Cervical dysplasia and cervical cancer in pregnancy: diagnosis and outcome

by

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This book is dedicated to all pregnant women with atypical cervical cytology and to my parents, Ingrid and Rolf

Abstract

Cervical dysplasia and cervical cancer in pregnancy: Diagnosis and outcome

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Gothenburg, Sweden, 2012

Cervical cancer is one of the most common types of cancer that is diagnosed during pregnancy. The primary aim in investigation of atypical cervical cytology during pregnancy is to exclude cancer so that further treatment of the lesion can be postponed until after delivery**.** However, there are difficulties in colposcopic evaluation of cervix due to specific changes and cancer can be overlooked if not multiple biopsies are obtained, but these invasive interventions may increase the risk of bleeding from the vascularized cervix and further obstetrical complications. Thus, there is need for means to reduce the number of biopsies and to find biomarkers that can exclude cervical cancer among pregnant women with atypical cervical cytology.

In this thesis, pregnant women were evaluated with the Swede score colposcopic scoring system, due to atypical cervical cytology, dysplasia in biopsy or signs of malignancy in a prospective clinical study. Five colposcopic variables, acetowhiteness, margins plus surface, vessel patterns, lesion size and iodine staining were scored. Colposcopically directed biopsies were taken from all lesions and histology was compared with the Swede score sum. All CIN2+ lesions and cancers had total scores of ≥5and ≥8, respectively. All variables except iodine staining were found significant predictors of CIN2+. In prediction of CIN3+, lesion size, vessel patterns and margins plus surface were significant factors.

In a prospective clinical study, surgical/obstetric complications due to colposcopically directed cervical biopsies, loop-biopsies, or LEEP-cones were evaluated. The histology results during pregnancy were compared to that after delivery to evaluate the natural course of dysplastic lesions. Obstetric outcome was recorded and compared to the 54919 other births in the same geographical area during the study period. Only a minor part (12.3%) of the dysplastic lesions showed progression during pregnancy with 54.6% and 33.1% showing persistence and regression, respectively. No surgically-related postoperative bleeding that needed surgical (diathermy/suture) treatment occurred. The miscarriage rate was low (0.8%). There were no differences in mode of delivery, rate of premature birth or other obstetrical variables between the study group and the control cohort.

In a retrospective clinical study, medical records were evaluated of all women with cervical cancer diagnosed during pregnancy or within 6 months after parturition between 1993 and 2008 in the Western region of Sweden. Cervical cancer was diagnosed in 47 women (15.6/100 000 deliveries). Sixteen women were diagnosed after abnormal vaginal bleeding and/or discharge. The other women were asymptomatic and diagnosed by abnormal cervical smear or clinical signs at vaginal examination. Nine women had ASCUS as presenting cervical atypia. Cancer was diagnosed in the 1st trimester in 2 women, in the 2nd trimester in 14, in the 3rd trimester in 5 and post-partum in 26 women. Twenty women underwent cesarean section due to cancer, combined with the Wertheim-Meigs procedure in six women. Sixteen women having stage IA1 cancer underwent conization as final treatment. Six women died of the disease.

Liquid-based cytology samples were analysed for high-risk-HPV DNA genotype (an In-house real-time DNA PCR assay and the commercial Linear Array®), high-risk-HPV E6/E7 mRNA (a recently developed In-house real-time mRNA PCR assay and the commercial PreTect^{TM} HPV-Proofer) and p16^{INK4a} immunocytochemistry in pregnant women with normal cytology and atypical cytology. This study followed an initial study of different HR-HPV tests in mainly non-pregnant populations. In pregnant women stepwise logistic regression analysis showed that the p16 ^{INK4a} test and the In-house real-time mRNA PCR test were the most suitable tests in detecting high-grade lesions.

In summary, the Swede score seems to be a useful tool in evaluating atypical cervical cytology in pregnant women and may reduce the need for diagnostic biopsies and analysis of p16 ^{INK4a} positivity and HR-HPV mRNA may be useful supplementary tests in pregnant populations with atypical cytology to accurately detect high-grade lesions. Investigation of atypical cytology during pregnancy with biopsy including large loop excisions is a safe procedure in regards to surgical complications and obstetrical outcome. There is a high rate of persistence and regression of dysplasia during pregnancy. Early detection of cervical cytological atypia and proper follow-up during pregnancy may lead to the detection of an increased proportion of stage I cancer, thereby avoiding radical operative procedures.

Keywords: Cervical dysplasia, cervical cancer, pregnancy, colposcopic scoring system, regression, persistence, progression, HPV DNA test, HPV mRNA E6/E7 test, p16^{INK4a}

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List of papers

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

I. **Histological diagnosis and evaluation of the Swede score colposcopic system in a large cohort of pregnant women with atypical cervical cytology or cervical malignancy signs**
Kärrberg C, Ryd W, Strander B, Brännström M, Rådberg T
Acta Ob Gyn Scand, 2012;91:952-8.

II. **Colposcopically directed cervical biopsy during pregnancy; minor surgical and obstetrical complications and high rate of persistence/regression**
Kärrberg C, Brännström M, Strander B, Ladfors L, Rådberg T
Submitted.

III. **Incidence and treatment of cancer of the uterine cervix in pregnancy, including all cases in the Western region of Sweden 1993-2008**
Kärrberg C, Rådberg T, Holmberg E, Norström A
Submitted.

IV. **Type-specific human papillomavirus E6/E7 mRNA detection by real-time PCR improves identification of cervical neoplasia**
Andersson E, Kärrberg C, Rådberg T, Blomqvist L, Zetterqvist B-M, Ryd W, Lindh M, Horal P
J Clin Microbiol, 2011;49:3794-9.

V. **Could p16 ^{INK4a} and high-risk HPV mRNA analysis of liquid-based cytology identify pregnant women at risk for cervical carcinoma?**
Kärrberg C, Andersson E, Ryd W, Dohse M, Brännström M, Rådberg T
Submitted.

Contents

Abbreviations

AIS	adenocarcinoma in situ
ASCUS	atypical squamous cells of uncertain significance
AUC	area under the ROC curve
CI	confidence interval
CIN	cervical intraepithelial neoplasia
CIN2+	CIN2, CIN3 or cancer
CIN3+	CIN3 or cancer
CIS	carcinoma in situ
CS	cesarean section
E1, E2, E4-E7	early proteins of HPV
FIGO	the International Federation of Gynecology and Obstetrics
HGL	high-grade lesion (CIN2, CIN3 and AIS)
HPV	human papilloma virus
HR-HPV	high-risk-human papilloma virus
HSIL	high-grade squamous intraepithelial lesion
LBC	liquid based cytology
LEEP	loop electrosurgical excision procedure
LGL	low-grade lesions (koilocytosis, CIN1 and glandular dysplasia lower than AIS)
LLETZ	large loop excision of the transformation zone
LSIL	low-grade squamous intraepithelial lesion
L1+L2	late protein components of HPV
MCM2	minichromosome maintenance complex component 2
NPV	negative predictive value
OR	odds ratio
Pap	Papanicolaou
PPV	positive predictive value
pRB	retinoblastoma protein
$p16^{INK4a}$	cyklin-dependent kinase inhibitor 2A $p16^{INK4a}$
ROC	receiver operating characteristics
SCC	squamocellular cancer
SIL	squamous intraepithelial lesion
TOP2a	topoisomerase (DNA) II alpha
TZ	transformation zone
W-M	Wertheim-Meig
WHO	world health organization

Introduction

Background

Cervical cancer is one of the most common types of cancer, that is diagnosed in association with pregnancy. However, the total incidence is low due to cervical cancer prevention screening programs with cervical cytological smear sampling. The incidence rate of atypical cervical cytology in pregnancy is considerably higher with a reported incidence in different countries between 0.5 and 6%. The primary aim in investigation of atypical cervical cytology during pregnancy is to exclude cancer so that further treatment of the lesion can be postponed until after delivery. A clinical difficulty is that the normal changes of the cervix during pregnancy, can in many way mask a cancer and the malignancy may thus be missed if not multiple or large biopsies are taken. Invasive interventions on the cervix may increase the risk of bleeding, secondary to the increased vascularity during pregnancy, and increased risks for obstetrical complications may also be present. In this thesis a colposcopic scoring system and different biomarkers were evaluated in order to improve the investigation of pregnant women with atypical cervical smear in order to select those women needing further investigation and to reduce the number of biopsies. Furthermore, the management of cervical dysplasia during pregnancy and the effect of screening on the cervical cancer incidence during pregnancy were evaluated.

The cervix

The anatomical structure that this thesis is focused on is the uterine cervix, which is the lower portion of the uterus. The cervix varies in size and shape depending on the woman´s age, parity and hormonal status. The most caudal part of the cervix protrudes into the upper vagina, and this lower half of the cervix is called portio vaginalis (ectocervix). Inside the cylinder-shaped cervix is the cervical canal (endocervix) (Fig. 1).

The cervix consists of fibrous connective tissue with muscle threads being arranged in a superficial longitudinal layer and an inner circular layer (1). The ectocervix is covered by a stratified squamous epithelium of approximately 0.5 mm thickness and composed of 15-20 layers of cells. From the deep layers towards the surface, these cell layers undergo maturation. This maturation process is characterized by an increase in size of individual cells and a reduction in nuclear size. The internal basal layer is formed by a single row of small, cuboidal-shaped cells. The intermediate layer consists of 5 or 6 rows of cells, which have clear cytoplasm and contain large amounts of glycogen.

The superficial layers are formed of 6-8 rows of cells that become progressively flatter towards the surface. The cytoplasm of these cells is almost entirely filled with glycogen (2).

The endocervix consists of a single epithelial layer of mucus-secreting columnar cells and a few ciliated cells, so called “reserve” cells from which the mucosa may regenerate. The glandular epithelium covers numerous stromal connective tissue papillae, forming glands when the epithelium becomes invaginated into the connective tissue (2).
When the cervix grows and enlarges under influence of estrogens after puberty and with higher estrogen levels during pregnancy, there will be an eversion of the columnar epithelium onto the ectocervix and the squamocolumnar junction will then become positioned on the ectocervix and will thereby be readily visible. A squamous metaplasia is then formed on the everted columnar epithelium. This immature metaplastic squamous epithelium is derived from the sub-columnar reserve cells and the region where squamous metaplasia occurs is named the transformation zone (TZ) (Fig. 1). The metaplastic process mostly starts at the original squamocolumnar junction on the ectocervix and proceeds centripetally towards the external os of the cervix through the reproductive period and develops into mature metaplastic epithelium. Human papilloma virus (HPV) of many different types may persistently infect the immature basal squamous metaplastic cells and transform these cells into atypical cells with nuclear and cytoplasmic abnormalities (3). After menopause and more or less after pregnancy the transformation zone is retracted into the endocervix.

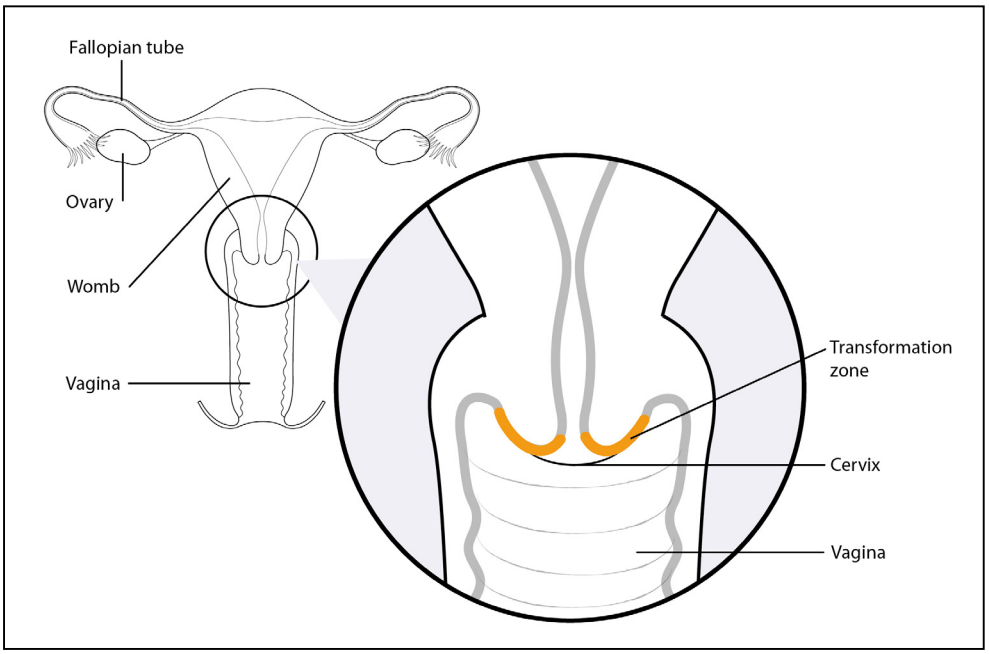

Figure 1. The uterine cervix

Human papilloma virus life cycle

Infection of the cervix with human papilloma virus is the underlying cause of cervical dysplasia and cervical cancer. The HPV particles consist of an around 8000 base-pair long circular DNA molecule wrapped into a protein shell that is composed of two molecules, late protein components of HPV (L1 and L2). The genome has the coding capacity for these two proteins and for at least six so-called early proteins (E1, E2, E4-E7) that are necessary for replication of the viral DNA and for assembly of new virus particles (Fig. 2).

