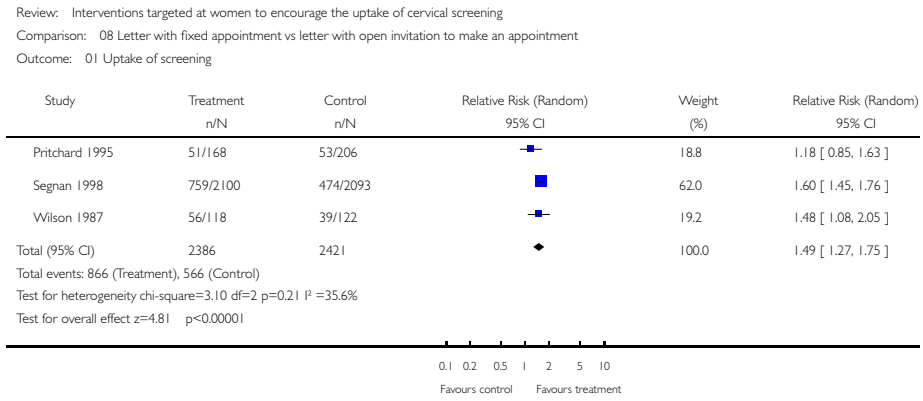

Fig 9. Comparison of letter with fixed appointment vs. letter with open invitation to make an appointment. Outcome: Uptake of screening. From Cochrane review of interventions to increase screening uptake[129].

Smeartaking

Studies on smeartaking and the actual importance of skill in smeartaking are scarce. A recent New Zeeland study found that 84% of women with cervical cancer who had a smear had correct cytological interpretation, indicating that the abnormal cells just were not in the sample[91]. In a study of video monitoring of smeartaking and relating the results to colposcopic and histological findings[131] location of the squamous columnar junction was not seen, a large transformational zone and small lesions were factors explaining disagreement between cytology and histology. An article based on a consensus conference underlines the need of training[132] and a Israeli study found the ability to sample adequately correlates directly with the total number of smears taken annually by the smeartaker[133]. There are detailed guidelines published on smeartaking but with few scientific references[134].

In an anonymous survey only 18.5% of doctors taking smears (gynecologists and general practitioners) in the Northwest region of England reported having had any supervision taking smears and only 60% had had any training in smear taking[135]. In another survey 28% of doctors and 25% of midwives in the North Times area used the round end of the Ayre's spatula to sample the portio and only 46% of the smear-takers used any device for endocervical samples in post menopausal women[136]). In a recent survey from West Sweden 267 out of 290 eligible smear-taking midwives in the screening program (92% response rate) described their experiences of taking smears. Only 54% had learned smear-taking from their midwifery education and 84% had learnt from colleagues on the unit (several alternatives were possible)[137].

In contrast to smear taking there are several studies comparing instruments used in smear-taking. A Cochrane review[138] and a systematic review[139] found that collection devices shaped for sampling endocervical cells not only collect endocervical cells to a higher extent but also gives a higher frequency of abnormal smears. These authors found that the presence of endocervical cells is a valid surrogate marker for the ability to detect abnormal cytology. The combination of Cytobrush with spatula, particularly with extended tip, was the best device for sampling.

The use or non-use of lubricant gel

In gynecological practice the use of a water soluble gel lubricant is routine praxis and makes the examination with speculum less uncomfortable. However it has been a common conception that gel lubricants should not be used in cytology screening because of their supposed negative effect on smear quality. This restriction has been frustrating to many smear-takers who want to be as gentle as possible when performing the examination. A number of studies have been published in recent years that have refuted this conception. A US study[140] randomized 5 public health planning clinics to use either gel or not during 7 months. 8534 Papaniculaou smears were collected, with 1440 using gel lubrication. The number of unsatisfactory smears as well as other parameters were practically identical between the methods; 1.4% for the gel group versus 1.3% (OR 1.1; 95% CI 0.6, 2.0). Another recent study from Texas[141] compared gel-lubricant with moistening the speculum with water. 6538 Pap smears were collected from 3460 patients. During the 4 months of gel lubricant use, the rate of unsatisfactory cytology was 1.1% compared to 1.5% during the 4 months of water lubrication OR 0.74; (CI 0.41-1.35) These results have been confirmed also in a smaller study from California[142].

Reasons for failure of cervical smear test[9]

* Patient very tense owing to failure of reassurance

* Cervix not visualised adequately

* Cervix not scraped firmly enough

* Transformation zone not completely scraped

* Material incompletely transferred to the slide

* Sample poorly spread (too thick or too thin or distortion due to excessive pressure)

* Smear allowed to dry before fixation

* Insufficient fixative used

* Smear consisting mainly of blood or inflammatory cell exudate, possibly associated with menstruation

* Contamination of the smear with lubricant, vaginal cream, or spermicide

* Menstrual smears containing large numbers of endometrial cells

Fig 10. From the British Society for Clinical Cytology. Taking cervical smears. Orpington: BSCC, 1989, cited in [98].

Laboratory analysis

Classification of cytological smears

From a quite unified use of the PAP-classification the development of cytological classification has produced different schemes that up to the year 2000 could schematically be described as in fig 11.

<table>
<tr><td>Classification System</td><td colspan="8">Cytology Classification</td></tr>
<tr><td rowspan="2">The Bethesda System</td><td rowspan="4"></td><td rowspan="2">Infection
Reactive Repair</td><td rowspan="2">ASCUS</td><td colspan="5">Squamous Intraepithelial Lesion (SIL)</td><td rowspan="4"></td></tr>
<tr><td colspan="2">Low Grade (LSIL)</td><td colspan="3">High Grade (HSIL)</td></tr>
<tr><td rowspan="2">Richart</td><td colspan="3" rowspan="2"></td><td colspan="5">Cervical Intraepithelial Neoplasia (CIN)</td></tr>
<tr><td>Condyloma</td><td>Grade I</td><td>Grade II</td><td colspan="2">Grade III</td></tr>
<tr><td>Reagan (WHO)</td><td>Normal</td><td colspan="2">Atypia</td><td colspan="2">Mild Dysplasia</td><td>Moderate Dysplasia</td><td>Severe Dysplasia</td><td>In situ Carcinoma</td><td>Invasive Carcinoma</td></tr>
<tr><td>Papanicolaou</td><td>I</td><td colspan="2">II</td><td colspan="3">III</td><td colspan="2">IV</td><td>V</td></tr>
</table>

Fig 11. Map of classification schemes for cervical cytology. ASCUS = atypical squamous cells of undetermined significance; WHO = World Health Organization. From Nanda 2000[143].

The Bethesda system, using a few categories has the advantage of better reproducibility compared with other systems. However it was modified 2001 adding the category of *ASC-H, Atypical squamous cells favouring high grade intraepithelial lesion*, in an attempt to reduce the high proportion of A*SCUS*[144]. The Bethesda system is today the most widely used and has been endorsed by the WHO in 2006[145].

In Sweden a modified classification is used, based on the WHO scheme but incorporating major aspects of the Bethesda classification[146]. The classification is transformable to the Bethesda system but has two distinctions. 1) *CIN1* and *Sign of HPV-infection* are not lumped together into *Low grade squamous intraepithelial lesion*. 2)

Adenocarcinoma in situ (AIS) and *Adenocarcinoma*, two very rare diagnoses, are reported as *Adenocarcinoma/AIS.*

Conventional PAP-smear

The performance in terms of sensitivity and specificity of the conventional PAP-test has been the subject of a great number of studies. The design of the studies have varied widely as well as sample sizes, locations, populations, thresholds, reference standards and follow up of cytology negatives (necessary for assessing sensitivity). Not surprisingly the results and conclusions have differed. In a meta-analysis of 59 studies Fahey and colleagues 1995 found that cytology had a mean sensitivity of 58% and a mean specificity of 69% with an astonishing range, 11 – 99% and 14 – 97%[147]. Another meta-analysis[143], applying more strict criteria and including studies published later found similar results. Only 12 studies meeting inclusion criteria were made in low prevalence settings that could correspond to screening. On the LSIL/CIN1 threshold the mean sensitivity was 47% (range 30% – 87%) and mean specificity 95% (86% - 100%). Data for the more relevant ASCUS/CIN2+ threshold was not presented.

A review in 2002[148] by Martin-Hirsch and collegues confirmed the wide range of sensitivity 31[149] – 99%[150] in 12 studies on low prevalence populations when a low threshold for referral were used (~ASCUS). When higher threshold was used (CIN) the range was 22[151] – 88%[152].

The often cited HART-study, performed within the English NHS screening program, found sensitivity for cytology, taken by general practitioners without endocervical brushing, to be 77% (95% CI 65 – 85%) with a specificity of 95.8% and a positive predictive value of 16[153]. Another British study found sensitivity to be 86% with border line changes as threshold and CIN2+ as endpoint[154].

Although prevalence of disease should not influence sensitivity, studies in populations with high prevalence of CIN, like in referral centers, often show better sensitivity[155].

Liquid based cytology

Liquid based cytology (LBC) aims to improve the quality of the conventional PAP-smear by improving the preparations of the slides. LBC slides are claimed to be more representative samples of the specimens and to reduce obscuring background material like blood, mucus and inflammatory cells. This should allow faster and more reliable examinations of the slides by the laboratory staff.

The idea with liquid cytology is not entirely new as some attempts to improve cervical cytology in similar ways have been made before the breakthrough in the late 1990:s. For example chemical depolymerisation of cervical mucin has been tried[156] as well as a sedimentation velocity separation method that was described in 1981[157]. A Swedish research team developed a pulse-wash method in 1986. Spatulas and brushes were replaced with ejecting liquid with high velocity and the resulting suspension was collected[158, 159]. The authors concluded ahead of their time "The results of this work suggest that the pulse wash technique gives a more representative cell sample than the Pap smear sampling technique, thus offering a simple method to decrease false negative diagnoses in the detection of carcinoma of the uterine cervix. Samples by the new technique give an abundance of cells for slide preparation for cytodiagnostic techniques as well as for additional cytochemical, immunocytochemical and microbiologic diagnostic techniques."

There are mainly three methods of liquid based cytology marketed in Europe:

The SurePath® test pack (BD, Franklin Lakes, NJ, USA) with the PrepStain® slide processor uses a plastic collection device to sample the cervix. The head of this device is detached into a vial containing a preserving transport fluid. In the laboratory, after vortex mixing, the suspension is treated through a density gradient centrifugation process to remove obscuring material. The centrifuge tube is loaded into a slide processor (PrepStain), handling 48 slides at a time, were cells are allowed to sediment under gravity to form a monolayer. The system is approved by the US Food and Drug Administration (FDA).

Using conventional cytocentrifuges has been proposed as an alternative to specially designed LBC systems. A few, but promising studies have been made using PAP Spin® (ThermoShandon Inc, Pittsburgh, USA)[160-162] and a single study could be found in which Turbitec® (Labonord, Templemars, France)was evaluated[161]. The sampling is identical with SurePath. Various fixatives are used. In the laboratory a cytocentrifuge/processor is utilized (e.g. CytoSpin), a device that is commonly used for other laboratory purposes. The preparations can be made at a relatively low cost but the processing capacity is low with 12 slides processed at a time. The technique has been evaluated for automatation[163].

The ThinPrep® (Cytec Corp. Marlborough, MA, USA) system is by far the best documented. The process is similar to SurePath. A plastic collecting devices, spatula, brush or "broom" (Cervex Brush), is rinsed thoroughly in a vial containing transport and preservation fluid. The device is then disposed of. Vials are processed in a ThinPrep processor. There is a choice between two processors. ThinPrep 2000 is semi-automatic, and prepares one slide at a time. ThinPrep 3000 is described as fully automatic, preparing 80 slides at a time. In the process a suspension is made breaking up cell clumps and mucus. Fluid is sucked thorough a filter, leaving cellular

material in the filter that is transferred to a glass slide. The slide passes a fixation processes and is then stained like a conventional smear.

For the smear taker there is little difference in using LBC compared to conventional cytology and learning is quick. Transferring cellular material into a vial instead of smearing and fixating it on a glass slide is not more complicated. It has been shown however that the rinsing must be meticulous and it seems to be especially difficult to transfer glandular cells as several studies have reported improved quality on all parameters except for the presence of endocervical cells[164, 165]. The bulk to transport to the laboratory is substantially larger when thin glass slides are replaced by cylindrical jars.

The main differences in processing slides occurs in the laboratory. The processing machines require investments and space. Training is necessary for those who process slides and also the cytotechnicians evaluating the microscopic picture. Benefits are that time needed by the cytotechnicians to evaluate the slide is reduced[166, 167] and savings of 60% in interpretation time have been reported[168, 169].

Numerous studies have compared the performance of cervical slides prepared using liquid based cytology with conventionally prepared slides. The wide variety of subject populations, sampling schemes, collection devices, follow up references and even type of LBC make meta-analysis and systematic reviews difficult and those that have been made have used different restrictions.

Liquid based cytology is marketed and used in many countries. In most of the few countries with national screening programs, the introduction of LBC into these programs has been considered and has been an important reason for performing Health Technology Assessments (HTA:s). However the results and conclusions have not been synonymous.

The New Zeeland Health Technology Assessment Clearing House published a systematic review 2001[170] (reviewed and commented in Läkartidningen[171]). Apart from liquid based cytology this review also included a few studies on automated cytology. It was based on 26 articles published after 1997. Works before that date were already evaluated in an Australian HTA review[172]. The NZ review used CIN2+ in histopathology as reference standard and found 13 papers with original data on LBC performance compared with conventional cytology. There were no randomized trials. Six studies used split sample techniques where the residual material in the collection devices after making a conventional smear is used for LBC. The remaining seven studies used different cohorts, usually separated in time, and compared these.

The New Zeeland review found no evidence for improved detection of high grade abnormalities (CIN2+). There was minimal evidence relating to test specificity and no

conclusions were drawn in that area. Regarding slide adequacy, the review found some evidence that LBC lead to fewer inadequate smears.

In England a HTA report[173] (reviewed and commented in Evidence-Based Healthcare[174]) found limited evidence that LBC may reduce false-negative results, unsatisfactory specimens and sample interpretation time. The report was updated in 2004[175], now with more explicit conclusions. Still there were no randomized controlled studies to include. When assessing effectiveness the HTA found a decrease in the proportion of inadequate specimens based on 35 studies, but found it hard to combine data as these were very heterogeneous. There was an improvement in sensitivity that varied between populations. In populations classified as ordinary (four studies[176-179]) the relative risk for false negative result was 0.55 (95% confidence interval 0.46 to 0.66) in favour of LBC corresponding to an increased sensitivity for LSIL+ of 18%. In high risk populations (10 studies) the relative risk was less, 0.76, but still significant (CI 0.60 to 0.98) corresponding to an increase in sensitivity of 4%. There was no separation done according to reference standard and study data were aggregated regardless of the type of liquid based cytology system used. Based on this report, LBC was implemented in the English national screening program[180] as it already had been in the Scottish program.

A Canadian HTA review[181] with similar conclusions led to the use of LBC in Canadian screening programs, while an earlier American review could not find any substantial difference between the methods[182]. A Danish HTA review[183] included only population based studies and had also the inclusion criterion that 10% of test negatives should have a verification by a reference standard. Only one original article was considered fulfilling the criteria for counting sensitivity and specificity[184], a split sample study with 1757 women and 35 outcomes in the form of CIN2+. Colposcopy supplemented with biopsy in selected cases was the reference method. Sensitivity was equally bad in the groups (51%, 95%CI 35 – 67%). This paper sparked a debate in the British Medical Journal and one of the co-authors publicly questioned the methodology, results and conclusion of the article[185]. The Danish review had also to rely on systematic reviews and other HTA-reports and concluded as two out of five former HTA-reports that there was not evidence for a difference in clinical effect between the methods.

Outside the HTA:s a number of meta analyses and systematic reviews have been made[143, 186-190] with various conclusions.

A study by Bernstein et al[186] was restricted to ThinPrep technology and included only studies using the Bethesda system for cytological classification. Only outcome in cytological diagnoses was measured. 25 articles were included and the authors concluded that ThinPrep improved sample adequacy and led to more diagnoses of low-grade and high-grade squamous intraepithelial lesions.

In 2003, Abulafia and co-workers[187] included 24 articles where ten used a reference standard other than the direct comparison of cytology diagnosis. Using a dichotomised outcome for cytology (normal/abnormal) they found an agreement of 92% with an adjusted kappa value of 73%. The total fraction of positive slides in this analysis was 8.4% for conventional PAP and 10.7% for Thin-Prep which was the LBC-method studied in this analysis. When comparing with reference standards they assessed the sensitivity to be higher for Thin-Prep (76%) than for conventional PAP (68%) corresponding to a 12 % increase. However the populations are not stated, and confidence intervals are not presented. Two studies are not paired but contain only Thin-Prep data. Among the paired studies 5 of them found LBC to have superior sensitivity and 3 studies favoured conventional cytology in this respect.

The Lancet published a meta-analysis in 2006 that included a large number of studies (N = 58) but rated them in 5 categories according to the authors view of quality. There were no "ideal" studies defined as "An independent randomized sample study with verification by a masked reference standard of at least all positive slides" Only 6 studies were considered high quality which required masked reference standard. 32 were medium quality and 19 lacked reference standard and were categorized as low quality. Of the high quality studies five were split sample and one used direct-to-vial were two samples were taken from each woman, and the order was randomized. This single study[191], with cone material as reference, showed a difference between second and first sample on the same cervix, supporting the view that split sample studies disfavour LBC that always is the second test. Counting only the first sample, sensitivity for conventional cytology with CIN2+ as threshold is 79% and LBC 89% ($p = 0.02$) displaying a difference in sensitivity of 13%.

