Cervical cancer prevention
Studies on outcome of cervical screening and on management of abnormal cytology findings

Lena Silfverdal
To all women with cervical cancer
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ABSTRACT

Background
Screening by cytology has been highly effective in reducing the incidence and mortality from squamous invasive cervical cancer (ICC), but the effectiveness is less established regarding non-squamous ICC and regarding women above screening ages and below 30 years of age. Cervical cancer still occurs despite the presence of an organised screening programme. A substantial proportion of screened women with ICC are reported to have had previous abnormal cytology findings. The significance of negative cytology with limited evaluation is not quite determined, the most effective management of women with low-grade abnormalities is controversial, and evaluation of long-term effect of different treatment methods is limited.

Aims
To identify possible areas of improvements in the prevention of cervical cancer by evaluating the effectiveness of the Swedish cervical screening programme, and by exploring risk factors for ICC in the cytological screening histories and in the management of women with abnormal cytology findings.

Methods
The screening histories of all ICC cases in Sweden 1999-2001 (n=1230) and of five population-based control women per case were reviewed, using data from the Swedish Cancer Registry, the national population register, the Swedish national cervical screening quality register, histopathological reports and questionnaires to clinicians. The risk of cervical cancer according to screening histories 0.5-6.5 years before cancer diagnosis was estimated as odds ratios (ORs) in logistic regression models with 95% confidence interval (CI) (Paper I). Risk related to different cytological reports was assessed in women below 67 years of age with cytology (n=572, n=3569) in Paper II. The initial follow-up of women with abnormal or unsatisfactory cytology reports (n=159, n=258) was evaluated in Paper III, and further investigation and treatment of abnormalities (n=143 cases, n=176 controls) in Paper IV.

Results
The cancer cases were above screening ages (31%), had not been screened according to recommendations (33%), had negative cytology (23%), or had previous positive screening tests (13%). No screening within the recommended interval increased the risk of squamous (OR 2.97, 95% CI 2.51-3.50) as well as non-squamous cancer (OR 1.59, 95% CI 1.20-2.11), and increased the risk in all ages. Negative cytology with partially obscuring factors and unsatisfactory
cytology increased the risk of subsequent early stage ICC. All cytological abnormalities increased the risk of ICC, and women with glandular atypia or atypia in cells of uncertain origin carried a particularly high risk (OR 11.69, 95% CI 7.02-19.46). After a low-grade squamous abnormal smear finding, further investigation with biopsy was more effective than repeated cytology (OR 0.46, 95% CI 0.24-0.89). Lack of biopsy increased the risk in women with both low-grade and high-grade squamous abnormalities. Neither repeat cytology, nor biopsy, decreased the risk in women with glandular atypia or atypia in cells of uncertain origin. Treatment decreased the risk, even when the biopsy before treatment was negative or showed low-grade atypia only. Ablative therapy was less effective than excision and laser conisation was the most effective therapy.

Conclusions

Improved adherence to screening recommendations and including older women at increased risk in the programme would have significant cancer preventive gains. Women with negative cytology with limited evaluation and with unsatisfactory cytology may need further evaluation. Assessment with biopsy should be recommended for women with low-grade as well as high-grade squamous abnormalities. The diagnosing of precancer lesions and the identification of women in need of treatment warrant improvements, in particular in cases of glandular or “other” atypia in cytology. Treatment techniques need further evaluation.

Key words

Cervical cancer, screening programme, effectiveness, risk factors, cytological screening history, management abnormal Pap smear, treatment, nationwide population-based case-control study
SAMMANFATTNING PÅ SVENSKA

Bakgrund

Syfte
Avhandlingens målsättning var att identifiera områden i screeningprogrammet som behöver förbättras för att antalet kvinnor som insjuknar i livmoderhalscancer ska minskas ytterligare. Delmålen var att utvärdera screeningprogrammets effektivitet på befolkningsnivå, och att undersöka risken för cancer efter olika kategorier av cellprovsvar och efter olika utrednings- och behandlingsstrategier vid ett avvikande cellprov.

Metod
Screeninghistoriken bland alla kvinnor i Sverige som insjuknat i livmoderhalscancer under åren 1999-2001 (1230 fall) granskades och jämfördes med screening historiken bland kvinnor som inte insjuknat (6124 kontroller) under 0,5-6,5 år före fallens cancerdiagnos. Fem åldersmatchade kontroller per cancerfall valdes slumpmässigt från befolkningsregistret. Data hämtades från cancerregistret, regionala onkologiska databaser, nationella kvalitetsregistret för gynekologisk cellprovskontroll, och enkäter till kliniker där kvinnor med cellförändringar utretts och behandlats. I delarbete I bedömdes risken för livmoderhalscancer utifrån om kvinnor deltagit i screening inom de rekommenderade intervallen eller ej. I delarbete II värderades risken för att utveckla cancer efter olika cellprovsresultat bland kvinnor under 67 år (572 fall och 3569 kontroller). I delarbete III utvärderades handläggningen av kvinnor med avvikande cellprov (159 fall och 258 kontroller), och i delarbete IV granskades den vidare utredning med vävnadsprov och behandling av kvinnor med cellförändringar (143 fall och 176 kontroller). Risken uppskattades genom att beräkna odds kvoter (OR) med 95% konfidensintervall (CI).
Resultat

Utav alla kvinnor med livmoderhalscancer var 31% mer än fem år över åldern för sista inbjudan till screening, 33% hade inte deltagit enligt rekommendationerna, 23% hade deltagit med normala cellprov, och 13% hade haft cellförändringar. Icke deltagande i screening inom de rekommenderade intervallen ökade risken för både skivepitelecancer och körtleccellsancer (OR 2.97, 95% CI 2.51-3.50, respektive OR 1.59, 95% CI 1.20-2.11). Riskökningen var signifikant för alla åldersgrupper. Deltagande i screening minskade kraftigt risken att utveckla cancer i avancerat stadium. Cellprov som inte var bedömbara, eller normala cellprov med nedsatt bedömbarhet pga inflammation eller blod, ökade risken för en påföljande livmoderhalscancer i tidigt stadium. Alla kategorier av cellförändringar i screeninghistoriken ökade cancerrisken. Den största riskökningen sågs bland kvinnor med tidigare körtleccellsförändring, eller cellförändring av oklar typ (OR 11.69, 95% CI 7.02-19.46). Utredning med vävnadsprov var effektivare än handläggning med upprepat cellprov efter en läggradig skivepiteleförändring (OR 0.46, 95% CI 0.24-0.89). Avsaknad av vävnadsprov ökade cancerrisken både efter en läggradig och efter en höggradig skivepiteleförändring. Varken upprepat cellprov eller utredning med vävnadsprov minskade risken för cancer efter ett cellprov med körtleccellsförändring eller förändring av oklar typ. Behandling minskade risken, även när vävnadsprovet före behandling varit normalt eller visat enbart läggradig cellförändring. Antalet cancerfall som tidigare behandlats för cellförändringar var mycket lågt (24 kvinnor). Behandling som skär bort vävnaden var effektivare än behandling som bränner, frysar eller förångar bort vävnaden, och laserkonisation var effektivare än konisation med elektrisk slynga.

Slutsats

LIST OF PUBLICATIONS

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


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### ABBREVIATIONS AND DEFINITIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AGC</td>
<td>Atypical glandular cells</td>
</tr>
<tr>
<td>AGUS</td>
<td>Atypical glandular cells of undetermined significance</td>
</tr>
<tr>
<td>AIS</td>
<td>Adenocarcinoma in situ</td>
</tr>
<tr>
<td>ALTS</td>
<td>ASCUS-LSIL Triage Study</td>
</tr>
<tr>
<td>ASCCP</td>
<td>American Society for Colposcopy and Cervical Pathology</td>
</tr>
<tr>
<td>ASC-H</td>
<td>Atypical squamous cells, high-grade squamous lesion cannot be excluded</td>
</tr>
<tr>
<td>ASCUS</td>
<td>Atypical squamous cells of undetermined significance</td>
</tr>
<tr>
<td>CGIN</td>
<td>Cervical glandular intra-epithelial neoplasia</td>
</tr>
<tr>
<td>CIN1-3</td>
<td>Cellular intraepithelial neoplasia grade 1-3</td>
</tr>
<tr>
<td>CIS</td>
<td>Cancer in situ</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EC</td>
<td>Endocervical cells</td>
</tr>
<tr>
<td>ECC</td>
<td>Endocervical curettage</td>
</tr>
<tr>
<td>Effectiveness</td>
<td>The extent to which the programme, when deployed in the field in routine circumstances, does what it is intended to do for a specified population</td>
</tr>
<tr>
<td>FIGO</td>
<td>International Federation of Gynecology and Obstetrics</td>
</tr>
<tr>
<td>HC</td>
<td>Hybrid Capture</td>
</tr>
<tr>
<td>HPV</td>
<td>Human papillomavirus</td>
</tr>
<tr>
<td>hrHPV</td>
<td>High-risk HPV type</td>
</tr>
<tr>
<td>HSIL</td>
<td>High grade squamous intraepithelial lesion</td>
</tr>
<tr>
<td>ICC</td>
<td>Invasive cervical cancer</td>
</tr>
<tr>
<td>IQR</td>
<td>Inter-quartile range</td>
</tr>
<tr>
<td>LBC</td>
<td>Liquid based cytology</td>
</tr>
<tr>
<td>Lead time</td>
<td>Period between the detection of a lesion by screening and the time point that it should have progressed, in the absence of screening, to a clinically recognised cancer</td>
</tr>
<tr>
<td>LEEP</td>
<td>Loop electrosurgical excision procedure</td>
</tr>
<tr>
<td>LLETZ</td>
<td>Large loop excision of the transformation zone</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>LSIL</td>
<td>Low grade squamous intraepithelial lesion</td>
</tr>
<tr>
<td>NETZ</td>
<td>Needle excision of the transformation zone</td>
</tr>
<tr>
<td>NPR</td>
<td>National population register</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative predictive value; i.e. the extent to which subjects are free of the disease in those that give a negative test result</td>
</tr>
<tr>
<td>Pap</td>
<td>Papanicolaou</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive predictive value; i.e. the extent to which subjects have the disease in those that give a positive test result</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised clinical trial</td>
</tr>
<tr>
<td>SAS</td>
<td>Statistical analysis system</td>
</tr>
<tr>
<td>SCR</td>
<td>Swedish Cancer Registry</td>
</tr>
<tr>
<td>SNCSQR</td>
<td>Swedish national cervical screening quality register</td>
</tr>
<tr>
<td>SNOMED</td>
<td>Systematized nomenclature of medicine</td>
</tr>
<tr>
<td>Sojourn time</td>
<td>Duration of the detectable pre-clinical phase.</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for the social sciences</td>
</tr>
<tr>
<td>Stage IA</td>
<td>Microinvasive</td>
</tr>
<tr>
<td>Stage IB</td>
<td>Localised cancer</td>
</tr>
<tr>
<td>Stage II+</td>
<td>Advanced cancer</td>
</tr>
<tr>
<td>TBS</td>
<td>The Bethesda system</td>
</tr>
<tr>
<td>TZ</td>
<td>Transformation zone</td>
</tr>
<tr>
<td>VIA</td>
<td>Visual inspection of the cervix after application of acetic acid</td>
</tr>
</tbody>
</table>
INTRODUCTION

Cervical cancer is largely preventable by cervical screening and treatment of screen-detected precancerous lesions.1 The objective of screening programmes is to reduce the mortality as well as the incidence of invasive cervical cancer.1-3

Epidemiology

Cervical cancer is the third most common malignancy among women in the world, with an estimated 529,000 new cases and 275,000 deaths in 2008.4 Over 85% of all cervical cancer and 88% of the deaths occur in the low income countries. The highest incidence rates are observed in sub-Saharan Africa, South-Central Asia and South America.4 Before the introduction of screening, the incidence in many European countries, such as Germany and Denmark, and in North America was similar to that seen in many low income countries today.1,5 In Europe, around 55,000 new cases are diagnosed each year and 25,000 deaths.6 The majority of the cases are seen in Eastern Europe where there are no cervical screening programmes.7 The lowest incidence and mortality rates in 2004 were observed in Finland.7 In Sweden, cervical cancer ranked 16th among the female malignancies in 2009,8 and accounted for 464 cases and 139 deaths in 2008.

Cervical cancer primarily affects younger women and therefore the total loss of years-of-life is relatively higher than for other cancers with later onset. The peak incidence was in the 40-49 age group among women treated at different centres throughout the world 1999-2001.9 In many countries the pattern of cervical cancer and its precursors has changed during the last two decades, with increasing incidence in younger age groups.1,7

Histopathological types and classification

The WHO classifies epithelial cervical cancer into squamous tumours, glandular tumours (adenocarcinoma), and “other tumours”. Among “other” epithelial tumours are adenosquamous carcinoma, neuroendocrine tumours, and undifferentiated carcinoma.1

The majority of all cervical cancer cases are squamous cell carcinomas (75-90%).1,9 The proportion of adenocarcinoma cases varies between 5% and 26% worldwide and is often higher in regions with effective screening programmes and low incidence of cervical cancer,1,10 which would reflect that cervical screening is less effective in preventing cervical adenocarcinoma.11-13 Apart from the relative increase, an absolute increase in rates of adenocarcinoma has been observed in some countries over the past two to three decades.14-16
Cervical cancer is staged according to the system developed by the International Federation of Gynecology and Obstetrics (FIGO) (Table 1). For adenocarcinoma, however, the term microinvasive is not distinct and is poorly reproducible.

Table 1. FIGO staging of invasive cervical cancer, and 5-year survival.

<table>
<thead>
<tr>
<th>Stage</th>
<th>5-year survival</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>98%</td>
<td>Cancer diagnosed microscopically, no visible lesion</td>
</tr>
<tr>
<td>IA1</td>
<td>98%</td>
<td>Invasion ≤3 mm deep and ≤7 mm wide</td>
</tr>
<tr>
<td>IA2</td>
<td>95%</td>
<td>Invasion &gt;3 mm to ≤5 mm deep, and ≤7 mm wide</td>
</tr>
<tr>
<td>IB</td>
<td>89%</td>
<td>Visible lesion limited to the cervix, or microscopic lesion &gt;stage IA</td>
</tr>
<tr>
<td>IB1</td>
<td>89%</td>
<td>Clinical tumour ≤4 cm</td>
</tr>
<tr>
<td>IB2</td>
<td>76%</td>
<td>Clinical tumour &gt;4 cm</td>
</tr>
<tr>
<td>II (IIA1-2, IIB)</td>
<td>66-73%</td>
<td>Tumour beyond the uterus, but not to the pelvic wall or to lower third of the vagina</td>
</tr>
<tr>
<td>III (IIIA, IIIB)</td>
<td>40-42%</td>
<td>Tumour extends to the pelvic wall and/or to lower third of the vagina and/or causes hydronephrosis or non-functioning kidney</td>
</tr>
<tr>
<td>IV (IVA, IVB)</td>
<td>9-22%</td>
<td>Tumour spread to the bladder or rectum and/or to distant organs</td>
</tr>
</tbody>
</table>

The overall mortality: incidence ratio in the world is estimated to 52%. Stage at diagnosis, the presence and quality of treatment, and the presence of lymph node metastasis are the most important factors affecting the prognosis. The overall five-year survival is 70% in Sweden, as well as for cases treated at different centres in the world. The stage distribution varies between centres in the world, which probably reflects the presence or not of screening programmes that detect early stage disease. Cases with microinvasive disease (stage IA) have low incidence of lymph node metastases (3.9-9.7%) and very good five-year survival (Table 1). The introduction of effective screening programmes may cause an overestimation of survival estimates because some cases will be diagnosed at an earlier stage (lead time bias). On the other hand, screening may prevent slow-growing tumours more than fast-growing tumours with a poorer prognosis.

**Treatment**

Early stage cervical cancer (I–IIA) has traditionally been treated by radical hysterectomy and pelvic lymphadenectomy that leaves women without their fertility, sometimes followed by radiotherapy and chemotherapy. Higher stages of cancer, and often stages IB2 and IIA as well, are best treated by radiotherapy and chemotherapy combined. Microinvasive cancer stage IA1 is treated by hysterectomy or, to preserve the fertility, by excision of the lower part of the cervix (conisation). Women at stage IA2 or IB1 (small tumour) may also be treated by fertility sparing surgery if there are...
no lymph node metastasis found at pelvic lymphadenectomy.²,²² Often the lymph nodes are taken away laparoscopically, and if negative nodes, the cervix is radically removed by a vaginal approach.²³ Another technique is the abdominal approach with pelvic lymphadenectomy and radical trachelectomy, leaving the uterus.²⁴ A careful selection of patients and considerable experience and specialisation are needed, and only a low-risk subgroup of young patients is considered eligible for this treatment. Recurrence rates are similar to those after radical hysterectomy in this selected group (4%).²⁵ The rate of second trimester losses and preterm deliveries due to cervical weakness is high.²⁶

Etiology

Human papillomavirus

Persistent infection with a carcinogenic papillomavirus (HPV) is a necessary cause of both squamous cell carcinoma and adenocarcinoma.²⁷-²⁹ Papillomaviruses are double-stranded circular DNA viruses that infect many species. Around 16 HPV types have been classified as high-risk (hrHPV) for carcinogenesis in humans, of which 12 are classified as definitely carcinogenic.²⁸,³⁰ HPV16 has a particular high potential for malignant transformation of infected cervical cells.³¹ HPV DNA has been found in almost all cervical cancers (99%).²⁷ A recent report shows that HPV types 16, 18, 31, 33, 35, 45, 52, and 58 account for 91% of all HPV DNA positive cervical cancers in the world, of which HPV16 and 18 are the most common (71%).³² HPV16, 18, and 45 were found in 94% of the adenocarcinomas.