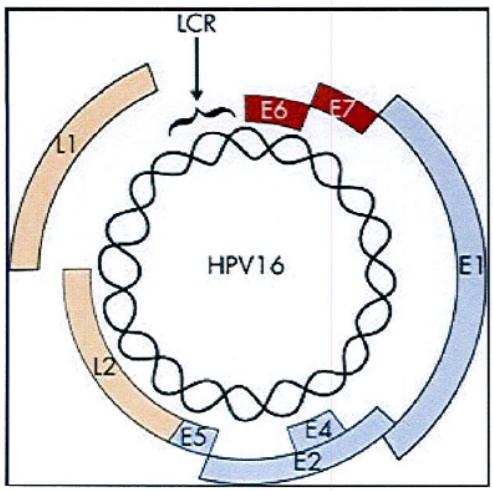

Figure 2. The human papilloma virus (HPV)

The life cycle of the HPV is initiated when virus-particles, through scratches in the epithelium, reach the basal cell layer, where they bind to and enter the cells. First, the viral genome is replicated to a copy number of about 100 and maintained for varying periods of time at this low copy number within the initially infected cell. The viral proteins E1 and E2 are essential for the replication of DNA in the basal cell. When the basal cells are pushed to the suprabasal layers they lose their ability to divide. The viruses replicates in this compartment and are released at the superficial layer (4, 5) (Fig. 3).

Transformation of the epithelial cell may only occur in persisting infection and the E6/E7oncogenes are required to induce and maintain neoplastic growth of cancer cells. The viral proteins E6 and E7 interact with a number of cellular proteins. The major interactions are 1) the E6 protein of the oncogenic HPV types supports premature degradation of the p53 tumor suppressor protein and thus interferes with apoptosis, cell

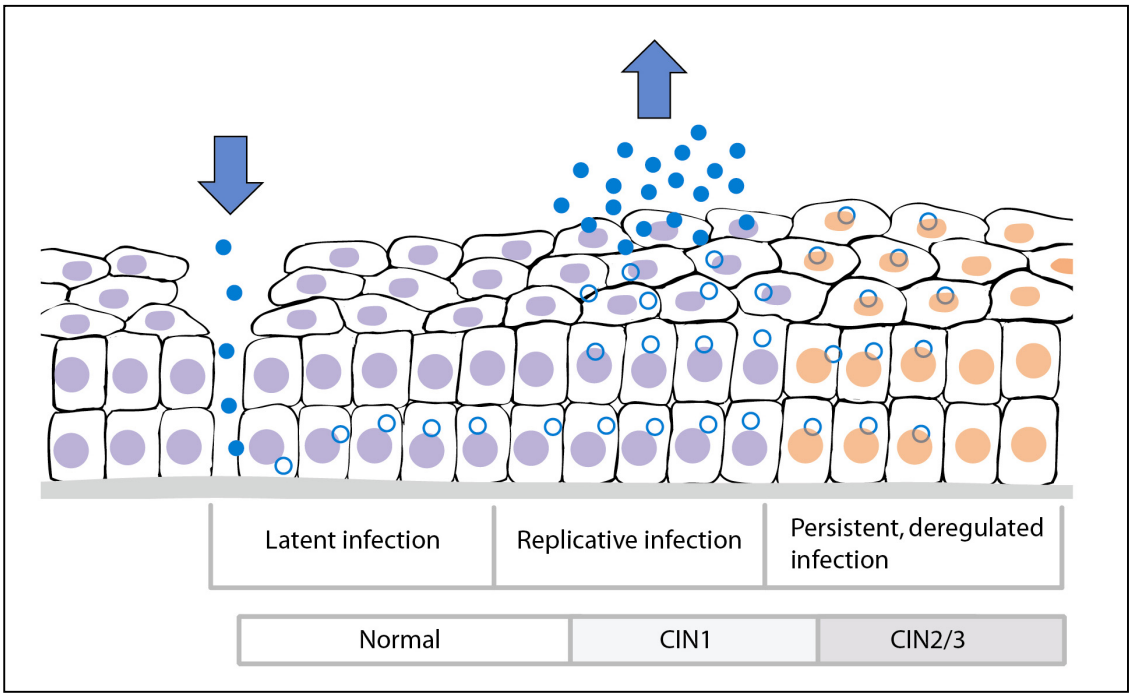

Figure 3. The life cycle of HR-HPV infections

cycle regulation and DNA repair, (6), 2) the E7 protein induces destabilization of the retinoblastoma protein complex and thus allows the cell to evade from the controlled cell cycle and differentiation control through the retinoblastoma-protein (pRB) pathway, (7), 3) both E6 and E7 induce substantial disturbances of the mitotic function by interfering with centrosome synthesis and function that results in desegregation of the chromosomes during mitosis and numerical and structural chromosome aberrations. (8).

As mentioned above, the E6/E7oncogenes are required to induce and maintain neoplastic growth of cancer cells. In this context the gene coding for the cyclin-dependent kinase inhibitor $p16^{INK4a}$ is of special interest in the epithelial cells with malignant transformation. The protein $p16^{INK4a}$ is a negative regulator of the cell cycle and contributes to the arrest of the cell cycle. The promotor of the $p16^{INK4a}$ gene is blocked by the pRB complex in normal cells and consequently the transcription of $p16^{INK4a}$ is inhibited. However, in infected, transformed epithelial cells the oncoprotein coded by the viral E7 gene interacts with the pRB and this induces a premature degradation of the pRB and the cells start to overexpress $p16^{INK4a}$ in proliferating parts of dysplastic epithelium. Occurrence of high levels of $p16^{INK4a}$ has been proposed as a biomarker for cervical dysplasia by the use of monoclonal antibodies which stain dysplastic cells whereas normal epithelium does not show any $p16^{INK4a}$ immune-reactivity (9) (Fig. 4).

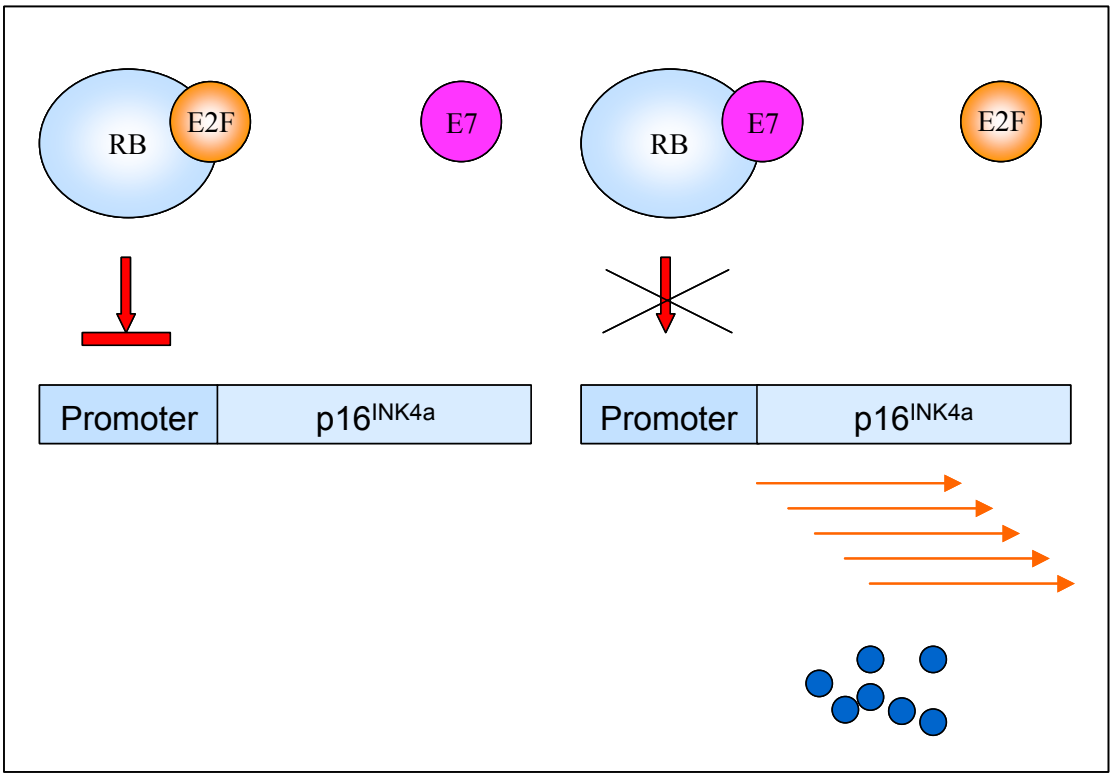

Figure 4. Schematic presentation of the mechanisms that induces $p16^{INK4a}$ over expression in proliferating epithelial cells that express the HR-HPV E7-protein

Human papilloma virus and cervical cancer

HPV causes virtually 100% of cases of cervical cancer (10). Cancer of the cervix uteri is, after breast cancer, the second most common cancer among women worldwide, with estimated 530 000 cases and 275 000 deaths in 2008 (11). About 86% of the cases occur in the developing countries. Worldwide, mortality rates of cervical cancer are substantially lower than the incidence with a mortality around 52% (11). The majority of cervicalcancer cases are squamous cell carcinoma while adenocarcinomas are less common (12).

The International Agency for Research on Cancer, IARC, is an extension of the World Health Organization (WHO). It has a program on the evaluation of the carcinogenic risk of chemicals, biological agents, physical agents, life-style and occupational factors, and to produce critically evaluated monographs on each carcinogenic agent/factor. The evidence relevant to carcinogenicity is classified into four categories: 1) sufficient evidence of carcinogenicity 2) limited evidence of carcinogenicity, subdivided into group A; probably carcinogenic, and B; possibly carcinogenic 3) inadequate evidence of carcinogenicity and 4) evidence suggesting lack of carcinogenicity. The HPV types that are carcinogenic to humans are the so-called high risk HPV which are HPV 16, 18, 31, 33, 35, 39, 45, 51, 52,

56, 58, and 59. HPV 68 is probably carcinogenic. HPV types 26, 53, 66, 67, 70, 73, 82 are possibly carcinogenic to humans and HPV types 30, 34, 69, 85, 97 are possibly carcinogenic to humans based on their phylogenic analogy to HPV types with sufficient or limited evidence in humans. HPV5, 6, 8 and 11 are not classifiable as carcinogenic to humans with the exception of HPV 5 and 8, which are possibly carcinogenic to patients with the skin disease epidemolysis verruciformis (13).

Worldwide HPV 16 and 18 contribute to over 70% of all cervical cancer cases, between 41-67% of high-grade cervical lesions and 16-32% of low-grade cervical lesions (14). The twelve most common HPV types identified in cervical cancer in the world are in order of descending prevalence HPV 16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51 and 56 (15).

For reasons that are not understood, persistent HPV infections cause cancers mainly at the TZ of the cervix (16). However, recent findings suggest a discrete population of squamocolumnar junctional cells with unique morphology and gene expression to be the target of HPV infection (17). Even though HPV is the essential cause of cervical cancer, it is also dependent on a number of cofactors that are necessary for progression from cervical HPV infection to cancer. Established cofactors are tobacco smoking (18), parity (19), oral contraceptive use (20), immunosuppression (21) and probable cofactors are co-infection with Chlamydia trachomatis (22) or herpes simplex virus type-2 (23) and certain dietary deficiencies (24). Genetic and immunological host factors and viral factors other than type, such as variants of type, viral load, and viral integration are likely to be important in malignant transformation of the cervix, but have not yet been clearly identified as such (5).
Worldwide among women having normal cytology smear the most common high-risk (HR) HPV types are HPV 16, 18, 31, 58, 52, but the ranking of the different HPV types varies in different parts of the world (25). In a Danish cohort of 11 000 women (mean age 36 years) with normal cytology the five most common HR HPV types were HPV 16, 31, 52, 51, 18 (26).

Classification of cytology

The classification of cervical cytology shows the same variations worldwide. In all Papers of the thesis a generally accepted classification system has been used. The Bethesda system, using a few categories is today the most widely used (27). In Sweden a modified classification is used, based on the WHO-classification and the Bethesda-classification (28) (Fig. 5). In Paper V in this thesis the terminology of the British Society for Clinical Cytology is used (143).

<table>
<tr><td>Classification system</td><td colspan="8">Cytology classification</td></tr>
<tr><td rowspan="2">The Bethesda system</td><td rowspan="3">Normal</td><td rowspan="2">Infection
Reactive
Repair</td><td rowspan="2">ASCUS</td><td colspan="4">Squamous intraepithelial lesion (SIL)</td><td rowspan="3">Invasive carcinoma</td></tr>
<tr><td colspan="2">Low-grade (LSIL)</td><td colspan="2">High-grade (HSIL)</td></tr>
<tr><td>Richart</td><td colspan="2" rowspan="2"></td><td>Condyloma</td><td colspan="3">Cervical intraepithelial neoplasia (CIN)</td></tr>
<tr><td></td><td>Grade I</td><td>Grade II</td><td>Grade III</td></tr>
<tr><td>Reagan (WHO)</td><td colspan="2">Atypia</td><td colspan="2">Mild dysplasia</td><td>Moderate dysplasia</td><td>Severe dysplasia</td><td>In situ carcinoma</td></tr>
<tr><td>Papanicolaou</td><td>I</td><td>II</td><td></td><td colspan="3">III</td><td colspan="2">IV</td><td>V</td></tr>
</table>

Figure 5. Classifications of cytological smears. Adapted from Nanda (29)

ASCUS=atypical squamous cells of uncertain significance
WHO=world health organization

Classification of histology

The classification of cervical intraepithelial neoplasia (CIN) by Richart is the most commonly used classification to define grade of dysplasia in histology. In this classification system, CIN1 represents mild dysplasia, CIN2 moderate dysplasia and CIN3 represents both severe dysplasia as well as carcinoma in situ (CIS) (30) (Fig. 6).
CIN 1 is defined as histology showing atypical cells in the lower third of the squamous epithelium. These lesions often include the so called koilocytosis in the upper part of the epithelium, reflecting the presence of a HPV infection in the epithelium. CIN2 has atypical cells in the lower 2/3 of the epithelium and in CIN3 atypical cells are found in more than 2/3 of the epithelium. The transition from CIN3 into microinvasive cancer is by definition when the basal membrane is penetrated by atypical cells (31).