The findings in the Lancet meta-analysis were that medium and high quality studies showed no difference in sample adequacy between the methods. In high quality studies conventional cytology picked up more HSIL in cytology and LBC classified more slides as ASCUS. The authors conclude that high quality studies do not provide evidence for LBC being more accurate than conventional cytology in detecting high-grade disease. Furthermore they called for randomized studies and proposed such studies to be integrated into routine services.

A debate broke out in the same journal and one of the controversial points that was observed[192] was the fact that the main study that the US FDA reputedly relied on for approval of liquid cytology[164] was rated low quality by the reviewers.

In 2007 the results of a large randomized controlled trial was published[193]. The trial was not designed or powered primarily for evaluating LBC, but provided data on this issue. Out of 58 700 eligible 45 307 woman (77%) were randomized to either liquid cytology (ThinPrep) or conventional cytology[194, 195] and 14 laboratories

participated. LBC-slides and conventional slides were read by the same cytopathologists. All women in the experimental arm were referred to colposcopy if the cytology showed at least ASCUS while this was the case for 72% of the women with conventional smears. CIN2+ lesions detected within a year of referral was the endpoint of the study. While colposcopy was not blinded for cytology or HPV-results the pathologists were blinded for cytology. Significantly more cytological abnormalities were found in the LBC arm; the relative frequency for ASCUS was 1.6, for LSIL 1.8 and for HSIL 1.6. The proportion of unsatisfactory cytology was reduced from 4.1% to 2.6%, due to a reduction of obscuring inflammation. Of 661 women who underwent colposcopy 84 women with CIN2+ in histopathology were detected with conventional cytology (0.37% of the arm) and of the 1337 women who had colposcopy in the LBC arm 99 had a CIN2+ diagnosis (0.44% of the arm) leading to a non significant relative sensitivity of 1.17. Including all CIN however gave a significant relative sensitivity for LBC of 1.68. Restricting analysis to the centers that used ASCUS as threshold for referral to colposcopy did not significantly change the result. The authors found no evidence of heterogeneity between the centers and no effect of time (learning curve). While there was no significant difference in detection of CIN2+ the positive predictive value for CIN2+ in this population was significantly lower for LBC (7.4) than the PPV for conventional cytology (12.7).

Liquid based cytology and automation

A computerised system for reading slides, the ThinPrep Imager (Cytyc) has been marketed linked to the ThinPrep system. The imager identifies 22 fields of interest most likely to contain abnormal cells. These fields are then examined by a cytotechnician. An Australian split sample study was published May 2007[196] where conventional cytology was compared with liquid cytology using the ThinPrep Imager system. 52 665 women were examined with two slides for each woman. The rate of unsatisfactory slides decreased with the imager LBC (from 3.1% to 1.8%) The proportion of non benign slides decreased (94.0 to 92.6%) and the yield of histopathological high grade lesions picked up within six months increased by 16%. The time for reading slides decreased by 56% from 10.6 minutes with conventional slides to 4.7 minutes with the ThinPrep Imager system[197].

The SurePath system is also offered with computerized analysis of slides. The BD FocalPoint system (formerly AutoPap) sorts out a pre-set fraction (25%) of the most normal slides and leaves the rest for manual review. For these the system provides maps (PapMaps) to identify the 15 most abnormal areas of the smear. The system has been found to reduce interpretation time by 40%, to be safe and with a high concordance with manual analysis (weighted $\kappa = 0.75$)[198].

The FocalPoint system is not confined to Liquid based cytology but can examine conventional smears as well and this was the technique that originally was approved

by the US Food and Drug Administration (FDA) in 1998. The ThinPrep Imager can only analyze LBC-smears.

Colposcopy and examination of women with abnormal smears

Fig 12. Modern colposcope.

A colposcope is a two-eye piece microscope with a magnification of 6 – 40 times. It is used for in vivo studies of the transformational zone of the cervix. The area of the highest degree of epithelial abnomalities can be located and biopsied. With experience it is possible to forecast histological diagnosis with reasonable accuracy. At initial observation a magnification of 6 – 12 is used and for detailed observation, mainly on angio-architecture and surface characteristics, the magnification is increased, seldom more than 24x. A green filter increases the contrast in the red part of the colour spectrum and can be of help for studying vessel patterns. A two-bladed speculum, in a size appropriate for the woman, is used. The portio and upper part of the vagina can be inspected as well as the distal part of the cervical canal as the speculum retracts the cervical lips somewhat. Initial investigation is often performed with saline, but the most important colposcopic findings are made after applying 3-5% acetic acid on the portio. Finally the reaction to iodine staining is assessed. If justified, biopsies are taken, normally without need of local anaesthetics as the cervical stroma has sparse innervation (as opposed to the vagina).

Colposcopy has been the international standard of care for the assessment of abnormal or persistently abnormal smears since at least twenty-five years. In some low resource settings with poor or unaffordable infra-structure, colposcopy has been recommended for a long time as a screening tool but international standards recommend colposcopy as a triage sorting out patients for treatment or surveillance in some form.

The history of colposcopy goes back to the earliest years of the past century. At that time cervical cancer was the most common cancer among women. Reports were published about white areas on the uterine cervix that was termed *leukoplakia* that appeared to sometimes turn into cancer[199, 200]. Otto von Franqué, professor in gynecology in Vienna, assigned his assistant Hans Hinselmann to do further investigations of leukoplakia. Hinselmann concluded (wrongly) that leukoplakia was a truly precancerous lesion and always progressed to cancer, but saw a need of closer examination with magnification in vivo. He had the idea that cancer could be detected in a treatable subclinical stage with limited size. With much effort he 1924 presented an instrument consisting of a Leitz dissecting binocular microscope combined with a light source that he termed a colposcope (colpo = vagina i.e. bay, cleft)[201]. Later on he succeeded not only to find small cancers but he also described intraepithelial cancers (carcinoma in situ) and other pre-cancerous signs and lesions[202, 203]. He used acetic acid to remove mucus but discovered also the acetowhitening of intraepithelial lesions. The existence of intraepithelial non-invasive cancers were known since the turn of the century and Hinselmann made very close and detailed studies on the histopathological correlations of colposcopical findings like punctuation and mosaic. He saw that these changes were found in a limited area of the portio that we today recognize as the transformational zone and named these lesions the matrix area of carcinoma. Initially it was thought that all colposcopic changes were part of a process leading to cancer and it was not until the 1950:s that the non-malignant characteristics of reactive changes of metaplasia were recognized[204]. Even before that colposcopic changes were divided into *simple atypical*, corresponding to what we now know as reactive changes and *marked atypical*. Pathologists subdivided marked atypical epithelium into subgroups a classification that many years later took the shape of the still used grading of CIN1, CIN2 and CIN3[205]. An important contribution came from Norway in 1972 with the presentation of a rich material in an atlas with the emphasis on describing vascular patterns in colposcopy[206].

Evidence accumulated that simple atypia was not precancerous and in a 23 year (!) follow up of patients with a simple atypical matrix zone Dietel could not find a single cancer[207]. The colposcopic differentiation of metaplasia, sometimes with underlying inflammation from lower grades of CIN still is a challenge where colposcopy often fails. This is a main reason for the poor specificity of colposcopy used as a screening tool.

The evolution of colposcopy was in many respects parallel with cervical cytology. The Romanian Aurel Babes documented cervical cytology to detect cervical cancer in 1926 and published this in French[208] some months before the legendary report of Georgious Papanicolaou, finding cancerous cells in the posterior fornix among women with cervical cancer[209]. During further development colposcopy turned out to have its most important role in detecting pre-invasive lesions, and that turned out to

be the case for vaginal cytology as well. Separate traditions started to develop with German influence in central and eastern European countries relying on colposcopy for examining symptom-free patients while cytology took the main role in this kind of screening in the US and later on also in Norway and Sweden. Colposcopy became wide spread in Latin America, not at least because of Hinselmann's own extensive travels there, but also because it provided a direct tool for screening without the need of elaborate cytology in a continent still struck by very high incidence of cervical cancer. However in countries were cytology services could be provided the performance of this technique often turned out to be superior as cytology is considered easier to learn, more economical[210] and often more specific. While proficiency could develop in large laboratories in some parts of the world it can be noted that cytology still is of debatable quality in countries that clung to colposcopy as a screening tool, eg. Germany[211, 212] were cytological diagnosis still can be an office procedure for gynecologists.

In the 1970:s colposcopy had its breakthrough also in the Anglo-Saxon world, where it came to be a necessary step for selecting those women who should receive treatment after having an abnormal smear (diagnostic colposcopy). In some parts of Sweden the internationally abandoned practice of treating all women with one or two abnormal smears without colposcopy have been retained. Due to the poor specificity of low grade cytology this leads to a substantial overtreatment.

Colposcopy as a screening tool

Colposcopy is still used as a screening tool in different parts of the world, not only in low resource settings. The possibility of an immediate diagnosis and the reduced risk of loss to follow up can be seen as advantageous compared with having to rely on laboratory diagnoses[213]. The performance however is not impressive. In a German study of 4700 women sensitivity for CIN2+ was only 13%[214].

How does colposcopy perform compared with histology?

In 1998 Michele Follen Mitchell and co-workers published a meta-analysis on the specificity and sensitivity of colposcopy[215]. Articles were included if colposcopy was used for investigation of an abnormal PAP-smear result, excluding colposcopy as a screening tool or investigations due to symptoms. Secondly data should compare colposcopy with the gold standard of histopathological evaluation. Out of 86 articles identified nine studies were included in the meta-analysis containing data from 6281 colposcopies. Two thresholds were used: 1) the ability to distinguish normal tissue from abnormal and 2) distinguishing CIN2+ in histopathology from normal tissue or lesser degrees of abnormalities, where eight studies comprising 5378 women provided data. When the first threshold was used the sensitivity was excellent with a weighted mean of 96% (95 % CI 95 – 97%) and a range of 87 to 99% between studies.

Specificity was 48% (CI 47-49%) with a greater range of 23 – 67%. When comparing high grade lesions or cancer with all other observations the sensitivity was somewhat lower; 85% (CI 84-86%, range 30 – 99%) and the specificity 69% (CI 68-70%). In the meta-analysis sensitivity was plotted against specificity in a ROC-curve and the area under the curve for all abnormalities was 0.80 and marginally better for high grade lesions (0.82). Likelihood ratios (see page 58) were also calculated for each individual study, but not for the whole material. Of the studies reviewed one showed a likelihood ratio of more than 10 for the ability of colposcopy to identify high grade lesions (for this study CIN3+)[216], one was in the range 5 – 10[217] and the remaining seven studies were in the range 2 – 5[218-223]. The authors concluded that colposcopy has a tendency of "overcalling" low grade lesions, classifying them as high grade but that specificity and sensitivity according to the area under the ROC-curve were comparable with other tools used in medical diagnostics.

Since 1998 a number of reports have been presented, not all of them reassuring.

In 2002 a study by Massad and Collins[224] investigated the correlation between colposcopic impression and histology in biopsy. The population consisted of 2406 women from a deprived area of the inner city of Chicago, examined between 1996 and 1999. Colposcopies were made by residents under supervision. Although the mean age was 33 years, 26% of colposcopies were unsatisfactory. Despites this they were included in the study. The analysis of the correlation was done with unweighted kappa statistics (see page 59). Although the association between colposcopic impression and histopathology was highly significant with chi^2 test ($p<0.001$) the kappa value was poor, only 0.2, when 6 different grades of histology were compared with the same grades in colposcopical assessment. These authors noted that colposcopic assessment more often overestimated than underestimated the severity of lesions and concluded that the results might reflect the standard of colposcopy and pathology outside academic centers. The authors calculated kappa values on the basis of data from other studies, and found similar poor results: Ferris and Miller[221] 0.16 and Lozowski[222] 0.26.

In most clinical settings biopsies are the result of a colposcopic evaluation and are taken from the area(s) that are colposcopically considered most severe. Different attempts have been made to reduce the risk of misclassification when biopsies are used as gold standard. In 1999 a study was published[225] where British researchers followed 255 women, included if they had a positive referral smear and negative colposcopy between 1988 and 1991. This group was drawn from a series of 1000 consecutive referrals during the period. The studied women were in retrospect followed up for at least five years and compared with a control group. This study showed a highly significant difference in subsequent findings of CIN in histopathology ($p <0.0001$). Statistics for CIN2+ are not presented but as basic numbers are presented the proportions can be calculated as 8.2% (21/255) high grade

lesions in the follow up of those with a negative colposcopy and abnormal index smear compared with 2.7% (20/726) in the control group with normal smear and without colposcopy (p=0.0002). In this study no difference could be seen in subsequent cytological or histological abnormalities among those who had a punch biopsy at the referral colposcopy and those who had not. The results support close follow up of cytology positive women with negative colposcopy.

In the Swedish randomised trial of primary HPV-screening, SwedeScreen, data from 195 colposcopies was evaluated[226]. Colposcopies were made in 100 women with persistent HPV-infection and 95 population controls. 8 colposcopists in 5 centers were involved. Two biopsies were taken when colposcopies were normal, otherwise biopsies were directed by colposcopy. Colposcopy was classified as abnormal in 16 out of 17 CIN3 cases (sensitivity 94%) and from the data in the study, with CIN2+ as threshold, the sensitivity can be calculated as 83%.

There are several studies which have assessed the accuracy of colposcopcially guided punch biopsies, without specifically recording colposcopic impression, and compared the histopathology result with subsequent histopathology from complete removal of the transformational zone. This can be used as a rough estimate of colposcopy performance as the sites of biopsy in these studies are determined by colposcopy. McIndoe et al found 10% (19/196) of punch biopsies to be false negatives, here defined as underrating the final histology report with two steps or more[227], but a study of 118 cases from Hammersmith Hospital in London[228] a false negative rate of as much as 54% was reported when punch biopsies were compared with subsequent laser cones. Recalculating their data however gives a sensitivity of punch biopsy, taken at a mean of 10 weeks ahead, of 80%. This study also compared colposcopy diagnosis with cone histology and calculations from this data show a sensitivity of 93% for squamous CIN2+ lesion at a threshold of CIN in colposcopy and 82% at a threshold of CIN2+. Another British study[229] found a higher grade of histopathology with LLETZ compared with punch biopsy in 24%, corresponding to a sensitivity for colposcopically directed biopsies of 76%. In a recent report from the Merck phase III HPV vaccine trials a similar design was used. 319 women had biopsies taken from the "worst" area detected by colposcopy and at the same visit LEEP was performed. The biopsy underestimated the worst pathology in 41% and made only 4.4% overestimations. 48% of the 84 CIN3 cases had biopsies less than CIN2.[230]. A South African study[231] followed up cytologically positive but initially colposcopically negative cases and performed LLETZ on the women with either positive repeat colposcopy or cytology. The sensitivity of the initial punch biopsy for CIN in this study can be calculated to 89% (123/138).

Gage and co-workers recently published follow up results from the large US trial on managing cytology showing atypical squamous cells of undetermined significance (ASCUS) or low grade squamous intraepithelial lesions (ASCUS-LSIL Triage Study –

ALTS)[232]. Of 5060 women enrolled 2773 had a colposcopic examination in conjunction with enrollment. The authors studied the influence on performance of the number of biopsies taken at colposcopy and the professional background of the colposcopists. CIN3+ was chosen as outcome. In a dichotomized fashion results were controlled for age (over/under 30), use of oral contraceptive pill (current user: yes/no), parity (< 3/≥3) cytology (HSIL/<HSIL), colposcopic impression (high grade/<high grade), and biopsy number (1/>1). Medical training was divided into nurse practitioners, general gynecologists, gynecologic oncology fellows and gynecologic oncologists and expectation of colposcopic performance was increased in that order. Nurses were more inclined to take multiple biopsies, (and were encouraged to do so) and the number of biopsies taken was correlated with medical profession and decreased in the order mentioned. More expertise - fewer biopsies. The number of biopsies was also correlated with lesion size and the colposcopic severity of the lesion. 81% of nurse colposcopies with impression of high grade resulted in more than one biopsy while gynecological oncologists took more than one biopsy in this situation in only 35 %.

In this study verification bias was reduced as the endpoint was not only the biopsies taken at the enrollment colposcopy but the result of two years close follow up in the study. The CIN3+ cases during follow-up were concluded to represent missed prevalent cases rather than incident lesions[233] as all participants had cytological abnormalities. 26 out of 408 women who were found to have CIN3+ in quality assured histopathology analysis during the study, had a normal colposcopy at enrollment and no biopsies taken. In addition 53 patients were biopsied with histopathology showing normal or benign abnormality. 57% of women who eventually had CIN3+ had CIN3+ in the biopsy at enrollment colposcopy. If initial biopsy result was expanded to include CIN2, which would lead to treatment, the sensitivity was increased to a still meager 70%. Those women with a final CIN3+ diagnosis who had only one biopsy taken at enrollment were less likely to be diagnosed as having CIN2+ on this occasion (68%) than those who had two or more biopsies (82 – 83%) ($p<0.01$). The authors state that this result is maintained after adjustment for confounders including colposcopic impression but data are not presented.

The ALTS study had three arms and CIN3+-cases were evenly distributed between them. In one of the triage arms all minor cytological abnormalities were examined by colposcopy, in another arm a smaller number of colposcopies were made after a repeat abnormal smear and in the third arm after a positive HPV-test. The sensitivity for colposcopy was significantly lower in the colposcopy arm (59%) than in the other two arms (80% and 81% respectively). The authors' conclusion was that the colposcopists' performances improved due to knowledge of the additional test results.