Co-factors or risk factors

Smoking,³³ long term use oral contraceptives (>5 years),³⁴,³⁵ and multiparity (>5 children),³⁶,³⁷ are co-factors and can increase the risk of precancer and cancer 2-5 times among women infected by HPV. The role of chronic inflammation is less certain, but infection with Chlamydia trachomatis and Herpes simplex type 2 might be co-factors as well.³⁸,³⁹ Decreased immunity, including HIV infection, is also associated with increased risk.¹ Squamous cell carcinoma and adenocarcinoma share most risk factors, with the exception of smoking and high parity which associate with squamous ICC mainly.³⁷

Other risk factors

HPV is sexually transmitted and sexual behavior therefore affects hrHPV exposure and risk of ICC. Lifetime number of sexual partners of the woman or her partner, and early age at first intercourse are significantly associated with risk of ICC.¹,³⁷,⁴⁰ Condom use has shown protection in some studies, but not significantly so in several studies.¹,⁴¹ Male circumcision reduces the risk of ICC in female partners.⁴²
An increase in risk of ICC has been observed in successive birth cohorts in the Nordic countries before the introduction of screening,\textsuperscript{5} and in successive generations born after 1930 in other European countries,\textsuperscript{7-43} something that would probably be explained by an increased prevalence of HPV and of other risk factors/co-factors.\textsuperscript{7,44-45} Other epidemiological factors that have been associated with incidence of ICC are social class, marital status, ethnicity, religion and occupation,\textsuperscript{1} and they are most likely reflecting differences in exposure and maybe immune response to HPV, and also differences in screening.

**Cervical neoplasia**

**Carcinogenesis**

HPV infection has a very high rate of acquisition,\textsuperscript{46} and the presence of HPV causes cytological abnormalities in 30\% of the cases.\textsuperscript{47} However, new HPV infections also have very high spontaneous clearance, regardless of the age of the woman.\textsuperscript{48} Half of the infections clear within 6 months, two thirds within 12 months, and around 80\% within two years.\textsuperscript{49} The longer the infection lasts, the higher the likelihood is that it will persist and cause precancer/cancer.\textsuperscript{49} Women with persistent infections over 12 months have been diagnosed with CIN2+ in 20-30\% within 30 months.\textsuperscript{49,50} The small proportion (about 10\%) of carcinogenic infections persisting for several years is strongly linked to a high absolute risk of diagnosis of precancer.\textsuperscript{47} The onset of microscopically detectable precancer may occur rapidly after infection, possibly within 5 years.\textsuperscript{47}

The carcinogenicity of HPV is related to the activity of two oncoproteins, E6 and E7. E6 inhibits p53 in the blocking of apoptosis, and E5 inhibits pRB (retinoblastoma suppression protein) in abrogating cell-cycle arrest.\textsuperscript{51} Both proteins are expressed at low levels during the infectious phase, but at some point in the progression to precancer the expression of E6 and E7 is deregulated, leading to their over expression and in unregulated cellular proliferation.\textsuperscript{47}

For unknown reason, persistent hrHPV infection causes cancer mainly at transformation zones (TZ) where two different kinds of epithelium meet, such as in the cervix, anus and oropharynx.\textsuperscript{47} In the cervix, the junction between the two epithelia is situated towards the periphery of the ectocervix in the puberty and with age, it gradually moves towards and into the endocervical canal as the glandular cells are replaced by squamous cells (squamous metaplasia).

The carcinogenesis in squamous lesion is characterised by three stages: 1) acute infection with hrHPV, 2) viral persistence rather than clearance and the development of precancer lesion, and 3) invasion through the basement membrane of the epithelium.\textsuperscript{30} The age peak of HPV incidence in a population occurs in the first years following the average age of women becoming sexual active.\textsuperscript{48} The age peak of precancer lesions is seen 5-15 years later, and the peak or plateau of invasive cancer
incidence occurs one or several decades later.\textsuperscript{47} The carcinogenesis of adenocarcinoma is less understood than that of squamous ICC, although it is also caused by hrHPV and arises from glandular cells adjacent to the TZ or in the endocervical canal.\textsuperscript{1}

**Pathology**

*Intraepithelial squamous lesions*

The preinvasive squamous cellular changes are graded into the histopathological classes mild dysplasia/cervical intraepithelial neoplasia grade 1 (CIN1), moderate dysplasia/CIN2, and severe dysplasia/CIN3/carcinoma in situ (CIS).\textsuperscript{5,52} The grading is inclined to high rates of inter- and intraobserver variability, and more so for CIN1 than for CIN3.\textsuperscript{53} It is considered that persistent HPV infection of the squamous epithelium may lead to two categories of intraepithelial lesions: 1) productive and self-limited HPV infection, classified as koilocytosis, condyloma and CIN1, and 2) lesions with the potential to progress to invasive squamous cell carcinoma, classified as CIN2-3/CIS, and associated with hrHPV.\textsuperscript{1} CIN1 has been associated with any anogenital HPV and is unlikely to act as a cervical cancer precursor.\textsuperscript{54} Although CIN2 and CIN3 have different risk of progression to invasive disease (intermediate versus high risk), the reproducibility of the distinction between CIN2 and CIN3 is poor.\textsuperscript{55} Also, the morphological appearances do not allow distinction of lesions that will progress from those that will not.

The histopathological findings are characterised by abnormal cellular proliferation and maturation, together with nuclear atypia. In CIN1, the changes occupy the lower third of the epithelium and marked HPV cytopathic effects (koilocytosis) are often seen. In CIN2, the changes occupy the lower two thirds of the epithelium, and in CIN3, they occupy the full thickness of the epithelium and the nuclei are dense and irregular. Precancer lesions are also characterised by intracellular hrHPV DNA and chromosomal instability.\textsuperscript{1}

In Sweden, 3\textsuperscript{275} women were diagnosed with cervix cancer in situ in 2009.\textsuperscript{8}

*Intraepithelial glandular lesions*

Adenocarcinoma in situ (AIS) is defined as a preinvasive cervical lesion, although natural history studies to confirm its potential to progress are lacking.\textsuperscript{1} AIS is much less commonly diagnosed than the corresponding squamous preinvasive lesions. In a US database, only 2\% of the cervical in situ lesions were AIS.\textsuperscript{1} No terminology of glandular lesions with lower degrees of nuclear atypia has been established because they have been rare in biopsies.\textsuperscript{1,18,56} Coexisting CIN2-3 or invasive squamous ICC has been found in nearly two thirds of cases with AIS.\textsuperscript{57}
Natural history

It has been suggested that only 1% of CIN1 and 5% of CIN2 would progress to invasive cancer if left untreated, whereas over 12% of CIN3 would progress.58 The overall persistence rate of CIN2-3 was 50% and the regression rate was 29%. However, these rates might have been underestimated because the rates as well as follow-up time varied greatly between the reviewed reports, and also the age groups may have varied.6 Others have estimated the progression rate of any CIN to be 19-38%,59 and higher rates in women aged 35 years or more.60 The rate of invasive cancer in women with untreated biopsy verified CIN3/CIS was 31% during 30 years of follow-up, and 50% in women with persistent CIN3 after two years.61 The natural history of glandular lesions are poorly documented.62

Primary prevention of HPV infections

Health education

Health education programmes to promote sexual risk reduction behaviour may have some effect in reducing the risk of cervical cancer, as shown in a Cochrane review.63 Health education has shown to be effective in the HIV prevention in several countries, most notable in Uganda.64 However, since the protection from condom use against HPV transmission is not 100% efficacious, the effect of educational interventions may have limited effect.

Vaccines

Large RCTs have proven that the two licensed HPV vaccines are highly effective in preventing new infections with HPV16 and HPV18, and in prevention CIN2-3 lesions.65,66 Cross protection against HPV31, 33 and HPV45 has also been demonstrated.65 The introduction of vaccination is especially important in low income countries, where ICC is most common and the impact of screening has been limited. In Sweden, the National Board of Health and Welfare decided that HPV vaccine should be included in the childhood vaccination as a nationwide programme targeting 12 year-old girls from 2010, as a part of the school-health programme.67 However, it will be several decades before most women will benefit from the vaccines, and until then, cervical screening will remain the primary preventive strategy. Some modifications of the screening programme may be considered for vaccinated cohorts in the future. Efforts may also be needed to maintain high levels of population coverage in vaccinated women.68
Cervical screening

**Principals of screening**

The World Health Organisation (WHO) defines screening as “the presumptive identification of unrecognised disease by means of tests or examinations that can be applied rapidly”. The screening test should allow as few as possible with the disease to get through undetected (high sensitivity) and as few as possible without the disease to be subject to further investigation (high specificity). The likelihood that a positive screening test will give a correct result (positive predictive value) strongly depends on the prevalence of the disease in the population. Cervical screening for precancer and early stage cancer fulfils the Wilson and Jungner criteria for appraising the appropriateness of a screening programme. Cervical cancer is associated with high morbidity and mortality, the natural history is well known, it has a long sojourn time of detectable precancer, and treatment of precancer or early stage ICC leads to better outcome than late treatment. Further criteria are that the test must be acceptable, easy, and safe; the intervals for testing are determined; the benefit from screening outweighs the physical and psychological harm; and that screening is cost-effective. Broad coverage and full follow-up of abnormalities are key requirements for reducing the incidence of cervical cancer by screening.

**Effectiveness**

Although there are no randomised trials that have evaluated the efficacy of cervical screening, there are numerous observational studies that have showed effectiveness. Time trends form the Nordic countries have shown that both the incidence and the mortality from cervical cancer decreased after the introduction of organised screening in the mid 60s. The decrease was largest in Finland, where the mortality rates decreased over 80%. Further evidence of the effectiveness of screening has come from the United Kingdom, where incidence rates decreased rapidly after the introduction of organised screening in 1988.

In Sweden, the incidence of cervical cancer declined by 65% over a 40-year period, from 20 cases per 100 000 women (world standard rate) in 1965 to 6.6 in 2009. During the last 20 years the rate of the decrease has leveled off and the decrease is now slightly over 1% per year. The decline has been entirely attributed to squamous cell carcinoma (Figure 1). The incidence of adenocarcinoma increased until the late 80s, thereafter the incidence has been stable. The mortality form cervical cancer has also decreased, as shown in Figure 1.
The effect of screening on the incidence of adenocarcinoma is less well established than for squamous ICC,\textsuperscript{15,73} although a decrease in mortality was reported from Finland.\textsuperscript{13} Reasons for less effectiveness could be that precancer lesions of adenocarcinoma are poorly sampled from glands within the cervical canal, that the lesions are poorly recognised at colposcopy,\textsuperscript{47} or of a shorter precancer phase.\textsuperscript{75,76} In a case-control study from Australia, the risk of adenocarcinoma was not reduced by screening,\textsuperscript{12} but other studies do show an effect.\textsuperscript{10,77}

Studies have also shown that the effect of screening programmes has varied among different countries, which may be attributed to differences in the implementation of screening and in coverage of the target population.\textsuperscript{1,43,78}

**Ages and intervals**

The EU established principles for organised population-based cervical screening in the first edition of the European Guidelines for Quality Assurance in Cervical Cancer Screening in 1993.\textsuperscript{79} In the year 2003, the Council of the EU recommended implementation of population-based cervical screening programmes to the EU member states, with quality assurance at all levels in accordance with the European guidelines.\textsuperscript{2} According to these recommendations, screening should start at 20-30
years of age and continue at 3-5 years intervals until the age of 60 or 65. The basis for these recommendations is a multi-centre study from 1986, in which the incidence of squamous ICC was followed in women who at the age of 35 had had two negative smears. There was no additional impact of starting at the age of 20 compared to at age 25, or to screen with shorter intervals than every three years. Screening every year prevented 93% of all squamous ICC, screening every third year prevented 91% and every fifth year 84%. The rational for the upper age limit of 60-65 has been studies showing that well screened women seldom get precancer or cervical cancer after the age of 50. However, firm evidence on the optimal age to stop screening is lacking and studies using mortality as outcome are needed. Recent studies have suggested that women above the age of 50 years with previous negative cytology may have similar risk of ICC as younger women.

Organisation

An organisation that reaches a high proportion of the women with screening is essential for reducing cancer incidence. High population coverage even with long screening intervals is more effective than screening of a lower proportion of the women at shorter intervals. The EU recommends that at least 85% of the population should be covered by regular screening. It has also been shown that organised population-based screening is more effective than opportunistic screening. In the UK, the mortality and incidence decreased by 7% per year after the initiating of an organised programme in 1988, whereas the decrease was 1-2% in the preceding years since the introduction of screening in the 1960s. In Finland the effect of participating in organised screening was almost two-fold higher than that of opportunistic screening. In the EU, nine out of the 27 member states have a population based nationwide programme, and in five of them there is a national screening registry available. In a few countries the programme is nationally governed and monitored, such as in Finland (1964), the UK (1988) and Norway (1997). In other countries, like Sweden, screening is organised on a regional or county level. Coverage of the screening test taken within population-based programmes was below 80% in all the EU states in a recent report.

Factors that have been shown to increase participation are knowledge about the screening test, invitation letters offering a fixed appointment rather than open-ended invitations, letters signed by the physician rather than by the programme staff, and telephone reminders. Other important factors that are thought to increase coverage are invitations at the end of screening intervals rather than of birth cohorts; accessibility for women to change the time of screening online; regular re-invitations of non-responders; and screening free of charge.
Cervical screening in Sweden

Population-based cervical screening was introduced in Sweden 1964 and in all counties by the year 1977. In the beginning, most counties invited women aged 30-49 years to cervical screening every fourth year. In 1985, the National Board of Welfare recommended screening every third year in ages 20-59, and in 1998 the recommendation was changed to every third year in ages 23-50, and every fifth year in ages 51-60. However, because cervical screening has been organised at a county level, intervals and age limits, as well as policies regarding the management of abnormal cytology results varied, in particular in the 1970s and 1980s, and some variations still exist. Most counties (70%) invite women on the basis of time interval since last registered cytology, including opportunistic tests, and most counties (74%) re-invite non-responders the following year or earlier. The participation in cervical screening was 79% in 2006 during 3.5 years follow-up in ages 23-50 and 5.5 years in ages 51-60. The variation between counties was large, from 65% to 91% in the ages 23-50 years.

Evaluation

A screening programme needs evaluation and quality assurance at all levels. It is recommended that the entire programme is monitored continuously, including invitation, attendance, compliance, quality, and impact. Also, systematic audits of all cancer cases among screened and non-screened women are recommended. The audit process allows evaluation of routine service screening as opposed to extrapolation from clinical trials. Hence, audits may allow an estimation of the extent of the cervical cancer morbidity that can be affected by changes in the organisation, screening methods, or management and treatment of women with abnormal findings.

Screening by cytology

Conventional cytology

Traditionally, cytology has relied on smears that are performed as described by Papanicolaou in the 1940s. Cells are collected from the surface of the uterine cervix and the cervical canal, smeared on a glass slide, and analysed in a microscope. A single cytology test has limited sensitivity for histologically confirmed CIN2+ (70-80%), although it may vary considerably due to low-moderate reproducibility. The specificity is high (92%-99%) and the positive predictive value (PPV) is fairly high (42%). The sensitivity of cytology increases with the number of tests, and cytology screening has been successful because cervical cancer develops slowly, and repetitive screening rounds catch precancer lesions as they grow. Well organised cytology screening can reduce the incidence of squamous ICC by at least 80%.
False negative tests could be due to sampling error. The full circumference of the TZ must be sampled. However, a sample centred at the cervical os may not always collect the optimal cells, as in cases where the TZ is in the endocervical canal, or at the periphery of the ectocervix. Both the ectocervix and the endocervix must be sampled by either using 1) the combination of a wooden spatula (Ayre or Aylesbury) and an endocervical brush (e.g., Cytobrush), 2) a cervical broom (e.g., Cervex-Brush), or 3) an extended tip spatula (Aylesbury). In a Cochrane review the combination of an extended tip spatula (Aylesbury) and the Cytobrush appeared to be the most effective sampling method.

**Liquid based cytology**

Liquid-based cytology (LBC) is a new technique for transferring the cellular material to the microscope slide. The cells are sampled with Cervex-Brush or with a plastic spatula and an endocervical brush. Instead of smearing the cells on a slide, the material is immersed in a container with a special liquid and sent to a specially equipped laboratory. The advantages are that the technique increases the likelihood of representative smears and of less obscuring factors (blood, mucus, inflammatory cells), the interpretation of samples requires less time, and other analyses (hrHPV, Chlamydia) can be done on the same sample. Also, as the epithelial cells are spread in a thin layer, automated screening devices could be suitable.

Although a meta-analysis of international studies found no evidence of improved accuracy (sensitivity and specificity) with LBC compared with conventional cytology in the detection histologically verified CIN2+, a Swedish RCT showed a 40% increase in sensitivity with LBC. Several, studies have shown that LBC reduces the proportion of unsatisfactory samples.