A new two graded classification, the Bethesda system, was introduced 1988. In this system low–grade lesion incorporates CIN1 (low grade CIN) and HPV-related changes. High-grade lesions (HGL) consist of CIN2-3 (high grade CIN) (32). Low-grade lesions (LGL) are also called LSIL (low grade squamous intraepithelial lesion) and high-grade lesions are also referred to as HSIL (low grade squamous intraepithelial lesion).

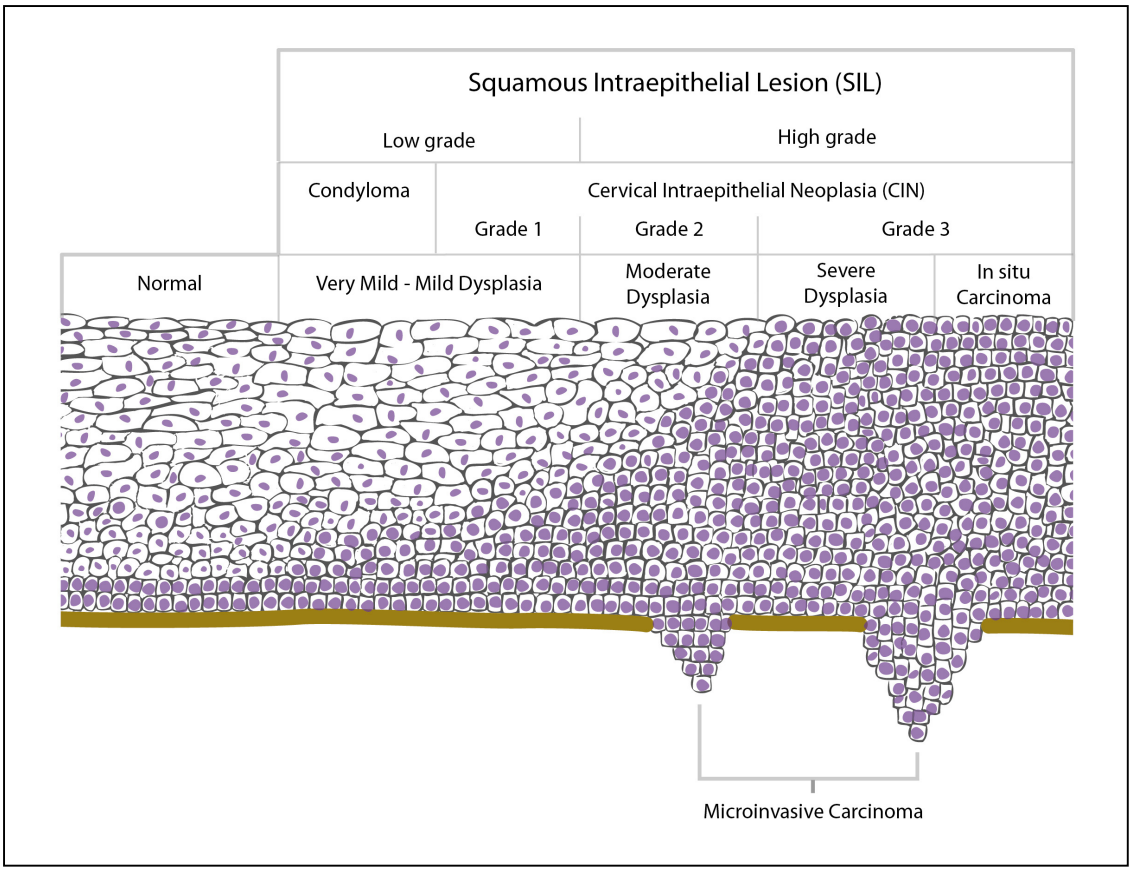

Figure 6. Classifications of cervical intraepithelial neoplasia

SIL=squamous intraepithelial lesion
CIN=cervical intraepithelial neoplasia

The colposcopic examination

Colposcopy, as a means of detailed examination of the cervix, was used in Paper I in the present thesis. Colpolscopy is the standard method to investigate the cervix in women having atypical cervical smears and was first described by Hans Hinselmann 1925. The colposcope is a binocular, stereoscopic microscope providing magnification between six- and 40 times (33) (Fig. 7). At initial examination of the cervix a magnification of 6-12 is commonly used and for detailed observations of exact structure and details of vessels, higher magnifications have to be used. Most often a green filter is used to identify the blood vessels and their angio-architecture. At colposcopic examination, the woman lies in a lithotomy position and a bivalve specula is inserted in to the vagina to separate the vaginal walls so that the cervix and the distal part of cervical canal can be visualized.

The colposcopic appearance of the tissue is caused by various factors such as the architecture of the epithelium and possible variations in its thickness and formation, the composition of the underlying stroma and the surface configuration of the tissue. At colposcopic examination, the most abnormal area is identified by estimating different parameters so that adequate biopsies can be taken from that specific area for further histological analysis (33).

Figure 7. The colposcope

1. General assessment of the cervix

The first step at colposcopical examination is to perform a general assessment of the external cervix and to identify the position of transformation zone and then estimate type of transformation zone (1-3) (Fig. 8) (34). Satisfactory colposcopy examination is defined as when the new squamocolumnar junction is seen at its full extent and when the entire area of abnormal (atypical) epithelium is visible at further colposcopic examination. After this first procedure a green filter is used to identify any signs of suspicion of invasive disease such as the structure of the vessels.

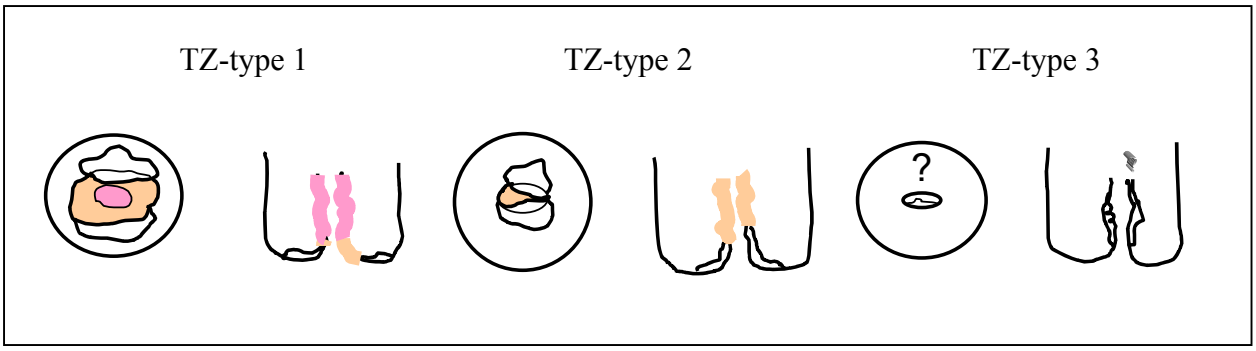

Figure 8. Types of transformation zones (TZ)

2. Application of acetic acid and assessment of acetowhiteness

To locate atypical epithelium, acetic acid of concentration between 3 and 5% is then applied on the cervix. The acetic acid makes atypical epithelium white or opaque and it is then quite easy to distinguish this from normal epithelium which appears pink. The mechanisms of this epithelial acteowhiteness, is a reversible coagulation of the epithelial and stromal cytokeratins. Furthermore, there is a swelling of the tissues (35). It has been shown that there is also an increase in the keratin filament proteins in epithelium, which turn white by acetic acid (35, 36). There is also a precipitation of nucleoprotein of the cells (33). However, it must be emphasized that this acetowhite appearance is not unique for neoplasia and will be seen on other occasions when there is increased nucleoproteins present e.g. during metaplasia, in columnar epithelium, at healing and at presence of virus or viral products (33).

When acetic acid is applied to areas of cervical intraepithelial neoplasia (CIN), the precipitated nucleoproteins within the neoplastic cells obscure the underlying vessels, the light is reflected and the epithelium appears white at colposcopy. With high-grade CIN, there will be an almost instant response within about 50-60 seconds and the acetowhiteness will appear markedly white as a greater part of the epithelium consists of atypical cells. The effect is slowly reversed because the acid is buffered and the nucleoprotein will no longer stay precipitated. The acetowhiteness will disappear within about 40 seconds. With low-grade CIN, the onset of white is delayed because the acid must penetrate into the lower half of the epithelium where the atypical cells are situated (33).

3. Assessment of margins and surface of the lesion

The margins of the atypical cervical epithelium are graded according to a number of features, which include sharpness, conformation and thickness of the border and the presence of internal margins in a lesion. In HGL, the margins exhibit distinct raised edges that may also be located within a larger low-grade lesion. In low-grade lesions, margins are usually described as irregular, geographic, and indistinct. Further-more, satellite lesions or exophytic micropapillous condylomas are regarded as LGL (37).

The surface contour of the lesions can be assessed by the stereoscopic magnification in the colposcope. The surface can be smooth, papillary, nodular, uneven or even ulcerated. In normal squamous epithelium, a smooth surface is seen while normal columnar epithelium has a grape-like and papillary shape. In high-grade CIN, particularly concerning CIN3 and early invasive cancer, the surface is uneven and slightly elevated while the frankly invasive lesions have nodular or polypoid lesions that develop to ulcerated or exophytic lesions (33). Another atypical sign is so-called cuffed glands, which describe that gland openings are surrounded by a cuff after application of acetic acid. These kind of findings indicate presence of high-grade CIN squamocellular epithelium in the cervical glands (2).

4. Assessment of vascular patterns

The two atypical vascular patterns found within abnormal epithelium are punctuation and mosaicism, which may also exist in combination. In epithelium stained with acetic acid, the intraepithelial atypical vessels will often be absent but sometimes these vessels appear. Punctuation is seen as red points throughout the epithelium and mosaic pattern has a wall-like honeycomb pattern.

In histological sections, punctuation consists of dilated and often twisted, irregularly terminating vessels that are of hairpin type and in a prominent punctate pattern. In the mosaic pattern the capillaries are arranged parallel to the surface, forming the honeycomb like configuration. When acetic acid is applied to this type of tissue a pattern of small white cobblestone is produced, each corresponding to an epithelial bud and surrounded by a red margin that corresponds to the blood vessels.

An important factor in assessment of mosaic pattern or punctuation is the intercapillary distance. This refers to the distance of two adjacent vessels or to the diameter of fields delineated by mosaic-like vessels. In normal squamous epithelium, the intercapillary distance averages about 100 µm, but the intercapillary distance increases as the preinvasive

or malignant nature of the lesion increases, where the epithelial pegs are wider, thicker and more irregular (3, 33).

It must be noted that punctuation and mosaic pattern may also be found in normal epithelium. The vessels are discrete and the intercapillary distances are variable but usually not excessive and can be distinguished from neoplastic lesion. The pattern of punctuation may also be seen when there is an inflammation of the tissue, especially in vaginal trichomonal infection and cervicitis.

Atypical vessels may often indicate invasive cancer and these vessels have different appearances (hairpin-, corkscrew-, tendril-, waste-thread-, willow-branch-like or in some cases root-like pattern that indicates adenocarcinoma in situ (AIS) as well as adenocarcinoma) (33). In the earlier stages of invasion it may be difficult to note the clear distinction between the vascular pattern of CIN (punctuation and mosaic pattern) and these atypical malignant vessels. In many cases high-grade CIN and early invasion are found together in the lesion where only a small focus of slightly atypical cells is seen in a more extensive area of punctuation and mosaic pattern. Coarse and irregular vessels with extremely large inter-capillary distances and variation between vessel characteristics, sometimes in addition to avascular whiteness appearing within the epithelium, indicate early invasion. As malignant cells proliferate a vascular areas get larger (38, 39).

During the course of development of adenocarcinoma or undifferentiated carcinoma a vascular pattern is frequently seen that differs to that of squamous lesions (39): atypical vessels that originate from the central capillary networks of the papillary columnar epithelium. The adenocarcinomas seem to derive their nutrition through the central capillary system; which is in contrast to the well-differentiated squamous cell cancers that seem to have microcirculation by peripheral vessels that surround the epithelial buds but do not seem to have any penetrating vessels. The undifferentiated lesions penetrating between the epithelial buds of malignant cells tend to have fine capillaries. Therefore, inter-capillary distances in these undifferentiated malignancies may be quite normal in many areas. Ueki et al. (40) proposed that these features are representative of these early stages. There may be a mixture of different types of vessels in adenocarcinoma in contrast to punctuation and mosaicism that is seen in squamous carcinoma.

5. Size of the lesion

The size of the lesion is related to the grade of dysplasia in histology. Kierkegaard et al. found that when the size of the lesion was evaluated at colposcopy, the accuracy of the colposcopy was improved and fewer lesions were underestimated concerning grade of dyplasia when compared to histology in biopsy samples (37). In another study, it was shown that in women having cervical smears with mild dyskaryosis, the size of the lesions were smaller than among those having severe dyskaryosis (41). Tidbury et al. showed that the mean size of CIN3 lesions containing parts of microinvasion was 7-fold greater than that for severe dyskaryosis without invasion and 100-fold greater than for mild dyskaryosis (42).

6. Assessment of iodine staining

The iodine test also named, Schiller´s iodine test, was originally introduced 1933 by the American pathologist Walter Schiller in order to detect preinvasive lesions. When the so-called Lugol´s solution, consisting of iodine and potassium iodine, is applied on normal cervical squamous epithelium a brownish stain develops due to glycogen content. This represents an iodine positive state. Atypical epithelium is free of glycogen and is stained yellow with the iodine solution and an area of atypical squamous epithelium can be outlined (33).