The main finding in this study according to the authors was that the sensitivity for colposcopically directed biopsies improved if more than one biopsy was taken, and that this finding was consistent regardless of training background.

In the county of Värmland, Sweden, the policy has traditionally been to treat women mainly based on cytological diagnosis with little role of colposcopy. As shallow LEEP/LLETZ has been standard for diagnosis/treatment it has offered a gold standard in a triage study[234]. Although the authors in this study discourage trust in colposcopy their results, recalculated, gives a fair sensitivity of 88% for colposcopy to find CIN2+.

The effect of training and professional background on colposcopic performance

In a recent retrospective study[235] the performance of colposcopy was compared between residents in different stages of their training and specially trained colposcopy nurses. Diagnostic agreement between histology and colposcopic finding within one step of CIN was 73 – 77% for doctors in residence training, and no difference related to number of years in training. Interestingly the specially trained colposcopy nurses had an accuracy of 92%, which could be interpreted as a documented result of colposcopy training.

The results from the ALTS contradict the notion that colposcopic expertise in the US is substantially higher among gynecological oncologists than among nurses doing colposcopy. The actual experience of colposcopy in the different professional groups was not accounted for, however.

Other studies have concluded that nurses perform as well as colposcopists although the number of inpatient procedures compared to outpatient procedures increased in one study[236].

Colposcopy and interobservatory agreement

Colposcopy is not only a subjective method but it is also an in vivo procedure. This makes it difficult to compare observations between different colposcopists. The method used in studies is photographic images, either in the form of cervicograms or less standardized colpo-photographs.

Sideri used kappa values to compare the colposcopic evaluation of 100 cervigrams, standardized photos of the cervix taken with application of acetic acid and with magnification[237]. Evaluation was made by nine experienced colposcopists. The classification was made according to the International Federation of Cervical Pathology and Colposcopy terminology[238]. Interestingly the ability to distinguish an

atypical transformational zone was highly reproducible (Kappa 0.70) while the identification of CIN had lower agreement (0.42) and the grading of CIN had quite poor reproducibility (0.30). These data were in agreement with an earlier study by the same authors[239].

It can, rightly, be argued that two dimensional photographs are a poor substitute for the real picture seen by the colposcopists and that this might underestimate the reproducibility of this diagnostic procedure. In a clinical setting the light is good, with possibility of variation. The picture is stereoscopic and green filter can be used. The amount of reflection in acetowhite areas can more easily be assessed when the focus and light can be manipulated and the surface structure of the cervix can be examined more accurately. Furthermore the dynamics in viewing the acetowhiteness appearing and sometimes disappearing and the ability to resolve vascular and epithelial changes are important features in colposcopy. Comparisons between colpophotographs and live, on-site colposcopy are sparse but the ability to assess whether a colposcopic examination is satisfactory appears to be better with live colposcopy[240].

The importance of lesion size in colposcopy

The notion that colposcopists are highly influenced by the diagnosis of the referral smear was addressed in a combined Chinese – Californian study[241]. The authors had observed that the colposcopic diagnosis was not only related to histopathology but also to the grade of the referral smear. In this study colposcopists were not blinded for referral smear. The sensitivity of a colposcopic diagnosis of CIN2+ in relation to a histopathology diagnose of CIN2+ was only 12% (62/529) if the referral smear showed LSIL or less but 55% (110/197) if the smear showed HSIL, a highly significant difference. Biopsies were taken from all four quadrants regardless of colposcopic impression, in a subset of this study population. The number of quadrant biopsies showing CIN2+ was used as a proxy for lesion size. When the grade of referral smear was adjusted for lesion size in a logistic regression model, the effect disappeared. The authors concluded that grade of referral smear was a confounder for lesion size, and that colposcopic impression of high grade lesion became more accurate when lesion size increased. Difference in lesion size could also explain higher accuracy in detecting CIN3 lesions and cancer compared with CIN2 lesions.

The relation between lesion size and histological grade has previously been studied. Lesion size was determined from colposcopical assessment[242-244] or from measuring length of CIN in laser conization specimen[245], all showing that the larger the lesion, the higher the grade of CIN. One study has also reported failure of colposcopy related to small lesion size[246].

A new dimension in colposcopy trials is noted in a very recent study made in China. Comparing colposcopy and four-quadrant biopsies the investigators found an association between false negative colposcopies and thin lesions[247]. The explanation could be that thin lesions are more transparent.

Scoring systems for colposcopy

All scorings systems use recognized features of colposcopic change, and the items of abnormal colposcopic findings in the classification of the International Federation for Cervical Pathology and Colposcopy (fig 13) are most often included[1].

I. Normal colposcopic findings
  - Original squamous epithelium
  - Columnar epithelium
  - Transformation zone

II. Abnormal colposcopic findings
  - Flat acetowhite epithelium
  - Dense acetowhite epithelium*
  - Fine mosaic
  - Coarse mosaic*
  - Fine punctation
  - Coarse punctation*
  - Iodine partial positivity
  - Iodine negativity*
  - Atypical vessels*

III. Colposcopic features suggestive of invasive cancer

IV. Unsatisfactory colposcopy
  - Squamocolumnar junction not visible
  - Severe inflammation, severe atrophy, trauma
  - Cervix not visible

V. Miscellaneous findings
  - Condylomata
  - Keratosis
  - Erosion
  - Inflammation
  - Atrophy
  - Deciduosis
  - Polyps

*Indicates the characteristics of high-grade changes.

Fig 13. The IFCPC classification of colposcopic findings and terminology 2002. From Walker et al 2003[1].

Reid's index is the only scoring system that has been used in clinical trials including colposcopy, such as in the ALTS study[232, 233] and the phase III Merck HPV vaccine trial[248]. In the ALTS trial an abbreviated form of Reid's index (RCI) performed poorly[249]. The iodine reaction was not included due to "its limited use in the US". 3549 colposcopies were analyzed. 87.8 % of all colposcopies scored 0-2 on the 6 grade scale and when colpophotographs were reviewed independently by reviewers as many as 96.2% were graded 0-2. Many CIN2-3 lesions received this low score and sensitivity to detect CIN3+ by a score of ≥3 was only 37.3%. Because of the low prevalence of disease and scores ≥3 the NPV was high however, 92.1%. The performance with the RCI did not differ between those colposcopists who had passed or failed an exam. It was inferior for the quality control reviewers who had to rely on colpophotographs (sensitivity to detect CIN3+ for a score of ≥3 p was 14%). When analyzing individual parameters the specificity for 2 points on any of them was high, 93 – 98%, but sensitivity for even 1p threshold was 46% for margins, 52% for vessels and 51% for colour in this scale.

Colposcopic sign	*Zero points*	*One point*	*Two points*
Colour	Less intense acetowhitening (not completely opaque). Indistinct, semi-transparent acetowhitening. Acetowhitening beyond the margin of the transformation zone. Snow-white colour with intense surface shine	Intermediate, shiny, grey–white shade	Dull, oyster white
Lesion margin and surface configuration	Feathery, indistinct or finely scalloped edges. Angular, irregularly shaped, geographical margins. Satellite lesions with margins well removed from the new squamocolumnar junction. Lesion with a condylomatous or micropapillary contour	Regularly shaped lesion with sharp, straight edges	Rolled, peeling edges. Internal margins separating lesions with differing scores, the more central one with the higher score tending to be nearest to the new squamocolumnar junction
Blood vessels	Fine punctation or mosaic pattern	Absent vessels (after application of acetic acid)	Coarse punctation or mosaic pattern
Iodine staining	Positive iodine staining (mahogany-brown colour). Negative iodine staining in an area that scores 3 or less on the first three criteria	Partial iodine uptake, giving a variegated pattern	Negative for uptake, giving a mustard yellow appearance in area that is significant (4 or more points) by the other three criteria

Fig 14. Reid's colposcopic index. A score of 0-2 is reported to correspond to metaplasia or CIN1; 3-5 to CIN1-2 and 6-8 to CIN2-3.

Treatment of dysplasia

CIN2-3 and AIS proven by histopathology should be treated[250-253]. With concordant diagnosis of CIN1 treatment can be refrained from, especially in young women with good possibilities of follow up. Treatment of CIN is surgical. Treatment is tailored after the grade and extent of the lesion, the size and type of transformation zone and age and history of the patient[204].

Knife conization

The method was once developed as a less radical alternative to hysterectomy. General anaesthesia is normally used and this is often an in-patient procedure. The excised tissue has the form of a cone, hence the concept conization that is often used even for resection methods where the excised tissue has other shapes. The area on the portio is delimited by iodine or colposcopy and the cutting is made by a surgical knife. Peri-operative and post-operative haemostasis can be difficult to achieve and various surgical techniques have been developed to reduce this. Sturmdorff sutures have often been used in western Sweden. Treatment success (i.e. no residual disease on follow-up of different lengths) of knife cone biopsy is reported to be 90 to 94%[254-256].

Laser conization

This procedure can be performed under general or local analgesia. A focused laser spot is used to make an incision around a circumference of the portio at a chosen depth. Small hooks or retractors are then used to manipulate the cone to allow deeper incision to complete the endocervical incision. Haemostasis if required is generally achieved by laser coagulation by defocusing the beam. Laser conization needs skilled operators, safety precautions due to the high energetic beam and moderately expensive equipment. Treatment success of laser cone biopsy is reported to be 93 to 96%[254, 255]. The major advantages are accurate tailoring of the size of the cone, low blood loss in most cases, and less cervical trauma than knife cones.

Loop excision

Large Loop Excision of the Transformation Zone is often abbreviated to LLETZ in the UK or LEEP (Loop Electrosurgical Excisional Procedure) in the U.S. A wire loop electrode on the end of an insulated handle is powered by an electrosurgical unit. The current has a high frequency so as not to induce myoclonal cramps and is designed to achieve a cutting and a coagulation effect simultaneously. Power should be sufficient to excise tissue without causing thermal artefact. The procedure is often performed under local analgesia. Treatment success of LLETZ is reported to be 91 – 98%[257-261]. Invented by Renee Cartier in 1970:s[262] LLETZ is now the dominating method of treatment in Sweden and all over the world. It is, for better or worse, easy to learn, seldom produces significant blood loss and with different sizes and shapes of the loops the resection can be tailored to the individual cervix. Disadvantages are difficulties in making the resection in one piece. This has led to the development of variants of the methods as straight wire excision and different cone shaped electrodes.

Multiple punch biopsies and diathermy

This approach, used without colposcopy, was the standard of treatment in many clinics in west Sweden during the 1980:s until it was replaced by laser conization in some clinics and later LLETZ in the remaining. It was a mixture between destruction and resection and was often used with iodine as demarcation of the transformational zone. There are no published results to be found.

Hysterectomy

This method has become rare as primary treatment but is an alternative when there are additional clinical conditions such as bleeding problems, or uterovaginal prolaps in women above child-bearing age. The method should be considered in older women with recurrent or residual CIN. Vaginal route is preferred[263].

Destructive methods

Destruction of tissue has the basic disadvantage of not producing sample for a histology report and destructive methods have almost entirely been replaced by resection methods. In cryosurgery a metal probe fitting the particular transformational zone is used and necrosis is induced by hypothermia. Carbon dioxide or nitrous oxide is used in the instrument and at the tip the temperature is -65° to -75°C. Failure rates for treatment of CIN3 have been reported between 9%[264] and 23%[265]. Elmfors reported 20% failure rate in his thesis[266]. Cryosurgery has the advantage of being a quick out-patient procedure and requires no anaesthetics. It could have a role in see and treat programs with Visual Inspection in resource-deprived settings in the underdeveloped world[267]. Tissue destruction can also be achieved by laser or diathermy.

A Cochrane review[268] could not find significant differences in performance between modes of treatment but concluded that cryotherapy did not appear to be an effective treatment for high grade disease. Four RCT:s compared LLETZ and laser conization. There was no significant difference with respect to residual disease but a tendency for better results with LLETZ (1.22, 95%CI 0.71 – 2.12) at follow-up. Laser conization takes significantly longer to perform , the depth of thermal artefact and incidence of significant thermal damage are all significantly increased.

In a recently published study Finnish researchers compared different treatment in retrospect[269]. Cold knife conization, used in Finland in the 70:s, they found to be inferior to other methods, after controlling for age at diagnosis and histopathology at treatment. They also found a tendency to a higher rate of post treatment CIN3+ in patients treated with cryotherapy rather than with LLETZ or laser.

In clinical practise resection and destruction are often combined. Laser conization is commonly followed by more or less extensive vaporisation, sometime with the purpose of hemostasis, and in concordance LLETZ can be followed by cautery. A randomized controlled trial showed LLETZ not followed by cautery to result in higher rate of satisfactory colposcopy at follow up and lower rate of cervical stenosis than when the procedure was ended with cautery[270].

Risks in subsequent pregnancy

The risk of cold knife conization causing prematurity has been known for some time. In a Göteborg study, using the same women before conization as controls, the incidence of late spontaneous abortions was increased seven-fold[271]. Larsson reported similar proportions[272] leading to 31% preterm deliveries after conization. Among several studies a Norwegian study found similar risks even with laser conization[273] and another lower birth weight[274] while Bekassy found no difference in follow up of

laser conization in women with minimal depth of treatment[275]. A recent meta-analysis[276] found an increased risk of preterm delivery and decreased birth weight with knife conization (RR = 2.6 and 2.6 respectively), with LLETZ, (RR= 1.7 and 1.8) and with laser conization preterm delivery just barely failed to be significantly increased (RR=1.71 CI 0.93 – 3.14). Stratifying for excisional depth with laser or LLETZ a depth of more than 10 mm gave a significant increased risk for pre-term delivery (RR=2.6) while the three studies that gave data to this comparison showed a higher point estimate for less than 10 mm without reaching significance (RR= 1.45, CI 0.55 – 3.86). A large and recent Danish study showed a risk of preterm delivery among women who had undergone LLETZ at 6.5% compared with 3.4 % among not treated women. (OR=2.0; 95%CI 1.8 – 2.2)[277]. Data on fertility after treatment are sparse and has not convincingly shown effects on fertility after treatment for CIN[278].

Follow up after treatment

The risk of failure in treatment for CIN

As more conservative treatments have been introduced the risk for incomplete treatment has been recognized. The risk after LEEP/LLETZ has been typically reported to be 3.7% – 9.1% with different times of follow up[279-289]. In West Sweden the proportion of re-treatments are 6.5% within one year and 7.8% within two years[99]

Several studies have been made evaluating the risk for residual disease and for risk factors. Today incomplete excision (CIN in specimen margins)[290-292], particularly of endocervical margin[280, 282, 293], older age (above 40-50)[285, 294-296] and lesion size[284, 285, 296, 297] are recognized risk factors as well as positive endocervical curettage[293, 295, 298, 299] and some authors have recommended the use of colposcopy in at least one follow up visit[284, 300], while others have found no advantage with colposcopy[301]. Smoking status has been found to be significantly associated with recurrence of CIN2+ in one study[302].

The risk of cervical cancer following treatment of CIN

For many years there has been concern about the long term risks for invasive cancer among women once diagnosed and treated for high grade cervical lesions. Kolstad issued a warning in 1976 that patients once treated for carcinoma in situ "should be carefully followed for a much longer time than the conventional 5 years"[303]. This was based on a study on a case series of 795 patients treated with cold knife conization of which 1% developed cancer after follow up for 5 – 25 years. Among 238 women treated with hysterectomy 2% developed invasive cancer. Andersch and Moinian[304] surveilled 429 women treated for CIS for 1 – 10 years. They made a distinction of remaining (residual) disease found within one year and relapses (recurrence) after more than a year. In this cohort, followed closely with cytology, 2 invasive cancers

and 36 high grade lesions (CIN 2+) were found as "relapses". Recalculating their data we find an incidence of 2 invasive cancers in 2 889 person years which equals 69/100 000. The authors recommended multiple punch biopsy and diathermy, guided by colposcopy and iodine, as standard diagnostic work up and treatment and long observation time for carcinoma in situ cases.

A number of case series have been reported from clinics often with close surveillance of their patients after treatment. Results are generally excellent, and few, if any, invasive cancers were reported at follow up[73-75, 256, 279, 300, 301, 305-312]. Cytology and sometimes colposcopy had been used in follow up. Dan Hellberg and Staffan Nilsson made an unusually long follow up of a cohort of 893 women treated for CIN, mainly with conization[313]. They found cure rates for conization similar to that of hysterectomy and attributed that to the consistent use of colposcopy in diagnosis and treatment. After 5- 20 years of follow up 2 microinvasive cancers were found.

In 1989 computerized records were used for the first time for assessing the long term risks for cervical cancer after treatment for CIS. Folke Pettersson and Birgitta Malker[314] linked the diagnosis in the Swedish cancer register where 56 116 women had contributed 453 362 person years. They found an increased risk, with a point estimate of observed/expected 2.40 after excluding recurrences the first year, and found older women to have an even higher risk. A similar slightly smaller study was made in Norway 1995[315]. Between 1970 and 1992 37 001 women with carcinoma in situ of the cervix contributed 336 798 person years. In the Norwegian cancer register a negative association was found for cervical cancer. Standardized incidence ratio (SIR) was 0.11 (95%CI 0.06 – 0.23). This study investigated the association with all sites of cancer. There were three times as many cancers in the combined report for vulva and vagina and the SIR was 4.04. There was no combined increase for all sites of cancer after a diagnosis of cervical CIS (SIR=1.04).