Despite the higher cost, LBC has largely replaced conventional cytology in several countries, and the method is recommended by the Swedish Society for Obstetrics and Gynaecology.

**Terminology**

**Classification systems**

The terminology for classifying interpretations of cervical cytology has changed several times and differs between countries (Table 2). To unify the terminologies, the Bethesda system (TBS) has been introduced and is now widely used. The European guidelines recommend that all classification systems should be translatable into TBS. The latest modification of TBS was done in 2001 (Table 2).
In Sweden, a uniform classification has been elaborated by the Swedish Society of Pathology and Clinical Cytology, which mainly follows the CIN (Richart) system (Table 2). In the Swedish system, AIS and adenocarcinoma are classified together as “adenocarcinoma/AIS”, and atypical glandular cells are not subdivided into “AGC” and “AGC, favour neoplastic” as in TBS (Table 2). The Swedish system also includes the category “atypia in cells of uncertain origin” which is not found in other systems. In the latest revision from 2006, the former category ASCUS was divided into “atypical squamous cells - undetermined significance” (ASC-US) and “atypical squamous cells – high-grade dysplasia cannot be excluded” (ASC-H) according to TBS. A wide variety of different terminologies and SNOMED codes have been in use in Sweden, but during the last decade the recommended classification has become widely accepted. However, the interpretations of the categories vary substantially between the laboratories, in particular regarding low-grade changes.

**Specimen adequacy**

Cytology samples may be unsatisfactory for evaluation because there are too few cells, the cells are poorly fixed or they are obscured by blood or inflammatory cells. The recommended minimum requirement for adequacy is 8000-12000 visible squamous cells on a conventional smear, and 5000 cells on a LBC preparation. Specimens with more than 75% of squamous cells obscured should be termed unsatisfactory. However, if atypical cells can be identified, the smear is reported as abnormal regardless of the number of visible cells. If a smear is judged as unsatisfactory for evaluation, the reason why should be stated. Evidence of TZ sampling (endocervical cells (EC)) should also be recorded as a quality indicator, but it is not a requirement of its own for adequacy.

The former category “satisfactory but limited by” was eliminated in the 2001 revision of TBS, because the term was considered confusing to many clinicians and prompted
unnecessary repeat testing. This category included samples lacking EC/TZ components (i.e. metaplastic cells) and samples that were partially obscured by factors such as blood or inflammation. In a “partially obscured” specimen, 50-75% of the epithelial cells would not be visualised. In Sweden, the categories “limited evaluation” and “absence of EC” were retained in the revision of the system in 2006, even though the use of the category “limited evaluation” was discouraged.

Previously, lack of EC was considered as a reason to repeat the test, but because longitudinal studies have failed to show an increased risk of future precancer/cancer, a repeat test is in general not recommended any longer. However, data are conflicting and a repeat test within 12 months has been recommended in some guidelines. Data on the significance of partially obscuring factors are very limited. Two studies have reported no association with false negative reports.

Negative cytology

In TBS 2001, the category “negative for intraepithelial abnormality” includes results with reactive or degenerative repair alterations. In other systems, like the Swedish, the terms “benign specimen” or “normal” are used.

Abnormal cytology

The three-tier system of abnormalities (mild-moderate-severe dysplasia/CIN1-3), and the corresponding TBS two-tier system (LSIL-HSIL), represent interpretations of cellular changes that indicate the presence of squamous intraepithelial neoplasia with increasing severity. TBS includes “condyloma/koilocytosis” in the LSIL category, while it is not included in the CIN1 category (Table 2). The category ASC-US should only be used when there is genuine doubt on whether the changes are reactive (e.g. due to inflammation) or neoplastic. The category ASC-H is a subgroup of borderline changes in which CIN2+ is suspected but the diagnosis is uncertain for some reason, such as too few cells. The Swedish category “atypia in cells of uncertain origin” includes cells that are clearly atypical/neoplastic but might be too few to permit the diagnosis of type, or includes atypical cells other than squamous or glandular. In cases of glandular atypia, the cytology report should define whether the cytological glandular abnormality relates to cervical or endometrial glands.

Cytological interpretation are subjective and they are not optimally reproducible. Since they are not always accurate, the diagnosis cannot be determined without histopathology.

Cytology findings

Among the 650,000 Pap smears sampled in Sweden in 2006, approximately 5% showed abnormalities and 1% was unsatisfactory for evaluation. In addition, 8% lacked endocervical cells and less than 0.5% were reported to have had the evaluation
limited by obscuring factors. The most common cytological abnormalities were ASCUS (2%) and CIN1 (1.4%), followed by CIN2 (0.5%), CIN3/CIS (0.4%), atypia in cells of uncertain origin (0.3%), AGC (0.2%), and AIS (0.03%). However, the proportions of ASCUS and CIN1 may vary considerably between laboratories (0.6-3.9% ASCUS, and 0.2-5.2% CIN1).

Histological correlations to cytology findings

In a meta-analysis, the prevalence of CIN2+ in women with ASCUS was 10%, and in the large ASCUS-LSIL Triage Study (ALTS) the cumulative incidence of CIN3+ within 2 years was 9%. In Sweden, the reported rates of CIN2+ have been higher (19-21%). The probability of progression of ASCUS to invasive disease over 24 months was 0.25% in a meta-analysis. In a Swedish study the rate was 0.8%. It is noteworthy that most ASCUS diagnoses in the US and the UK were scored as normal in Sweden in a study in which references slides were exchanged for blind control evaluation.

In women with LSIL, the prevalence of CIN2+ was 17% in a meta-analysis. In the ALTS study the cumulative rate of CIN3 was 14-18% within 2 years. The reported rate of invasive cancer has been 0.15% within 2 years in, and 0.4% within 10 years. In Swedish studies, 21-55% of women with CIN1 have been found to have CIN2+, and 0.3-0.6% ICC.

In women with cytological CIN2-3, 77-90% have been reported to have histopathological CIN2+. The reported rate in Sweden is 79%. Four Swedish laboratories reported that 3.4% of the women had underlying ICC, while others have reported a rate of 1.4-1.6% ICC within two years.

In a systematic review, women with AGC had a rate of 14% underlying preinvasive high-grade lesion and 5.2% invasive cancer. The invasive cancers included endometrial adenocarcinoma (58%), cervical adenocarcinoma (24%), ovarian and fallopian tube carcinoma (6%), squamous cell carcinoma of the cervix (5%), and other cancers (7%). Data from four Swedish laboratories show 26% precancer and 18% invasive cancer. The corresponding rates for cytological adenocarcinoma/AIS were 20% preinvasive and 66% invasive lesions. In women with atypia in cells of uncertain origin, 26% were found to have preinvasive disease and 11% invasive cancer.

Screening by HPV testing

Primary cervical screening for hrHPV DNA is more sensitive in identifying CIN2+ than cytology, but it is less specific. In a low-resource setting, a single round of HPV DNA testing reduced the numbers of advanced cervical cancer and deaths from ICC significantly, while no such effect was observed in the cytologic-testing group or in the VIA group. A Swedish study has evaluated the efficacy of different screening
strategies based on HPV DNA testing. Primary hrHPV DNA screening with cytology triage and repeat HPV DNA testing of cytology-negative women was found to be the most effective strategy. Compared with cytology, this strategy increased the CIN3+ sensitivity by 30%, while a high PPV was maintained and the number of screening tests were only moderately increased (12%). With HPV DNA based screening the intervals may be extended because of the very high negative predictive values for precancer/cancer and a prolonged duration of protection compared to screening by cytology. Before implementation on a national level, pilot projects have been recommended to assess the appropriate age-groups, screening intervals, adverse effects and cost.

Management of cytology findings

Unsatisfactory cytology

Women with a Pap smear that has been unsatisfactory for evaluation need a repeat cytology. Although the necessity of early repeat testing after an unsatisfactory smear has been questioned, several studies have shown that women with unsatisfactory cytology have increased risk of subsequent precancer and cancer. It is recommended that the test should not be performed less than 3 months after a previous smear because the cervical epithelium needs time to regenerate. Treatment of infection is indicated before re-testing if there is suspicion of infection, and topical oestrogenic treatment is recommended if there are signs of atrophy.

Abnormalities

An abnormal cervical cytology report indicates that a precancer lesion might be present, which may progress to a life-threatening cancer if left untreated. The diagnosis of precancer has traditionally relied on colposcopy of the TZ and biopsy of suspected lesions. There is international agreement on that women with high grade squamous abnormalities and with glandular abnormalities need colposcopy, while evidence are lacking regarding the optimal management of women with low-grade abnormalities/CIN1.

Equivocal and low-grade squamous abnormalities

Because mild lesions are very likely to regress spontaneously, cytological surveillance (repeat cytology) of women with ASCUS or CIN1 has been the recommended or accepted management, such as in the current EU guidelines and in the former Swedish guidelines. The Swedish guidelines have recently been revised by the Swedish Society of Obstetrics and Gynaecology Working group for Cervical Cancer Prevention, in which repeat cytology is no longer recommended. The main reasons behind the new recommendation are the high prevalence of CIN2+ in women with
ASCUS and CIN1 in Swedish reports,\textsuperscript{119,120,141} and the risk of decreased compliance with cytological surveillance over time, as showed in a meta-analysis of RCTs.\textsuperscript{142}

**HPV DNA testing**

HPV testing has been introduced in many settings as a management strategy to identify those women with low-grade abnormalities who need colposcopy and biopsy. In a meta-analysis of RCTs, triage with hrHPV DNA testing was more effective than repeat cytology in women with ASC-US.\textsuperscript{117} The sensitivity and specificity for CIN2+ of Hybrid Capture 2 (HC2) was 95\% and 67\% respectively. Repeat cytology with ASCUS as threshold showed a sensitivity of 82\% and a specificity of 58\%. In the ALTS trial, repeat cytology referred more women for colposcopy than did triage with HPV testing during 2 years of follow-up (73\% vs 53\%).\textsuperscript{118} In the same trial, immediate colposcopy showed a lower sensitivity for cumulative CIN3 than HC2.

In women with CIN1, however, HPV testing was not enough selective in a meta-analysis of RCTs.\textsuperscript{123} Repeat cytology and HC2 showed the same sensitivity for CIN2+ (92\% vs 95\%) and a low specificity for both methods (42\% vs 33\%). In the ALTS trial, both repeat cytology and HPV triage referred the majority of women for colposcopy during two years of follow-up (89\% vs 85\%).\textsuperscript{124} They concluded that LSIL is best managed by colposcopy initially.\textsuperscript{124}

In a Swedish RCT, hrHPV testing increased the sensitivity for CIN2+ by 32\% compared to repeat cytology in women with ASCUS or CIN1.\textsuperscript{141} In this trial, most of the women had ASCUS (79\%), the overall HPV positivity was 60\%, and 33\% of all women were diagnosed with CIN2+. The histological diagnosis was based on a cone/LLETZ biopsy of all women with a positive HPV test and/or a positive repeat Pap smear. In another Swedish RCT, triage with hrHPV testing detected a similar proportion of CIN2+ as did direct referral to colposcopy (23\% vs 20\%) in women with ASCUS or CIN1.\textsuperscript{125} The HPV positivity was 64\% in ASCUS cases and 77\% in CIN1 cases, with higher prevalence in younger women. In another Swedish study the hrHPV positivity was 49\% for ASCUS and 71\% in LSIL cases, with similar prevalence after the age of 25 years.\textsuperscript{143}

**Recommendations**

In the Swedish guidelines, the same recommendations are given for women with ASCUS and CIN1 because of the heterogeneity between the laboratories in the use of the terms, and also because of similar risk of CIN2+ in some laboratories.\textsuperscript{3,126} Triage with hrHPV DNA testing is recommended in older women with referral for colposcopy in HPV positive cases. Direct referral for colposcopy is recommended when HPV testing is not used. The age limit for HPV testing will depend on the cost of the test, as well as on the HPV prevalence in different ages in women with ASC-US/CIN1. Women who are hrHPV negative in triage are recommended repeated cytology after one year.\textsuperscript{2,3,144}
In the EU guidelines, HPV DNA testing is the recommended management for women with ASC-US. Cytological surveillance is an acceptable option to HPV testing, with referral to normal screening if the repeat smear after 6-12 months is negative. For women with LSIL/CIN1, acceptable management is repeat cytology at 6 months interval with two subsequent negative smears before referral to normal screening. However, it is pointed out that potential loss to follow-up should be taken into account and referral to colposcopy is an option, as well as hrHPV testing of older women.

A policy of immediate treatment without histological verification of CIN (“see and treat”) in women with low-grade squamous abnormalities is not recommended. In a large RCT it led to overtreatment and more after effects, such as severe bleeding, and to no difference in the cumulative incidence of CIN2+ compared to a policy of biopsy and selective recall for treatment.

Normal colposcopy/biopsy or CIN1 in biopsy

In younger women with histological CIN1 and a satisfactory colposcopy, observation is often the preferred management in order to avoid adverse reproductive outcomes. Women with persistent CIN1 are recommended treatment.

Women with normal colposcopy and biopsy are recommended follow-up during two years. The ALTS trial found equivalent risk of a subsequent diagnosis of CIN2+ during two years follow-up in women with LSIL and HPV positive ASCUS, regardless of whether the initial colposcopy results were CIN1, negative biopsies, or normal colposcopic impression leading to no biopsies.

High-grade squamous abnormalities and ASC-H

Women with CIN2-3 and ASC-H should always be referred for colposcopy and biopsy according to guidelines. The vast majority of women with CIN2-3 are hrHPV positive (90-100%), and the majority show CIN2+ upon histology. When the colposcopic impression correlates to high-grade dysplasia, a “see and treat” strategy may be considered. In cases of fully visible TZ and negative colposcopy and biopsy, the cytology should be reviewed. If the cytological interpretation is upheld and no lesion is found upon colposcopy of the vagina, a diagnostic excision of the TZ and endocervical curettage (ECC) is recommended. If the colposcopy is unsatisfactory, the TZ needs to be excised including the lower third of the cervix and an ECC of the remaining cervix.

Glandular atypia and atypia in cells of uncertain origin

Women with atypical glandular cells or atypia in cells of uncertain origin are recommended further investigation with colposcopy. Repeat cytology is insufficiently sensitive. If no lesion is detected with colposcopy, or if the colposcopy
is unsatisfactory, ECC or a diagnostic conisation is recommended. In older women (>35-40 years) or in women with abnormal bleeding, an endometrial biopsy should be taken as well. Triage with hrHPV testing may distinguish between risk of endometrial cancer and cervical cancer, particularly in women 50 years of age or older. When no cervical or endometrial malignancy is found, the possibility of an ovarian or fallopian tube cancer should be considered.

**Colposcopy**

The aims of colposcopy are to: 1) determine the position of the TZ, 2) confirm or refute the suspicion of CIN, 3) recognise or rule out invasive cancer or glandular disease, 4) facilitate treatment, and 5) monitor progression or regression of CIN.

The colposcope is a microscope that allows the cervix to be viewed with a magnification of 6-40 times. After application of 3 or 5% solution of acetic acid onto the cervix, precancer lesions usually appear “acetowhite”. However, also immature squamous epithelium appears acetowhite, as well as healing epithelium and HPV infections. The entire TZ needs to be identified and it is recommended that the size, position and visibility of TZ are classified into three different types (Table 3). In TZ type 1, the TZ is fully visible on the ectocervix. If the TZ extends into the cervical canal (TZ type 2-3), an endocervical speculum can be used to visualise the lower part of the endocervix. If the squamo columnar junction is not fully visible (TZ type 3), the examination is judged unsatisfactory.

<table>
<thead>
<tr>
<th>Type of TZ</th>
<th>Ectocervical size</th>
<th>Site</th>
<th>Visibility</th>
<th>Adequacy colposcopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>Small or large</td>
<td>Completely ectocervical</td>
<td>Fully visible</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>Type 2</td>
<td>Small or large</td>
<td>Partially endocervical</td>
<td>Fully visible</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>Type 3</td>
<td>Small or large</td>
<td>Partially endocervical</td>
<td>Not fully visible</td>
<td>Unsatisfactory</td>
</tr>
<tr>
<td>Type 3</td>
<td>-</td>
<td>Totally endocervical</td>
<td>Not fully visible</td>
<td>Unsatisfactory</td>
</tr>
</tbody>
</table>

The following features of the epithelium in colposcopy related to the architecture of the blood vessels have been considered as indicators of precancer: coarse punctuation, coarse mosaic, atypical irregular vessels (suggestive of ICC), dense aceto-whiteness, and sharp borders of the lesion. In some cases, the CIN is located in the gland clefts and may show a white rim around the gland opening (umbilical mosaic). The evaluation is subjective and poor inter- and intraobserver agreement has been reported. The assessment may be more difficult in postmenopausal women who are not on oestrogen therapy, as well as falsely negative in case of glandular cervical lesions or in case of endocervical location of CIN.