There are a number of false positive results of Schiller´s test that are not uncommon and these are particularly likely to be produced when there is much immature metaplasia. Other examples of false positivity of Schiller´s test are the state of the cervix after a recent pregnancy, after menopause and in inflammatory lesions. Furthermore, normal columnar epithelium is not normally stained by Lugol´s solution (33).

Colposcopic scoring systems

In the present study, a specific colposcopic scoring system was evaluated in a pregnant population (Paper I). Several attempts have been made to make the colposcopic evaluation of the cervix more structured, instead of only being a subjective assessment of the overall cervix. In the early days of the history of coloposcopy, it was generally thought that the colposcopic possibility to observe the ectocervix and the transformation zone at high magnification, in comparison to observation by the naked eye, would make the diagnosis of different grades of dysplasia and also of microinvasive cancer fairly easy and straightforward. However, at an early stage it became apparent that reproducibility and accuracy of the colposcopic evaluation was dependent on that separate variables were

determined in a structured manner, and hence different colposcopic scoring systems were presented. In the section below some of the currently most frequently used scoring systems are described.

Reid´s colposcopic index

Reid´s index is the most well-known colposcopic scoring system and it consists of four parameters that are graded 0, 1, or 2 (43). This colposcopy scoring index was created by Richard Reid in the beginning of the 1980s to differentiate benign HPV infections from high-grade CIN. In the original study, 72 women with colposcopic changes and satisfactory colposcopic examination were consecutively investigated in a prospective manner. Biopsies were obtained from the most atypical area of each transformation zone. The study was designed to evaluate the novel colposcopic sign (sharpness of peripheral margin) and to compare this new colposcopic variable with five previous criteria i.e. apparent thickness, exact color of any acetowhitening, surface contour of abnormal epithelium, precise pattern of vascular atypia and iodine staining reaction. A variety of minor patterns of both peripheral outline and lesion shape, each identifying minor histologic disturbance were found. In contrast, middle grade lesions were found to display much greater regularity of shape and outline. The best markers of CIN2 and 3 were the findings of rolled or peeling edges (a reflection of friability of neoplastic epithelium) and the occurrence of internal demarcation between lesions of different colposcopic appearances.

As mentioned previously, Reid´s colposcopic index, differentiated the colposcopic findings into either 0, 1 or 2. The definitions were as follows: A zero score for each variable was given when each parameter showed 1) all condylomatous or micro papillary lesions; 2) all faint acetowhitening with indistinct margins; 3) definite acetowhite lesions with broken flocculated borders; 4) sharply outlined lesions with frilly, feathered edges; 5) irregularly shaped lesions with jagged outlines and; 6) satellite lesions, not continuous with new squamocolumnar junction or acetowhite epithelium situated distal to the peripherial margin of the TZ. One point was assigned for sharply defined lesions with smooth, straight peripheral borders. Two points were given for (dull), oyster- white epithelium with rolled, peeling edges, reflecting the fact that neoplastic epithelium of reduced tensile strength is easily detached during colposcopic examination and the characteristics of proximal lesions, in which the distal margin represent an internal border between two colposcopically different grades of acteowhite epithelium. The specific characteristics of the parameters color (acetowhitening), vessels and iodine for each score are given in Table 1.

Table 1. Reid's colposcopic index (43)

Colposcopic Sign	Score		
	Zero points	**One point**	**Two points**
Margin	Condylomatous or micropapillary contour Indistinct acetowhitening Flocculated or feathered margins Angular, jagged lesions Satellite lesions and acetowhitening that extends beyond transformation zone	Regular lesions with smooth, straight outlines	Rolled, peeling edges Internal demarcations between areas of differing appearance
Color	Shiny, snow-white color Indistinct acetowhitening	Intermediate shade (shiny grey)	Dull, oyster-white
Vessels	Fine-caliber vessels, poorly formed patterns Condylomatous or micropapillary lesions	Absent vessels	Definite punctation or mosaic
Iodine	Positive iodine staining Minor iodine negatively	Partial iodine uptake	Negative staining of significant lesion

In the Reid study, colposcopic scores were prospectively recorded from each woman and correlated with the histologic findings. These findings were categorized histologically as either HPV infection or CIN 1 to 3 according to previously validated criteria.

The differences in pattern of the peripheral margin were predictive throughout the morphologic spectrum and these histologic associations of the different categories of the peripheral margins were statistically significant. Minor patterns of peripheral margin indicated benign, viral proliferation or mild dysplasia. Straight borders indicated middle-grade lesions, while both rolled and internal borders were predictive of full thickness intraepithelial dysplasia i.e. CIN3.

Combining the new colposcopic sign of peripheral margin with acetowhite color, vascular pattern and iodine staining in the index increased the predictive power to approximate histology and the predictive accuracy to approximate histology rose to 97%. When adding the scoring points at colposcopy of all cases, scoring with two points or less were either benign, HPV or mild dysplasia and 13 /14 women scoring three to five points were either mild or moderate dysplasia. In 30 out of 31 women scoring six to eight points, CIN 2 or 3 were found.

Stellato Paavonen colposcopic scoring system

In 1995, Stellato and Paavonen published a study of a colposcopic scoring system that evaluated three parameters at colposcopy with purpose of distinguishing low-risk from high-risk lesions by using cervical cytology and a HPV-test (44). One hundred and fifty-nine HPV DNA positive women (seven with HPV 6/11, 51 with HPV 16/18, 52 with HPV 31, 33 and 35 with mixed HPV types and 24 with HPV not categorized) were investigated alongside 69 age-matched HPV DNA negative women as control group. Cytological samples from the endocervix and the ectocervix, were stained with a modified Papanicolaou (Pap) stain, and at last evaluated and classified in either of the cytological categories negative, atypical or dysplastic.

At colposcopy, the parameters included and scored were: borders of the lesion, tone of acetowhitening and vascular atypia. These parameters were given a value of 1 to 3, with the grading so that a higher score was associated with a higher risk of having a higher grade of dysplasia (Table 2). Colposcopical, cytological and HPV DNA examinations were performed in the study group with four months intervals, with a mean follow-up-time of around 12 months. The presence of atypical cervical smear consistent with CIN represented the study endpoints and 26 of the women had the endpoint criteria and underwent colposcopic directed biopsy.

Table 2. Stellato Paavonen colposcopic scoring index

Index	**1**	**2**	**3**
Borders	Diffuse	Irregular, sharp with fingerlike projections	Sharp, regular
Color tone	Transparent	Faint	Opaque
Vascular Atypia	Spiderweb Capillaries	Fine, regular	Coarse, regular

Presence of atypical TZ at the first study visit was observed in 52% of these women and in 45% of the controls. In the HPV 6/11 positive women, atypical TZ was seen in 43%, in the HPV 16/18 positive women in 59%, in the HPV 31/33/35 positive women in 54%, in the mixed HPV DNA group in 56%, and in the HPV DNA group not categorized in 33%. The mean colposcopic score derived from the characteristics of atypical TZ was 4.9 in the HPV DNA positive women and 4.3 in the control group. The mean colposcopic score was 3.3 for those with HPV 6/11, 4.5 for HPV 16/18, 5.3 for HPV 31/33/35, 5.0 for mixed HPV types and 4.0 for the HPV DNA not categorized. Moreover, 64% of the HPV DNA positive cases and 80% of the controls had normal cytologic cervical smear at first study visit. Benign atypia was observed in 36% of the cases and in 20% of the controls.

During the follow-up-period, atypical cytology that was consistent with dysplasia was observed in 16% of HPV DNA positive women, and in 1% HPV-negative women. CIN in histology was detected in ten HPV DNA positive women of whom eight belonged to the HPV 16/18 or HPV 31/33/35 cohorts. In the group with dysplasia, the mean score was 4.3 compared to 2.2 among women without dysplasia. In women with biopsy proven CIN, the score was 5.1 compared to 2.3 in women without CIN. The mean score was higher but not significantly in women having CIN2 (5.6) compared to women having CIN1 (4.6). The only study published in the English language where this scoring system has been applied on pregnant populations is a study by Marana et al. (45).

Swede score

Some years ago the Gothenburg group of colposcopists introduced a new colposcopic scoring system named the Swede score (46), with addition of the parameter size of the lesion to the four classical parameters of acetowhitening, margins and surface, vessel pattern and iodine staining. In this scoring system the observations concerning these parameters are graded with 0, 1 and 2 points. Thus, the maximum score is 10 (Table 3) (46).

This new colposcopic scoring system was introduced in a prospective study of 297 non-pregnant women referred for further investigation or treatment due to atypical cytology. All women were scored according to the protocol at colposcopy. Biopsies were taken from the most atypical part of the lesions or large loop excision of the transformation zone (LLETZ) were performed as treatment of dysplasia. The results of the histological analysis were compared to the scoring sum of each patient. Multiple logistic regression analysis showed that all parameters contributed to this model. For each score, there was a

certain probability to find or exclude high-grade lesions. This has been summarized as sensitivity and specificity for HGL at different threshold scores in Table 4.

In the study (46), no CIN2, CIN3 or cancer (CIN2+) lesions were found in women having four points or less. A score of 8 or higher had a specificity of 90% for CIN2+ in histology with a sensitivity of 52%. All women with adenocarcinoma in situ were scored eight to 10 scoring points.

Table 3. Variables and scores of the Swede score system (46)

Score	**0**	**1**	**2**
Acetowhiteness	0 or transparent	Cloudy, milky	Distinct, opaque white
Margins plus surface	0 or diffuse	Sharp but irregular, jagged, ”geographical”, satellites	Sharp and even, varying surface level (including cuffing)
Vessel patterns	Fine, regular	Absent	Coarse or atypical
Lesion size	<5mm	5-15 mm or 2 quadrants	>15 mm or 3-4 quadrants, or endocervically undefined
Iodine staining	Brown	Faint or patchy yellow	Distinct yellow

Table 4. Colposcopy score for all observations (*n*=297) with sensitivity and specificity for HGL (*n*=135) at different threshold values (46)

Score	**All** (*n*)	**HGL** (*n* and percentage of all)	**Sensitivity**	**Specificity**
0	0	0 (0.0%)	1.00	0.00
1	10	0 (0.0%)	1.00	0.00
2	5	0 (0.0%)	1.00	0.06
3	13	0 (0.0%)	1.00	0.09
4	23	0 (0.0%)	1.00	0.17
5	38	2 (5.3%)	1.00	0.31
6	57	20 (35.1%)	0.99	0.54
7	65	43 (66.2%)	0.84	0.77
8	47	36 (76.6%)	0.52	0.90
9	25	20 (80.0%)	0.25	0.97
10	14	14 (100%)	0.10	1.00

Ten invasive cancers were diagnosed and all were the International Federation of Gynecology and Obstetrics (FIGO)-stadium I. Four were adenocarcinomas and one contained both adenocarcinoma and squamous cell carcinoma. One of the invasive cancers scored seven points and the other cancers received scores of eight to ten. It should be noted that all cancers scored two points for margin and size. One cancer received one point for acetowhitening and the rest two points for this variable.

The importance of size of lesion was originally described in a large research study by Kierkegaard et al. (37), who investigated 755 women by colposcopy and expressed the size of the lesion and the size of the TZ as a percentage of the visible part of the cervix. Directed biopsies were taken of the TZ from any area with abnormal colposcopic characteristics or if the cervix appeared normal at 6 and 12 o´clock positions on the cervix, and in addition endocervical curettage was taken from the non-visible parts. When each single finding was analyzed, the size of TZ, the size of the lesion, margins, micro papillae, vascular pattern and acetowhitening, and extension beyond TZ were registered. All these parameters were included in a multiple-ordered polychotomous logistic regression to evaluate the findings in combination. Both large-and medium-sized lesions showed independent increased risks for having a higher grade of CIN with an odds ratio (OR) of 3.6 confidence interval (CI) 95% (2.1-6.3) for large lesions and 2.0 (OR 1.3-3.0) for medium-sized lesions.

In another study, Tidbury and coworkers (42) reviewed 39 cervical cone specimens which fulfilled the criteria of microinvasive cancer according to definitions of Burghardt (47). The perimeter lengths of lesions were measured and it was found that the mean size of CIN3 lesions, showing microinvasive cancer, was 7-fold greater than those showing severe dysplasia without invasion and 100-fold larger than lesions with mild dysplasia.
In another study by Shafi et al. (48), the relation between size of colposcopic transformation zone abnormality and histology of specimens obtained at diathermy excision of the TZ was analyzed. There was a positive correlation between number of sectors involved in the specimens and the histological grade of CIN.

The Swede score system has also been evaluated by Bowring et al. in a prospective study on 200 women in London, who were attending diagnostic colposcopy clinics or undergoing inpatient treatment of CIN (49). The scores were recorded and correlated with the histological diagnosis after either a directed punch biopsy or excisional cervical biopsy. Performance of each scoring point was assessed for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) (Table 5). A score of eight or higher had a specificity of 95% for CIN2+ with sensitivity of 38%, a PPV of 83% and a NPV of 70%.

In the latter British study, there were altogether 7 colposcopists involved in the examination of the 200 women with four of the colposcopists being fully trained and accredited and 3 colposcopists still under training, according to the criteria by the British Society for Colposcopy and Cervical pathology. A subanalysis was done in order to investigate whether any typical learning curve was present when using the Swede score and in this analysis the first 100 examinations were compared with the subsequent 100 examinations. The trained and accredited colposcopists were compared against the un-accredited trainee colposcopists. Using the cut off Swede score of 8 or higher in predicting a CIN2 or higher, their PPVs did not differ significantly. This can be interpreted that the Swede score is relatively easy to learn. The trainees however had a greater NPV when scoring 3 or less.