Patrick Soutter and co-workers published the first meta-analysis on the subject of long term risks after treatment in 1997[316, 317]. They pooled five British studies and added some new data. Around 12 000 women were observed for a total of 44 699 women year. The authors found 33 cases with cancer, an incidence of 75 per 100 000 women years and a constant increased risk of "about five times" for at least eight years after treatment with a cumulative risk for cervical cancer of 5.8 per 1000 women during this period of time.

Kalliala and co-workers[318] studied a cohort of 7564 women treated for all grades of CIN in the Helsinki area. The number of person years was 97 556. Data were linked to the Finnish cancer register and, in contrast to the Norwegian study, there was an overall significant risk for all cancers (SIR 1.3), an increased risk for lung cancer and other smoking related cancers, an increased risk for cervical (SIR: 2.8, 95%CI 1.7 to

4.2) vulvar (SIR: 4.1, 95%CI 1.5 to 8.9) anal (SIR: 5.7, 95%CI 1.2 to 17.0) and a dramatic increase of vaginal cancer (SIR 12.0, 95%CI 3.9 to 28.0).

Soutter, Sasieni and Panoskaltsis published a new meta-analysis in 2006. This had the ambition to cover the entire research field. 1848 possible articles boiled down to 25 from ten different countries. In this ten Scandinavian studies (six Swedish, three Norwegian, and one Danish) were included. The study from the Swedish cancer registry[314] represented more person time than all the other studies lumped together and for that reason had to be handled separately in some analyses. The smaller studies were in agreement with Pettersson on an incidence rate for cervical cancer of around 55 per 100 000 person years more than ten years after treatment of CIN. The study found no relationship between year of study and rates of cancer. There were higher rates of cancer in studies containing more CIN3 and an increased risk of around 2.8 times the background at least for 10 years and "probably for up to 20 years". There was no significant differences in risk in the series in which women were treated with hysterectomy and those were localized methods were used, and the data did not provide support for differences between the various conservative methods. The risk for post treatment CIN was also analysed. This was lower in women treated with hysterectomy, no difference related to grade of CIN at treatment and a decreasing trend with time.

The risk for vaginal cancer after CIN

Cancer appearing in the vaginal vault after hysterectomy for CIN can be classified as vaginal cancer. In smaller series the risk for vaginal intraepithelial neoplasia (VAIN) or cancer is 30 - 90 per 100 000 women years[307, 310, 319, 320](Data from Gemell et al[310] recalculated from 10-years follow up). Burghardt found four times fewer recurrences when patients were treated for CIN with vaginal hysterectomy compared with abdominal hysterectomy[263].

Data from register studies in Norway and Finland have been described[315, 318]. A Swedish study[321] used data from the Swedish cancer register linking to other registers making adjustment for age, social status, calendar period, and smoking (data retrieved from maternity records). The risk for vaginal cancer was elevated 6.69 times, for vulvar cancer 2.64 and for anal cancer 2.81 compared with the general population.

Effect of follow up on subsequent cancer stage

The stadium for invasive cancer is rarely reported in follow up studies, and comprehensive cancer registers seldom contain these data, despite the crucial importance for survival. (FIGO stage for gynecological cancer cases is included in the Swedish cancer registry from 2004[322]). An English study[323] reported 33 cases of

cervical cancer that had previously undergone treatment for CIN and was classified as interval cancers – having a smear within five years before cancer diagnosis. At a mean time from treatment of 40 months 91% of the cancers were found in stadium 1 (44% of squamous carcinomas in stadium 1A1) indicating that cytological follow up after treatment of CIN results does not give full protection against cancer but leads to a down-staging of cancer cases.

The role of HPV-testing after treatment for CIN

Many studies have been performed evaluating sensitivity for HPV at follow up after treatment for dysplasia but with few exceptions follow up has been for a short period of time. Alonso et al followed 203 women with CIN 2-3 at treatment for a mean of 20 months[324] and found HPV-testing at 6 – 12 months to have a higher sensitivity than single or repeated cytology (97.2% vs. 83.3% and 94.4% respectively) but a lower specificity (81.4% vs. 92.2% and 82.6%).

Three reviews, one including a meta-analysis, and another separate meta-analysis have been published in this area. In 2004 Bornstein and co-workers[325] included seven studies evaluating HPV-testing post treatment. They suggested that HPV-testing should not be done earlier than 6 months post treatment and preferably at 12 months. The same year Paraskevaidis and collaborators published a systematic review[326] abstaining from pooling data as the 11 studies included were too heterogeneous. The authors concluded cautiously that data suggest a role for HPV-testning after treatment. Zielinski and her team[327] reviewed 20 papers and chose to include 11 of these in a meta-analysis comparing cytology with HPV-testing post treatment. They found sensitivity for cytology post treatment to be 62% (95% CI 53-71%) and specificity 89% (CI 87-92%). The corresponding sensitivity for pooled HPV-data was : 91% (CI 86-95%) and specificity: 79% (CI 76-92%). They also extracted data for the combination of cytology and HPV-test and found sensitivity 96% (CI 89-99%) and specificity 81% (CI 77-84%). Finally Arbyn, et al[328] pooled nine studies in a meta analysis finding a superior sensitivity of HPV-testing versus cytology in follow up with relative sensitivity of 1.27 (95% CI 1.06 – 1.51) and a drop in specificity with border-line statistical significance (relative specificity of 0.94, CI 0.87 – 1.01).

In their review/meta-analysis Zielinski et al recommend HPV testing to be introduced in conjunction with cytology in a 6 months follow up visit, calculating that 70% of women will be negative in both tests[327]. These women should not have to be re-tested until 24 months post treatment. The follow up of positive women is not discussed. An almost identical strategy is further evaluated in another Dutch study using Markov modelling[329]. In the recommended strategy HPV test would be taken at 6 months and combined cytology/HPV at 24 months. Effectiveness was measured as new disease discovered within 5 years from initial treatment of CIN2-3. This strategy in the setting of the 5-year interval Dutch screening program could find an otherwise

missed CIN2/3 per 196 initially treated women and be cost effective from a societal point of view (patients indirect costs included, like loss of salary, transportation etc). It would also lead to fewer colposcopies and thus a lower burden for the women.

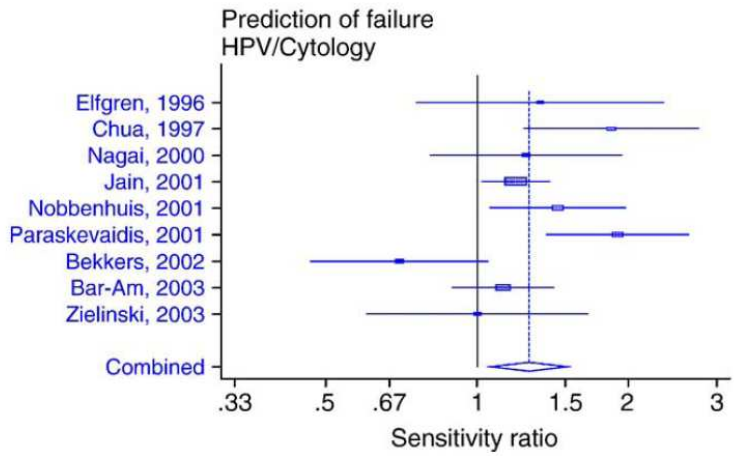

Fig 15. Meta-analysis of the sensitivity of HPV-testing relative to follow-up cytology to detect residual or recurrent disease after treatment of high-grade CIN. From Arbyn et al[328].

On the basis of database searches throughout the years including one in September 2007, earlier reviews and follow up of Pub-med links and references, 24 articles that provide data on specificity and/or sensitivity (or making these calculation possible) for HPV-testing post treatment have been found. These are listed in Table 1. The table also includes data, when available, on follow up time and interval between HPV-test and recurrence of cervical dysplasia. In case control studies follow up time is the time the disease-free controls have been observed. Time between last HPV test and the event (recurrence of disease) are seldom stated in the publications and are calculated from the follow up scheme presented and the average follow up time of cases. "Short" means less than a year in cohort studies with regular testing at least once a year. PPV and NPV are not calculated due to very heterogeneous populations and study designs. End-points differ too as some studies have included all CIN both in first treatment and in outcome, and some are restricted to CIN2+. Types of HPV test differ as well. Some studies have included only women who were HPV-positive in histopathology at baseline (first treatment).The studies by Lin, Jain and Chao are from the same institution and may contain identical patients.
Studies by Negri[330], Aschkenazi-Steinberg[331], Elfgren (2002)[332], Söderlund-Strand[333] and Izumi[334] were not included in the table as no fitting data was presented.

Reference	N	Events (new CIN)	Follow up time in months	Interval: Test to event	HPV-test Sensitivity (%)	Specificity (%)	LR neg test	LR pos test
Acladious[302]	116	47	≤ 24	<18 mo	47	94	0.56	7.83
Almog[335]	63	14	53	< 6 months	100			
Alonso[324]	203	24	20	≤ 8 mo	97	81	0.04	5.11
Bar-Am[336]	67	9	63	short	100	69	0.00	3.23
Bekkers[337]	90	7	32	< 3 months	70	66	0.45	2.06
Bodner[338]	37	3	24		100	79	0.00	4.76
Bollen[339]	43	16	48	concurrently	100	44	0.00	1.79
Chao[340]	442	46	17	14 - 3 mo	88	49	0.24	1.73
Chua[341]	45	24	71	18 months	92	100	0.08	
Cruickshank[342]	205	108	60	years(?)	29	87	0.82	2.23
Debarge[343]	205	26	15	< 3 - 6 months	81	72	0.26	2.89
Elfgren 1996[344]	23	4	16 - 27	short	100	95	0.00	20.00
Hernandi[345]	61	4	26	≤ 21 mo	100	91	0.00	11.11
Jain[346]	79	32	2	2 weeks	100	44	0.00	1.79
Kjellberg[347]	88	0	35	-		97		
Kucera[348]	84	0	12	short		94		
Lin[349]	75	27	1.5	concurrently	100	48	0.00	1.92
Nagai[350]	56	5	32	short	100	88	0.00	8.33
Nobbenhuis[351]	184	26	24	< 21 months	90	92	0.11	11.25
Paraskevaidis[352]	123	38	60	?	93	84	0.08	5.81
Strander[353]	567	189	165	56 mo	24	89	0.85	2.18
Verguts[354]	72	6	< 24	≤ 6 mo	100	77	0.00	4.35
Zielinski[355]	108	5	29	9 months	100	81	0.00	5.26

Table 1. List of studies with data presented that allows calculation of sensitivity and/or specificity for HPV-testing in finding recurrent disease after treatment.

In an era where we learn more about the epidemiology of the different HPV-types Gök and his Dutch co-workers recently have reported that carriers of HPV16 post treatment have a higher risk for recurrence with CIN3 than women infected with other HPV-types[356].

Program monitoring and quality assurance

In a manual for establishing and surveilling cervical screening systems the World Health Organisation (WHO) 1992 proposed an integrated information system as the ideal support for program monitoring and program operation[357]

"The goals for informations systems for cervical cancer screening programmes are:

1. To enrol the at-risk population. Data on the entire target population, including women who have never been screened, must be stored on the database.
2. To maintain information. Information on the screening history of each woman must be maintained on the database; in addition, the information must be organized and the data elements defined to facilitate analysis and planning.
3. To provide follow up. The information system must support communication with individuals concerning smear results, the need for screening, rescreening or follow up.
4. To support quality assurance. The design of the information system must permit qualitative assessment of the over all programme.
5. To track utilization. A critical measure of the value of the information system will be its capacity to monitor screening patterns to determine levels of both underscreening and overscreening,
6. To monitor compliance. Compliance with recommended screening and appropriate follow up must be monitored by the information system to assist in evaluating the success of the overall programme as well as targeted outreach programmes."

The regional database on cervical screening in West Sweden is a comprehensive register containing data on most activities of the screening programmes in the region of Västra Götaland (VGR) and the county of Halland. The source of the data are 1) the cytology and pathology departments of the hospitals in Skövde, Trollhättan, Borås, Göteborg (Sahlgren's University hospital) and Halmstad. 2) Medilab, provider of cytology, pathology and screening administration like invitation to parts of the region in the 1990:s. 3) Reports from operating wards and gynecology offices in the area. 4) The population register of the catchment area.

The data collected are a) relevant data on each smear, from structured referral notes and the laboratory report b) Data on all histology reports from cervix uteri (T83) and malignant and premalignant diagnoses from vulva, vagina, uterus, ovaries and fallopian tubes c) data on all invitations to screening sent from the laboratories and

d) reports on treatment (LLETZ, laser, cryodestruction, diathermy, hysterectomies). Data on cytology have retrospectically received a unified classification, allowing multiple diagnosis on the same smear when applicable. All histology related to the uterine cervix have been classified in regard to cancer or precancer of the cervix. All clinics sending material to the labs have also undergone a structured classification indicating size, type of ownership (private/public) and activity (antenatal care unite, general practice, ob/gyn etc.).

The database, inaugurated in 2005, has three purposes:

1) To monitor screening activities and relate performance to quality parameters (corresponding to WHO points # 4 – 6 above). Reports are made yearly. Attendance, coverage, smear quality, reason for smear taking (eight alternatives including screening) distribution of the resulting histopathological diagnosis for each cytological abnormality, outcome of treatment (including proportion of resections without CIN) are among these parameters and each community get their own edition with local data.

2) To facilitate screening and health care. Laboratories are provided with comprehensive data on smear taking within and without the program in order not to send out invitations to women already screened within a screening round (Point # 1). Records for individual women containing all screening activities in the region (smears, biopsies, treatments) can be obtained on the internet by gynecologists, with special security measures resembling communications with banks, and after the permission of the woman (Point # 2 and 3). Every six month a "larm-list" is sent out to the laboratories to be forwarded to smeartakers about high grade abnormalities that are not followed up within 7 months by biopsy or another smear.

3) To be a source for investigations and research. With a complete coverage for more than 15 years and around 2 million entries the database has shown its value in supporting several studies including papers I, II and IV in this study.

On the national level England[130], Denmark[145] and Norway[104] are currently the only countries with national comprehensive, up-to date databases on cervical cytology and pathology. In England and Norway these are also the base for nationally run screening programs while the Danish database gives directly operational support to the regional programs. Finland has a national database for invitations and cervical cytology within the screening program[102].

HPV

HPV is a small, non enveloped virus with a double stranded DNA genome, coding for 8 proteins. The late (L1, L2) genes, or ORF:s (open reading frame), codes for the capside protein. L1 is the protein that is synthesised as virus like particles (VLP) in the prophylactic vaccines. The six early (E) genes encode for proteins that manage DNA synthesis. The E6 and E7 proteins induce proliferation of epithelial cells and inhibition of the cell cycle regulatory proteins. Foremost p53 is inhibited by E6 which blocks apoptosis whereas E7 inhibits pRB (retinoblastoma tumour suppression protein) and cancels cell cycle arrest[358], thereby inducing oncogenesis.

HPV initially infects the basal cells of squamous epithelium[359]. Epithelial cell differentiation and maturation is then linked to the intricate process of viral replication and assembly. Infectious virions with full antigenic capacity are only produced in terminally differentiated cells that eventually are shed. This hides the virions from the human immune system and avoids a significant host immunity inflammation response[360]. However, almost all HPV-infections are eventually cleared, a process where T-cell induced immunity play a crucial role[360]. Those infections with high risk types who are not cleared can result in neoplastic change[361] and eventually cancer.

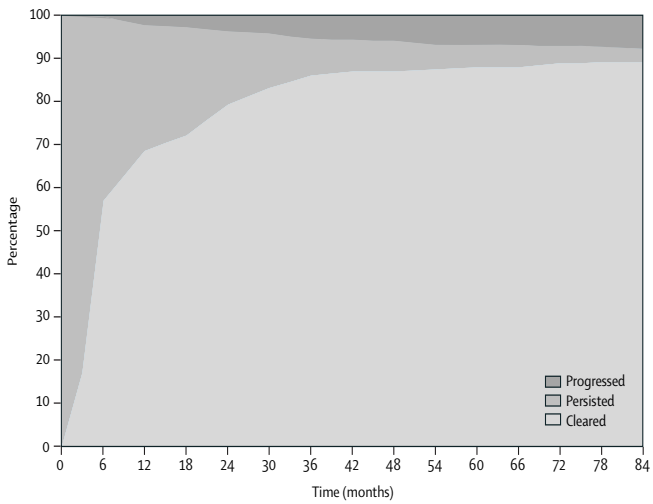

Fig 16. Average clearance, persistence, and progression to CIN of high risk HPV infections. Diagram of 777 infections found at enrolment visits of a large population-based cohort study (Guanacaste, Costa Rica) From Schiffman et al 2007[3].

HPV is typed, based on DNA sequencing. A type differs from other types when 10% or more of the nucleotide sequence of the L1 gene differs[362]. Overall, 40 types have affinity for the anogenital tract. They all belong to the order (genera) alpha papillomaviruses. 12 – 15 types have been classified as high risk types, regarding their potential to induce cancer. There are also a number of low risk types that have not been associated with malignant development. Smaller variations (< 2% of the genome) are classified as variants. Variants of hrHPV are not unusual[363] which makes studies on HPV-persistence somewhat unreliable as some cases can be the effect of re-infections.

From a phylogenic standpoint HPV has been classified into several species. Types belonging to the same species share more of the DNA, have been separated later in the evolution and share to some extent properties of oncogenisis.

Species A7 and A9 contain high risk viral types. The A7 species include HPV 18 and 45. The A9 species includes HPV 16, 31, 33, 52 and 58.