Meta-analysis of earlier studies of colposcopy has estimated a high sensitivity for detecting CIN2+ (98%) and a moderate specificity (48%). However, more recent studies have shown that the sensitivity of colposcopy is substantially lower than...
Introduction

Many earlier studies have been biased by the fact that the histological verification of the “true” diagnosis has depended on colposcopy itself. Colposcopy has often guided the site of the biopsy and colposcopically negative cases have often been considered as truly negative without histological confirmation, thus inflating the estimate of sensitivity. In a study with less verification bias, the sensitivity of colposcopically directed biopsy for CIN2+ was 57% in women with satisfactory colposcopy. In this study, the histological diagnosis was verified by biopsies taken also from the four quadrants of the TZ, as well as endocervical curettage. In the ALTS trial, the initial colposcopy identified 54-56% of cumulative CIN3+ cases diagnosed over 2 years in women with ASCUS or LSIL. Excluding women with unsatisfactory colposcopy increased the sensitivity to 70%. Also, it has been demonstrated that the detection of CIN2+ is more related to the number of biopsies taken, rather than to the training of the colposcopist, and that taking random biopsies in four quadrants increases detection.

Studies have shown a poor correlation between colposcopic impression and histological diagnosis. In order to standardise the terminology of colposcopy findings, the International Federation for Cervical Pathology and Colposcopy introduced a classification system in 2002. The use of this system has shown to improve the agreement between colposcopic interpretation and histology.

Scoring systems have been elaborated to further assist in the prediction of histological diagnosis, of which the Reid index is the most well known. However, the effectiveness of colposcopic grading for identification of CIN2-3 has been questioned in studies. It has been proposed that only the degree of acetowhite change is able to significantly predict CIN, and that all acetowhite lesions should be assessed with biopsy to maximise sensitivity. On the other hand, recent data indicate that the acetowhite changes may be related to HPV type, as well as that CIN2-3 lesions may have thinner epithelium than CIN1 making the lesion less white. These findings would explain why not all high grade lesions have acetowhite changes. It has also been shown that CIN3 lesions missed by colposcopy are smaller than those detected visually.

A new scoring system, the Swede score, has been proposed by Strander et al (Table 4). In this system, the size of the lesion is included in the grading. A total score of 8 or more had a specificity of 90% for CIN2+ and no CIN2+ scored less than 5. It has been suggested that a cutoff value of 8 might be used for the selection of women to “see and treat”. A recent UK evaluation found the Swede score simple to use with no major learning curve, that it was a useful training tool, although the accuracy was somewhat lower than in the Swedish study.

In a Cochrane review, it was concluded that colposcopy has an established role in determining the most suspicious areas for colposcopically directed biopsies and in planning effective treatment, but that it was not a diagnostic test and cannot substitute reliable histological evaluation.
Table 4. The Swede score system.172

<table>
<thead>
<tr>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetouptake</td>
<td>0 or transparent</td>
<td>Shady, milk</td>
<td>Distinct, stearin</td>
</tr>
<tr>
<td>Margins and surface</td>
<td>0 or diffuse</td>
<td>Sharp but irregular, jagged, geographical. Satellites</td>
<td>Sharp and even, difference in surface level including 'cuffing'</td>
</tr>
<tr>
<td>Vessels</td>
<td>Fine, regular</td>
<td>Absent</td>
<td>Coarse or atypical vessels</td>
</tr>
<tr>
<td>Lesion size</td>
<td>&lt;5 mm</td>
<td>5–15 mm or 2 quadrants</td>
<td>&gt;15 mm or 3–4 quadrants or endocervically undefined</td>
</tr>
<tr>
<td>Iodine staining</td>
<td>Brown</td>
<td>Faintly or patchy yellow</td>
<td>Distinct yellow</td>
</tr>
</tbody>
</table>

**Cervical biopsy**

A biopsy of the cervix is taken under colposcopic vision from the most abnormal area/s and sometimes randomly from the TZ when no abnormality can be identified.2,161 Punch biopsies are taken by specially designed cervical biopsy forceps including both the epithelium and the underlying stroma. A punch biopsy is only a few millimeters large and is often too superficial for ruling out microinvasive carcinoma, in which case the use of a small diathermy loop for taking small biopsies is superior.2 Local anesthesia is needed if loop diathermia is used, and may also be used in punch biopsy taking to reduce the discomfort.2

**Endocervical curettage**

Endocervical evaluation by cytology or curettage is sometimes used when colposcopy is unsatisfactory or when an endocervical lesion is suspected. However, the significance of adding ECC to biopsy to improve sensitivity of colposcopy is controversial.159 Histological results of ECC are often inconclusive because the specimen is superficial.174 Also, ECC distorts the architecture of the tissue, making it difficult to distinguish AIS from invasive lesion.2 Performance under local anesthetic might improve the sampling.175 Without anesthesia, sampling with an endocervical brush may decrease the false negative rate.174

Studies have shown that ECC detects 2-6% of all CIN2+ that otherwise would have been missed by cervical biopsies alone.161,176,177 However, in older women above 40 years of age ECC increases the sensitivity by 13-18%.176,177 Thus, the utility of ECC would be greatest among older women, but on the other hand, a diagnostic conisation or LLETZ is usually preferred when an endocervical lesion must be ruled out.2,3
**New technology**

Optical spectroscopy is a new technique to enhance the diagnostic accuracy of colposcopy, although further evaluation is needed. In one study, spectroscopy worked as well as, or slightly better, than colposcopy for the detection of CIN 2+. New biomarkers are also currently evaluated to identify more specifically women who are at risk for progression to cancer. The most studied is the cellular tumour suppressor p16, and it has shown promising outcomes in terms of its ability to identify high-risk cases among women with equivocal and low-grade abnormalities, as well as in improving the interobserver agreement for histology among pathologists. Other progression markers that may assist colposcopy are HPV E6/E7 mRNA, HPV DNA typing, type-specific viral load, and DNA methylation. The adoption of a risk estimation model to guide clinical decisions has been suggested. Such a tool would assign a risk based on each combination of a woman’s age, current clinical test results and past results, and would account for the lack of complete sensitivity of the colposcopic biopsy.

**Treatment of CIN**

Women with histological CIN2+ need treatment. However, in very young women with CIN2 in biopsy, observation and careful follow-up may be considered, because CIN2 lesions in adolescents and women below 25 years of age show high rates of regression (85%).

Treatment can be ablative/destructive or excisional. Although there is no obvious superior method for eradicating CIN, excisional methods are preferred in most cases. The presence of a high-grade lesion indicates that the entire TZ is at risk and therefore the entire TZ needs to be removed.

The choice of therapeutic approach depends on the suspected diagnosis, the size and type of TZ, and on age and fertility aspirations. Excision permits a histological evaluation of the excised tissue. The excisional techniques used are large loop excision of the transformation zone (LLETZ), cold knife conisation, laser excision and needle excision of the TZ (NETZ). In LLETZ, which is synonymous to the North American term LEEP, a diathermy loop is used. This method has largely replaced the laser conisation technique in Sweden during the past 10-15 years because it is rapid and considered easy to learn.

Ablative therapy destroys the lesion and the TZ by using radical diathermy/electrodiathermy, laser vaporisation, or cryotherapy. The methods are considered to be safe if the following criteria are met: a histological diagnosis before treatment; the entire TZ is visible; no glandular atypia or suspicion of invasive cancer; treatment is performed under colposcopic control; and no history of previous treatment.
Cryotherapy should not be performed in women with large lesions, and it is not effective in CIN2+ according to a recent systematic review. Hysterectomy may be performed in cases of recurrent CIN or when there are other indications for hysterectomy as well. However, invasive disease must be ruled out before hysterectomy, because ICC cases may need radical hysterectomy and extirpation of pelvic lymphglands.

**Complications**

Treatments for CIN have very low rates of complication in the short term. There may be severe bleeding, discharge or infection in a few percent. Long term complications include cervical stenosis and cervical insufficiency causing mid-trimester abortions or preterm delivery. All excisional treatments were associated with increased risk of subsequent premature delivery in a meta-analysis, and cold knife conisation, laser conisation and radical diathermy have associated with increased risk of severe preterm delivery (<32 weeks). Laser ablation and cryotherapy did not increase the risk of severe serious adverse pregnancy outcomes. Recent studies on the risk of preterm birth after LLETZ have shown conflicting results. The results of one study suggest that the risk of preterm birth may be intrinsic to the women who develop CIN.

**Follow-up**

In 90% of cases treatment is effective in eradicating CIN. Failures rates increase in cases of involved excision margins, older age or glandular involvement. If CIN is found in the endocervical sample taken after treatment, or at follow-up, a re-conisation should be considered. There is no consensus on the optimal follow-up policy, interventions or frequency in surveillance after treatment. HPV testing after treatment has shown to predict residual/recurrent CIN with higher sensitivity than both cytological follow-up and histology at the section margins, and is therefore recommended in the Swedish guidelines.

Women treated for CIN have an increased long-term risk of ICC for at least 20-25 years. They may also have increased long-term risk of mortality from ICC, as shown in a large cohort study from Finland. However, in a case-control study from the university hospital of Helsinki, treated women had no increased risk of mortality from ICC. These results underline that women treated for CIN need careful follow-up and long term surveillance.
Adverse effects of screening

The benefits of screening are counteracted by the adverse effects of testing large populations of healthy women to prevent significant disease in a few. The negative effects may be psychological consequences of positive tests (anxiety and fear); misunderstanding of the meaning of negative test (interpretation as “no risk” instead of “low risk” which could lead to underinvestigation of symptoms); false positive test results leading to unnecessary investigations and costs; false negative test results leading to delayed intervention against ICC; overdiagnosis and overtreatment of lesions that would never have progressed to ICC; and treatment complications such as pregnancy related morbidity. For very young women, the risk of harm from screening may be greater than the risk of benefit. In the future, new biomarkers may be used for the selection of women with lesions who would need treatment, thus reducing over- and undertreatment.

Challenges in cervical cancer prevention

Globally there is an enormous challenge to implement organised cervical screening in all countries and HPV vaccination of all school girls, particularly in countries with the highest incidence and mortality rates of ICC.

In Sweden, as well as in other countries with organised cervical screening and low incidence of ICC, the challenges are on the one hand to optimise the efficacy of the screening programme and to prioritise the most effective preventive methods as new technology is emerging. On the other hand, the challenge is to do as little harm as possible by avoiding unnecessary investigations and treatment, without putting women at increased risk of ICC.

Although nonparticipation in screening has been found to be the major reason for the remaining morbidity and mortality caused by cervical cancer in countries with screening programmes, cervical cancers do occur among screening participants. In settings with organised cervical screening, up to one half of all cancer cases have been reported as adequately screened, and this proportion rises as coverage increases. Studies have shown that a substantial proportion of screened women with ICC have had abnormal Pap smear findings more than 6 months before cancer diagnosis, with rates of 36-70% in some studies. Therefore, the reasons for why women still develop ICC despite having had a previous positive test need to be investigated.

The effectiveness of cervical screening is dependent on a chain of procedures. Each step in the programme needs auditing in order to identify weaknesses and possible areas for improvements. In the present study, the effect of the screening programme has been evaluated, as well as the significance of different cytology reports and the management of women with abnormal findings.
AIMS OF THE THESIS

The overall objective was to explore risk factors for cervical cancer associated to the cervical screening programme, hence to identify possible areas for further improvements in the prevention of cervical cancer.

The specific aims were:

- To evaluate the effectiveness of the cervical screening programme by comparison of the screening histories of women with and without invasive cervical cancer.
- To evaluate different cytological screening reports in terms of subsequent risk of invasive cervical cancer.
- To evaluate the risk of invasive cervical cancer related to management of women with an abnormal cytology finding.
- To assess the impact of histological investigation and treatment of women with abnormal cytology on the subsequent risk of invasive cervical cancer.
METHODS

The key features of the methods used are presented in Table 5.

Table 5. Overview of methods used in the case-control studies I-IV (Paper I-IV).

<table>
<thead>
<tr>
<th>Paper</th>
<th>Research question</th>
<th>Setting and participants</th>
<th>Data sources</th>
<th>Main exposure variables</th>
<th>Main outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Why does ICC occur in Sweden? What is the effectiveness of the cervical screening programme?</td>
<td>All cases of ICC in Sweden 1999-2001, aged-matched controls, screening histories ≤6.5 yrs before ICC.</td>
<td>SCR, NPR, SNCSQR, clinical records.</td>
<td>Screened or not; negative or abnormal results, abnormal results with or without biopsy.</td>
<td>Screening history: ICC risk stratified on squamous/non-squamous ICC, age-groups, and FIGO stages.</td>
</tr>
<tr>
<td>II</td>
<td>What are the risks of ICC related to cytological results in the screening history?</td>
<td>Cases and controls in study I, &lt; 67 yrs with cytology reports 0.5 to 6.5 yrs before ICC.</td>
<td>SCR, NPR, SNCSQR.</td>
<td>Category of cytological reports; cytological diagnosis at first abnormal smear.</td>
<td>ICC risk stratified on squamous/non-squamous ICC and on FIGO stages.</td>
</tr>
<tr>
<td>III</td>
<td>What are the risks of ICC related to management of an abnormal smear finding?</td>
<td>Cases and controls in study II with a report of abnormal/unsatisfactory smear.</td>
<td>SCR, NPR, SNCSQR.</td>
<td>Cytology only, histology, or no morphology ≤2 yrs of first abnormal/unsatisfactory smear.</td>
<td>ICC risk stratified on cytological abnormalities.</td>
</tr>
<tr>
<td>IV</td>
<td>What are the risks of ICC related to histological investigation and treatment in women with cytological abnormalities?</td>
<td>Cases and controls in study III with a report of abnormal smear.</td>
<td>SCR, NPR, SNCSQR, questionnaire, histopathological reports.</td>
<td>Type of histological investigation after a report of abnormal smear; and type of treatment.</td>
<td>ICC risk stratified on cytological abnormalities and on histological results before treatment.</td>
</tr>
</tbody>
</table>

Abbreviations: ICC, invasive cervical cancer; FIGO, International Federation of Gynecology and Obstetrics; NPR, national population register; SCR, Swedish Cancer Registry; SNCSQR, Swedish national cervical screening quality register.

Study design and participants

A nationwide and population-based case-control study design has been used for all four studies. The studies in Paper II-IV form parts of the large audit of the Swedish cervical screening programme presented in Paper I.

The study base was the whole female population of Sweden, which in the midpoint of the study (i.e. year 2000) was approximately 3.7 million women. All cases of primary epithelial invasive cervical cancer diagnosed between January 1, 1999, and December 31, 2001 were included in study I. To identify the case women, we retrieved all cases of invasive cervical epithelial carcinoma and unspecified uterine cancer reported to the Swedish Cancer Registry, and their archival histological
specimens were obtained and reexamined by a reference pathologist. When no archival histological specimens were available the clinical and pathology records were obtained.

For each case subject, five age-matched control subjects were randomly selected from the national population register. The matching was made by year of birth at the date of the cases’ cancer diagnosis. The control subject had to be alive at the date of cancer diagnosis for the respective case subject and not diagnosed with invasive cervical cancer up to that date.

In Paper II we included the subgroup of case and control women in the audit who had cervical cytology registered 0.5 to 6.5 years before the date of the cases’ cancer diagnosis and were younger than 67 years of age. The age limit 67 was chosen to include only the recommended screening ages plus recently tested women above 60. Nationwide data on screening were available for a minimum of 6.5 years prior to cancer diagnosis (from mid 1992 to 2001). The time limit 0.5 years was set to not include a test that had led to the detection of invasive cancer.

In Paper III the inclusion criteria were having had an abnormal and/or an unsatisfactory cytology result 0.5-6.5 years before cancer diagnosis and being under 67 years of age, and in Paper IV we included the case and control subjects who had an abnormal cytology result during the same period and who were below 67.

**Ethics**

The studies were approved by all seven regional ethical review boards. Since the studies were based on data from registers and patient records the participants were not at risk of suffering from the studies. When analysing the data the researchers could not identify the participants by name or personal identification numbers. Therefore, it was approved that the studies could be performed without informed consent of the participants. It was anticipated that the studies would contribute to the understanding of why cervical cancer still exist in Sweden and assist in improving the effectiveness of the screening programme.

**Outcome variables**

The main outcome studied was invasive cervical cancer. In study I and II we also divided the outcome in squamous and non-squamous cell carcinoma, as well as in FIGO stages in Paper II. Screening history, age, clinical tumour stage and histological type were outcomes in the description of the cancer cases in study I.
Exposures variables

**Screened or not screened**

A history of having had a cytology test registered or not, within the recommended screening interval, was studied in Paper I. Last screening interval was defined as 0.5-3.5 years before cancer diagnosis in women 53 years old or younger, and as 0.5-5.5 years before cancer diagnosis in women 54-65 years of age. In women 66 years or older exposure was any cytology registered 0.5-6.5 years before cancer diagnosis.

In the analysis of the effect of screening on tumour stage (Paper I), having had cytology within 6 months but more than one month before cancer diagnosis or not was also included as a variable. Cases with cytology 1-6 months before diagnosis were considered as presumed screen detected cancer cases, and those without as presumed symptomatic cases.

**Adequate, partially adequate or non-adequate screening results**

All the reported cytological diagnoses in a woman’s screening history 0.5-6.5 years before the cases’ cancer diagnosis were analysed in Paper II. The screening history regarding adequacy was categorised into: 1) all smears negative without limited evaluation; 2) all smears negative, any with partially obscuring factors; 3) all smears negative, any with absent EC/TZ cells; and 4) a smear unsatisfactory for evaluation at least once and no abnormal smear. “Negative” was defined as the absence of atypia or koilocytosis.