Table 5. Prediction of HGL (≥CIN 2) by Swede score (49)

Score	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
≥2	100 (96-100)	12 (6-17)	43	100
≥3	98 (91-98)	32 (23-40)	48	95
≥4	91 (83-96)	53 (44-62)	56	90
≥5	75 (64-84)	73 (65-81)	65	81
≥6	65 (53-75)	82 (75-87)	70	78
≥7	45 (34-57)	89 (84-95)	73	71
≥8	38 (27-49)	95 (89-98)	83	70
≥9	29 (19-40)	99 (95-100)	96	68
10	10 (4-19)	100 (97-100)	100	63

Values in parentheses are 95% CI
NPV=negative predictive value; PPV=positive predictive value

Changes of the cervix during pregnancy

The present thesis deals with the pregnant cervix. It is important to keep in mind that the gross appearance and histology of the cervix changes during the transition from a non-pregnant to a pregnant state. During pregnancy there are certain changes of the appearance of the cervix. Especially among primigravida there is an eversion of the endocervical columnar epithelium on to the ectocervix. This occurs in the majority of cases during the second and the third trimesters. The mechanisms behind this eversion have been suggested to be related to retraction of sphincter-like portion of the cervical musculature (50). In some women, although less frequently in primigravida, there is no noticeable eversion of the columnar epithelium onto the ectocervix and the squamocolumnar junction

is located at the external cervical os even in late pregnancy. However, in some rare cases the eversion of the endocervical epithelium may develop as far as to the vaginal fornices (51). In parallel with the progression of pregnancy and subsequently to the eversion of the columnar epithelium, immature metaplastic squamous epithelium develops on the ectocervix and a new TZ appears. The proportion of columnar epithelium that generates metaplasia varies among pregnant women. This pregnancy-related development of metaplasia has its highest activity during the third trimester, but it is described that some further metaplasia may sometimes occur up to six weeks post-partum (51). During the further post-partum period, the eversion with the new squamous epithelium retracts more or less into the cervical canal. Thus, after pregnancy some women will show a total persistence of the everted state while in other women a reverse process of inversion occurs with complete retraction into the cervical canal (51). In subsequent pregnancies there is generally less eversion of the endocervix and just a minority of multi-parous women show any eversion. The eversions during the subsequent pregnancies are covered with metaplastic squamous epithelium, which have been developed during the first pregnancy rather than by columnar epithelium (51).

The bluish color of the cervico vaginal mucosa during pregnancy is due to blood congestion, especially the venous plexuses, of the lesser pelvis and its organs and there is also an increase in size and number of blood vessels leading to hyperemia of the cervix. The increase in lymph vessels of the cervix during pregnancy may also contribute to these changes. Furthermore, the stroma becomes softened and edematous due to fluid retention with concomitant hyperplasia of the endocervical mucosa (3). Proliferation of the columnar cells leads to enlargements of columnar villi, with further development into glandular crypts with formation of numerous secondary clefts and tunnels by fusion of the villi (1) and the glandular tissue may present as “honeycomb “ appearance (3).
A characteristic change during pregnancy is a decidual reaction of the stroma which may be limited and focal or may be quite extensive and produce polyp-like lesions, referred to as decidual polyps (3).

The consistency and appearance of the cervical mucus also undergo characteristic changes during pregnancy, with transformation into more viscous and turbid, with whitish or yellowish color and containing threads and particles (3).

Specific pregnancy-related colposcopic characteristics

The main purpose at colposcopy during pregnancy is to exclude presence of invasive cancer. Atypical colposcopic findings during pregnancy are generally assumed to be more difficult to evaluate due to the normal pregnancy-related metaplastic changes that can easily resemble CIN after application of acetic acid (52). Typical changes during pregnancy are cyanosis of the cervix and both hyperthrophy and edema of the endocervical glands. Furthermore, extensive immature metaplasia, especially in primigravida, often produces an intense acetowhitening, after application of acetic acid. There is also an increased vascularity on the cervix and fine punctuation as well as mosaic patterns are seen in the acetowhite areas of metaplasia. These changes can easily be misinterpreted as HGL.

The decidual reaction of the pregnant uterus is an effect of the high progesterone levels that render cells of both the endometrium and endocervix to enlarge. The reaction is a prerequisite for implantation and further progression of the pregnancy. This decidual reaction of the endocervical glands around the cervical opening in combination with vascular changes may mimic invasive cancer. However, the colposcopic appearance of HGL may often be easy to recognize. The acetowhite epithelium is then dense with distinct margins that are prone to lift and spontaneously peel off in pregnancy. The coarse mosaic pattern and punctuation of high-grade squamointraepithelial lesion (SIL) can usually be distinguished from physiologic changes.

As mentioned above, the primary goal of colposcopic evaluation during pregnancy is to diagnose early invasive cancer with high accuracy, so that biopsies or small cones are avoided and that no cancer cases are missed. The use of directed biopsy would decrease the risk of missing an invasive cancer. However, results of a biopsy may not necessarily reflect the dysplasia/cancer state of the cervix and larger wedge biopsy may be necessary if any suspicion of early invasive disease or progression of dysplasia. Marked oedema and vascularity of the cervix contribute to significant bleeding and this fact has to be taken into account (53).

It is well-known that it is easier to examine the cervix after about 16 to 18 week of gestation, than at early pregnancy, because of the eversion of endocervical columnar epithelium when the TZ is more accessible for colposcopic examination. It is my general observation that in a majority of women, the TZ can be fully visualized by the 20th week of gestation (51).

Colposcopic assessment and use of colposcopic scoring systems during pregnancy

There is a need to develop colposcopic scoring systems that are applicable also during pregnant state. It may also be that the use of a good scoring system is especially valuable in pregnancy as further discussed below.
Even an experienced colposcopist may overestimate or underestimate colposcopic lesions due to the pronounced structural and functional changes of the cervix during pregnancy (54, 55). An illustration of the difficulty is that some colposcopists also have reported false negative cases in their antenatal colposcopic assessment of malignancies (56-58). Thus, there is often a need to obtain biopsies to get accurate histologic diagnosis in order to exclude microinvasive cancer during pregnancy. However, when the cervix is prone to bleed at invasive procedures, due to the increased vascularity, there is a need to reduce the number or extent of tissue excision. Thus multiple or large biopsies as well as conization should be avoided. Furthermore, there is an increased risk for miscarriage and obstetric complications, at least after extensive conization (59-62).

Several studies suggest that a colposcopic scoring system, to standardize the investigation, can improve the accuracy at colposcopic investigation. Reid's index is the most established among the colposcopic scoring systems and it has been extensively tested in non-pregnant women showing an accuracy of predicting histological diagnosis between 97% in the original study (43) and 87% in a later study (63). The recently introduced Swede score, with the added parameter of lesion size, has been found to detect or exclude HGL with high accuracy as well as to reduce the need for biopsies in about 17% of non-pregnant women of fertile age (46). Its usefulness in a pregnant population is tested in this thesis.

There is only one study published in the English language on the use of colposcopic scoring systems in pregnant populations (45). The small study of 20 pregnant women had the aim to distinguish LGL from HGL and the results were that a three-variable score (44) detected HGL among pregnant women with a sensitivity of 100%, a specificity of around 92%, a PPV of 89% and a NPV of 100%. It was concluded that colposcopic indices may be a tool in selecting those pregnant women who should be further investigated with directed biopsies and thus reduce the need for biopsies in pregnant women with atypical cytology. It is especially important to find a highly reproducible and accurate scoring system in pregnant women, to avoid invasive procedures which may be connected to complications later in that pregnancy or in subsequent pregnancies.

Management of cervical dysplasia during pregnancy

The optimal management for dysplasia has been the subject of debate during many years. It is known that the prevalence of atypical cervical cytology is increased among pregnant women compared to non-pregnant populations (64). The reported rates of atypical cervical cytology in pregnancy vary between 0.52% and 6.8%, with the disparities most likely reflecting differences in study populations (65-71). Concerning Scandinavian countries, the prevalence of atypical cervical cytology during pregnancy has been reported to be around 1.4% in a Danish population (67). Most cervical abnormalities in pregnancy are identified as a result of routine screening at the initiation of antenatal care and some authors suggest that pregnancy is an opportunity to detect abnormal smears (72-74).

Atypical cervical cytology in pregnancy should be investigated further to rule out the presence of invasive cervical cancer. Treatment of cervical dysplasia can be postponed until after delivery. Different strategies in management and follow-up during pregnancy have been suggested. Colposcopic evaluation during pregnancy is recommended but requires a high degree of skill and should be performed by an expert colposcopist with knowledge of the specific changes of the pregnant cervix (53-55, 75). The primary goal of colposcopy is to exclude the presence of invasive cancer. However, even in experienced hands there may be overestimation of cervical lesions as well as underestimation (54, 55, 76) in evaluation of colposcopic findings and cervical cancer may then be overlooked (56-58, 75). Cytological accuracy in predicting histological diagnosis during pregnancy was estimated by Kashimura et al. to be 73% (77). Furthermore, some reports have documented rates of moderate to severe dysplasia in histology of around 30% among pregnant women having atypical squamous cells of uncertain significance (ASCUS) and L-SIL (67). Therefore, colposcopy directed biopsies are recommended to verify the colposcopic findings (54, 67, 76, 77). However, there are other reports suggesting investigation only by colposcopy and cytology unless invasive cancer is suspected or if there exist disagreement between findings of colposcopy and cytology (78, 79). The strategies of management and follow-up during pregnancy also differ. In a large retrospective multicenter cohort study of 1079 pregnant women in the U.S.A. by Fader et al., approximately 90% did not undergo follow-up with repeated cytology and colposcopy during pregnancy. Post-partum follow-up was done >six months after delivery with a regression rate of cytological abnormalities to normal in 64% of women having ASCUS/LSIL during pregnancy and a regression rate of 53% to normal among women having high-grade squamous intraepithelial lesion (HSIL) during pregnancy. Among women having ASCUS/LSIL during pregnancy 29% had persisting CIN1/ASCUS/LSIL

and 6% progressed to CIN2/CIN3/HSIL. Of the women having HSIL during pregnancy 31% were persisting as CIN2/ CIN3/HSIL. One case having ASCUS/LSIL during pregnancy progressed to microinvasive cancer (79). In an Australian retrospective study of 811pregnant women with atypical cytology all except those with normal colposcopy were biopsied (74%). Thirty-six percent of the women attended the clinic for a second visit around the 28th week of pregnancy. The results of the previsit cytology were concordant with in one degree of severity with the colposcopicallydirected biopsies in 87% of the women. The agreement was low when the cytology revealed atypia only. Among these women 14.5% had biopsy proven CIN2 or CIN3. The colposcopy tended to overestimate the predicted severity of abnormality; 19% of the women estimated to have CIN3 had normal histology in biopsy and 52% had CIN3 in histology. However, at post-partum follow-up with biopsy only 14.5% of the 811 women had CIN (55). In an US prospective study 279 pregnant women with biopsy verified CIN2-3 were reevaluated by colposcopy in the 28th and 32nd week of pregnancy. In that study women with CIN2-3 were reevaluated at three and six months after delivery with cervical smear and colposcopically directed biopsies if indicated. The regression rates were 68% and 70% for CIN2 and CIN3, respectively and no lesions progressed to cancer (80). In a Greek retrospective cohort study of 208 women colposcopically directed biopsies were obtained from lesions with suspicion of malignancy or CIN2-3. All women, biopsied or not, were followed up every 8-10 weeks during pregnancy with colposcopic reevaluation 8-12 post-partum. No cancer was found. Of the women with biopsy verified CIN2-3 38% persisted and 62% had regressed to LGL (73). In a Danish retrospective cohort study of 305 women all women having colposcopical abnormalities were biopsied. If atypical cytology only were demonstrated at investigation, repeated cytology and colposcopy were performed with reevaluation with colposcopy, biopsies and endocervical curettage eight weeks after delivery. When the histology in biopsies taken during pregnancy were compared to histology in samples taken post-partum persistence was found in 47%, progression was found in 28% and 25% showed regression. At follow-up post-partum progression to cancer was found in two cases (67). Recently Coppolillo and co-workers reported a study of 56 women having CIN2-3 in histology during pregnancy. Thirty women were followed up after delivery and 13.3% progress to cancer (81).

In the scientific literature, there is a general agreement that a lesion with suspicion of microinvasive cancer and frank invasive during pregnancy should be biopsied by multiple biopsies or cone biopsies i.e. loop electrosurgical excision procedure (LEEP) - or laser cone to verify the diagnosis. However, it is important to select those women who should be biopsied. In a pregnant woman, in comparison to a non-pregnant woman, the invasive diagnostic procedure of a cervical biopsy is more demanding since colposcopic

examination of the uterine cervix is a procedure of lower accuracy during the pregnant state and that the pregnant cervix has considerably increased vascularity (53).

It is well described that conization during pregnancy may lead to complications such as bleeding, miscarriage and premature rupture of membranes. When the procedure of colposcopically directed biopsy was introduced cone biopsies were performed on all pregnant patients with atypical cervical cytology and any of the following criteria: non visualization of the entire TZ, biopsy verified CIN3 taken at colposcopy or unexplained cytologic findings (persistence of abnormal findings on repeated cervical smears of either CIN3 or frank invasive disease) (59). An abortion rate of about 27%, blood loss >500ml in 7% of women who underwent conization and a perinatal death around 5% have been reported (60). However, modern conization techniques with CO_2 laser or LEEP seem to be connected with only minor risks of obstetric complications. Still, Robinson et al. (61) reported major complications in LEEP conization during pregnancy as high as 25% and Schaefer et al. (62) reported 22% experiencing premature labour, incompetent cervix or missed abortion after LEEP procedure. Furthermore, in the latter study, 59% of the margins of the specimen were positive. Thus, conization should be avoided during pregnancy if microinvasive cancer of frank invasive cancer can be ruled out with a high accuracy. However, Paraskevaidis et al. reported no cases of bleeding when loop biopsies were performed with the smallest electrode (75).