The viruses are transmitted by contact of skin or mucosa. The rate of transmission is not known but estimates are that it is high[364] A computer simulation study found probability of HPV-transmission per coitus ranging between 5 – 100% with a median of 40%[365]. Data based on seroprevalence suggest partner to partner transmission to be 60%[366].

High risk HPV has been recognized by the IARC[367, 368] and the scientific community as a cause for development of cervical cancer and has been claimed to be the first "necessary" cause found for a cancer[369]. This is based on the now classical finding of hrHPV in 99.7% of cervical squamous carcinomas[370] and 94 – 100% in adeno/adenosquamous carcinomas[371, 372]. As reviewed by Bosch and co-workers[7] the association fulfils basic criteria of causality[373]: strength, consistency, temporality, biological gradient (dose-response), plausibility, coherence with other data and experimental evidence.

In the most recent classification (2005) IARC recognizes 13 mucosal HPV-types as carcinogenic[368]. These are HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.

Vaccines against HPV and cervical cancer

In September 2006 a new era in cervical cancer prevention started as the first vaccine against HPV-infection and cervical cancer, Gardasil® (Merck), was approved for use in Europe. A year later a second vaccine, Cervarix® (GlaxoSmithKline), was approved for marketing.

The most important breakthrough in the development of HPV-vaccines was the discovery that viral capside proteins have the intrinsic capacity to self assembly into Virus like particles (VLP)[374, 375]. These VLP, based on the L1 protein of the specific HPV types are the basis of both HPV vaccines. VLP:s are morphologically mimicking the virus, have high immunogenic capacity but are empty shells without DNA and lack ability to replicate.

For obvious ethical and practical reasons cervical cancer could not be the end point of the efficacy trials. Thus, the World Health Organization and the US Food and Drug

administration have previously recommended the use of CIN 2 or 3 as surrogate outcome for cervical cancer in the vaccine trials[376].

Gardasil is a quadrivalent vaccine that offers protection against HPV types 6 and 11, which are claimed to be responsible for 90% of genital warts, and HPV types 16 and 18, which are associated with around 70% of cervical cancers. This vaccine is formulated with a classic alum adjuvant. Data on this vaccine's safety, immunogenicity, efficacy and effectiveness are now available from 5-year phase II[377-379] and 3-year phase III trials[248, 380-382] that included 20 000 participants.

Cervarix is a bivalent vaccine that protects against HPV types 16 and 18. The vaccine is formulated with a new ASO4 adjuvant that contains monophosphoryl lipid A, a derivative of bacterial cell walls. Data are published from 5-year phase II and 15-month phase III trials that included 18 000 participants.[383-385].

Immunogenicity

Both Gardasil and Cervarix are highly immunogenic, achieving vaccine-induced antibody titres that are many times higher than those induced by natural HPV infections. Gardasil-induced antibody titres reach their highest level 7 months following the first vaccine dose. The titres then decline, reaching a plateau 18–24 months later. This plateau is maintained for at least 5 years, with 5-year levels that are similar to the titres naturally induced by HPV types 6 and 18 and that are higher than the titres naturally induced by HPV types 11 and 16.[379] At 24-months follow-up, over 96% of participants in the Gardasil trial were seropositive for HPV types 6, 11 and 16; however, only 68% of participants were seropositive for HPV type 18[248] The significance of this reduction remains unclear as immune memory is induced by the vaccine[386]. No signs of vaccine breakthroughs have been reported although efficacy data have shorter follow up than the immunogenicity data.

Cervarix-induced antibody titres follow the same profile as Gardasil. However, the 18-month plateau level is many-fold higher than the levels induced by natural infection and, after 51–53 months, 100% of women were seropositive for both HPV types 16 and 18[384]. Both vaccines are highly immunogenic in young adolescents (aged 9 - 13), with titres that are 1.7 to 2.4 times higher than those among women aged 16–26 years.[387-389] This is in concordance with high immuno-response of other vaccines in children[387].

Efficacy data on high grade lesions

Among the per-protocol populations, a full series (three injections) of both vaccines is highly efficacious against lesions containing the same HPV-types as the vaccines. Vaccine efficacy for precancerous lesions (cervical intraepithelial neoplasia, grade 2 or higher) containing HPV types 16 or 18 is 98% for Gardasil[248] and 90% for

Cervarix[385]. In the Cervarix trial the 10% loss from 100% efficacy was shown to be caused by multiple infections where HPV16/18 did not seem to be the causative infectious agent. Gardasil offers 100% protection against vulvar intraepithelial neoplasia (grade 2–3) and vaginal intraepithelial neoplasia (grade 2–3) caused by HPV types 16 and 18[381, 382].

In the Cervarix trials, modest cross protection was documented against 12 months persistent infection of HPV type 45 (vaccine efficacy 60%) and for all oncogenic HPV (38.2%)[385]. Gardasil has been reported to have an efficacy of 62% for HPV 31/45 related CIN2-3/AIS and 43% for HPV 31/33/45/52/58 related high grade lesions[390].

The efficacy, in the Gardasil phase III trial population, without sign of exposure for the vaccine types, is suprisingly reported as only 27% (95%CI 4 – 44) for all high grade lesions regardless of HPV-types[248]. Corresponding figures for high grade lesions have not been presented for Cervarix.

The intention to treat population in the Gardasil phase III trial included all women who met the inclusion criteria (e.g. maximum four life-time sexual partners) and 27% had been infected with HPV types covered by the vaccine at or before trial enrolment and who may have received fewer than 3 vaccine doses (few cases). In this population, resembling an ordinary population of young women around 20 years of age,vaccine efficacy against cervical disease was low (vaccine-specific types 44%–55%, all types 17%–20%)[248], thus demonstrating that the vaccine have good effectiveness only if it is given to women/girls before exposure to HPV. No such data have been presented for Cervarix. These results highlight that the vaccines are prophylactic and therefore should be offered to females before they are at risk of HPV infection.

Safety

Both vaccines have a good safety profile. Local reactions are fairly common but there was no difference noted in the frequency of systemic adverse events among those who received the vaccine or placebo[385, 391].

The vaccines are not approved for pregnant women, although data on 1900 women in the Gardasil trials who became pregnant during the vaccine trials and 1350 pregnancies in the Cervarix trials indicate that pregnancy outcome (including congenital anomalies) were similar among recipients of the vaccine and placebo.[385, 391].

Gardasil and Cervarix are both virus-like particle vaccines that were created using recombinant technology. Both vaccines are safe. Both vaccines provide protection against infection by HPV types 16 and 18, two oncogenic HPV genotypes that cause about 70% of cervical cancers. Both vaccines protect against high-grade cervical disease (cervical intraepithelial neoplasia, grade 2 and higher). In addition, Gardasil

trials have demonstrated that this vaccine is efficacious against high-grade vaginal and vulvar lesions. Gardasil also offers protection against the two HPV genotypes that are considered responsible for about 90% of genital warts. Efficacy for type-specific condylomas are close to 100% and the effect on all condylomas, regardless of HPV-type was 82%[392] in the HPV-naive population and in the unrestricted intention to treat group it was 51%[381].

Future questions about vaccines

There are unanswered questions however. The efficacy on cervical cancer is based on assumptions and surrogate markers in the trials. Only long term follow up of well registered vaccinated cohorts can give the answer regarding the true effect. How long the effect of the three injection series will last and if booster vaccination will be needed is not known. Health economic analyses suffer from this uncertainty. Will the causative role of HPV 16/18 in oncogenisis be replaced by other high risk virus types when the former are eradicated? No proof of this exists but the high proportion of multiple infections found with modern sensitive HPV-detection methods raise concern.

Trials of the vaccines among men are under way, and there are plans to test a 3-dose regimen of Gardasil among people who are immunocompromised and to test a 2-dose regimen among adolescents. Both companies are conducting trials involving older women and are planning trials of second-generation vaccines that will offer protection against additional high-risk HPV genotypes[388].

Need for screening and effect on screening programs

Non-virginal women who chose to be vaccinated can count on only limited protection from the vaccines, and obviously need full protection from cytological screening programs. When young girls are vaccinated before their sexual debut they will still benefit from the additional cancer protection of screening for two reasons. First their estimated protection is 70% which is lower than what is expected from participating in screening program. They have insufficient protection from the non 16/18 oncogenic HPV-types. Secondly, so far we don't know how long the protective effect of vaccination will last.

Even in a situation of mass vaccination of young girls the screening programs will be important over a foreseeable future. The screening programs include women up to 60 years of age and is the protection offered for the 45 – 50 age cohorts that will not be affected by possible mass vaccination. Age-cohorts that are vaccinated will still need additional protection from screening, but the programs might be redesigned, considering among other things a loss of specificity in both cytology and HPV-testing if HPV 16 and 18 are more or less eradicated from targeted populations.

Notes on statistical methods

Logistic regression

Regression analysis can be thought of as finding the best mathematical model for predicting one variable from another. A dependent variable varies according to one or more independent variables.

Logistic regression is a powerful and useful tool, that has been called "the Swiss army knife of statistics"[393]. The method is valid for binary, continuous or categorical predictor variables but the outcome is binary, most often disease/no disease. It can be used to estimate the outcome after adjusting simultaneously for a number of potential confounding factors. It can also give an assessment of effect modification (interaction). The risk of developing an outcome (i.e. disease) is expressed as a function of predictor variables. Mathematically the outcome expressed as a probability should only have values between 0 and 1. This is satisfied by logistic regression as the dependent variable is defined as the natural logarithm (ln) of the odds of the variable (disease) or the logit.

One of the major reasons the logistic regression model has become so widely used in epidemiologic research is the ease of obtaining adjusted odds ratios when sampling is made conditionally from the outcome, as in case control studies. The odds ratio is simply the antilogarithm of the slope of the regression curve. As odds ratios are calculated the models can also be used in cross sectional studies and cohort studies [394-396].

ROC-curve

The receiver operating characteristic (ROC) analysis is a procedure originally developed to study the detection of electronic signals (e.g. radar) in the 1950:s as a mean to assess relation between signal and noise. The trade offs between the true positive fraction (sensitivity on the Y-axis) and the false positive fraction (1 minus specificity on the X-axis) can be established by varying the decision thresholds. In a scoring system this will be represented by different scoring points. The curve represents the *characteristics* of a diagnostic procedure. The *receiver* of the information can choose to *operate* anywhere by applying different decision thresholds[397]. The performance of the diagnostic procedure is reflected in the area under the curve as a proportion of the entire diagram, the larger the better.

Likelihood ratio

The likelihood ratio of a test tells us basically how much the pre-test chance of a specific diagnosis increases or decreases by using the test. The positive likelihood ratio indicates how much more likely it is to get a positive test in the diseased as opposed to the non-diseased group and the negative likelihood ratio shows how much more likely it is to get a negative test among those who are non-diseased compared with the group of diseased[398]. Likelihood ratios of less than 0.1 or more

than 10 generate large and often conclusive changes from pre test to post test probabilities. Ratios of 0.1 – 0.2 and 5 – 10 generate shifts that are considered moderate, 0.2 – 0.5 and 2 – 5 small (but could still be clinically important) and 0.5 – 2.0 generate negligible shifts[399].

Kappa value

Kappa statistics are sometimes used to measure repeatability or reproducibility of a test when used in similar circumstances. Intra-observer or inter-observer reliability can be assessed. Kappa statistics indicate how much the actual agreement beyond chance (Observed minus Expected) represents relative to the potential (1 minus Expected)[400]. A kappa value of 1.0 shows a perfect agreement, the test always predicts the outcome, and is never seen in reality. A kappa value of 0.8 or more is considered almost perfect and above 0.6 substantial. Between 0.4 – 0.6 the agreement often is considered moderate and below 0.4 poor to fair[401]. A kappa value of 0 is equal to random distribution. To adjust for level of agreement in ordered category data arbitrarily selected weights are often used. The Kappa value can show that agreement is above chance but the use of kappa values to quantify agreement have been critizised as being methodilogically questionalble and sometimes underrate level of agreement[402].

HPV-analysis

There are several detection systems for HPV-DNA that are commercially available for clinical purposes and/or use in research. The Digene Hybrid Capture II (HCII) (Digene corp Gaithersburg, Maryland USA) is the most widely used method and the only one approved by the US Food and drug administration. It is a immunoassay that uses a cocktail of two different probes, one targeting against 5 low-risk HPV genotypes (HPV 6, 11, 42, 43 and 44) and the other against 13 high-risk HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68)[403]. Recently a PCR based broad-spectrum commercial standardized test has been marketed, the Amplicore HPV (Roche Molecular Systems, Alameda, California, USA) targeting the same 13 high risk types as the HCII. Roche also provides the Linear Array test, a PCR based test that identifies 37 individual genotypes. There is good concordance between the three methods (HCII versus Amplicore, $\kappa = 0.64$) and especially between the two PCR methods ($\kappa = 0.94$) which seem to be more sensitive but less specific than HCII[404, 405].

There is also one method on the market detecting HPV mRNA, the Norchip HPV-Proofer. It identifies high-risk HPV E6 and E7 expression. This assay can actually determine whether these transforming genes are present and active, which could be

more clinically valuable to identify at-risk patients as compared to available methods[406-408].

In research however PCR (polymerase chain reaction) is more commonly used in the form of in-house non-standardized assays. PCR methods are simple and sensitive but demand laboratory skills and high standards above all for controlling the risk for contamination. The DNA is amplified in repeated cycles and the content of DNA is doubled for each cycle, making the method highly sensitive. The most widely used consensus PCR primers are MY09/11[409] and GP5+/6+[410] but there are also PGMY[411] and SPF10[412]. These primers target a number of conserved and type-variable regions in the HPV L1 open reading frame (ORF). Quantitative PCR for determination of viral load by real-time PCR has been developed but its use is purely in research. In this method DNA is quantified after each round of amplification.

Determining HPV-type is necessary for measuring persistence of virus infection as well as multiple co-infections. Based on a PCR analysis, several methods can be used. The genotypes can be detected by reverse line blotting (RLB)[413] restriction fragment length polymorphism (RLFP) and sequencing[414] which is regarded as the gold standard. The traditional electrophoretic sequencing methods, that rendered Frederick Sanger a Nobel prize in 1980, have developed since, but the real-time method by pyrosequencing has shown good possibilities for further automatation and for the detection of multiple co-infections in a single sample of HPV[415, 416].

Pyrosequencing uses a "fire-fly" approach. Sequencing is made by synthesizing a new molecule from a template. When a nucleotide is incorporated into the DNA template a pyrophosphate molecule is released and converted to ATP. This reaction provides energy for luciferin to be oxidated by luciferase, a light is emitted and registered by a CCD camera or photomultiplicator. In a computer, characteristic patterns for different HPV-types are detected as pyrograms®, and with the development of pattern recognition even complex multiple infections can be detected[417].

Aims of the study

The aims of the present study were to search for and evaluate some possible improvements in the Swedish screening program against cervical cancer.

Paper I: To compare the performance of liquid based cytology with the presently used conventional PAP-smear in a Swedish screening setting.

Paper II: To develop and test the performance of a scoring system for colposcopic diagnosis of CIN2+ in order to systematize observations, facilitate learning and training in colposcopy and to record and evaluate the performance of colposcopists.

Paper III: To evaluate the long term risk of acquiring cervical or vaginal cancer after treatment of carcinoma in situ of the cervix, in order to assess the need for long term follow up after treatment.

Paper IV: To evaluate the ability of HPV- testing, done within a year after treatment for CIN2-3, to predict the long term development of new high grade lesions or cancer.

Methods

Epidemiological design

Paper I is a randomized controlled trial comparing two methods of cytological screening. Randomization was performed by allocating screening method depending on the period of time (week) the sample was taken. Paper II is a clinical trial evaluating a diagnostic method - a scoring system. The scoring system was tested against a reference standard, histopathology, and modified to give the best performance in regard to sensitivity and specificity, measured as the area under the ROC-curve. Paper III is a cohort study where a cohort of women diagnosed with Carcinoma in situ/CIN3 was obtained from the Swedish cancer registry. The risk of developing invasive cancer was compared with the general population and Standardized incidence ratios (SIR), were calculated. SIR was calculated as the ratio of the observed to the expected number of new cases of cancer. The expected number was based on the age-specific rates for all Swedish women. Paper IV is a case-control study that could be considered a nested study as both the cases and the controls were derived from a cohort of women treated for CIN2-3. Exposure was measured as presence of HPV in cytological smears taken 3 – 24 months after inclusion (first treatment).

Ethics

All studies were approved by ethical committees - study I, II and IV in Göteborg and study III in Stockholm.

Paper I

All women attending cervical screening at five screening units (antenatal care units) in the greater Göteborg area were included for a period of one and a half years. The screening units represented areas that differed in socioeconomic characteristics. Altogether 13 484 smeartaking procedures from 13 427 unique women were randomized to either sampling by liquid cytology (Thin Prep®) or conventional Pap-smear. Randomization was made by time of sampling as the midwives changed method from LBC to Pap each calendar week. The quality of the smears were compared out of proportions of smears judged *inadequate for diagnosis* and the presence or absence of endocervical cells, that is cells from the endocervical canal or metaplastic cells from the transformational zone. The cytological diagnoses were compared as well between the groups. The main outcome however was the histopathological diagnosis that were the result of the diagnostic procedures following atypical cytological samples. There was no blinding in the first steps of the study. The midwives of course knew what sampling method they used and there is no way of blinding the diagnostic procedure in the lab. However the gynecologists examining the women with atypical smear were blinded for the type of screening

sample as well as the histopathologists analysing the biopsies or cone material. Their examinations constituted the reference standard for the main outcome. The follow up of atypical smears was done entirely as routine procedure within the screening program of Western Sweden[251].