**Normal or abnormal screenings results**

A history of negative or abnormal screening results were exposures studied in both Paper I and Paper II, but from slightly different views. In Paper I, having had a negative test only within the recommended interval was compared with having had an abnormal finding within the interval. A report “unsatisfactory for evaluation” was classified as abnormal unless it was followed by a normal result.

In Paper II, having had negative and satisfactory cytology 0.5-6.5 years before cancer diagnosis, was compared with having had an abnormal finding, regardless of interval. The screening history regarding abnormalities was categorised into: 1) low grade squamous cell atypia only at least once; 2) high grade squamous atypia at least once (no glandular/“other” atypia); 3) glandular or “other” cell atypia at least once (no high-grade squamous atypia); and 4) abnormal smears at least twice with different cytological categories of abnormality. “Low grade squamous cell atypia” was defined as koilocytosis, ASCUS, and CIN1; and “high grade squamous cell atypia” as CIN2 and CIN3/CIS. “Glandular or “other” cell atypia” was defined as AGC, adenocarcinoma in situ, and atypia in cells of uncertain origin. When there was more than one cytological diagnosis in the same report the “most severe” was considered as
the main result. Severity was ranked as follows: koilocytosis, ASCUS, CIN1, atypia in cells of uncertain origin, AGC, CIN2, CIN3/CIS and adenocarcinoma in situ.

**Management of abnormal cytology findings**

**Completeness of follow-up**

A history of having had a first, second, and third follow-up visit or not, with morphology within 25 months after an abnormal finding, was studied in Paper III.

**Repeated cytology or biopsy**

Management by repeat cytology only, or by biopsy, after an abnormal cytology was addressed as exposures in Paper III. Management was defined as the presence of cytology only or of any cervical histopathology within 25 months after the first recorded abnormal Pap smear. The time limit within 25 months was chosen because low-grade abnormal smears could be kept under cytological surveillance until 2 years according to former guidelines. In women under cytological surveillance only, the number of repeated tests and the number of negative results were also examined.

**Histological investigations**

Having had a biopsy or not after abnormal cytology was studied in Paper I, as well as in paper III-IV. In Paper III, we also investigated the time between the first abnormal smear report and the date of the first histological sampling, and the proportion of all histological assessments that were done at the first follow-up visit. In Paper IV, we assessed the following means of histological investigations: 1) punch biopsy and/or cervical curettage, or cone biopsy/LLETZ at first histological investigation (“see and treat”), 2) adding endocervical curettage or not to the investigation, and 3) use of colposcopy or not, and if colposcopy had been satisfactory or not.

**Treatment**

In Paper IV we also included exposure variables regarding any treatment in connection with or following a histological sampling up until 2 months before the cases’ cancer diagnosis. The variables were: 1) treatment or not, 2) treatment by excision/conisation, ablation only, or hysterectomy, 2) LLETZ or laser conisation among those treated only once, 3) dysplasia involvement or not in resection margins and 4) retreatment or not.

**Management of unsatisfactory screening results**

Follow-up or not within 13 months after an unsatisfactory cytology result was investigated in Paper III. Follow-up was defined as the registration of a repeat Pap smear and/or of cervical histopathology.
Potential confounders and effect modifiers

Age was regarded as a potential confounder and/or effect modifier in all the studies, and FIGO stage at cancer diagnosis was considered as a potential effect modifier in Paper I and II. In Paper II, we also considered as potential confounders number of negative result in those without abnormal cytology, number of negative results before the first abnormal cytology, time since last negative result, and whether screening was done within the recommended screening interval or not. In Paper IV, time from first abnormal cytology to cancer diagnosis, category of cytological abnormality, highest grade of histopathology before treatment, and level of treatment centre (when comparing LLETZ versus laser conisation) were considered as potential confounders.

Data sources and collection

For the case subjects, data on age at cancer diagnosis and on histological type of cancer were collected from the Swedish Cancer Registry, and information about the FIGO stage was collected from regional databases or from clinical records.

Data on cervical cytology and histology were obtained from the Swedish national cervical screening quality register. The registry has collected data from all 30 histopathological and cytological laboratories in Sweden. The completeness of the registry regarding cytology was 90% in 1991 to 1993, and 98% in 1994 to 2001. Conventional cytology was used in Sweden during this period. The following data were available for each report: the personal identification number of the tested woman, date of test, specimen number, analysing laboratory, and one or more diagnostic codes. All the 159 different diagnostic codes, and combinations of codes, used in the reports were converted to the 14 Systematized Nomenclature of Medicine (SNOMED) codes that are currently in use in Sweden.

In study IV, we identified all study participants who had histology from the cervix or uterus registered within 25 months after an abnormal cytology report, and the results of all their histology registered until 61 days prior to the date of cancer diagnosis were collected. For each histopathological report, a questionnaire regarding examinations and treatment was then sent to the clinic where the woman had been managed (see Appendix A). For patients managed at clinics no longer in operation, data were requested from medical records filed at the County Council Archive. When the medical record was missing, as well as for patients who had undergone excisional treatment, the full histopathological report was requested from the laboratory.
Statistical methods

Independent T-test and Mann-Whitney U test were used for testing equality of means. Non-parametric median test for independent sample was used for testing equality of medians. Pearson Chi-Square test and Fisher’s exact test for small samples were used for test of independency when proportions were compared and for test of homogeneity. All tests were two-sided and we considered statistical significance as \( p < 0.05 \).

To estimate the risk of ICC associated with different exposures, we compared the presence of exposure/s in cases and controls with a chosen referent. Odds ratios (ORs) were considered to approximate to relative risk and were calculated in logistic regression models with 95% confidence intervals (CI). The regression analysis was conditional in Paper I, and it was unconditional in Paper II-IV, as well as in the sub-group analyses in Paper I.

In the unconditional regression analyses, adjustment for age at cancer diagnosis was done in Paper I-II and for age at the earliest recorded abnormal finding in Paper III-IV. Since the relation between age and the outcome was non-linear, age was categorised in 5-year classes in Paper I and in 10-year classes in Paper II and IV. Likewise, in Paper II, number of cytology results was grouped into four categories and time since last negative result was grouped into seven. The selection of further covariates to be included in the model, in addition to adjustment for age, was based on significant changes (>10%) of any OR.

Interaction was tested in logistic regression models to estimate p-values for homogeneity over age categories (Paper I-II) and FIGO stage (Paper I). In Paper III-IV, the effect of management was stratified on category of cytological abnormality and in paper IV also on grade of histological result before treatment.

SAS statistical software (version 9.1; SAS Institute, Cary, NC) was used for the analyses in Paper I, and SPSS Statistics (version 15.0.1, 17.0 and 18) was used in Paper II-IV.
RESULTS

Characteristics of cases and controls

Figure 2 shows the numbers of individuals that were confirmed eligible for the audit and the numbers that were included in each study.

**Figure 2.** Flow diagram of case and control subjects included in Paper I-IV.

Among all potentially eligible cases, 194 were not included because histopathological reexamination showed that they had not primary cervical carcinoma, and 26 of the controls were excluded because they had previous ICC (Paper I). In total, we found 1230 cases of primary epithelial invasive cervical cancer women, and 6124 eligible control women were included. Seventy percent of the cancer cases were younger than 67 years of age at diagnosis and thus had evaluable screening histories. Among them, two-thirds had cytology reported and were included in Paper II. Among those with cytology, 28% of cases had a history of an abnormal or unsatisfactory finding and were included in study III and the 25% with abnormalities were included in Paper IV. The corresponding numbers of control subjects included in the Papers II-IV are
shown in Figure 2. The proportions in Figure 2 are those out of all case and controls subjects in the audit. In Paper IV we found that 55 case women and 103 control women had histology registered and so we sent a total of 248 questionnaires to gynaecologists at 83 different clinics. Information about the clinical management was fully obtained in 96% of the cases and in 95% of the controls.

The main characteristics of the study participants are summarised in Table 6.

### Table 6. Main characteristics of the study participants in Paper I-IV.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Paper I</th>
<th>Paper II</th>
<th>Paper III</th>
<th>Paper IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>1230</td>
<td>6124</td>
<td>572</td>
<td>3569</td>
</tr>
<tr>
<td>Median age at date of cancer diagnosis(\text{a}) (IQR)</td>
<td>51 (39-71)</td>
<td>51 (39-71)</td>
<td>43 (35-51)</td>
<td>44 (36-52)</td>
</tr>
<tr>
<td>Squamous ICC (%)</td>
<td>921 (75%)</td>
<td>-</td>
<td>380 (68%)</td>
<td>-</td>
</tr>
<tr>
<td>Non-squamous ICC (%)</td>
<td>309 (25%)</td>
<td>-</td>
<td>182 (32%)</td>
<td>-</td>
</tr>
<tr>
<td>FIGO stage, N (%): IA</td>
<td>251 (23%)</td>
<td>-</td>
<td>184 (32%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>IB</td>
<td>502 (41%)</td>
<td>-</td>
<td>295 (52%)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>477 (38%)</td>
<td>-</td>
<td>33 (16%)</td>
</tr>
</tbody>
</table>

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; IA, microinvasive; IB, localised cancer; II or higher, advanced cancer; N, number of women; IQR, inter-quartile range.

\(\text{a}\) Corresponding date for the control women.

\(\text{b}\) Adenocarcinoma, adenosquamous carcinoma, poorly differentiated, small cell, and neuroendocrine carcinoma.

### Ages

The youngest cancer case was 21 years of age at diagnosis and the oldest was 95. Median age for all cases was 51 years, and for squamous and non-squamous ICC it was 52 and 50 years respectively. One out of four cases was younger than 40 years of age, but few cases were below 30 (5%). The age distribution of the case women and their tumour stage at diagnosis are shown in Figure 1 in Paper I. The median ages in the studies II-IV were the early forties for both cases and control women.

### Histopathology and FIGO stages

The majority of the cancer cases had squamous ICC; one in four had non-squamous cancer. Details on the histological types and their FIGO stages are presented in Table 1 in Paper I. Most of the non-squamous ICCs were adenocarcinomas (79%).

Out of all cases, 39% were found in an advanced stage (FIGO II+) at diagnosis and the proportion of advanced stages increased with increasing age (Figure 1, Paper I). Above the age of 65, two-thirds had an advanced disease and below the age of 30 almost half of the cases had a microinvasive cancer. In total, one out of five cancer cases was diagnosed in a microinvasive stage (Table 1, Paper I), but this was less common in non-squamous ICC compared to squamous ICC (14% vs 23%). Among the case participants in studies II-IV, 32-38% had microinvasive cancer (Table 6).
**Screening histories of the cancer cases**

Half of cancer cases had no test at all and another 11% had not been screened within the recommended interval (overdue, negative cytology), which is illustrated in Figure 3. Slightly less than one forth had been screened within the interval and had negative cytology. In total, 13% had a history of abnormal Pap smear findings, of whom the majority had been screened within the recommended interval. A history of abnormal smear findings was equally common in cases with squamous cell carcinoma as in cases with adenocarcinoma, among those that had cytology (Table 1, Paper II).

**Figure 3.** Screening histories of the cervical cancer cases 0.5–6.5 years before diagnosis (n=1230).
Effect of screening on tumour stage

The tumour stage distribution of ICC related to screening history, as illustrated in Figure 4. Tumour stage was significantly shifted towards lower stages in cases screened within the interval compared to those not screened, in cases with previous abnormal smears compared to those with negative cytology, and also in presumed screen detected cancer cases compared to presumed symptomatic cancer cases (Table 2, Paper I). Among cases in screening ages with previous abnormal cytology, microinvasive disease was significantly less common in adenocarcinomas compared to squamous cell carcinomas (15% vs 47%, Paper II). Quite one half of the cases with previous abnormal smears in screening ages was presumed screen detected (n=75/143), of whom quite one half was in early stage (IA, n=39/75).

Figure 4. FIGO stage distribution of invasive cervical cancer related to screening history (n=1230).
Results

Effect of screening on cancer risk

Screened or not screened

Having had no screening within the recommended interval increased the risk of ICC 2.5 times compared to having had screening (Table 3, Paper I). Screening had effect on squamous (OR 2.97, 95% CI 2.51-3.50) as well as on non-squamous ICC (OR 1.59, 95% CI 1.20-2.11). The effect of screening was not modified by age groups (\( P_{\text{homogeneity}} = 0.96 \)) but it was strongly modified by FIGO stages (\( P_{\text{homogeneity}} < 0.001 \)) with an almost five fold increased risk of advanced ICC without screening.

Adequate, partially adequate or non-adequate cytology

A history of ever having had cytology with limited evaluation, due to lack of EC/TZ cells or due to obscuring factors, or an unsatisfactory smear, was present in 20% of the cases and in 17% of the controls with cytology and no abnormal findings. After adjustment for age and screening intensity, negative cytology with partially obscuring factors increased the risk of squamous ICC, compared to negative and adequate results (OR 2.13, 95% IC 1.22-3.74). However, the risk was significant only for microinvasive cancer (IA) in the multinomial regression analysis (Table 2 and Table 3, Paper II). A result unsatisfactory for evaluation (no abnormal smears) also increased the risk of ICC (OR 1.94, 95% CI 1.07-3.54). There was a tendency towards increased risk of localised cancer (stage IB) after negative cytology lacking EC/TZ cells (OR 1.46, 95% CI 0.97-2.21) (Table 3, Paper II).

Normal or abnormal Pap smears

Women with abnormal smear findings had a 7.5 times higher risk of ICC than women with negative smears (OR 7.55, 95% CI 5.88-9.69) (Table 3, Paper I). In screening ages, previous abnormal cytology increased the risk of squamous ICC six times and of non-squamous ICC seven times (Table 2, Paper II), and the risk was strongly associated to all tumour stages (Table 3, Paper II). A history of low-grade squamous atypia only increased the risk of squamous ICC strongly (OR 4.84, 95% CI 2.94-7.95), as did a history of high-grade squamous atypia (OR 6.89, 95% CI 3.71-12.76). Nonsquamous/"other" atypia associated very strongly with non-squamous ICC (OR 20.5, 95% CI 10.78-39.1) and also with squamous ICC (OR 7.36, 95% CI 3.97-13.64). Age below 40 years, compared to 40 years of age or older, increased the risk in non-squamous/"other" atypia (\( P_{\text{homogeneity}} = 0.019 \)), but not significantly in other abnormalities (Table 2, paper II). Out of all different cytological diagnosis, atypia in cells of uncertain origin, atypical glandular cells, and CIN3/CIS showed the highest risk estimates for ICC (Table 4, Paper II).
Effect of management of cytology abnormalities

Table 7 shows a summary of the management within two years of women with abnormal smear findings.