Natural course of dysplasia during pregnancy

The fact that a proportion of dysplastic lesion may spontaneously regress or progress must also be considered in the management during pregnancy. Still, there exist large differences in reported rates of dysplasia progression during pregnancy and those may be due to different definitions of regression and progression, where either changes in degree of dysplasia in cytology or changes in degree of dysplasia in histology or a combination of cytology and histology is used. Other explanatory background factors may be related to disparities in HPV exposure or prevalence of different HPV types in specific populations.

Furthermore, there may also be differences in screening intervals, coverage of screening in the various populations and, naturally, skills and experience of colposcopists, which will influence accuracy of the biopsies. In non-pregnant populations, the rates of regression and progression of HGL have been reported to be around 3% and 14%, respectively (82). However, after delivery a regression rate have been reported between 8-

70% (67, 80, 83, 84). Yost et al. found a regression rate of 68% and 70% in biopsy verified CIN2 and 3, respectively, (80) and Ackerman (84) found a regression rate of around 34% CIN3 lesions when histology from colposcopically biopsies were compared (84). Furthermore, Coppola et al. found a regression rate of only 8% in a small study of 25 women having CIN 3 (83). Palle et al. found a regression rate of 25% in all kinds of biopsy verified dysplasia. In that study regression was defined as regression to normal histology (67).
Progression rates after delivery have been reported to be around 3-28%. Palle et al. reported a progression rate of 28% in degree of CIN. This is in the same range (10%) that was shown in a study of HGL during pregnancy in Argentinian women (81). Seven percent progressed from CIN2 to 3 in another study (80). In one study on a US population (85), the results was entirely based on cytology, and a Spanish population (86), which to a large extent relied on cytology, progression rates between 3% and 4%, were reported.

Progression to cancer was not registered in the prospective studies of Yost et al. among the 279 women with CIN2 and 3 in histology (80) and in an Italian prospective study of 78 women with biopsy verified CIN (70). In the large US study of 1079 pregnant women where investigation during pregnancy in the majority of cases were performed by colposcopy and cervical smear, none of the women developed cancer at follow-up post-partum (79). However, in the recently published Argentinean study of 30 women, invasive cervical cancer was diagnosed in one woman post-partum (3.3%) (81). Furthermore, in the Danish study of Palle et al. two microinvasive cancers were found post-partum (1.1%) (67) and in a Czech prospective study of 167 pregnant women with CIN diagnosed by colposcopically verified biopsies, six cases of micro-invasive cancer (3.6%) were detected post-partum (87). The lesions with highest risk to progress to cancer are the CIN3 lesions. Ackermann et al. could demonstrate in a prospective study of 76 pregnant women with biopsy verified carcinoma in situ (CIS) in histology, progression to microinvasive cancer in 2.6% (84) and Kaplan et al. showed progress to micro-invasive cancer in 3/28 (11%) of women with antepartum HSIL in cytology (68). These findings stress the importance of follow-up after delivery.

It has been discussed whether the route of delivery influence the regression rate. In some studies, the regression of the lesions was more frequent in patients undergoing vaginal delivery than those who delivered by cesarean section (CS). Coppolillo et al. found a persistence and progression of CIN2-3 in 12% of women who had delivered vaginally, in contrast a persistence rate of 76% among those who had undergone caesarean section (CS) (81).

These results are in concordance with those of Ahdoot (88) and Paraskevaidis who found a higher regression rate among women who had delivered vaginally (75, 88) compared to the CS group. However, studies also point towards that no differences in progression or regression rates exist when comparing mode of delivery (80).

Cervical cancer in pregnancy

Cervical cancer is, after breast cancer, the malignancy which is most commonly diagnosed during pregnancy (89) and the reported incidence varies between 12 and 45 per 100 000 pregnancies (90, 91). In the western region of Sweden an incidence of 11.1 cancer cases per 100 000 was found during the period 1973-1993 (92). Screening programmes for detecting pre-stages of cervical cancer was initiated in Sweden in 1964 with implementation of nationwide screening in 1977. The incidence of cervical cancer overall was 20 cases per 100 000 women in 1965 and 6.6 cases per 100 000 women in 2005 (93). Pettersson et coworkers performed a study on cervical cancer associated with pregnancy by reviewing all cancer cases that had been referred to Radiumhemmet in Stockholm, Sweden, during the 90-year period between 1914 and 2004. The incidence of cervical cancer decreased by 66% among all women in the age group <50 years during the study period an apparent downstaging of cancer during the period and the proportion of pregnant women with cervical cancer among all cervical cancer cases was reduced from around 4% to 1.3% (94).

Significant factors in the apparent decrease in pregnancy-related cervical cancer incidence and mortality in Sweden during the last century are most likely a general improvement of the health system, and most importantly implementation of screening programs to detect cervical dysplasia or early cancer.

According to the current national guidelines, screening with cervical cytological smear is initiated at 23 years of age with screening every 3rd year. Pregnant women, not being screened during the previous 2.5 years before pregnancy, are recommended to be tested in early pregnancy at their first visit to the antenatal care unit (95), which usually takes place around week 10-12 of pregnancy.

Symptoms and signs of cervical cancer during pregnancy

It has been reported that cervical cancer in pregnancy is detected by cytological cervical smear in 46-69% (72, 92, 96). The incidence of bleeding as a symptom of cervical cancer during pregnancy seem to vary between 41-63% (90, 91, 96). Lee et al. reported that women with stage IA cervical cancer did not experience any symptoms (97). Among women with stage IB, 43% had no symptoms. In advanced stages (≥IIA) a greater percentage had vaginal bleedings, but still a small portion of women were without symptoms (97). As the symptoms of cancer may be similar to those of an uncomplicated pregnancy with episodes of bleeding the diagnosis may be delayed. The average duration of a period of symptoms before diagnosis was estimated to be around 4.5 months (98). Sood et al. reported that persistent post-partum bleedings led to cancer diagnoses among women diagnosed after delivery in 56% (72).

Table 6. FIGO staging of invasive cervical cancer (99)

Stage	Description
IA	Cancer diagnosed microscopically, no visible lesion
IA1	Invasion ≤3 mm deep and ≤7 mm wide
IA2	Invasion >3 mm to ≤5 mm deep, and ≤7 mm wide
IB	Visible lesion limited to the cervix, or microscopic lesion >stage IA
IB1	Clinical tumour ≤4 cm
IB2	Clinical tumour >4 cm
II (IIA1-2, IIB)	Tumour beyond the uterus, but not to the pelvic wall or to lower third of the vagina
III (IIIA, IIIB)	Tumour extends to the pelvic wall and/or to lower third of the vagina and/or causes hydronephrosis or non-functioning kidney
IV (IVA, IVB)	Tumour spread to the bladder or rectum and/or to distant organs

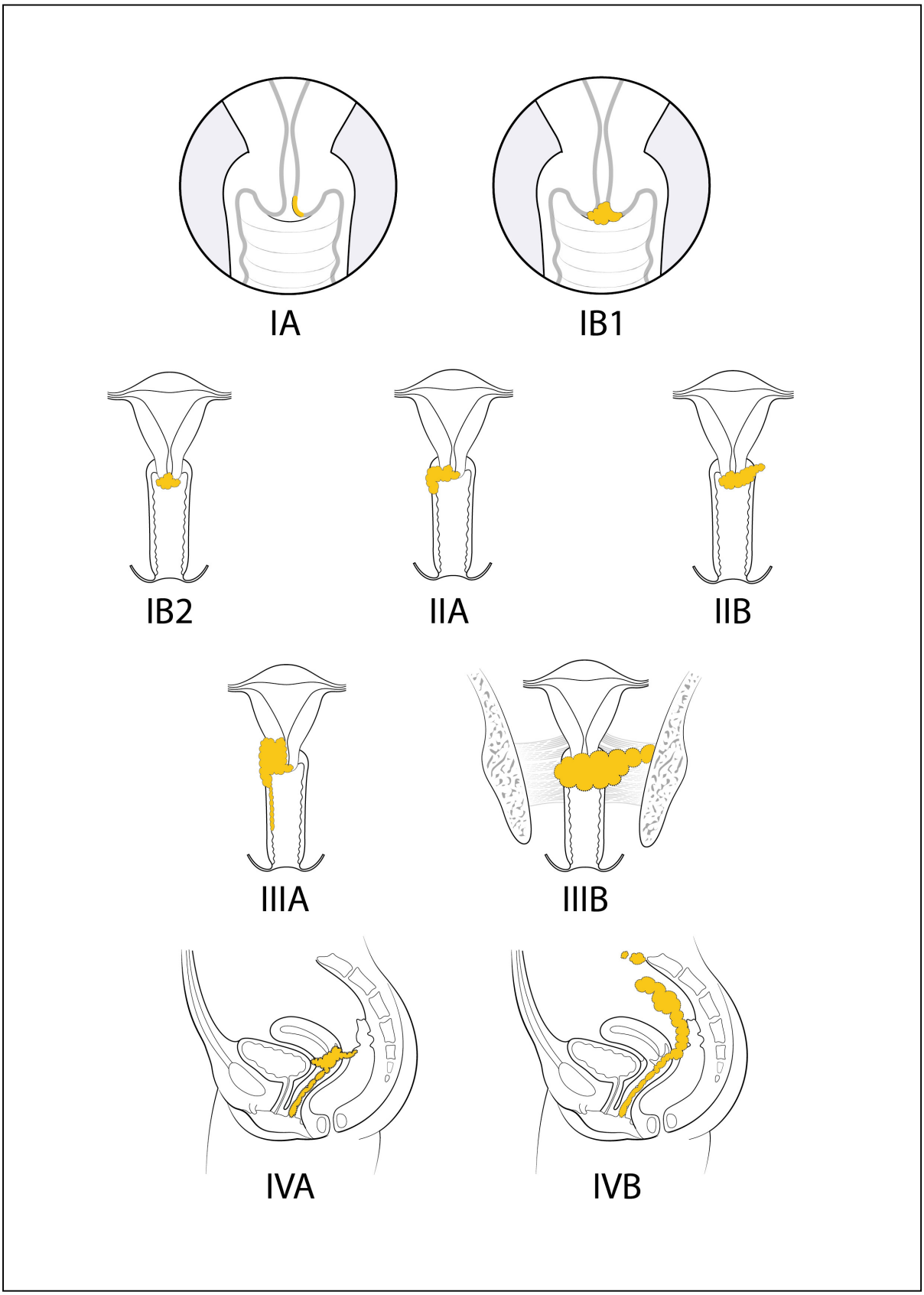

Figure 9. FIGO staging of invasive cervical cancer (99)

Cervical cancer in pregnancy; stages and histology

Overall the most frequent cancer stages detected during pregnancy are stage IA and B (100). Sood et al. reported from a cohort of 83 women in the U.S.A. that the cancer cases diagnosed during pregnancy were dominated by stage IA and IB1, whereas stage IB2 or more advanced stages were most frequent among women diagnosed post-partum. The proportions of women having stage IIB diagnosed in the first two trimesters, in the third trimester or during the post-partum period were estimated to be 3%, 14% and 26% respectively (72).

The most common histological cell type of cervical cancer during pregnancy is squamous cell carcinoma with a reported incidence between 56-96% (72, 90, 100). This dominance of squamous cell carcinoma is similar to that in non-pregnant populations (12). Adenocarcinoma and adenosquamous carcinoma were reported with incidence figures of 7-11% and 5-33%, respectively (72, 90).

Staging and treatment of cervical cancer during pregnancy

The staging of cervical cancer according to FIGO-classification is based on clinical examination and there is no distinction between non-pregnant and pregnant women (Table 6, Figure 9). However, when magnetic resonance imaging is available this modality is usually added as an extra help to decide if parametrial extension exist.
There are no strict guidelines concerning treatment of pregnant women with cervical cancer, as the disease is so seldom encountered and that the evidence based knowledge on this subject is low. Thus, no controlled randomized controlled trials including this patient group has been conducted. This is also true for most types of surgical interventions concerning gynaecologic oncology in general. However, principles for treatment of non-pregnant women can be performed with consideration to length of pregnancy and ethical, religious and cultural implications as well as to woman´s attitude.

Stage IA
All pregnant women with suspicion of microinvasive cancer should undergo conization (74) or extended large biopsies. Any of these two restricted and fertility preserving treatment is recommended for women with SCC (74). Women having adenocarcinoma IA, which often are multifocal, should be treated by the recommendation for squamous cell cancer stage IB cancer (74). Concerning stage IA2 cancer in pregnancy, radical trachelectomy during ongoing pregnancy and with live birth has been described (101).

Stage IB1

In pregnancies prior to around 20 weeks of gestation, radical hysterectomy with fetus in situ should be recommended. In pregnancies where cervical cancer is diagnosed later than 20 weeks of gestation consideration to reach fetal maturation versus risk for progression of disease should be considered. In the cases where continuation of pregnancy is recommended CS, followed by radical hysterectomy, is the recommended treatment strategy (74, 102, 103). It is recommended that CS should be performed with a vertical incision on the uterus to avoid division of the uterine vessels in the lower segments and thus to avoid possible dissemination of cancer cells. The surgery around the cervix, including ureter dissection, would also be easier if there is no incision close to the dissection planes. The CS should be followed by radical hysterectomy (74, 96). Delay of treatment for stage IA and stage IB has been reported up to forty weeks with no increased maternal risk for progression of disease (72, 90, 100, 104).

≥ Stage IB2

In treatment of advanced cervical cancer with a fetus of a gestational length that is pre-viable, a combination of radiation and chemotherapy followed by medical or surgical termination is recommended. If the fetus is of viable gestational length, CS followed by neo-adjuvant chemotherapy or radiation treatment and chemotherapy have been suggested. Significant gains in fetal outcome can be achieved in gestational weeks between 28 and 32, but it is not recommended that treatment should be delayed beyond pregnancy week 32-34. Delays of up to four weeks may not have significant impact on the prognosis of the mother in terms of survival (74).