Despite efforts to get an adequate randomization the procedure limped somewhat. Some screening units had almost a 50-50% distribution of their smears while others ran into problems. As both age and screening unit were potential confounders this was adjusted for in a logistic regression model.

The database for cervical cancer prevention in Western Sweden has data on all cervical samples, cytological and histopathological, in the region. This database was searched to find the histopathological diagnosis on two occasions. The first was 8 months after the study was closed which gave a mean follow up time of 1.5 years. The minimum range was 9 months which would be sufficient for all atypical smears to be followed up, including those who had a repeat smear. The second follow up was made at a mean follow up time of 3 years and 7 months after the index smear was registered in the study. As the screening interval for women 23 – 47 years of age is 3 years this gave most, but not all, women the chance to enter a second round of screening, then made with the standard technique of PAP-smear.

Results for the main effect parameter were presented as relative sensitivity and odds ratios. We abstained from trying to calculate absolute sensitivity as the true number of high grade lesions among the cytology negative was not known.

Paper II

Five possible variables of potential importance to find high grade lesion (CIN 2, CIN 3 or cancer) with colposcopy were selected based on our own experience and studies in the literature. We described three potential levels, in an assumed ordered fashion, for each variable which created the scheme below:

Variabel\Level	A	B	C
Aceto uptake	0 or transparent	Shady, milk	Distinct, stearin
Margins and surface	0 el diffuse	Sharp but irregular, jagged, "geographical". Satellites	Sharp and even, difference in surface level
Vessels	0	Fine, regular	Coarse or atypical vessels
Lesion size	<5 mm	5-15 mm or 2 quadrants	> 15 mm or 3-4 quadrants or endocervically undefined
Iodine staining	Brown	Faintly or patchy yellow	Distinct yellow

We used this scoring system for 297 consecutive routine colposcopic evaluations, examining the same number of unique women. We excluded women with atrophy (postmenopausal and puerperal), pregnant women and of course women with unsatisfactory colposcopy (when the entire transformational zone or the whole extent of a lesion could not be seen). Only evaluations that resulted in a histopathological sample, biopsy or cone, were included.

All histopathological diagnosis were classified into one of nine categories and the distribution is shown in this Table 2:

PAD	Number	Per cent
0. Benign	40	14 %
1. Chronic inflammation	27	9 %
2. Cervicitis	22	7 %
3. Koilocytosis without signs of dysplasia	26	8 %
4. CIN 1	47	16 %
5. CIN 2	37	13 %
6. CIN 3	84	28 %
7. High grade glandular dysplasia/AIS	4	1 %
8. Cancer	10	3 %

Table 2. Classification and distribution of histopathological findings in paper II.

All observations were dichotomized as high grade lesion (categories 5 – 8)/ not high grade lesion (categories 0 – 4) and a multiple regression model was created with category C as the reference level.

The parameter estimates were used for making an ”ideal” model that from this material could assign each value a weight that would give the best performance for specificity and sensitivity measured by the area under the ROC-curve.

The categories A, B and C were replaced with discrete numerical values that corresponded roughly to the weights and a number of models were tested by comparing the area under the ROC-curve with the “ideal” model that made the best use of the data.

In the latter phase of the study we also classified 199 consecutive lesions from an overall judgement of the colposcopic appearance, not based on scoring. This subjective impression was classified into three categories: Benign, low-grade lesion or high-grade lesion, which included cancer or AIS.

Paper III

The Swedish cancer registry was established in 1958 and has ever since included data on women diagnosed with carcinoma in situ and severe dysplasia of the cervix, equvivalent to CIN3. We created a cohort of all the women with this diagnosis between the foundation of the register 1958 and the end of 2002. Data was linked to the National Swedish Causes of Death Register, the National Swedish Population Register of deaths and emigration could be accounted for. The final cohort included 132 493 women with a total number of person years of 2 315 724. The cohort was then linked back to the Cancer register and diagnoses of invasive cervical cancer and vaginal cancer were searched for in the cohort. To exclude obvious treatment failures and account for prevalent cases, diagnoses of cancer less than one year after CIS were excluded. Using the entire population of women as reference, standardized incidence ratios were calculated. The influence of birth cohort, time-period (i.e. in what decade the diagnosis of CIS was made), age at the time of the CIS-diagnosis, and time interval between the diagnosis of CIS and invasive cancer, were explored. A multivariate regression model was created including time-period, age and time to cancer diagnosis. The calculations were done for cervical cancer but were also done separately for the two dominant histological types, and for vaginal cancer. SIRs were calculated as well for the combined risk to develop either vaginal or cervical cancer, after censoring for double diagnosis. Absolute risk difference were calculated as difference in incidence, also within the different strata of age at CIS diagnosis, follow up time, birth cohort and time period for CIS diagnosis.

Paper IV

The base for this case-control study is the women who had a histological sample of high grade dysplasia (CIN2, CIN 3 or AIS) registered in the database of the Department of pathology at Sahlgrenska University hospital up to May 2000. From this cohort of 4 526 women, cases and controls were selected. A case was defined as a woman who had a second diagnosis of high grade cervical dysplasia or cervical cancer more than 2 years after the initial diagnosis, and had 2 cytological samples registered 3 – 24 months after the original diagnosis of high grade dysplasia. Records were checked to confirm that all women had their dysplasia treated. Controls were matched 2:1 to each case and were selected as the two women with the same age at treatment (+/- 2.5 years) who were closest in time for treatment. With this matching we controlled for the potential bias of age and period of time, which we consider a decisive factor influencing the mode of treatment. Cases were excluded as controls as well as the women who had no proof of follow up around the time the matching case had her second diagnosis of dysplasia. Women that were hysterectomized during this time were excluded as controls as well.

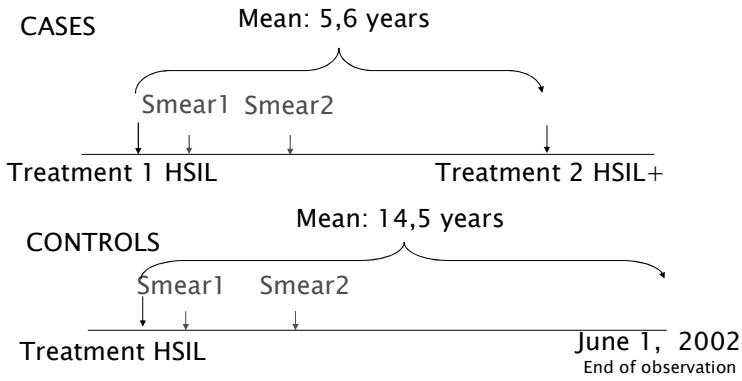

Fig 17. Flow chart Study IV.

The slides for the cases and the controls were retrieved. Before this stage we could choose a new control if a person did not meet the inclusion criteria. However when the process of finding almost 1200 glass slides, some of them more than 20 years old, had started we had to accept three cases and two controls with only one smear instead of two. Thus 99.6% of all smears intended to analyse were retreived.

As the morphological picture of the slides would be destroyed in the process to follow, they were all "photographed" with the PAP-Net procedure, to allow re-analysis in the future.

The cover glasses were removed with solvent, stains used in the cytological laboratory removed with alcohol and the biological content of the slides were removed with enzymatic, chemical, physical and mechanical methods. Pellets of DNA were extracted and dissolved in sterile water. PCR was performed targeting the human gene S14 as control for DNA-quality[418] Detection of HPV-DNA was done with the general primer GP5+/6+. Assay quality controls were performed with HPV-positive controls and blanks. Genotyping of the HPV-DNA was made with pyrosequencing and the specimens that could not be typed were cloned, amplified and re-sequenced again with pyrosequencing. The procedure is described in detail in Paper IV as well as other works by the co-author responsible for the laboratory work[33, 341, 419].

As the controls were matched to the cases conditional logistic regression was used where each stratum was a sample, containing the case and the matched controls. Exposure was defined as a woman who had at least one cytology sample positive for HPV-DNA. Odds ratios were calculated as the ratio between the odds of the exposed to have a recurrence (becoming a case) and the odds for the exposed not having recurrent disease (becoming a control).

Results and discussion

Cervical cytology

The conventional PAP-smear has been the cornerstone and the basic tool in one of the most successful medical interventions made on a population level in the western world. The method is labour intensive and based on the subjective judgement of the cytotechnician or the doctor. In Sweden the large screening programs were launched in the 1960:s and 1970:s. Laboratories were equipped and staffed with young people who have now spent a work-life behind the microscopes. Recruitment of new staff is not easy today. Education posts have been limited and an expected shortage of cytotechnicians is already apparent. Conventional cytology has remained basically the same method since the 1950:s although the vast increase in knowledge of interpretation of abnormalities, natural history of CIN etc has made the method more accurate. Sensitivity and specificity have been studied extensively but these studies have inherent methodological problems, foremost having a reference standard for the cytological negatives. In high risk populations and small series made before planned conizations, hysterectomies etc. this can be made and these studies often show sensitivity around 80%[191]. In population based studies however it is impossible to use an adequate reference standard for the entire population, not only ethical reasons prevent researchers from colposcoping and biopsing thousands of cytology negative women. This is one reason for the shortage of this kind of study. A striking feature in studies on accuracy of cytology is the range of performance measured as specificity and sensitivity. In the most recent meta-analysis[143] sensitivity was 18% - 98% and specificity 17% - 99% using ASCUS/CIN1 as threshold! Reasons for this could be the subjectiveness of the method, the dependence and differences in manual "craftsmanship", differences in national and local standards besides heterogeneity in designs of studies. This questions the generalisability of studies on cytology.

Liquid based cytology offers a development in cytology. There seem to be a general agreement that it has some advantages. It reduces the evaluation time for the cytotechnicians[420]. It is the preferred method by laboratory staffs[421, 422]. It provides the possibility for automatisation by computerized picture analysis[196]. The residual liquid can be used for microbiological testing, foremost HPV-DNA or RNA testing without having to re-sample the women who could benefit from such testing.

In paper I we tested liquid based cytology in the existing screening program of West Sweden, implemented in Göteborg and South Bohuslän. Within the program the women sampled with ThinPrep showed a substantial and significant difference in yield of high grade lesions documented by histology (42% increase, $p = 0.05$)

compared with conventional histology. The uptake of both low and high grade squamous intraepithelial lesions in cytology was increased with LBC but the concordance of cytology and histology tended to be somewhat better with conventional cytology, although this was not statistically significant. The difference between the methods regarding the uptake of high grade lesions in histology can mainly be attributed to a difference in the uptake of low grade squamous intraepithelial lesions in cytology. LSIL in cytology accounted for 27% of the histological CIN2+ diagnoses in the LBC group and 12% in the conventional cytology arm. While there was no important difference with regard to cytological HSIL diagnosis in this respect, 29% of the CIN2+ diagnoses were derived from ASCUS in the conventional arm and 20% in the LBC arm. (Data not shown in paper I). This can cautiously be interpreted as a tendency with LBC to classify a higher number of truly high grade lesions as LSIL instead of ASCUS. The important implication however is that with either method all cytological abnormalities must be carefully followed up.

The LBC arm had a higher percentage of ASCUS diagnosis but the difference was not statistically significant. This difference diminished during the progress of the study and during the last third of the study the rate was similar. (Data not shown in paper I). This implicates an effect of learning on the accuracy that has been documented in other studies[420, 423].

The proportion of inadequate smears is generally low in Swedish laboratories. Other studies have suggested that this rate could be lowered with LBC only in laboratories who report inadequacy rates of several percent[424]. However we found a significant difference of smears labelled as inadequate for analysis from 0.7% in the PAP arm to 0.3% in the LBC arm. In Western Sweden with around 100 000 smears yearly this would correspond to 400 women not having to be re-tested.

As other investigators[164, 425] we found a larger proportion of samples lacking endocervical cells when LBC was used. The clinical implication of this is unclear. LBC has been suggested to even have a better performance in finding high grade glandular lesions of the cervix[426]. However, the smear taker's ability to get smears clearly representing the transformational zone is the main quality indicator for cytological sampling, and this does not seem to be a valid variable with LBC. "Sip volume", the volume of fluid let through by the filter in the processor before it clogs, has been proposed as an indicator for cellular[427] and sampling quality, but this will not show the extent of sampling the transformational zone.

The study design was to allocate the women to the two arms in a random fashion according to the week they attended. The computerized invitational system in the laboratory allocated a time for appointment to the women that was random considering age and area of living. Randomization was not made on an individual basis but there was not a cluster randomization as the design was that both methods should be used in all clinics. In the study design all patients had a known and equal

chance to receive either sampling that could not be predicted by the patient. It could not either be predicted by the sampling midwife who did not know in advance what patient she would meet. We therefore consider the classification of the allocation in the design as randomization is justifiable, as it fulfils the basic criteria[428, 429].

Unsatisfactory monitoring and logistic problems were the reasons for the defects in the randomization procedure of this the study. As the adherence to the randomization procedure differed between the centers and the populations between the centers could differ this factor was adjusted for in a logistic regression model, however this made only a small contribution to the model. We also adjusted for age as it differed between the arms. There is no obvious reason for this difference and it can hardly be attributed to the problems in randomization as the age of the women was randomly distributed in invitations with no connection to what week the women were invited or attended.

The logistic regression model showed an even more profound difference between the methods regarding the main end point in the study - sensitivity for histological CIN2+. The odds ratio for the influence of the type of smear after adjusting for age and screening center was 1.6 (95%CI: 1.12 – 2.28). This implies an increase in sensitivity of around 60% which is higher than what is usually reported.

In the LBC arm the proportion of non benign smears was 4.5 % and in the conventional arm 3.5%. The difference of 30% implies a loss of specificity. With ASCUS as threshold for cytology and Low grade lesions for histology and with the uncertain assumption that all atypical smears that have no corresponding histology are negative for the reference method the specificity for conventional cytology in this setting was 98.5% and for LBC 97.7%. The corresponding positive predictive values were 48% and 44%. Using threshold of ASCUS/CIN2+, specificity was 98.0% and 97.2% and PPV showed no difference between the methods: 30%. (Data not shown in paper I). This is an excellent performance for both methods, especially when the assumption is considered. Histological follow up of some of the omitted women would only increase specificity and PPV further. The loss in specificity with LBC could correspond to an increase of around 800 - 1000 more women needing follow up in west Sweden during one year but with a gain in sensitivity and approximately the same extra number of precancerous lesions found.

The analysis made after another two years and one month, allowing a large proportion of the women to enter a second screening round indicate that this did not alter the conclusion that LBC has a higher sensitivity in finding high grade lesions within the screening program. If the difference between the methods had levelled out this could have suggested earlier and more reliable detection of lesions that would have been detected by a second screening round[36]. This could have given support to a lengthening of the screening interval. Although we find no support for this,

another follow up of this cohort, allowing all women to be invited to a new screening round will give more information.

Our results on sensitivity differ from what was found in the large Italian randomized trial that was published almost simultaneously[193]. The reason for this is unclear. The Italian study had a design similar to ours, nested in a screening program. It was more than three times as large counting numbers of smears and the randomization seem to have run smoothly. However there are some differences that might influence the diverging results. There is a surprisingly small yield of high grade lesions at colposcopy in the Italian study, less than 10%. The rate of high grade lesions between the studies also differed substantially 0.4 % compared with 1.0% in our study. The number of endpoint events (CIN2+ in histopatology) are actually similar – 183 in the Italian study vs. 131 in the short follow up and 206 in the long follow up in our study. The positive predictive value for CIN2+ is only a third of the very conservatively measured PPV in our study. This can be interpreted as a difference in the populations or different standards of colposcopy, both reasons implying that comparisons between studies should be made with utmost caution. The well documented Italian study offers an additional insight. As 14 laboratories were involved and interlaboratory differences can be substantional[192, 430, 431] a set of test slides were circulated between the laboratories[432] and results for 13 laboratories were accounted for. While kappa values were fair or good for LSIL, HSIL and negative slides they were poor for ASCUS (0.31) with a range between laboratories of 0.09 – 0.87. Although not explicitly stated it can be inferred from the tables that more than 20% of high grade lesions in the Italian study seem to have been detected by ASCUS cytology which makes it an important diagnosis in finding high grade lesions. Although there are inter-observatory disagreements within one laboratory this is more limited than between laboratories, suggesting at least more consistent diagnoses in our study. In assessing both studies it should also be considered that despite the large number of participants the number of cases is limited and only a fraction of all smears are actually followed up.

The study by Davey[196], published in the same issue of the British Medical Journal, was primarily designed as a study for comparing conventional cytology with manual reading of liquid cytology smears with the aid of computerized detection of the most abnormal area. With a split sample design that probably disfavours LBC they found a significant increase in the yield of high grade lesions with LBC at a rate within the confidence interval of our findings.

In conclusion there are some fairly consistent results in trials comparing LBC with conventional cytology. Inadequacy rates improve, reading time and professional satisfaction improves, more abnormalities are found in cytology and more CIN in histopathology are picked up. Data on the positive predictive value for atypical cytology and sensitivity for high grade lesions in histopathology differ. However studies done in different populations with different prevalences of HPV, disease and

former screening exposure, with different follow up schedules, different colposcopy services and different laboratory standards must be compared with caution. Or as expressed in a recent BMJ editorial on the subject "These variables mean that results of the most rigorous study in one setting may not be directly applicable in another"[433].