**Table 7. Summary of follow-up and management of women with abnormal cytology findings.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (N=143)</th>
<th>Controls (N=176)</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>First abnormal cytology:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median time to cancer diagnosis (months) (IQR)</td>
<td>33 (13-61)</td>
<td>48 (30-62)</td>
<td>II</td>
</tr>
<tr>
<td>Low-grade squamous atypia, N (%)</td>
<td>67 (47%)</td>
<td>111 (63%)</td>
<td></td>
</tr>
<tr>
<td>High-grade squamous atypia, N (%)</td>
<td>24 (17%)</td>
<td>35 (20%)</td>
<td></td>
</tr>
<tr>
<td>Glandular or <em>other</em> atypia, N (%)</td>
<td>52 (36%)</td>
<td>30 (17%)</td>
<td></td>
</tr>
<tr>
<td><strong>Follow-up, N (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-grade squamous atypia 1st visit</td>
<td>56/64 (88%)</td>
<td>104/110 (95%)</td>
<td></td>
</tr>
<tr>
<td>2nd visit</td>
<td>34/64 (53%)</td>
<td>78/110 (71%)</td>
<td></td>
</tr>
<tr>
<td>3rd visit</td>
<td>18/64 (29%)</td>
<td>45/110 (41%)</td>
<td></td>
</tr>
<tr>
<td>High-grade squamous/glandular/&quot;other&quot; atypia 1st visit</td>
<td>54/73 (74%)</td>
<td>56/64 (88%)</td>
<td>III</td>
</tr>
<tr>
<td>2nd visit</td>
<td>38/73 (52%)</td>
<td>46/64 (72%)</td>
<td></td>
</tr>
<tr>
<td>3rd visit</td>
<td>19/73 (26%)</td>
<td>36/64 (55%)</td>
<td></td>
</tr>
<tr>
<td>Cytological surveillance of low-grade squamous atypia</td>
<td>35/64 (55%)</td>
<td>45/110 (41%)</td>
<td></td>
</tr>
<tr>
<td>Mean number of repeated smears</td>
<td>1.7</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>≥2 negative repeated smears</td>
<td>10/35 (29%)</td>
<td>17/45 (38%)</td>
<td></td>
</tr>
<tr>
<td>Subjects assessed with histology, N (%)</td>
<td>55/143 (38%)</td>
<td>103/176 (59%)</td>
<td>IV</td>
</tr>
<tr>
<td>Low-grade squamous atypia</td>
<td>23/68 (34%)</td>
<td>61/111 (55%)</td>
<td></td>
</tr>
<tr>
<td>High-grade squamous atypia</td>
<td>10/24 (42%)</td>
<td>30/35 (86%)</td>
<td></td>
</tr>
<tr>
<td>Glandular or &quot;other&quot; atypia</td>
<td>22/51 (43%)</td>
<td>12/30 (40%)</td>
<td></td>
</tr>
<tr>
<td>Median time to first histology (months) (IQR)</td>
<td>2.5 (1.4-6.6)</td>
<td>2.3 (1.5-3.6)</td>
<td></td>
</tr>
<tr>
<td>Biopsy at 1st visit:</td>
<td></td>
<td></td>
<td>III</td>
</tr>
<tr>
<td>Low-grade squamous abnormality</td>
<td>14/21 (67%)</td>
<td>44/59 (75%)</td>
<td></td>
</tr>
<tr>
<td>High-grade squamous/non-squamous/&quot;other&quot; atypia</td>
<td>21/28 (75%)</td>
<td>33/43 (77%)</td>
<td></td>
</tr>
<tr>
<td>Mean number of histological assessments (SD)</td>
<td>1.6 (0.9)</td>
<td>1.6 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Colposcopy</td>
<td>45/55 (82%)</td>
<td>80/103 (78%)</td>
<td></td>
</tr>
<tr>
<td>Mean number of examinations</td>
<td>1.3</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>TZ entirely visible</td>
<td>24/45 (53%)</td>
<td>38/80 (48%)</td>
<td></td>
</tr>
<tr>
<td>Visibility of TZ not reported</td>
<td>9/45 (20%)</td>
<td>22/80 (28%)</td>
<td></td>
</tr>
<tr>
<td>Subjects assessed with histology and treated, N (%)</td>
<td>24/55 (44%)</td>
<td>66/103 (64%)</td>
<td>IV</td>
</tr>
<tr>
<td>Treatment centre</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>5 (21%)</td>
<td>12 (18%)</td>
<td></td>
</tr>
<tr>
<td>County/district hospital</td>
<td>16 (67%)</td>
<td>46 (70%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3 (13%)</td>
<td>9 (12%)</td>
<td></td>
</tr>
<tr>
<td>Treatment following high-grade dysplasia in biopsy</td>
<td>12/18 (67%)</td>
<td>18/22 (82%)</td>
<td></td>
</tr>
<tr>
<td>Ablation only</td>
<td>8/24 (33%)</td>
<td>14/66 (21%)</td>
<td></td>
</tr>
<tr>
<td>Conisation/LLETZ/hysterectomy</td>
<td>16/24 (67%)</td>
<td>52/66 (79%)</td>
<td></td>
</tr>
<tr>
<td>LLETZ only</td>
<td>10/14 (71%)</td>
<td>11/40 (28%)</td>
<td></td>
</tr>
<tr>
<td>Laser conisation only</td>
<td>4/14 (29%)</td>
<td>29/66 (43%)</td>
<td></td>
</tr>
<tr>
<td>High grade dysplasia in resection margins</td>
<td>5/14 (36%)</td>
<td>7/43 (16%)</td>
<td></td>
</tr>
<tr>
<td>Retreatment</td>
<td>0/9 (0%)</td>
<td>5/7 (71%)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; IA, microinvasive; IB, localised cancer; II or higher, advanced cancer; N, number of women; SD, standard deviation; IQR, inter-quartile range.

* From the first abnormal cytology result. Corresponding observation time for the controls.
+ The total numbers of women with abnormal cytology in Paper II were the denominators.
- The first abnormal smear finding in women with abnormal cytology and histology in Paper III.
\+ If treated more than once the latest treatment is counted.
\- No retreatment.
\# In primary cone specimen with dysplasia.
Follow-up or no not

The vast majority of both cases and controls had follow-up with a repeat test and/or a biopsy after a low-grade abnormal smear finding (88% and 95%). However, 39% of the cases and 25% of the controls with initial follow-up had no second visit with morphology. Among those with high-grade squamous or non-squamous/"other" atypia, 26% of the cases and 12% of the controls had no further assessment. Women with no morphological assessment at all after a high-grade squamous abnormality carried a very high risk of subsequent invasive cancer (OR 12.52, 95% CI 1.42-infinitive) (Table 1, Paper III).

Repeated cytology or biopsy

Compared to cytological surveillance only, histological assessment of low-grade squamous abnormalities strongly reduced the risk (OR 0.46, 95% CI 0.24-0.89) (Table 1, Paper III). The numbers under each cytological diagnosis were small and significant correlation was only found in CIN1 in which a nearly 70% risk reduction was observed. Among women under cytological surveillance of ASCUS/CIN1, the mean number of repeated smears was similar between cases and controls, and we found no significant difference regarding the proportions having presented with two negative, and no atypical, smear results (Paper III). Very few women with high-grade squamous abnormalities had been managed by repeated cytology only, and they had all CIN2 in the index smear. In women with glandular or "other" atypia, repeated smear was not associated with a lower risk compared to no follow-up at all.

Histological investigations

Lack of histological assessment was associated with increased cancer risk, both after low-grade (OR 2.37, 95% CI 1.27-4.43) and high-grade squamous atypia (OR 8.26, 95% CI 2.37-28.8), but not after glandular or "other" atypia (OR 0.90, 95% CI 0.36-2.27) (Table 2, Paper IV). The majority of the case and control women had the biopsy taken at the first follow-up after any category of abnormality, and delaying the biopsy to 7-12 months after a low-grade squamous abnormality increased the risk (Table 3, Paper III). Diagnostic cone biopsy ("see and treat") showed a tendency towards decreasing the risk in women with low-grade squamous and non-squamous/"other" atypia compared to biopsy (Table 2, Paper IV). Adding endocervical curettage to punch/cone biopsy did not decrease the risk.
Colposcopy

Among women with histology, we found no differences between cases and controls regarding the use of colposcopy and in the reported visibility of the TZ. However, among those with a history of high-grade squamous or non-squamous abnormality, and a negative or CIN1 biopsy without excisional treatment, 11 out of 22 cases, and 4 out of 11 controls had no or inadequate colposcopy.

Management of unsatisfactory results

No difference was found between cases and controls in the follow-up of an unsatisfactory smear result within one year; 77% of the cases and 68% of the controls (OR 1.61, 95% CI 0.54-4.83) had a repeated smear and/or a biopsy (Paper III). Only three case women and no control women were absent of any following cytology during the observation time up until 0.5 years before cancer date.

Effect of treatment on cancer risk

Among women with histology, cases had received treatment to a lower extent than the controls, and lack of treatment increased the risk of ICC nearly 4 times (OR 3.68, 95% CI 1.53-8.84) (Table 2, Paper IV). Laser conisation decreased the risk compared to LLETZ, even after adjustment for level of treatment centre (OR 0.06, 95% CI 0.01-0.37). One in three treated cases had ablative therapy only, and this was associated with increased risk compared to conisation/LLETZ (OR 3.82, 95% CI 1.01-14.4). Dysplasia in the resection margins followed by no retreatment was more common among cases than controls (Table 7). Lack of treatment after a negative/low-grade atypical biopsy increased the risk (OR 3.57, 95% CI 1.18-10.8); both for women with a high grade squamous/“other” atypical cytology, and for women with low-grade squamous cytology, although not significantly so in the last case (Table 3-4, Paper IV).

Adherence to guideline recommendations

Table 8 presents a summary of adherence to the recommended management of women in screening ages with abnormal cytology findings according to contemporary guidelines.140 In total, 62% of the case women and 35% of the control women with an abnormal or unsatisfactory Pap smear had not been followed-up or managed according to the recommendations.
Table 8. Summary of adherence to guideline recommendations in the follow-up of women with abnormal or unsatisfactory cytology.

<table>
<thead>
<tr>
<th>Management</th>
<th>Cases (n=146)</th>
<th>Controls (n=176)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Managed according to recommendations</td>
<td>56 (38%)</td>
<td>114 (65%)</td>
</tr>
<tr>
<td>Not managed according to recommendations</td>
<td>90 (62%)</td>
<td>62 (35%)</td>
</tr>
<tr>
<td>No repeat test after unsatisfactory cytology</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>No follow-up after low-grade squamous atypia</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>No 2 negative repeat tests upon surveillance of low-grade squamous atypia</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>No biopsy after high-grade squamous atypia</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>No biopsy after non-squamous/&quot;other&quot; atypia</td>
<td>29</td>
<td>18</td>
</tr>
<tr>
<td>No colposcopy or diagnostic conisation after high-grade squamous/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-squamous/&quot;other&quot; atypia and negative biopsy</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>No treatment of histologically verified high-grade CIN</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

Summary of the results

Out of all cancer cases, 33% were in screening ages but not screened or with negative screening overdue, 28% were above screening ages and had no cytology reported, 3% were above screening ages with negative cytology, 23% had been screened within the recommended interval and had negative cytology, and 13% had previous abnormal cytology findings of which more than half of those in screening ages had not been managed according to recommendations.

Cervical screening with cytology was effective in reducing the risk of squamous, as well as non-squamous invasive cervical cancer, and it was effective in all ages. Screening had also a down staging effect on tumour stage at diagnosis, in particular for squamous cervical cancer.

Women with negative cytology with a history of partially obscuring factors or of unsatisfactory cytology had increased risk of early stage ICC.

Previous abnormal cytology was as common in women with adenocarcinoma as in women with squamous cervical cancer. All cytological abnormalities increased the risk of all stages of invasive cervical cancer. The risk was at least as high in women below 40 years of age, as in women aged 40 years or above. Women with glandular atypia or atypia in cells of uncertain origin showed the highest risk estimates.

In women with low-grade squamous abnormal smear findings, biopsy was more effective than repeated cytology. Lack of histological investigation increased the risk in women with both low-grade and high-grade squamous atypia. Neither repeat cytology, nor biopsy decreased the risk in women with glandular atypia or atypia in cells of uncertain origin. Treatment decreased the risk, even when the biopsy before treatment was negative or showed low-grade atypia only. Ablative therapy was less effective than excision, and LLETZ was less effective than laser conisation.
DISCUSSION

Key findings

Three equally large groups of screening related reasons for ICC were found; non-adherence to screening recommendations, no testing in women above screening-ages, and insufficiency of the screening test or of the management of screen positive women (Figure 5). Screening was effective in both squamous and non-squamous ICC as well as in women of all ages, and screening had a favourable effect on tumour stage at diagnosis. Furthermore, all types of cytological abnormalities in the screening history were associated with an increased risk of ICC, in particular glandular and “other” cell atypia. Further investigation with biopsy was more effective than repeated cytology in women with low-grade squamous abnormalities. Treatment decreased the risk, even after negative/low-grade atypical biopsy, and laser conisation was the most effective treatment.

Figure 5. Cervical cancer in relation to screening history 0.5-6.5 years before diagnosis (n=1230).
Strengths and limitations

The present study is the first nationwide audit of an organised cervical cancer screening programme, and also the first nationwide case-control study on cancer risks related to the whole spectrum of different cytological diagnoses and to the management of abnormal smear findings. The major strength is access to data on a non-selective sample of women with and without ICC, having been derived from very accurate national population registers. The cases in all four studies were identified from a study base including all new cases of ICC in Sweden during a three year period, and their histopathological specimens were reviewed. By using the same source of screening history data for the case and control subjects, selection and recall biases were avoided. The access rate to data from all eligible cases and controls was high and was estimated to be at least 93%, as data were lacking from only seven out of 30 laboratories 1992-1993 and from only one laboratory 1994-1997. Also, the data on cytology were collected from a national database that included cytology taken outside the organised screening. Further, in Paper IV the response rate on the questionnaire was ≥95% for both cases and controls, and since the number of missing data points was low, the content validity should be considered as optimal. As the actual clinical routine in the entire country was the study base, generalisability of the results is high, and since invasive cervical cancer was used as endpoint, the relevance of the studies is also high.

However, the studies have some limitations as well. A potential bias in Paper I was that screen detected cancer cases were included in the analyses, but the screening test that led to the detection of their cancer was not included. This could have led to an overestimation of the effect, something that is further discussed below. In Paper II, a weakness would be that the cytological slides were not reviewed. A re-examination of the slides would probably have resulted in upgrading of negative/unsatisfactory and low-grade abnormal results of the cases. On the other hand, the study correctly assesses the risk of cancer related to the cytological diagnoses reported in routine practice. Both cytological under- and over diagnosis is a reality in regular practice, especially regarding the equivocal and low grade cytological diagnoses. Further, not all cytological laboratories in Sweden estimated and reported the quality of a negative smear during the observation time. Out of 30 laboratories, 27 used the term “endocervical/TZ cells absent” but only 13 before 1994. The term “evaluation limited by partially obscuring factors” was used by 19 laboratories and only 15 before 1994. This implies that the risk associated with these diagnoses could have been underestimated. The risk might also have been underestimated because we investigated these associations in women with a history of negative cytology only, excluding those that had subsequent abnormal findings.

The matching on age at cancer diagnosis, rather than on screening histories, means that the cases and controls were not individually matched in the studies of Papers II-IV. However, age differences between cases and controls in Paper II-IV were small, and were adjusted for in the analyses. In Paper III and IV, the observation time from
first abnormal cytology to the date of cancer diagnosis (corresponding date for the controls) were longer in control women than in case women. This may indicate a protective effect by the management of the controls, but it also means that the cases had less time for exposure which could have led to an overestimation of the effects. In the categories low-grade squamous and “other” atypia, the proportions of the different cytological diagnoses were equal. However, in the category high-grade squamous cytological atypia there was a higher proportion of CIN2 and a lower proportion of CIN3 among the controls compared to cases, which means that the effect of the management among the controls might have been underestimated in this category.

In Papers III-IV, the main limitations were the few numbers of women with abnormal cytology and the few numbers of women treated. Nevertheless, several associations of increased risk of ICC were revealed in these studies, although the sample sizes did not allow risk estimations in different age-groups. In subjects without histology, data were not available on colposcopic examination and on ablative treatment. Hence, we could not evaluate the contribution of colposcopy in the management of women with abnormal Pap smear and no biopsy, neither that of histological assessment without treatment. However, it is unlikely that ablative treatment without biopsy had occurred to any appreciable extent, as it has not been acceptable management.3,4,60

As in all observational studies, there is a possibility that the observed associations may be attributable to confounding that could not be adjusted for. Women less likely to participate in screening may also be exposed to other risk factors for ICC,1,2,208 thereby inflating the effect of screening and other exposures studied.

**Interpretation and comparison with other studies**

This audit identified three large groups of screening related explanations for the remaining morbidity and mortality from cervical cancer in Sweden (Figure 5). Each group represents different types of failures in the prevention of ICC. Our comparison of the screening histories of the cancer cases with that of control women who did not develop ICC, exposed several areas of possible improvements that will be discussed below.

**Cervical cancer among women not screened according to recommendations**

The first group of screening failures relates to women in screening ages non-adherent to screening recommendations, of which two thirds of the cancer cases had no test and one third had a test that was overdue (Figure 4-5). Our finding that non-participation within the recommended intervals increased the risk of ICC by 2.5 times corresponds to a 60% reduced risk for the participants (1/OR), in accordance with previous studies.10,209-211 Our result, however, is not as good as the 85% risk reduction by screening within 4 years reported from Australia.212 The Australian case-control
study included women 20-69 years, but it was biased by the fact that the control subjects were selected from a register of screened women, even though some of them were unscreened during the observation time.\textsuperscript{170} Another explanation for their better results would be an actual difference in the effectiveness of the screening programmes. The Australian programme recommends shorter screening intervals (every 2 year) and broader age-groups (20-69 years), compared to the Swedish programme.\textsuperscript{3,86,170} On the other hand, in a recent modelling analysis from Australia, it was concluded that lengthening the recommended screening interval to 3 years was not predicted to result in increases in rates of cervical cancer and was predicted to decrease the number of women undergoing diagnostic and treatment procedures.\textsuperscript{212}

In our study, non-participation increased the risk of both non-squamous and squamous ICC, although the effect was less prominent on non-squamous ICC (37% vs 66% decrease in risk). The majority of the non-squamous ICCs were adenocarcinoma. The effectiveness of screening on adenocarcinoma has been less clear than that on squamous ICC.\textsuperscript{1,11-13} One reason why adenocarcinoma has not decreased over time might be that the incidence without screening would have increased for both adenocarcinoma and squamous ICC, if background risks for ICC have increased, and that screening reduces the risk of squamous ICC more than that of adenocarcinoma. Our results are in concordance with other recent studies showing effect of screening on adenocarcinoma. In a case-control study from the UK, 3-yearly screening associated with a significant risk reduction of both squamous ICC and adenocarcinomas (75% and 43% respectively),\textsuperscript{213} and a meta-analysis showed similar risk reductions of 54% and 32% respectively.\textsuperscript{10} In both these studies, the effect on adenocarcinoma was significantly less than that of squamous ICC. It has also been shown that the duration of the effect is shorter for adenocarcinoma.\textsuperscript{10,213,214} By contrast, in the above mentioned report from Australia, the effect on non-squamous ICC was high (79% risk reduction) and was not significantly different from that on squamous ICC.\textsuperscript{170}

No screening within the interval increased the risk of all stages of ICC, with the exception of very early non-squamous cancer, and in particular, the risk of advanced stages decreased in accordance with the results of other studies.\textsuperscript{43,215,216} However, in the Australian study the effects on localised and on advanced cancer did not significantly differ.\textsuperscript{170}

We also found that screening was effective in the youngest age group (<30 years), which is supported by the results of one other study that showed a 76% risk reduction,\textsuperscript{170} while others have not demonstrated any effect.\textsuperscript{210,217} A reason for the lack of effect in the large case-control study from the UK could be that in that study, the effectiveness of screening was evaluated with more extended screening intervals (5-8 years),\textsuperscript{210} which might not be as protective as the 3-year interval in our study. This was also indicated by the results of further analyses of the data in our audit, as well as of those in a study by Zappa et al.\textsuperscript{214,218} As has been pointed out by others,\textsuperscript{210} in the current study the effect of screening could have been overestimated because the screen detected cases were less likely to have had a smear within the window of 0.5-
In our study, half of the case women and one third of the control women in the screening ages had not participated within the recommended intervals, but it is unknown to what extent this is explained by eventual lack of invitations to screening or by non-adherence to the invitations. Fear of cancer and anxiety are important reasons for non-attendance in many studies. In Sweden, time consuming barriers and economic barriers have associated with non-attendance, while knowledge about the recommended screening intervals, as well as a perception of screening as being beneficial, have been found to increase the probability of attendance. The result of our study, showing a risk reduction for ICC as well as a down staging effect of the cancers detected by screening, is important knowledge to communicate to the population as well as to health care providers, and may increase the motivation for participation and high quality screening performance. Further, a large Swedish RCT showed that combinations of modified invitation, written reminder, and phone reminder almost doubled attendance within 12 months, and the number of detected cytological abnormalities was more than tripled. An additional way of increasing screening compliance could be to offer self-sampling of cervicovaginal specimens for hrHPV testing to women who have not attended regular screening, and this method is currently being evaluated in a Swedish RCT. In one study, a reminder letter as well as offering self-sampling increased the attendance, but the effects on coverage were similar in both groups.