Mode of delivery

Sood et al. evaluated 56 women with cervical cancer diagnosed during pregnancy or within 6 months after delivery in a matched case-control study (72). Among women who delivered by CS 14% developed local and distant recurrence whereas 56% of the women who delivered vaginally developed recurrence. In that study, a multivariate analysis showed that vaginal delivery was the most significant predictor for recurrence (72). Still, Lee et al. and Van der Vange et al. have not registered any differences in mode of delivery regarding recurrence rate (97, 105). However, CS is suggested to be most appropriate to deliver due to the reduced risk for recurrence (74). It should also be noted that episiotomy site recurrence has been found in four women (106).

Prognosis

The knowledge concerning prognosis after treatment for cervical cancer during pregnancy is limited, but it has been assumed that the overall survival profile is approximately the same as among non-pregnant women (72, 74). In a series of 26 women with 18 women having stage IB2 or more, the 5 years survival rate was 62%. There but there was no difference compared to a non-pregnant control group (72). In contrast to that study, Takushi et al. reported one recurrence and death in a study of 28 pregnant women with a delay of treatment up to 25 weeks (100). Importantly, survival of women diagnosed post-partum was lower compared to a non-pregnant control group matched on age histology, treatment and time of treatment in relation to time of diagnosis (72).

Biomarkers for prediction of HGL

High risk (HR) HPV is a necessary factor for the development of cervical cancer but presence of HR HPV DNA does not invariably lead to disease. Detection of HR HPV DNA provides high sensitivity but has a lower specificity than cytology to identify high-grade cervical lesions (107). Thus, tests with improved specificity and retained high sensitivity are desirable.

During recent years several assays of new potential biomarkers have been developed. (108, 109). Assays of biomarkers can be grouped into three different groups: 1) markers of increased HPV oncogene expression, such as HPV oncogene mRNA and protein; 2) markers of increased cell proliferation such as the $p16^{INK4a}$ protein, antigen Ki-67 protein, minichromosome maintenance complex component 2 (MCM2), DNA topoisomerase II alpha (TOP2a); 3) markers of chromosomal instability, such as a gain of chromosome arm 3q and HPV integration. (108, 109). Liquid-based medium for sampling of cytological specimens has allowed other molecular techniques to be evaluated more easily as adjunctive or triage tests.

Szarewski et al. compared different predictors for detecting HGL among a screening population and in women referred for further investigation of atypical cytology (110, 111). The evaluated tests in these studies were different HPV DNA-based genotype tests that were compared to different HPV mRNA-based tests and immunostaining with $p16^{INK4a}$. These studies showed that HPV mRNA-based test and immunostaining with $p16^{INK4a}$ had a higher specificity compared to the HPV DNA tests.

In this thesis HPV E6/E7 mRNA tests and $p16^{INK4a}$ cytology, were tested in order to compare two HPV E6/E7 mRNA tests and immunostaining for $p16^{INK4a}$ to two HPV DNA tests in pregnant women and to evaluate their ability to identify HGL in this patient group.

Linear Array HPV genotyping test (Roche Diagnostics, Branchburg, NJ, USA) is a DNA-based genotyping assay detecting 12 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and 25 low-risk HPV genotypes. The test utilizes amplification of target DNA by PCR and nucleic acid hybridization using probes on strips to detect the L1 gene of the different genotypes (112). This test has shown a sensitivity of 0.98, a specificity of 0.33 and a PPV of 0.38 in detecting CIN2+in histology in a study of 953 women referred for colposcopy in the UK (110).

A Taqman real-time PCR genotyping assay targeting the genome segment of E6/E7 of 12 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and two low-risk (6 and 11) HPV types was recently developed in Gothenburg (113). The method can be used for relative quantitative estimates of HPV copies/cell by measuring differences in so-called threshold (C_t) values (113). The test showed 100% proficiency in the WHO LabNet proficiency panel study in 2009 and is the currently used assay at the laboratory of virology at Sahlgrenska University Hospital in Gothenburg (143).

PreTect^{TM} HPV-Proofer (NorChip AS, Klokkarstua, Norway) is a RNA-based detection assay. The test is a real-time multiplex NASBA (nucleic acid sequence based amplification) assay for isothermal amplification of E6/E7 mRNA expressed by 5 high-risk human papilloma virus (HR-HPV) types (16, 18, 31, 33 and 45) using proprietary primer sets (114). The PreTect^{TM} HPV-Proofer assay has a sensitivity of 0.74, a specificity of 0.73 and a PPV of 0.52 in detecting CIN2+in histology in a large study in the U.K. on of 953 women referred for colposcopy (110).

The In-house real-time mRNA PCR test is a Taqman real-time PCR assay that targets 12 HR HPV-types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and two low-risk HPV-genotypes (6 and 11) using E6/E7 region primers and probes in a duplex format as in the protocol for the In-house real-time DNA PCR test. In order to detect mRNA, a DNase digesting step and a reverse transcription step have been added to the analysis.
$p16^{INK4a}$ is a surrogate marker of HR-HPV infection and CIN. The $p16^{INK4a}$ immuno-staining method is based on a specifically designed antibody to identify $p16^{INK4a}$ in

cytological samples by staining cells containing high levels of $p16^{INK4a}$. This method has shown to have a better predictive value than HPV DNA tests in predicting CIN2/3 in LGL cytology samples (115). Furthermore, it has been shown that $p16^{INK4a}$ improves identification of women having HGL in cytology samples (116) with a high sensitivity and a high interobserver agreement (117). It has been shown that use of nuclear scoring system for interpreting the $p16^{INK4a}$ immunochemistry can improve the sensitivity and the specificity of the method (116). In this thesis intensity the $p16^{INK4a}$ nuclear immune-staining was scored 1-3 and the $p16^{INK4a}$ reaction was interpreted as positive if a minimum of one dysplastic cell had nuclear-and/or cytoplasmic staining, according to the methodology of a previous study (117). The CINtec cytology kit (DAKO, Glostrup A/S, Denmark) was used.

Aims of the thesis

The aims of this thesis "Cervical dysplasia and cervical cancer in pregnancy; diagnosis and outcome", were to evaluate current management of pregnant women with cervical dysplasia, the effect of cervical cancer screening on cancer incidence and staging during pregnancy and potential improvements in investigative management of women with atypical cervical cytology.

Specific aims were:

- To evaluate the Swede score colposcopic scoring system in pregnant women with atypical cervical smear and to investigate if the accuracy in colposcopy can be improved in order to reduce the number of biopsies.
- To evaluate the incidence of complications due to invasive interventions in colposcopical investigation of pregnant women with atypical cervical dysplasia and the natural course of dysplastic lesions during pregnancy regarding progression and regression in histology.
- To evaluate the effect of improved strategies in cervical cancer screening program, and in management of cervical dysplasia and cancer, including stage of cancer, during pregnancy in comparison to previous two decades.
- To evaluate the use of different biomarkers in investigation of pregnant women with atypical cervical cytology.

Material and methods

Ethics

All the studies were approved by the Regional Ethics Committee in Gothenburg.

Study population

Paper I

In paper I, 281 pregnant women were investigated by colposcopy at the Gynecology Division at Sahlgrenska University Hospital, between March 15, 2001 and June 30, 2009. The indications for colposcopic investigation in this study were any, or a combination, of the four defined entities: 1) atypical cervical cytology, mainly taken at an early antenatal clinic visit during the 10^{th}-12^{th} week of pregnancy according to The Swedish National Guidelines; 2) dysplasia in biopsy, taken for investigation of atypical cytology just before or during the first weeks of the current pregnancy; 3) suspicion of neoplasm on ocular inspection of the cervix; 4) recurrent, non–obstetric bleeding.

Fourteen women were initially excluded, since no biopsies were taken due to normal/fully benign colposcopic findings, which included polyp, typical metaplasia and/or decidua. Additional four cases could not be scored due to an invisible TZ or in conclusive histology. Two other cases were lost to study follow-up due to coding error. The remaining 261 women, with a mean age of 29.6 years (range: 20-42) and being at gestational age from 6 to 26 weeks at the time of examination, were included in paper I.

Paper II

In paper II, the study population of 251 pregnant women with atypical cytology in cervical smears consisted of a sub-population of the 261 women of paper I. Those women of paper I with cervical malignancy signs, such as repeated bleeding, were excluded in paper II. Cervical smear were mainly taken at an early antenatal clinic visit during the 10^{th}-12^{th} week of pregnancy (mean 10.5 week of pregnancy (range 0.4 -19.4)); or taken just before or during the first weeks of the current pregnancy according to The Swedish National Guidelines. The mean age of the study population was 30.0 years (range: 20-42). The gestational age at the time of colposcopical examination varied from 5 to 24 weeks.

The control group, concerning obstetric outcome of paper II, was made up of the 54 919 patients that delivered during the same study period, with exclusion of the study population (n=251) and only inclusion of the first pregnancy in cases of several deliveries

during the study period. The control group was extracted from the generated database from electronic medical records used for all deliveries (Obstetrix; Siemens AG, Munich, Germany). The mean age of the control group was 30.7 years (range 14-53).

Paper III

The 47 women included in this study were diagnosed with invasive cervical cancer during pregnancy or within 6 months after parturition between 1993 and 2008. The study group was identified from the database of women diagnosed with invasive cervical cancer or CIN3 at the Regional Cancer Centre Western Sweden Healthcare Region, which administers the registration and follow-up of pre-invasive and invasive diseases in Western Sweden. The medical records and further identification of each single woman were achieved after searching in various registers (Swedish Cancer Register; Swedish Medical Birth Register; West Sweden Regional Cervical Cancer Prevention Quality Register).

Paper IV

The study population consisted of 204 women, who were attending gynecological screening (n=51; 26 pregnant) or had been admitted to a referral center for investigation due to atypical cervical cytology (n=153; 25 pregnant). The age of the women ranged between 21 and 79 with a mean age of 34 years, respectively. Five of the women were sampled two or three times, resulting in a total of 210 samples.

Paper V

One hundred thirty-two women were included in the study of Paper V. The patients were divided into five different study groups; pregnant women with normal cytology (n=26; mean age 30), pregnant women with atypical cytology (n=26; mean age 32), non-pregnant women with normal cytology (n=25; median age 32), non-pregnant women with atypical cytology (n=30; mean age 31) and non-pregnant women with biopsy verified invasive cancer (n=25; median age 49). Gestational age of the pregnant women varied between pregnancy week 6 and 23. All women, including the cancer cases, were also included in paper IV. All non-cervical cancer patients were seen at routine cytology sampling. The indications for sampling were either participation in the national screening program for prevention of cervical cancer, which concerning pregnant women usually is performed during the first-trimester or in the beginning of the second trimester if cytology sampling has not been done during the previous 2.5 years, further investigation of cytological atypia taken just before pregnancy, or as study sampling of women from the Western region of Sweden, admitted for clinical staging or surgery of invasive cervical cancer.

Study variables

Paper I-II

Swede score

All women were examined with colposcopy and scored according to the five-variable Swede score system (46).

Procedure at colposcopy

At colposcopy, standard methods, with application of acetic acid (5%) and Schiller´s solution were used.

Cytological cervical smear

At the time of investigation a conventional cervical cytological smear was taken from the TZ with a wooden Ayre´s spatula and from endocervix with an endocervical brush (Cytobrush® Plus GT, Medscand, Medical, Trumbull, CT, USA).

Biopsy and LEEP-cone

Colposcopy directed punch biopsies were taken from atypical cervical lesions with the greatest degree of abnormality, by the use biopsy forceps with estimated biopsy size of 4x5mm. In 56 women showing colposcopically identified lesions with suspicion of micro-invasive cancer (lesions with atypical vessels, intense acetowhiteness with sharp elevated margins and large size) or lesions with tumour-like appearance, colposcopically directed loop-biopsies or LEEP-cones (UtahLoop®; Utah Medical Products Inc, Midvale, UT, USA) were obtained.

Paper II

The study population of Paper II was initially examined in the same way and with the same methods as in Paper I, but was then followed-up during and after pregnancy.

Follow-up during the study

In the majority of cases, both women with HGL (HGL i.e. CIN2, CIN3 and AIS) and cancer were re-examined around the 28th week of pregnancy with colposcopy and with an additional cervical smear to investigate if there any progress of the lesions towards micro-invasive cancer was present. When LGL was diagnosed in early pregnancy, no follow-up was performed during the present pregnancy.

All women were examined within 12 months after delivery. Women with HGL during pregnancy were treated with LEEP-cone. Women with LGL and women who were biopsied with colposcopically directed loop-biopsies or women treated with LEEP-cones due to suspicion of microinvasive cancer were examined by colposcopy and cervical smear. If colposcopically identified lesions then were seen among the women with LGL

during pregnancy, biopsies were taken and LEEP-cone was performed if histology showed HGL. LEEP-cone was performed if there was any dysplasia left among women with micro-invasive cancer during pregnancy.

Evaluation of natural history in histology

Evaluation of histological samples taken post-partum was done concerning persistence, regression or progression of dysplasia. For comparison of histology ante-partum and post-partum in this study the outcome of histological analyses were divided into the four categories: benign, LGL (i.e. koilocytosis, CIN1 and glandular dysplasia lower than AIS), HGL (CIN2, CIN3 and AIS) or cancer. Regression and progression were defined as changes to a lower or higher category, respectively. In 31 cases with LGL during pregnancy, investigation post-partum was done with cervical smear only as no lesions were seen. In these cases, regression to benign was defined as presence of three consecutive normal cervical smears within two years from the time when the atypical smear in early pregnancy was taken. Persistence of atypia was defined as no change of cytological diagnosis compared to the cytological diagnosis during pregnancy. In the events when follow-up cervical smear was atypical, colposcopy was performed and biopsies were taken.