Colposcopy and scoring

Colposcopy as a diagnostic tool shows considerable variation in performance between studies. In this it resembles cytology and histopathology and the methods share the common property of being subjective methods. However there seem to be a limit for the performance of colposcopy even in settings with experienced colposcopists when adequate reference standards and adequate follow up of negative cases are present.

In Paper II we constructed a scoring system for colposcopy. The odds ratios reflect the probabilities for a specific score to correspond to a histopathological verified high grade lesion compared with the value of "C" that was used as a reference standard (Table 3).

Variabel	Level "A"	Level "B"	Level "C"
Aceto uptake	<0.001 (n.a) , p=n.a	0.21 [0.12, 0.47], p=<0.0001	1.00
Margins and surface	<0.001 (n.a) , p=n.a	0.23 [0.17, 0.61], p=0.0005	1.00
Vessels	0.63 [0.08, 1.04] , p=0.0586	0.17 [0.06, 0.84] , p=0.0250	1.00
Lesion size	0.09 [0.03, 0.43] , p=0.0010	0.47 [0.18, 0.65] , p=0.0011	1.00
Iodine staining	0.31 [0.06, 3.39] , p=0.4305	0.25 [0.21, 0.76] , p=0.0051	1.00

Table 3. Logistic regression odds ratio estimates with 95% confidence intervals. NA = not applicable, as there were no observations of HGL:s with this score.

We found that all variables contributed to the model. Level A was better correlated than level "B" to a high grade lesion for "Vessel" and "Iodine". In the latter case there were few observations with level A, hence the wide confidence interval and we decided to keep the original order. For "Vessels" the order between level A and B was reversed in the final score. In this respect our system will also resemble Reid's index who came to this conclusion with other scientific methods[434]. Obviously, seeing no vessel architecture gives a higher risk for a high grade lesion than seeing a regular fine mosaic or puncture that is more associated with low grade lesions. The reason is that thick, acetowhite high grade lesions do not display vessel architecture. An effect of this observation is that most colposcopically benign cervices will be attributed one point in scoring due to absence of vessels which also is a feature of colposcopy within normal limits.

In shaping the final scoring system the predictive ability had been highest if we had attributed a unique score for each square in the table and used decimals. We had then extracted maximum power from the collected and calculated information. However such a system would have been impractical and far from "user friendly". We tested a range of different values for specificity and sensitivity, as ROC-curves and the areas under the curve, and found a very small difference between the "ideal" impractical score and this score that we settled for:

Variabel\Score	**0**	**1**	**2**
Aceto uptake	0 or transparent	Shady, milk	Distinct, stearin
Margins and surface	0 el diffuse	Sharp but irregular, jagged, "geographical". Satellites	Sharp and even, difference in surface level
Vessels	Fine, regular	0	Coarse or atypical vessels
Lesion size	<5 mm	5-15 mm or 2 quadrants	> 15 mm or 3-4 quadrants or endocervically undefined
Iodine staining	Brown	Faintly or patchy yellow	Distinct yellow

We found a good and graded agreement between colposcopic assessment done from this score and histopathology. The area under the ROC-curve (0.87) is a high value and larger than was found in the meta-analysis by Michele Mitchell and co-workers (0.82)[215].

Scoring systems are not new but it is our experience that they are seldom used in clinical routine. Most colposcopists probably rely on their impression. We compared the scoring with the impression of the colposcopist, recording both in the latter two thirds of the study. We found a good correlation, although it can be argued that the assessments are not independent of each other. To really test if there is a difference in performance between impression and the scoring system a randomized trial should be made.

		Histopathology			Score, mean (median)	
		Benign	**Low grade**	**High grade**		**N**
Colposcopic impression	**Benign colposcopy**	31	10	1	4.2 (5)	42
	Low grade colposcopy	25	42	17	6.0 (6)	84
	High grade colposcopy	3	3	67	8.0 (8)	73
Score, mean (median)		4.8 (5)	5.8 (6)	7.7 (7)		
N		59	55	85		199

Table 4. Cross tabulation of colposcopic impression vs. histopathology classified in three categories.

Using the suggested thresholds in the scoring system we could reach 100 % sensitivity for high grade lesions. If we should have abstained from biopses based on the impression of benign colposcopy we would have missed one high grade lesion (98.8% sensitivity) but if the threshold had been low grade lesion sensitivity would have been reduced to 78.8%. However the comparison limps slightly as the scoring system was developed to dichotomously predict HGL/not HGL while in grading impression three levels were possible. Based on impression 70% of the diagnoses were correct hence the accuracy was high. Kappa value κ was 0.55. Only 4 out of 199 colposcopic diagnoses were more than one step inaccurate, which is not considered in an unweighted kappa value.

A weakness in this study is that the reference in many cases is dependent on the method that should be evaluated, i.e. when histopathology is the effect of a biopsy this is guided by the colposcopic picture and taken from the colposcopically "worst" area. An estimated 50% of the diagnoses come from LEETZ or laser cones that contain the entire transformational zone but the rest were biopsies and we have not done separate analyses. This verification bias is however adjusted for by the follow up in the comprehensive regional register for additional high grade lesions within the studied population. This search was made with a mean observation time of 2 years and 3 months and a minimum time of 1 year 4 months. This allowed lesions missed by colposcopically directed biopsies to display, as three negative smears within two years are required after positive cytology with negative biopsy[251]. This follow up actually strengthened the colposcopy results as three of the additional five high grade lesions found corresponded better to the colposcopy score than the initial diagnosis. In none of the cases the score was below five, maintaining the sensitivity of 100% when this score is used as cut off.

After Paper II was published the results of the Reid's scoring system in the US ALTS trial have been published[249]. As presented in the background section (page 41) the

performance of the RCI was more or less a catastrophy as sensitivity to detect CIN3+ for a score of ≥3 p was only 37.3%. Sensitivity for CIN2+ can be calculated as 26%. This means that the Reid's colposcopic index is practically useless as predictor of high grade disease. 63% of the CIN3 and 74% of the CIN2 received a score of 0-2 in the ALTS study underlining our concern in paper II about the RCI scoring values being too severe and insensitive.

Our study shows a superior performance of colposcopy than what is common in the literature. This adds to the bulk of evidence that performance differs between settings and data is difficult to generalise. This emphasizes the necessity for each colposcopist to evaluate his/her performance, and in this the proposed scoring system may be of help.

Risk of cervical and vaginal cancer after treatment of high grade CIN

In paper III we show that women once treated for CIN3 constitute a high-risk group for cancer of the cervix and vagina. The combined risk for the two sites of cancer is increased 2.5 times over all strata. Most women get effective care and protection from cancer when they participate in the offered screening procedure, precursor lesions are detected and treated and they presumably participate in follow up programs. However this increased risks forces us to reconsider and re-evaluate parts of the screening programs.

It is reasonable to combine data for cervical and vaginal cancer for three reasons: 1) There is a classification problem where squamous cell carcinoma in the upper part of vagina occurring among hysterectomized women after earlier high grade CIN can be classified either as cervical or vaginal carcinoma; 2) There is to a large extent a common background in persistent high risk HPV that stands for > 95% of cervical cancer and 60% of vaginal cancers[41, 435] ; and 3) precursors are detectable with cytology in routine screening and thereby the cancers are preventable. The combined relative risk, expressed as standardized incidence ratios, as well as absolute risks, expressed as incidence per 100 000 person years are presented in table 5 (these data are not included in paper III).

We found the risk to be particularly high in women over 50 years of age at treatment for CIN3 with astonishing incidence ratios. There is an increase from 127 per 100 000 women years in the age bracket 50 – 59 to over 400/100 000 above 70 (Table 2). The clinical implication of this is that gynecologists should have a more liberal view on hysterectomy for high grade CIN in this age-group, assuming that hysterectomy gives a better protection. Data on this are sparse. In their meta-analysis Soutter et al[436] found a relative risk of 0.69 when hysterectomy was compared with conservative

		Observed cases	Expected	Person year	SIR (O/E)	95% confidence interval	Incidence per 100 000
All		990	399	2 314 648	2.48	2.33 to 2.64	43
Birth Cohort	<1915	119	15	52 393	7.97	6.6 to 9.53	227
	1915-1929	268	91	375 478	2.95	2.6 to 3.32	71
	1930-1939	184	79	476 556	2.34	2.01 to 2.7	39
	1940-1949	207	117	761 197	1.77	1.54 to 2.03	27
	1950-1959	163	75	462 490	2.17	1.85 to 2.53	35
	1960+	49	23	186 534	2.16	1.6 to 2.86	26
Age at CIN3 diagnosis	<20	3	3	29 464	0.92	0.19 to 2.69	10
	20-29	169	117	778 899	1.45	1.24 to 1.68	22
	30-39	319	153	897 887	2.09	1.87 to 2.33	36
	40-49	241	91	455 514	2.64	2.31 to 2.99	53
	50-59	145	26	114 409	5.59	4.72 to 6.58	127
	60-69	78	7	29 927	10.75	8.5 to 13.42	261
	70-79	31	2	7 686	17	11.55 to 24.13	403
	80+	4	0	861	24.19	6.59 to 61.93	464
Period of CIN3 diagnosis	1958-70	288	135	647 416	2.14	1.9 to 2.4	44
	1971-80	341	152	924 714	2.24	2.01 to 2.49	37
	1981-90	269	88	569 006	3.06	2.7 to 3.45	47
	1991-2002	92	24	173 512	3.77	3.04 to 4.62	53
Time since CIN3 diagnosis	<1	124	22	130 587	5.56	4.62 to 6.63	95
	1 - <2	84	22	126 757	3.81	3.04 to 4.72	66
	2 - 4	184	64	357 904	2.89	2.49 to 3.34	51
	5 - 9	266	95	527 950	2.8	2.48 to 3.16	50
	10 - 14	189	78	446 526	2.42	2.09 to 2.79	42
	15-19	114	59	353 456	1.94	1.6 to 2.33	32
	20-24	79	41	253 712	1.93	1.53 to 2.4	31
	25+	74	41	248 344	1.82	1.43 to 2.28	30

Table 5. Combined risks for invasive cervical cancer and vaginal cancer among women with a prior carcinoma in situ (CIN3) diagnosis. Standard incidence rates (SIR) and incidence per 100,000 person year for the whole population and stratified for birth cohort, age at CIN3 diagnosis, period for CIN3 diagnosis and time (number of years) between diagnosis of CIN3 and invasive cancer.

methods of treatment, but this result did not reach statistical significance as confidence intervals were 0.27 to 1.44. This wide confidence interval suggests insufficient data.

We also found an increased risk over the time periods and women treated for CIN3 in the 1990:s have a higher risk for cancer than women treated in the 1960:s. This we attribute to a switch to more conservative treatments over the years as all other parts of the screening program have decreased the cancer risks. Screening intervals have

been shortened, age limits increased and the population has been better covered, for longer time with more tests for each woman throughout life. In the general population this has more than compensated for a higher exposure for HPV over the years, but the effects of this must also be considered in this particularly vulnerable group of women.

All the data we present are not easy to interpret. Due to statistical interaction between Period and Time since CIS-diagnosis we stratified the material and found an increasing trend with time 1958 - 1970 and a decreasing trend after that. The main difference is to be seen the first years after treatment for CIS. Could the lower detection of cancer in the 60:s be attributed to the very fact that treatment was done with more extensive surgery? This could have delayed a cancer diagnosis. The most important finding in this stratified analysis however, is that the long term effects with an increase in cancer incidence of 50 – 100% seem to be quite similar in the different strata. The data for 25+ years among women treated later after 1970 lack statistical significance but it must be considered that we make this group small when we stratify for period.

A limitation in our study is that we have no data on cancer staging. Even deficient follow up that do not protect women from cancer can save lives in finding cancer in a treatable stage.

Our findings about an over-all increased long term risk for women treated for high grade CIN are supported by the studies presented in the background section – except for the Norwegian study[315]. Their finding of a strong significant decreased risk is quite puzzling. The findings that also the risk for cancer in the corpus uteri is lowered and the increased risk for vaginal/vulvar cancer (that unfortunately are not separated in the analysis) in Norway at that time suggest a high rate of hysterectomies in this cohort. Also the reporting of CIS can be questioned as it fluctuates over time. Still the low incidence rates for cervical cancer are in sharp contrast to the rest of the literature and lack explanation.

We do not have data on how follow up actually have been carried through which is a weakness in our study. There are three possible reasons for our findings, related to follow up in the screening program: 1) The used method, cytology, has been insensitive; 2) Women have been offered screening but have not participated; or 3) Women have not been offered screening.

1) Cytology has limited sensitivity and sensitivity is often considered even lower in older age groups[437]. This view is supported by a study showing a higher rate of HPV-positive/cytology-negative (in comparison with HPV+/Cyt+) women above 60 years of age compared with lower age groups[438]. Concomitantly specificity has been reported to be a problem above 50 years of age as there seems to be an over representation of ASCUS lesions[439, 440]. However the recent "overview" by Cuzic[212]

found equal sensitivity and specificity above and below 50 years of age in one study[153] and increased sensitivity for CIN2+ in three[211, 214, 441]. If screening has been offered there is no clear evidence that the method (cytology) have been less suitable for this age group.

2) There is a shortage of data about participation. However in Sweden the participation rate within the screening program among older women is higher than in younger women[99]. There is a general knowledge, although not documented, that women who have been treated for pre-cancerous lesions in general are highly motivated to follow up.

3) The Swedish screening program is phased out at fifty years of age and recommendations[97], that also are followed[251], are that one screening round should be offered at 55 and the last one at 60 years of age. This might be adequate for the general low-risk population but is clearly not sufficient for women treated for high grade cervical lesions. We consider this to be the main reason for the substantial increased risk for women treated in older age – they are simply not followed up as long as their younger counterparts as they fall out of the screening program reaching an age limit that has been set for the ordinary population. This is not only overlooked by screening authorities in Sweden but also by a number of authors who recommend "back to screening" after ruling out residual disease for a couple of years[285, 288, 327, 328, 352, 442].

Although our study has been limited to women with CIN3 other studies have shown higher(!), similar or slightly lower long term risks for women treated for lesser grades of CIN[269, 318, 436]. Long term follow up should preferably also include CIN2, a smaller group that has some overlapping risk profile with CIN3. The two diagnoses are regarded in international nomenclature as high-grade and usually not separated.

Testing for HPV in long term follow up

The different backgrounds and epidemiology in short term and long term incidence of new CIN or cancer are often recognized[279] and an increased long term risk has been acknowledged for some time. However our study (Paper IV) is the first, to our knowledge, to address a specific strategy for long term follow up. After excluding all CIN2+ lesion appearing within two years after treatment, we found an overall odds ratio between the HPV positive and the HPV-negative women to get a new CIN2+ lesion of 2.5. There was a consistent and expected decrease of OR when time passed and the protective effect of a negative HPV-test could not be seen 6 years and 6 months after the initial treatment for CIN2-3. A striking observation, which actually is somewhat outside the range of a case control study, was that 76% of the cases were HPV-negative on both tests taken within the first two years.

As can be seen in Table 1 (page 50) the studies reporting more favourable sensitivity data for HPV have far shorter follow up time and much narrower intervals between the testing and the event of new CIN2+. Actually none of the published meta-analyses or reviews has considered follow up time or time interval in their evaluations. It should also be noted that several of the studies in Table 1 are made exclusively in women that are high risk HPV-positive at treatment.

Lower sensitivity has not been reported in the literature for archive smear studies but we took the precaution to include two smears for every case and control, classifying exposure as HPV-positive in any of the two smears. The fact that PCR on archive samples has high sensitivity in several studies with short term follow up[339, 341, 352] provides further confidence in the method. Aside from our study the study by Cruishank[342] with a low sensitivity for HPV from archive samples (29%) has the longest follow up and interval test-to-outcome and their results are consistent with our findings.

There were 28 women who developed cancer. We have not calculated total person time for the cohort, but a fair estimate is that it is close to the observation time for the controls thereby generating 66 532 person years. This is equal to an incidence of 42/100 000 person years, supporting our findings of an excess risk. It should be noted that this cohort not only includes women with CIN3 but also CIN2, and that the analysis could be an underestimation since the other inclusion criteria should be met. It should also be noted that follow up was an inclusion criterion, implying that these women either have recieved sub standard care or that the method used (cytology) was insufficient.

In the selection of controls for the study in paper IV we did not keep record of the number of possible controls that were discarded due to lack of smears to analyse. We can now only note for the record that they were few. Another minor flaw was that cases should have been allowed to be controls before the time they became a case. This could theoretically introduce a bias as these cases-to-be have an increased exposure of HPV and the difference between cases and controls could have been diminished even more. However a sensitivity analysis did not alter the overall odds ratio by more than 0.1 and would have no bearing on our conclusions.

Our conclusion is that we cannot rely on a negative HPV-test taken within 2 years post treatment to exclude future follow up although there is a certain protective effect. We did no comparison with cytology. In short term studies comparing HPV with cytology in a single test HPV shows a better sensitivity, an important characteristic in follow up as a high risk population is addressed. Two studies have compared two serial cytologies with one HPV-test. One found[337] NPV for cytology (100%) actually was slightly higher than that of HPV testing (95%) while the other[324] presented slightly lower NPV for cytology (98.6%) compared with HPV-testing (99.3%) Specificity was similar in this study (54% vs. 53%). The largest difference in

sensitivity seems to appear a short time after surgery. Nobbenhuis[351] and to some extent Nagai[350] have provided data for a consecutive row of tests, finding that cytology after 12 months seems to be as sensitive as HPV-testing.