**Cervical cancer in women above screening ages**

The second group of cancer cases comprises women that are not included in the screening programme (≥66, that is 5 years above the age of the last invitation to screening). Only 8% of the case women while 19% of the control women in this group had a test (Table 3, Paper I), and our risk estimates indicate that the protection provided by screening was as high among these older women as among the younger. Our result is supported by studies from the UK and Australia, showing that screening in ages 55-69 was highly effective, and by a study from Finland showing a 54% risk reduction for screening attendees aged 55-79 years. It is possible that the effect in our study, as well as in those of others, could have been overestimated to some extent by the “healthier women” effect, i.e. if the control women with screening were at lower risk of ICC because they had participated more in screening before the age of
Discussion

60, and/or had lower prevalence of other risk factors. It is not known in this study to what extent the cases and controls had been screened before the age of 60. Although earlier studies have reported a low risk of precancer and cancer among previously well screened women above the age of 50, recent studies have shown continual risk of CIN and similar risk of ICC as in younger women.

The cervical screening programme in Sweden was meant to protect all women, because women with normal cytology up to the age of 60 years were considered to be protected after this age. However, invitations have in general stopped at the age of 60 regardless of screening history. This is a major weakness of the Swedish cervical screening programme. Since almost one third of all cancer cases, and more than half of all advanced stages of cancer, occurred in women ≥66 years of age, extending the programme to include older women at increased risk would be appropriate. Women previously treated for CIN3/CIS and women with no previous regular screening are identified risk groups that are recommended surveillance above 60. Audits including long term screening histories of cases and controls may help to further identify women that would benefit from screening after the age of 60. It has also been suggested that “exit” hrHPV testing of all women at the last recommended screening occasion might be useful to identify women in need of continued surveillance.

Cervical cancer in screening participants

The third group of cancer cases consists of women who developed ICC despite having had a negative screening test within the recommended interval, or despite having had an abnormal smear more than six months before diagnosis. They constitute half of all cancer cases in screening ages, of which one third had an abnormal test. These cases represent failures of the screening test, as well as failures in the diagnosis and treatment of precancer. Since screened cases presented with significantly lower tumour stages at diagnosis than non-screened cases (19% vs 50% in stage II+), women with screen detected cancers would still have benefited from screening through better chance of survival from cancer. This is supported by findings from Finland, showing a stronger effect of screening on mortality than on incidence of ICC (RR 0.31 vs RR 0.52). In our study, women with a previous abnormal smear finding presented with even lower tumour stages; 37% microinvasive and 14% advanced stage. The interpretation is that, because of a positive screening test, many of them would have been kept under surveillance and therefore the ICC was found in early stages. It can be argued that stage IA disease should be considered as a success of screening, rather than as a failure, which would be valid if one looks at the low rate of mortality in women with microinvasive stages of cancer. Yet, those with stage IA2 still need radical surgery, with loss of or impaired fertility, something that probably could have been prevented in cases with a previous abnormal smear finding. Although screening participants would have better chances of survival from ICC than non-participants, they have nonetheless suffered because of shortcomings or failures
of the screening programme, and therefore efforts to make improvements are needed.

An important observation during the study was the heterogeneity between the laboratories in the terminology and coding of cervical cytology, as well as lack of indications of the quality of the smear (present EC/TC cells) in some laboratories. More than 150 different diagnostic codes were found, and the meaning of the same code was not always identical between laboratories. It is possible that these differences might have led to some misunderstanding by the clinicians on how to interpret cytology reports. It also hampered the straightforward pooling of data in our study. This highlights the importance of uniform terminology and methods of data collection for monitoring quality assurance.3,52,93

**Negative screening results**

A negative screening test during the last screening interval before diagnosis of ICC reflects the moderate sensitivity of cytology screening.2,96,97 There are several reasons for failure of the test: the precancer lesion may have been missed by poor sampling of the TZ, the abnormal cells may have been overlooked or obscured on the cytology slide, or no precancer lesion was present in cases of rapidly developing lesions.225 In our study, it was not evaluated whether abnormalities were missed or if nothing was detectable. Others have reported that one third of previous negative slides in cervical cancer cases have been reinterpreted as abnormal, and 33-50% of unsatisfactory as HSIL, upon re-examination.201,206

Women with partially obscuring factors in any negative smear, and no abnormal results, had increased risk of squamous cell carcinoma, which was unexpected as the use of this terminology is no longer recommended.52,26 This finding is in disagreement with two retrospective studies showing no significant relationship between partially obscuring factors and false negative reports in women with CIN.115,116 Apart from these reports, data are limited on risks related to smears with partially obscuring factors.113 In our study, the association was significant only for microinvasive cancer, which might be explained by an increased co-existence of cervical inflammation/infection in younger women, compared to older, as microinvasive disease was more likely to occur among younger women. Cervical inflammation or infection has also been suggested to be a co-factor in the cervical carcinogenesis.226,227 Our finding of increased risk is supported by studies showing association between dysbacteriosis as well as cervical infection and subsequent high-grade squamous abnormalities and cervical cancer.226,228

Negative Pap smear lacking EC/TZ cells showed a tendency towards increased risk of ICC. Both a Cochrane review and several cross-sectional studies have shown that the presence of EC/TZ cells increases the detection of cervical abnormalities,99,113,114 but longitudinal studies have not shown increased risk of subsequent precancer or cancer.109-112 Most studies on risk of cervical abnormalities after negative cytology absent of EC/TZ have focused on squamous precursor lesions and few studies have
had invasive cervical cancer as outcome.\textsuperscript{13} On the other hand, Mitchell et al reported a relationship between the number of negative smears with endocervical components and reduced risk of adenocarcinoma.\textsuperscript{77} This was not confirmed in our study, although there was a trend.

The low numbers of cancer cases with a history of negative tests and any with partially obscuring factors or absent EC/TZ cells in our study (16\% of cases with negative cytology), indicate that these factors would be of minor importance for screening failure. However, the risk associations might have been underestimated because of the infrequent use of these qualitative terms by the laboratories, and also because women with abnormal cytology were not included in the analyses. Our result support the current recommendation that the absence of EC/TZ cells should be reported and that as few samples as possible lacking EC/TZ cells should be aimed at by the smear taker. A repeat test of all women lacking EC/TZ would probably not be cost-effective, considering the high frequency among the control women and the lack of significant increased risk in our study. The reporting of partially obscuring factor might also be justified, as it could aid the clinician in evaluating the risk of a woman, such as in cases of a history of previous abnormalities or of other risk factors. These women may need further evaluation, such as hrHPV testing. Due to the limitations of this study, further studies would be needed to further clarify the significance of partially obscuring factors.

A history of an unsatisfactory cytology, and no abnormal smears, was associated with a two-fold risk compared to a history of negative and adequate smears, which is in accordance with the results of others.\textsuperscript{102,109} In a Norwegian study, the risk of ICC was 1.6-4.0 times higher in women with an unsatisfactory Pap smear, compared to a negative smear.\textsuperscript{138} The reason behind would be missed abnormalities due to inadequate cervical sampling or due to obscuring inflammatory and/or blood cells. A repeat smear is recommended after an unsatisfactory smear result.\textsuperscript{5,3} However, we found no correlation between the initial management of women with an unsatisfactory smear and prevention of ICC, which is in disagreement with the Norwegian study showing that the increased risk was mostly attributable to women who did not have a repeated smear within 2 years.\textsuperscript{138} This difference might be due to our smaller numbers, or to the shorter follow-up time of one year in our study.

Our results support that adequate sampling is important for the sensitivity of cytology screening. The combined use of an extended tip spatula and the Cytobrush have shown to increase the rates of adequate smears, as well as the detection of abnormalities.\textsuperscript{99} The current introduction of LBC in many laboratories in Sweden would decrease the number of partially adequate and non-adequate samples,\textsuperscript{101-103} and increase the sensitivity for CIN2+.\textsuperscript{101} It has been further suggested that computerised automation-assisted screening of cytological slides might reduce the intraobserver variability,\textsuperscript{22} but a large RCT from Finland comparing this new technology with conventional cytology screening showed equal sensitivity for CIN2+ and no differences in the incidence and mortality patterns of ICC.\textsuperscript{211,229} Implementation of primary HPV testing, however, would increase the sensitivity of
screening, and give very high negative predictive values for ICC. HrHPV testing of women with unsatisfactory or partially obscuring negative tests might be useful for identifying those who would need further assessment.

**Abnormal screening results**

Among women screened within the recommended interval, 32% of the cases and 6% the controls had a history of an abnormal Pap smear more than six months before the cases’ cancer diagnosis. Others have reported a rate of 36-70% with previous smear abnormalities in screened women with ICC. In our study, a history of low-grade squamous abnormality or glandular/“other” atypia was the most typical among cases; 39% and 34% respectively.

Previous abnormal cytology was as common among women with adenocarcinoma as among women with squamous ICC, confirming that adenocarcinomas are preventable by screening in the same way as squamous ICC. However, previous high-grade abnormalities were very rare among women with adenocarcinoma. No one had AIS. The most frequent abnormalities were mild/moderate glandular atypia and atypia in cells of uncertain origin. These results are supported by findings that HPV18, accounting for one third of the adenocarcinomas, causes a disproportionately low fraction of high-grade lesions and that it in turn is associated with low-grade cytological abnormalities. This means that the adenocarcinomas caused by HPV16 might have been prevented to a higher extent, as HPV16 infections more often reveal themselves with high-grade abnormalities in cytology screening. In our study, the median time from the earliest recorded glandular/“other” atypia to cancer diagnosis appeared to be somewhat shorter than that of squamous abnormalities (Table 1, Paper II), which would be another reason for the weaker protective effect of screening on non-squamous ICC. A shorter interval implies that early glandular lesions are more difficult to sample, and/or that adenocarcinomas may have a shorter preinvasive phase. Moreover, in cases with previous abnormal findings, microinvasive disease was significantly less common among adenocarcinomas than among squamous carcinomas (47% vs 15%), also implying more rapidly developing tumours. Alternative explanations would be that the term microinvasive ICC was less used due to lack of distinct histopathological features of this term, or that the microinvasive lesions were not detected at further assessment of Pap smear abnormalities.

Our findings of increased risk of ICC in women with previous abnormal cytology were expected and in agreement with results of others. Our estimate of an almost eight-fold increased risk among women screened within the interval with a positive test, compared to those with a negative test, is higher than the RR of 5.8 in a cohort study of screening participants in Finland. The difference could be due to the fact that ORs usually tend to overestimate the relative risk and also due to the longer follow-up of 5 years in the Finnish study, as the risk of ICC has been found to be greatest during the first years of follow-up. The higher risk in younger women with glandular/“other” abnormalities in our study is also in agreement with the results
from Finland, showing higher risk in women below 40 years of age with abnormal Pap smears, compared to older women. The five-fold risk increase of squamous ICC associated with low grade squamous abnormalities is slightly higher than the three-fold risk reported from a Canadian cohort study, which could be due to differences between the study populations in relation to screening intensity, or to further investigations and treatment. The high risk of squamous ICC associated with high grade squamous abnormalities, and in particular CIN3/CIS, is supported by others who have shown a RR of 10-40, and an absolute risk of 31% in women with histologically verified CIN3 without treatment. The highest risk estimates were seen in women with a history of glandular or "other" atypia, who had a 20-fold increased risk of non-squamous ICC. This is consistent with another case-control study showing strong association between a history of AGC and subsequent non-squamous ICC, and with a cohort study showing an 18-fold increased risk of ICC. Atypia in cells of uncertain origin is strongly associated with both squamous and non-squamous ICC, although the majority of the cancer cases, 65%, developed squamous ICC. The increased risk with this cytological diagnosis is concordant with the high rates of underlying preinvasive high-grade lesion (26%) and cancer (11%) reported from Swedish laboratories.

In the analyses of risks associated with abnormal Pap smear findings we did not include in the model any variables in the pathway between exposure and outcome, such as further assessment and treatment after an abnormal cytology result, and thus the risk estimates reflect the relative risk in the population with a background of different management. The high risk estimates in women with AGC and atypia in cells of uncertain origin, for example, would be explained by suboptimal management. Unless a positive screening test signals an already invasive cancer, having an abnormal Pap smear should not necessarily be associated with an increased risk of ICC, because the subsequent assessment and possible treatment should prevent cancer, as this is the very foundation of screening. Thus, most of the cases that developed ICC in spite of having had a previous abnormality should be considered as failures of the screening programme.

The failure in preventing ICC could be grouped into those with a subsequent biopsy (38%) and those who lacked a histopathological diagnostic assessment. Among women without a biopsy, one third of the cases and one fifth of the controls had no follow-up at all (Table 1, Paper III), which could be explained by a non-compliance of the patient to further assessment, or by failure of the screening programme to send an invitation for repeat testing or to colposcopy. More than half of the case women that had been managed by cytological surveillance had a low-grade squamous abnormality at the index smear, of which 29% only had the minimum requirement of two or more subsequent negative tests. The proportion of women that underwent colposcopy with normal findings and no biopsy is not known. Among those with a punch biopsy and/or ECC, approximately one third of the cases and one fifth of the controls had histological high grade CIN, of whom 67% and 82% respectively received treatment. Thus, in total 62% of all cancer cases and 35% of the control
women with an abnormal Pap smear had not been managed according to contemporary guidelines (Table 8), representing 11% of the cases and 1.5% of the controls in the screening ages. Our findings are similar to those reported from a case-control study in the UK, in which 13% of the cancer cases and 1% of the control women under the age of 70 years had encountered suboptimal standard of care. However, 56 case women in our study had appropriate initial management, although the follow-up after treatment for the 12 women that had been treated for high-grade CIN is not known. By comparing the management of the case women with that of the control women, some strategies were found to be more protective against ICC than others.

**Management of low-grade squamous abnormalities**

Histological assessment reduced the risk of ICC by approximately 50% in women with a low-grade squamous abnormality compared to repeated cytology. Similar to our results, a case-control study from the UK found no significant difference between the proportions of cases and controls with an abnormal smear result followed by two negative results. Our results are further supported by studies showing high rates of underlying CIN2+ in women presenting with a single smear of ASCUS (19-36%), and CIN1 (31-49%), indicating that cytology may be insufficient in the management. Soutter and Fletcher showed, in a reanalysis of long-term studies, that women with low-grade abnormal smear findings who had been surveilled cytologically had a 16-47 times higher incidence of invasive cancer than the general population. In a Norwegian study, women with ASCUS followed by a normal smear finding had increased risk of ICC within a seven-year follow-up period compared to women with negative screening.

A large RCT from the UK compared cytological surveillance with a policy of direct referral for colposcopy and found that direct colposcopy detected more CIN3+ in women with ASCUS/CIN1, supporting our results. In this trial, the non-attendance rates were low in both study arms. Other RCTs, however, have demonstrated similar levels of detection of CIN3 within 2 years. In our study, we found that although most women with a low-grade abnormality had an initial follow-up, a high rate of both case and control women had no second follow-up visit, something that would explain the low mean number of repeat tests among those under cytological surveillance; 1.7 for cases and 1.8 for controls. Similar high rates of non-attendance rates in women under cytological surveillance, as among our case women, were reported from two RCT (20-25%). Therefore, the increased risk from cytological surveillance could partially be due to loss to follow-up. This is supported by the results of a meta-analysis of RCTs showing that compliance with follow-up declines over time, and concluding that cytological surveillance of low-grade abnormalities puts women at risk. Diagnostic assessment with colposcopy and biopsy at the first visit would thus be safer, but the drawbacks with this strategy would be overloaded colposcopy clinics as well as unnecessary investigations in a large number of women.
However, triage with hrHPV testing could indicate with high negative predictive value those who would not need further investigation.\textsuperscript{191}

Another important aspect of delayed assessment is the risk of negative psychological side effects. It has been shown from a Swedish study that the intermediary period between the notification of an abnormal smear result and the clarification of the nature and the degree of the pathological findings is particularly stressful for many women.\textsuperscript{242} In one RCT comparing immediate referral for colposcopy vs cytological surveillance, no difference in the proportion of women anxious or depressed was observed.\textsuperscript{145} However, in a recent RCT addressing the issue of psychosocial outcome specifically, it was shown that HPV triage was better for women’s psychosocial health than repeat cervical smear over 12 months of follow-up.\textsuperscript{243}

**Management of high-grade squamous abnormalities**

The very high risk associated with being lost for further assessment after a high grade squamous abnormality, as well as with having no histological assessment, was expected since the majority of these women have underlying CIN2+,\textsuperscript{128,129} and would need treatment. In our study, 42% of the case women and 86% of the control women with CIN2-3 in cytology had histology. Surveillance by repeated cytology without biopsy occurred rarely and only in women with CIN2. The high risk associated with no further assessment support current recommendations of a “fail-safe” system that notifies missed cases and re-invites them to investigation.\textsuperscript{2}

However, our data also indicate that colposcopic punch biopsies suggesting CIN1 or no atypia may be unreliable, as treatment reduced the risk of ICC in women with a history of high-grade squamous or non-squamous/”other” atypia and negative/CIN1 biopsy. These results are supported by others that have compared the histopathology of loop excisions with that of punch biopsies before treatment, showing CIN2-3 in 23-55% of women with negative/CIN1 biopsy.\textsuperscript{244} In one Swedish study, 36% of the women with CIN2+ on LLETZ had negative histopathology or CIN1 on biopsy before treatment.\textsuperscript{128} In our study, half of the biopsied non-treated cases with high-grade abnormalities had unsatisfactory colposcopy and would have needed a diagnostic conisation.\textsuperscript{53} In cases with satisfactory colposcopy, the deviation may be explained by the weak correlations between visual changes and disease severity in several studies,\textsuperscript{150,163} and the poor interobserver agreement in the interpretation of cervical histopathology.\textsuperscript{53}

Excisional treatment of women with a high-grade squamous abnormality, without prior biopsy to confirm histological CIN (“see and treat”), was not associated with reduced risk in our study. A “see and treat” strategy is considered to be justified in settings with high PPVs of a cytology or colposcopy findings suggesting high grade CIN, and in older women without childbearing interest,\textsuperscript{22,149,150} as it reduces the number of visits and the risk for women who might default from next visit. In young women, the taking of multiple biopsies, even from normal colposcopic areas, might improve the sensitivity of colposcopy performance.\textsuperscript{161,162}
Our results underline the need for improvements in the diagnosis of precancer lesions. The Swede score system for colposcopy findings might be useful in the selection of women for “see and treat” as it has shown high specificity for CIN2+. Optical spectroscopy may improve the diagnostic accuracy of colposcopy. New biomarkers, maybe combined with the use of a risk estimation model, may in the future identify women in need of treatment. Our results suggest that women with a high-grade squamous cytological abnormality, or a non-squamous/“other” abnormality, are at high risk and would score high in a risk model.

Management of glandular atypia or atypia in cells of uncertain origin

According to guidelines, women with glandular or “other” atypia should be referred for colposcopy and histological assessment and therefore the low rate of biopsy in controls (40%), was unexpected and indicates a poor adherence to recommendations. Neither repeat cytology, nor histological assessment reduced the risk of ICC. The lack of effect of repeat cytology in these women is in agreement with previous studies. As quite half of the case women with glandular or “other” atypia developed adenocarcinoma, the absence of risk reduction from biopsy could be explained by the lack of specific colposcopic signs of glandular abnormalities, as well as of poorly established histological criteria for glandular lesions. Another reason would be that glandular lesions are more often located into the endocervical canal and therefore not always visualised. The sensitivity of colposcopy in detecting endocervical lesions was only 9.8% in one study. Endocervical sampling is recommended in cases of glandular abnormality, but our data indicate that ECC may also lack sensitivity for identifying lesions. We found no risk-reducing effect of ever having had this investigation, in addition to punch/cone biopsy. This is supported by a RCT showing that the histological results of ECC frequently were inconclusive.

The number of women managed by a diagnostic conisation, or other treatment, was too small to allow for conclusions, although the trend was towards a protective effect. The reduced risk of cancer by treatment observed in women with a history of glandular/“other” or CIN2-3 and negative/CIN1 biopsy, also suggest that diagnostic conisation could be effective, as proposed by others. Such a policy is supported by the high rates of underlying precancer/cancer in many studies, the high PPV (81%) of abnormal endocervical cells for precancer/cancer during the following years in one study, and the low sensitivity of colposcopy and ECC. Diagnostic conisation is recommended in the European guidelines in cases of negative colposcopy and “AGC suggestive of neoplasia”. The use of hrHPV testing in women with glandular abnormalities has not been fully evaluated, but it has been suggested that it may distinguish between risk of endometrial cancer and cervical cancer, particularly in older women.
Treatment of precancer lesions

Our finding of a protective effect of treatment was expected and in accordance with previous studies, although the risk reduction in our study (73%) was not as high as the 95% reduction reported by Soutter et al. One reason for this difference would be that the data in the report of Soutter were obtained from teaching hospitals with a special interest and skill in gynaecological oncology, while our data were derived from routine practice in outpatient clinics, county hospitals and specialised departments. Another reason might be that the treated participants in our study were older than those in the study of Soutter et al; (34% vs 11% above 39 years of age), and older age increases the risk of insufficient treatment, maybe due to the higher location of CIN in the endocervical canal.

In our audit, only 24 case women had received treatment for CIN, corresponding to 17% of case women with abnormal Pap smears and to 2% of all cancer cases. This low number means that treatment in general is effective and that the absolute risk of ICC within the first 2-3 years after treatment would be very low, since several thousands of women are treated for CIN yearly in Sweden. However, compared to the general female population in Sweden, the risk of ICC after treatment of CIN was more than two-fold, with a persistent increased risk after 25 years in one study.

Despite the low numbers, statistically significant differences between cases and controls were observed regarding treatment. Ablative treatment was associated with increased risk compared to excision, and laser coagulation was more effective than LLETZ. This is not in line with previous studies showing no evidence for superiority of any of the conservative surgical techniques for eradicating CIN, or in future risk of ICC. However, in the Cochrane review of RCTs it was found that no real conclusions could be drawn regarding effectiveness, because the trials were grossly underpowered to demonstrate significant differences between the techniques, and that more research was needed. Also, long term data were lacking as most studies had a follow-up of 12 months or less. In the retrospective cohort study by Soutter et al on future risk of ICC after different treatment techniques, comparison was difficult because excisional methods were used in older women with more severe lesions. In another retrospective cohort study, which adjusted for age and grade of CIN, there was some tendency of LETZ, cryotherapy and laser vaporisation or coagulation being equally good methods of treatment, while cold knife treatment was less effective. However, our result of increased risk with ablative technique is supported by a RCT trial of Dey et al, showing higher failure rates compared to LLETZ, and by a large population-based study of women treated for CIN showing that the long-term risk of ICC was highest for women treated with cryotherapy. In the Cochrane review, cryotherapy was found to be effective for low-grade CIN, but not for high grade CIN. Our data indicate that ablative therapy was less effective in treating CIN2-3 as the point estimate was very high although the CI did not reach significance (Table 3, Paper IV). Excision, rather than destruction of the tissue, may provide more protection because it allows a comprehensive histological evaluation of the excised tissue, including recognition of a microinvasive cancer, and of margin involvement.
Another possible explanation would be that the ability to adequately sample the TZ after treatment may differ between excisional and ablative therapy, and, if so, residual CIN might be harder to detect on follow-up after ablation.

A better effectiveness of laser conisation than LLETZ might be explained by a greater depth with laser conisation than LLETZ,\textsuperscript{186} and by a larger volume removed as shown in a recent study.\textsuperscript{251} This is also supported by the finding in a meta-analysis that laser conisation, but not LLETZ, was significantly associated with an increased risk of serious pregnancy outcome,\textsuperscript{147} and by a study showing that the depth of the excision associated with preterm delivery.\textsuperscript{252} Another possible reason for our findings is a difference in skills. The laser conisation technique demands more training,\textsuperscript{150,186} whereas LLETZ might have been introduced as an easy and rapid mode of treatment that even clinicians with less experience could master. Since LLETZ allows less tailoring of the excision compared to laser conisation, in particular regarding the depth in the endocervical canal, the method could lead to incomplete treatment if performed by inexperienced performers. In the study by Soutter et al, it was observed that there was a tendency for inappropriately short LLETZ biopsy to be taken in older women in whom more severe disease lay higher.\textsuperscript{247} Adjustment for treatment centre, however, did not change the results in our study, which might be due to the small numbers treated at larger centres. The implication of our results would be that treatment performance of LLETZ, just as laser conisation, requires professional training, and that clinicians must be trained in adjusting the treatment technique according to the extent and localisation of the TZ.\textsuperscript{53} The risk of incomplete treatment should be observed and careful follow-up after treatment is needed. HrHPV testing has been shown to be effective for identifying women at risk of incomplete treatment,\textsuperscript{191,253,254} although HPV status 6-12 months post treatment has shown limited value for predicting risk of recurrence of CIN on long-term.\textsuperscript{255}

High-grade CIN in the resection margins of a LLETZ/cone specimen is a known risk factor for residual disease,\textsuperscript{5-55} something that was indicated by our data although the numbers were small. However, noteworthy is that most of the case women who had excisional treatment and CIN2+ in the cone/LLETZ specimen, had no report of involved margins, confirming the findings of other that this is an unreliable predictor of treatment success.\textsuperscript{190,256}
CONCLUSIONS AND IMPLICATIONS

- The screening histories of the cervical cancer cases show that they were above screening ages in 31%, had not been screened according to recommendations in 33%, had negative cytology within the recommended screening interval in 23%, or had previous abnormal cytology findings in 13% of which more than half had not been managed according to recommendations.

- Cervical screening within the recommended intervals was effective in decreasing the risk of both squamous and non-squamous ICC, and had a favourable effect on tumour stage at diagnosis. Improving adherence to screening recommendations is therefore essential.

- Screening was also effective in older women. Screening beyond the age of 60, or surveillance of older women at increased risk, would have significant impact on the cervical cancer morbidity and mortality.

- Negative cytology with partially obscuring factors and unsatisfactory cytology increased the risk of ICC, suggesting that women with such reports need further evaluation. Adequate sampling and improved technology (LBC) may have further impact on the effectiveness of cytology screening.

- A previous abnormal cytology finding of any kind increased the risk of subsequent ICC.

- After low-grade squamous abnormal cytology, further assessment with biopsy was more effective than repeated cytology in preventing ICC and should be recommended.

- After high-grade squamous abnormal cytology, no further assessment at all, as well as no biopsy, associated strongly with increased risk of ICC, and calls for quality improvements and the use of a “fail-safe” system.

- Glandular atypia, or atypia in cells of uncertain origin, in cytology associated strongest with subsequent ICC. Neither repeat cytology, nor biopsy decreased the risk, and further research on the most effective management of women with these abnormalities is greatly warranted.

- Treatment after a biopsy of normal or low-grade atypical histopathology decreased the risk, and therefore, improvements in the diagnosing of precancer lesions, and in identifying women in need of treatment, are demanded.

- Treatment with ablative therapy was less effective than excision, and LLETZ was less effective than laser conisation, suggesting that there is a need for further evaluation of treatment techniques and for proficiency training in CIN treatment.

- Audits need to become routine within cervical screening programmes and compliance with recommended management of women with abnormal cytology needs monitoring.
RESEARCH RECOMMENDATIONS

- Future audits would need to include data on invitations to screening to investigate whether non-attendance is due to failure in issuing invitations or to non-response to the invitations, as well as reviews of the negative slides of women who develop ICC.
- Studies on prognosis of cervical cancer in relation to screening history and mode of detection, with control for lead time bias, are warranted.
- Studies on reasons for non-response to invitations, on strategies to increase attendance, and the role of self-sampling for testing are needed.
- The optimal age to stop screening needs to be determined by studies including long-term screening histories of older women, and the efficacy of “exit” hrHPV testing at the last recommended screening occasion needs to be determined.
- To improve the sensitivity of screening, the implementation of primary HPV screening in routine practice needs to be evaluated. New technology, such as automated cytological reading, could be evaluated specifically on false negative slides, and the efficacy of HPV testing women with an inadequate/partially adequate smear needs to be assessed.
- Studies to improve the diagnosing of precancer lesions, and in particular in cases of glandular/"other" cytological abnormalities, are warranted, as well studies on how to ensure compliance of clinicians to guideline recommendations.
- Larger studies are needed to further evaluate the effectiveness of ablation and LLETZ, and the most optimal strategy for long-term surveillance of women after treatment of precancer needs to be determined.
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APPENDIX A

Questionnaire: Investigation and treatment of dysplasia.

Name of the Clinic: ................................................................. Patients ID-number: ....................

Information about the patient:
Please see the information on the attached page about cytology(ies) and histopathology(ies) with the date of registration/assessment.

INVESTIGATION

INVESTIGATION  Date of investigation: ......................

1. Reason for the investigation?
   □ Abnormal cytology at screening
   □ Other reason: ..............................................................
   □ The patient has not been to our clinic for investigation

2. Was colposcopy used?
   □ No. No colposcopy was used/no information about colposcopy
   □ Yes, the entire transformation zone was visible
   □ Yes, but the entire transformation zone was not visible
   □ Yes, but there is no information given on the visibility of the transformation zone

3. Type of biopsy?
   □ Punch biopsy of the cervix
   □ Cervical curettage
   □ Punch biopsy of the cervix + cervical curettage
   □ Cone/ring biopsy
   □ No biopsy taken

TREATMENT

4. Was treatment of dysplasia given?
   □ Yes  Date: ..............................................................
   □ No

5. Type of treatment?
   □ Resection:
     □ LEEP
     □ Laser conisation
     □ Knife conisation
     □ Hysterectomy
     □ Other method: ..............................................................
   □ Ablation:
     □ Laser evaporation
     □ Cryotherapy
     □ Diathermy
6. Further investigation at treatment?
   □ Cervical brush
   □ Cervical curettage
   □ Endometrial biopsy
   □ Curettage of the uterus
   □ No further investigation

FOLLOW UP

7. Has the patient been followed up?
   □ Yes, at the present clinic.
   □ Yes, at another clinic: ............................................................
     At which hospital? ............................................................
   □ We lack information on follow up

Name of the clinician responding: ..................................................... Date: ......................

After having filled in the questionnaire please send it to: Cancer screening unit, Oncologic Center M8, Karolinska University Hospital, 171 76 Stockholm. Telephone: 08/517 731 94, Telefax: 08/32 77 60
Utöver insändes blanketten till: Enh. för hälsoundersökn. Onkologiskt centrum, M8, Karolinska Universitetssjukhus, 171 76 Stockholm. Telefon: 08/517 731 94, FAX 08/32 77 60

Var god se uppgifter om cytologi(er) och histopatologi(er) med registreringsdatum / provtagningsdatum på bifogat blad

Uppgiftslämnare (namn): ................................................... Datum: .......................................

Uppgifter om patienten:

KLINIK / MOTTAGNING

Patient id

UTREDNING

Undersökningsdatum:........................................

1. Orsak till utredning?
   - Patologiskt cellprov taget i hälsokontrollsyfte
   - Annan orsak: ..............................................................................................................
   - Patienten är inte utredd vid kliniken

2. Gjordes kolposkopi?
   - Nej. Ingen kolposkopi gjordes/uppgift saknas.
   - Ja, hela transformationszonen bedömbär
   - Ja, men hela transformationszonen ej bedömbär
   - Ja, men uppgift saknas om transformationszonen

3. Typ av biopsi?
   - Px portio
   - Cervixskrap
   - Px portio + cervixskrap
   - Ringbiopsi / slyngkon
   - Ingen biopsi genomförd

BEHANDLING

4. Utfördes dysplasiebehandling?
   - Ja. Datum: .................................................................
   - Nej.

5. Typ av behandling?
   - Resektion:
     - Slyngdiatermi
     - Laserkonisering
     - Knivkonisering

   - Destruktion:
     - Laserevaporisering
     - Kryo
     - Diatermi

6. Ytterligare diagnostik i samband med behandlingen?
   - Cervixborste
   - Endometriebiopsi
   - Corpusabrasio
   - Ingen ytterligare diagnostik

UPPFÖLJNING

7. Har patienten följts upp?
   - Ja. Vid denna klinik
   - Ja. Vid annan klinik: .................................................................
   - Vilket sjukhus? .................................................................
   - Vi har inge uppgift om att patienten följts upp.

Efter fyllande insändes blanketten till: Enh. för hälsoundersökn. Onkologiskt centrum, M8, Karolinska Universitetssjukhus, 171 76 Stockholm. Telefon: 08/317 731 94, FAX 08/32 77 80

Endometriebiopsi
Corpusabrasio
Ingen ytterligare diagnostik