For short term follow up, within two years from treatment, the main purpose of follow up is to find treatment failures – residual disease. Here HPV-testing has performed well and introducing HPV-testing into programs can be justified. In the Swedish recommendations for follow up one of the three visits recommended within two years could be omitted if a previous HPV-test was negative. However the savings estimated in Holland[329] will not be found in Sweden as several of these visits are cheaper, run by mid-wives.

In the longer term follow up including HPV-testing is attractive due to the need for more sensitive analysis, but there is so far no support for lengthening screening intervals for the high-risk group of women treated for high grade lesion, even if they are HPV-negative. On the contrary it is an urgent task to include these women in long term screening programs, surpassing age-limits set for normal low risk populations.

Conclusions

- Liquid based cytology in the screening program of West Sweden increases the uptake of high grade lesions compared with conventional cytology. This will most probably reduce the incidence of cervical cancer among the participants.

- 30% more women need follow up with liquid based cytology but there are small if any, reductions in positive predictive values compared with conventional cytology.

- There is no evidence to this day that the screening intervals can be lengthened with LBC but longer follow up of the studied cohort can provide important data on this issue.

- Using a newly developed scoring system colposcopists can accurately identify or exclude high grade lesions.

- This scoring system can possibly standardize records and nomenclature for colposcopy in screening programs and contribute to learning and quality assurance.

- Treatment for CIN3 has generally been effective but after treatment there is still a substantially increased risk of acquiring cancer in the cervix or the vagina compared with the general female population in the same age.

- This risk has increased over the decades and is also positively correlated with age at treatment. There is an excess risk, compared with the normal population, more than 25 years after treatment.

- Women treated for CIN3 should be offered follow up for a long time, regardless of age.

- HPV-testing within one year after treatment for high grade cervical lesions does not seem to contribute substantially to strategies of long term follow up.

- If tested, women who would test negative for HPV-DNA within one year after treatment, still would need follow up after two years post surgery.

Summary in plain Swedish

Att förebygga cancer i livmoderhals –

Undersökningar av möjligheter till förbättring

När det svenska cancerregistret startade 1958 var livmoderhalscancer den fjärde vanligaste cancerformen hos kvinnor men genom den omfattande verksamheten med gynekologisk cellprovskontroll har denna sjukdom blivit ovanlig och hamnat långt utanför ”10-i-topplistan”. Trots det är det fortfarande varje år många kvinnor som har deltagit i kontrollerna men ändå drabbas av cancer. Denna avhandling är en genomgång av några möjliga metoder för att förbättra kvalitén i gynekologisk cellprovskontroll.

Ett vanligt cellprov tas med spatlar och borste som stryks mot kvinnans livmodertapp och i livmoderhalsen. Sedan stryks provtagningsintrumenten mot en glasskiva som fixeras med alkohol och studeras i mikroskop i ett laboratorium. Vid en nyare metod, vätskebaserade cytologi, tas provet på samma sätt men vispas ner i en burk med vätska. I laboratoriet processas det uppslammade provet i en maskin som ger ett tunt och jämnt cellprov för cytodiagnostikern att titta på i mikroskop.

Det finns många undersökningar gjorda i andra länder om effekten av att använda vätskebaserad cytologi. De flesta har visat fördelar med vätskebaserad cytologi men alla undersökningar har begränsningar. Exempelvis: 1) De kan vara gjorda i länder med dålig provtagningsmetodik där många prover är otillräckliga. När man då påvisar en förbättrad kvalité i proverna är det inte säkert att det kan överföras till Sverige som t.ex. har knappt en tiondel så många obedömbara prover som i England. 2) De kan vara gjorda i utvalda grupper av kvinnor, exempelvis med hög andel cellförändringar. Det är få undersökningar gjorda av vanliga grupper kvinnor, s.k. populationsbaserade studier 3) De kan vara gjorda med olika tekniker där man antingen har tagit det vanliga cellprovet först och det vätskebaserade provet efteråt på samma kvinna eller jämfört resultaten före och efter man infört den nya tekniken. Sådana undersökningar är inte lika tillförlitliga som att använda slumpmässig fördelning mellan de kvinnor som ska ta prov på det ena eller andra sättet (s.k. randomiserade studier) 4) Slutsatserna har varierat en hel del mellan olika undersökningar.

I den första studien (paper I) redovisar vi en randomiserad studie som gjordes vid fem mödravårdscentraler i Göteborg och Södra Bohuslän. Ena veckan skulle barnmorskorna ta prov och stryka ut dem på glasskivor som vanligt, nästa vecka skulle man använda vätskebaserad cytologi och på det sättet växla metod varje vecka. Resultaten jämfördes där det viktigaste fyndet inte var skillnad i andelen

cytologiska cellförändringar i dessa, eftersom det är ett osäkert mått på förändringar som riskerar att utveckla sig till cancer. De viktigaste fynden finner man när sedermera en utredning av de avvikande cellproverna hade gjorts. Denna analys grundade sig på vävnadsprov som knipsats eller opererats bort vid uppföljning av avvikande prover.

13 484 prover togs sammanlagt. Efter utredning med vävnadsprov hade 1,20% av kvinnorna i vätskebaserade gruppen och 0,85% av kvinnorna i gruppen med vanliga cellprov höggradiga cellförändringar eller cancer (CIN2+). Skillnaden är statistiskt säkerställd (mindre än fem procents risk att skillnaden beror på slump). Det visade sig att genomsnittsåldern mellan de två grupperna skilde sig med nästan 3 år. Detta missgynnar den metod som är använd för den något äldre gruppen kvinnor. Vi gjorde en statistisk justering för ålderskillnaden i en s.k. regressionsanalys och justerade också för vilken mödravårdscentral som hade tagit proverna. Denna analys visade en relativ skillnad (odds ratio) på 60%. Andelen obedömbara prover minskade också men andelen prover som behövde utredas ökade med 30% vilket är en nackdel. Båda testerna hade ungefär samma positivt prediktiva värde, d.v.s. möjligheten att ett prov som visar avvikelse efter utredning faktiskt visar att patienten har sjukdomen, i detta fall minst medelsvåra cellförändringar (CIN2+).

En svaghet i undersökningen är att fördelningen av prover inte blev som vi hade tänkt. Främst på grund av att vi inte givit mödravårdscentralerna tillräcklig uppbackning med påminnelser etc (monitorering) hade fler prover tagits som vanliga cellprover än som vätskebaserade. Detta stör tolkningen, som får göras med större försiktighet än vad som annars hade behövts. Vår slutsats är att vätskebaserad cytologi skulle leda till att fler höggradiga förändringar skulle upptäckas och att detta rimligen skulle leda till ett bättre skydd mot livmoderhalscancer.

När cellprover är avvikande behöver kvinnorna utredas. Detta görs med kolposkopi, en undersökning som görs av gynekologer. I samband med en gynekologisk undersökning använder läkaren ett mikroskop med liten förstoring, vanligen 6 – 24 gånger. Med detta kolposkop undersöks livmodertappen och i de flesta fall kan befintliga förändringar upptäckas. I den vetenskapliga litteraturen har det tidigare beskrivits hur läkaren systematiskt kan gå till väga vid en underökning, men vår uppfattning var att många läkare gör det lite mer på känn vilket riskerar att minska träffsäkerheten vid undersökningen. Vi gjorde ett schema för kolposkopiundersökning som vi ville skulle vara till hjälp för att den ska bli systematisk och som innehöll en poängbedömning (scoring) som vi ville göra så noggrann att undersökningen skulle kunna förutsäga om förändringar på livmodertapp var höggradiga eller inte. Denna studie redovisar vi som paper II.

Vi valde fem bedömningsgrunder: 1) Vilken färgreaktion som uppträder när gynekologen baddar området med utspädd (5%) ättika 2) Hur kanterna på förändringen framträder och hur förändringen avviker i höjd och djup från

omgivande normal vävnad 3) Hur blodkärlen ser ut i förändringen, om det går att se dem överhuvud taget 4) Hur stor del av livmodertappens s.k. övergångszon som förändringen upptar och 5) Hur färgreaktionen blir när man penslar livmodertappen med jodlösning. Varje bedömningsgrund (variabel) kunde ges en gradering av tre möjliga. Vi undersökte 297 patienter. I en statistisk analys undersökte vi hur väl de 5 x 3 olika möjliga graderingarna sammanföll med att ett samtidigt taget vävnadsprov som visat CIN2+. Vi kunde se att alla variablerna hade betydelse och att scoringsystemet skulle vara sämre om vi uteslöt någon av variablerna. Vidare fick vi en skala som gav ett exakt förhållande mellan de olika stegen för varje variabel. Vi gjorde en förenkling och avrundning och prövade med den enkla skalan 0, 1 och 2 för varje variabel. Detta användarvänliga system gav en obetydligt sämre förmåga att förutse CIN2+ jämfört med om vi hade använt bästa möjliga modell med olika skalor och decimaler för varje variabel. Vi hade då ett system som kunde ge 0 till 10 poäng och vi kunde hitta tröskelvärden. Ingen höggradig förändring hade fått under fem poäng och vi fann att 8 – 10 poäng motsvarade detta höggradiga förändringar i 90% av observationerna. Med sådana höga poäng krävdes ingen ytterligare utredning utan vi hade direkt kunnat fatta beslut om behandling. I de fall som var 5 – 7 poäng behövdes utredning med vävnadsprov.

Vi tror att detta scoringsystem kan vara till hjälp för träning i kolposkopi så att gynekologer kan göra undersökningar systematiskt och kolla hur säkra bedömningar de gör. Detta har vi dock inte undersökt vetenskapligt.

Förändringar på livmodertappen som är höggradiga behöver behandlas och behandlingen går ut på att det lilla men känsliga område som kallas transformationszonen (övergångszonen) tas bort eller förstörs. Det bästa är att ta bort området vilket kan göras med ett enkelt ingrepp som ofta kallas konisering. Det som tas bort från livmoderhalsen är oftast en liten skiva, 7-8 mm tjock och stor som en femtioöring eller enkrona.

Vi ville sedan ta reda på om de kvinnor som haft allvarliga cellförändringar verkligen var skyddade efter genomgången operation. Vi använde oss av cancerregistret som innehåller uppgifter om de mest allvarliga förstadierna till cancer (CIN3) och samtliga fall av cancer i livmoderhalsen. En del kvinnor får livmodern bortopererad och då tas nästan alltid livmodertappen med. Får de en cancer i övre delen av slidan kommer denna att kallas slidcancer (vaginalcancer) och vi ville undersöka risken för slidcancer också. Den undersökningen utgör arbete III. Först skapade vi en studiebas (cohort) av alla kvinnor som haft CIN3 sedan cancerregistret skapades 1958 fram till 2002. Det är 132 493 stycken. Sedan undersökte vi vilka av dessa som fick cancer i livmoderhals eller slida mer än ett år efter sin behandling (vi ville inte ha med dem som uppenbart hade fått en cancer felaktigt diagnostiserad som CIN3). Det var 990 stycken. I samband med detta fick vi kontrollera och göra justeringar för dem som lämnat landet eller livet. Eftersom det alltid och överallt i Sverige varit självklart att behandla CIN3 gjorde vi antagandet att alla blivit

behandlade när väl deras tillstånd upptäckts. Vi hade hela den kvinnliga befolkningen i motsvarande åldersgrupper som jämförelse. Vi kunde dra flera viktiga slutsatser. 1) Kvinnor var i allmänhet väl skyddade av behandling. 2) Trots det är det 2,5 gånger så vanligt att kvinnor som har haft CIN3 utvecklar cancer i livmoderhals eller slida jämfört med hela befolkningen. 3) Risken för att utveckla cancer minskar högst obetydligt med tiden efter behandling. När vi delade upp materialet utifrån när behandlingarna gjordes fanns det en något tydligare tendens till sänkt risk många år efter behandling om denna gjordes senare än 1970, men det fanns ändå en överrisk för cancer 20 – 25 år efter behandlingen. 4) Risken ökade brant för kvinnor som var över 50 år när behandlingen/diagnosen av CIN3 gjordes 5) Risken för cancer har ökat måttligt årtionde efter årtionde sedan 1960-talet och är dubbelt så hög för kvinnor som behandlats på 1990-talet jämfört med 1960-talet. 6) Dessa risker kvarstod när vi med en statistisk metod som heter multipel regression kunde kompensera för det dolda samband som t.ex. kan finnas mellan årtal för behandling och ålder.

Våra tolkningar av dessa fynd är att kvinnor som behandlats sedan måste följas med cellprover eller möjligen virustestning under många år. Detta gäller särskilt kvinnor som är över 50 år då behandlingen görs, i synnerhet som de riskerar att bli utan provtagning vid 60 års ålder som är den övre gräns som satts för gynekologisk cellprovskontroll i Sverige. Denna gräns verkar vara säker för kvinnor som inte har haft höggradiga förändringar på livmoderhals, men är helt klart inte acceptabel för dem som haft sådana förändringar.

I delstudie IV gjorde vi en undersökning av en ofta förespråkad metod för att kunna följa kvinnor efter behandling. Eftersom vi idag vet att cancer i stort sett aldrig uppträder utan förekomst av så kallade högrisktyper av humant papillomvirus (HPV) skulle man kunna göra glesa kontroller av dem som inte har sådana virus och tätare tester av dem som är bärare. HPV-testning verkar kunna vara av viss nytta för att hitta höggradiga förändringar inom ett par år efter behandling, vilket kan tolkas som att man hittar de kvinnor där behandlingen har varit ofullständig. Vi var intresserade att se om HPV-test tagen efter behandling skulle kunna göra nytta för att välja ut de som behöver följas med täta kontroller och de som kan följas med flera års mellanrum. Vi ville också veta hur länge en negativ HPV-test ger ett skydd, alltså en slags bäst före datum.

Vi gjorde en så kallad fall-kontrollstudie. I patologlaboratoriets vid Sahlgrenska Universitetssjukhuset databas hittade vi 4526 kvinnor som hade genomgått behandling för höggradiga cellförändringar (CIN2+). Vi letade sedan efter dem som hade fått ny CIN2+ mer än två år efter den första behandlingen. Dessa utgör fallen. För varje kvinna som blev fall letade vi fram två kvinnor som också hade behandlats för cellförändringar men inte fått återfall. De skulle ha utfört behandling vid samma tidpunkt och vara i samma ålder. Dessa blev kontroller. Vi tog fram två arkiverade cellprovsglas vardera för både fallen och kontrollerna. Dessa prover skulle vara

tagna 3 – 24 månader efter behandlingen. Glasen fotograferades så att de skulle kunna studeras igen vid behov. Därefter löstes cellmaterialet av från glasen och analyserades med känslig så kallad PCR-teknik för högrisk-HPV. För de prover som innehöll HPV undersöktes också vilken eller vilka HPV-typer som förelåg.

Vi hittade 189 fall och kunde ta fram 2 kontroller till varje, alltså 378. Nästan alla cellprovsglas kunde tas fram och analyseras, även de som var över 20 år gamla. Fallen fick återfall i höggradiga förändringar i vävnadsprov från livmoderhalsen i genomsnitt 5 år och 8 månader efter den första behandlingen, men den genomsnittliga uppföljningstiden var 14 år och 7 månader. Det första cellprovet var i genomsnitt taget 6 månader efter behandlingen och det andra 12 månader efter behandling. Det fanns en skillnad mellan fallen och kontrollerna i förekomst av HPV i de prover vi hade analyserat. Denna skillnad uttrycks som odds ratio som ungefär betyder hur många gånger vanligare det var att hitta virus i den ena gruppen än i den andra. HPV var uttryckt på detta sätt 2,5 gånger vanligare bland de kvinnor som hade fått återfall, än bland de kvinnor som inte fick återfall. Det är alltså klart ovanligare att senare i livet få återfall i höggradiga förändringar om man är fri från högriskHPV 6 – 12 månader efter behandling. Denna skillnad kvarstod upp till 6½ år efter behandling, efter det kunde vi inte se någon skyddande effekt av en negativ HPV-test (d.v.s. där man inte fann några tecken på virus). Men det som var mest anmärkningsvärt var nog att 76% av dem som senare utvecklade nya förändringar hade negativa HPV-tester. Vår tolkning av detta var att man dessvärre inte tycks kunna använda resultatet av HPV-test tagen inom närmaste två åren efter operation till att sortera upp kvinnor som behöver följas tätare och kvinnor som kan följas glesare med kontroller i framtiden.

Kortfattade slutsatser av våra fynd

- Vätskebaserad cytologi i västra Sveriges screeningprogram ökar fynden av höggradiga förändringar i livmoderhals, påvisade med vävnadsprov, jämfört med konventionella cellprover. Detta kommer troligen att minska förekomsten av livmoderhalscancer bland dem som deltar i cellprovskontroll.

- Ett nytt scoringsystem för kolposkopi kan med god träffsäkerhet identifiera eller utesluta höggradiga förändringar.

- Detta scoringsystem kan troligen vara till nytta för att standardisera beskrivning av kolposkopiundersökning och bidra till inlärning och kvalitetssäkring.

- Behandling av höggradiga förändringar har i allmänhet varit effektiv men det kvarstår en ökad risk att utveckla cancer i livmoderhals eller slida jämfört med hela befolkningen.

- Denna risk har ökat sedan 1960-talet och är också ökad för kvinnor som är över femtio år då de behandlas. Det finns en riskökning mer än 25 år efter behandling.

- Kvinnor som behandlas för höggradiga förändringar bör erbjudas kontroll under lång tid.

- HPV-testning inom ett år efter behandling förefaller inte ge underlag för att kvinnor med olika testresultat ska ha olika långtidsuppföljning.

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