Registration of complications

Data concerning complications such as bleedings after punch biopsies, colposcopically directed loop-biopsies or LEEP-cones, occurrence of miscarriages, premature delivery (i.e. delivery before gestational week 37), gestational length, mode of delivery (i.e. vaginal delivery or CS) and indication for CS, were extracted from routine medical records.

Collection of data concerning parity, smoking habits, previous cervical smear

Data on parity, smoking-habits, and previous cervical smears for cervical dysplasia were obtained from medical records and the Swedish National Cervical Cancer Prevention Quality Register/ process databases.

Parameters for comparison to the control group

Parity, smoking-habits, incidence of premature delivery, gestational length and mode of delivery in the present patient material were compared to a control group (n=54 919) consisting of all delivery cases, adjusted for above mentioned parameters, in the Gothenburg area during the study period.

Paper III

Collection of data

The 47 patients were identified from the database on women of the Western region of Sweden and diagnosed with invasive cervical cancer or CIN 3 at the Regional Cancer Centre Western Sweden Healthcare Region. The medical records and further identification of each single woman were achieved after searching in various registers (Swedish Cancer Register; Swedish Medical Birth Register; West Sweden Regional Cervical Cancer Prevention Quality Register).

Investigation of cancer

The diagnosis of cervical cancer was made from directed punch or cone biopsies taken under colposcopy at follow-up of women having atypical cervical smears, symptoms and/or clinical signs of suspected neoplastic disorder. In all cases, the initial histological diagnosis of cancer was confirmed by an expert pathologist at the Sahlgrenska University Hospital.

Colposcopical evaluation

At colposcopical evaluation, LGL (koilocytosis, CIN1 and glandular dysplasia lower than AIS) was considered in cases having low-moderate acetowhiteness without abnormal vessel pattern. Abnormal vessel pattern in conjunction with intense acetowhiteness, sharp margins and/or elevated and varying surface level of the lesion indicated HGL (CIN2, CIN3 and AIS). Coarse and atypical vessels in addition indicated cancer. Colposcopy was considered incomplete in cases the TZ (squamocolumnar junction) could not be visualized or local bleeding made adequate evaluation impossible.

Different treatment of cancer

Surgery:

Radical hysterectomy was done according to Wertheim-Meigs (W-M) preserving the ovaries in all women except one. CS was performed prior to the W-M operation via a corporeal uterine incision in the majority of cases. Vaginal trachelectomy was combined with laparoscopic pelvic lymph node dissection. One woman underwent total pelvic excenteration due to recurrence of disease.

Radiotherapy and chemotherapy:

Radiotherapy and chemotherapy, as single therapy or combined, were given as neoadjuvant therapy in women having advanced cancer or as adjuvant therapy postoperatively in women having lymph node metastases or vascular invasion. Radiation was administered vaginally using 192-iridium or 60-cobolt and/or as external pelvic irradiation.

Combined bleomycin, oncovin and paraplatin were mainly used in women having SCC (squamocellular cancer) and paraplatin was combined with farmorubicine or holoxane in women having adeno- or adenosquamous carcinoma.

Paper IV-V
Liquid based cytology (LBC) samples were obtained from all women in paper IV-V to acquire material for the different analytical methods.

In Paper IV, 153 of the 204 women investigated for atypical cervical cytology underwent colposcopy and directed biopsies or were treated by conization.
In Paper V, colposcopically directed biopsies were obtained or conization was performed in all women with atypical cervical smear and cervical cancer to verify the diagnosis of cervical dysplasia or cancer, respectively. Thus, no biopsies were taken from women with normal cytological smears in Paper IV or in Paper V.

Histological and cytological analyses

Paper I-II and Paper IV-V: Histopathologic analyses were performed, according to clinical routines. Biopsies were fixed in 4% buffered formaldehyde, dehydrated, embedded in paraffin, sectioned and stained with haematoxylin and eosin followed by examination under light microscopy.
Paper I-II: Cytological smears were fixed in 95% ethanol and stained according to Papanicolaou.
Paper IV-V: LBC was performed according to the manufacturer´s protocol.
Paper I, II, III, IV: The cytological samples were classified according to a Swedish modified classification based on the WHO scheme but incorporating major aspects of the Bethesda classification(28).
Paper V: The cytological samples were classified according to the terminology of the British Society for Clinical Cytology (142).
Paper I-V: The histological samples were classified according to the WHO scheme (28).

<u>Evaluation of histology</u>
Paper I-II: After the study was completed, 232 (89%) of the 261 samples underwent blinded reassessment by one expert pathologist, resulting in revision of the histological diagnosis in 14.2% of the cases. The remaining samples (11%), not reassessed by the

expert pathologist, were included in the study, and the original histological diagnosis was regarded as the final histological diagnosis in these cases.
Paper IV-V: An expert pathologist re-evaluated all histological samples. If the second diagnosis differed from the original diagnosis by more than one level of severity, the expert pathologist consulted a third pathologist and in all cases the diagnosis of the expert pathologist were confirmed.

Molecular markers

Paper IV-V

The In-house HPV DNA test

The In-house real-time DNA PCR test is a Taqman real-time PCR assay that targets 12 HR-HPV-types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and two low risk (6 and 11) HPV-types using E6/E7 region primers and probes in a duplex format. Detection of the human gene betaglobin serves as a control of sample sufficiency. The detailed performance of the assay is described by Lindh et al. (113).

The In-house HPV mRNA test

The In-house real-time mRNA PCR test is a Taqman real-time PCR assay that targets 12 HR-HPV-types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and two low risk (6 and 11) HPV-genotypes using E6/E7 region primers and probes in a duplex format as in the protocol for the In-house real-time DNA PCR test. Detection of the human gene betaglobin serves as a control of sample sufficiency.

PreTect™ HPV-Proofer

Detection of E6/E7 mRNA of the high-risk genotypes 16, 18, 31, 33 and 45 with PreTect™ HPV-Proofer (NorChip AS, Klokkarstua, Norway) was performed according to the manufacturer's guidelines. The analysis is based on NASBA (nucleic acid sequence based amplification) technique with isothermal amplification of mRNA in a duplex format, measured in real-time.

DNA and RNA extraction

The presence of HR-HPV E6/E7 mRNA was analysed both with an in-house real-time PCR method ("In-house real-time mRNA PCR test") and the commercial test PreTect™ HPV-Proofer (NorChip AS, Klokkarstua, Norway). In both the DNA tests and both the mRNA tests, DNA or nucleic acids were initially extracted using a MagNA Pure LC instrument (Roche). For mRNA analysis, 3-5 mL of the LBC sample were centrifuged (7 min, 1200 rpm) and pelleted cells were re-suspended in 1 mL of RLT lysis-buffer

(Qiagen, Hilden, Germany) for extraction with the total nucleic acid Large Volume protocol. To assure the quality of mRNA, the LBC samples were stored less than 30 days before re-suspension in lysis-buffer and total nucleic acid extraction. The extractions were stored at -70°C.

Paper V

The Linear Array ® HPV genotyping test

The Linear Array ® HPV genotyping test detects 12 high risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and 25 low risk HPV genotypes. The test utilizes amplification of target DNA by the PCR and nucleic acid hybridization and was performed according to the manufacturer´s protocol.

The $p16^{INK4a}$ method

The $p16^{INK4a}$ method is based on a specifically designed antibody (the CINtec cytology kit, DAKO A/S, Glostrup, Denmark) identifying $p16^{INK4a}$ in cytological samples by immunocytochemistry and was performed according to the manufacturer´s protocol. Examination of the samples was done in a blinded fashion and interpreted as positive if a minimum of one dysplastic cell had nuclear-and/or cytoplasmic staining, according to the methodology of previous study (117).

Statistical analyses

Paper I

Univariate and multiple logistic regression analysis were performed in order to predict the dichotomized histopathologic outcomes CIN2+ or CIN3+ (CIN3 or cancer) vs. LGL with the independent variables (acetowhiteness, margins plus surface, vessel patterns, lesion size, iodine staining) and total Swede score. Area under the Receiver Operating Characteristic (ROC) area under the ROC curve (AUC) with 95% confidence interval was calculated using the linear trapezoidal method for description of discriminating strength variables. The OR with 95% CI was calculated for each variable. Sensitivity and specificity were calculated for different cut-off points in the total Swede score.

Paper II

In order to adjust for age, parity and smoking when the study population was compared to all delivery cases in the Gothenburg area analyses of covariance was used for analyses of continuous outcome variables and multiple logistic regression for analyses of dicho-

tomous outcome variables. For comparison between the study group and the control group Mann-Whitney´s U-test was used for continuous variables, Mantel-Haenszel´s Chi-square test for ordered categorical variables and Fisher´s exact test for dichotomous variables. For comparison of regression persistence and progression between the groups biopsy, loop-biopsy/LEEP-cone and cytology Kruskal-Wallis´ test was used for continuous variables.

Paper III

Fisher´s exact test was used to calculate the differences in cancer stages between the first eight-year period and the second eight-year period and for calculation of the differences in cancer incidence between present study and previous study (92).

Paper IV

The sensitivity, specificity, PPV and NPV of each test algorithm were calculated with histologically confirmed CIN2+ as gold standard, but calculations were also made for CIN3+. Calculations of 95% CI were based on the normal approximation to the binomial distribution as suggested by Harper and Reeves.

Paper V

Stepwise logistic regression analysis was performed to detect the two most suitable tests to detect CIN2+ among pregnant women with atypical cytology. P-value was calculated to estimate the statistical significance between pregnant women with normal cytology and non-pregnant women with normal cytology compared with pregnant women with atypical cytology and non-pregnant women with atypical cytology. Weighted kappa was calculated to give measures of agreement between the tests.

Results and comments

Paper I

Background

Colposcopy is the standard diagnostic method for evaluating the cervix among women having atypical cervical smear and/or signs and symptoms indicating suspicion of malignancy. Colposcopy enables obtainment of directed biopsies of a dysplastic area and/or the establishment of the margins for cervical conization and can thereby reduce the size and/or required number of biopsies (54, 55), after cytology have indicated HGL (i.e. CIN2 or 3 or AIS). According to current Swedish guidelines, all women with atypical cervical smear should undergo further investigation by colposcopy (95).

The prevalence of atypical cervical cytology is markedly higher among pregnant women than among non-pregnant women of similar ages and populations (64) and even pregnant women should be further investigated by colposcopy to obtain biopsies if there are any atypical signs indicating HGL or cancer. During pregnancy there is enlargement of the cervix, evasion of the cervical epithelium, intense squamous cell metaplasia and a decidual reaction, which may be difficult to distinguish from a precancerous/cancerous lesion. Furthermore, there is an increased vascularity due to physiological changes and there may be an increased risk of major bleeding if multiple/large biopsies are taken or after conization, also including LEEP (61, 62). Still, some other authors have reported only minor risk for bleedings (118, 119). Furthermore, there may be a risk of miscarriage and other obstetrical complications such as preterm birth (59-62) if conization is performed during pregnancy.

There exist large international variations in the management of atypical cytology during pregnancy, with some recommending directed biopsies (54, 55) and others recommending repeated colposcopy and cytology only (79). It has further been proposed that the use of colposcopic indices may be beneficial to select patients that should undergo further examination by biopsy or conization. Reid´s index is the most well-known colposcopic scoring system (43) and was created in order to differentiate benign papilloma viral infections from high-grade CIN. This scoring system demonstrated an accuracy of 97% to predict HGL in histology and in another study it could identify HGL with an accuracy of 87% (63). The Reid´s index (43), as a tool to reduce the need for biopsies, has not been properly evaluated in pregnant populations.

The Reid's index was later simplified by Stellato and Paavonen by excluding iodine staining as a variable and this modified Reid´s index (margins/borders, color, vessels/

vascular atypia) also demonstrated that a high score was indicative of cervical dysplasia (44). The recently introduced Swede score (46) also adds lesion size as an extra variable, in comparison to the original Reid´s index.

Size of the lesion may be essential in colposcopic evaluation of the cervix also during pregnancy, since it has been reported that HGLs are larger than LGLs (37, 48) and since large CIN3 lesions have an increased risk of containing micro-invasive foci (42). The Swede score has been found to detect or exclude HGL with high accuracy as well as to reduce the need for biopsies in about 17% of non-pregnant women of fertile age (46).

To my knowledge, there is only one study in the English language on the use of any colposcopic scoring systems in pregnant populations. That small study (45) of 32 pregnant women found that a three-variable score (44) detected HGL with a sensitivity of 100% and a specificity of around 92%.

The aim of this study was to test the Swede score in a large population of pregnant women with atypical cervical cytology and evaluate whether use of Swede score could decrease the number of unnecessary biopsies. In addition the article also represents a description of the distribution of histology verified dysplasia in pregnant women with atypical cervical cytology.

Results

This study group of 261 pregnant women were referred for colposcopic investigation due to any of the four entities: 1) atypical cervical cytology (69% LGL and 31% HGL) 2) dysplasia in biopsy; 3) suspicion of neoplasm on ocular inspection of the cervix; 4) recurrent, non–obstetric bleeding. All women were scored according to the Swede score system and biopsies were taken at the part of the lesion, which was judged, by the experienced colposcopist, to be of the highest severity. The histological analyses showed that around 20% were non-dysplastic/non-koilocytotic lesions of inflammation or polyp and that the proportions of CIN1, 2 and 3 in the material were 24%, 15% and 36%, respectively. The rates of glandular dysplasia (of lower grade than AIS) and AIS combined with CIN3 were 0.5% and 1%, respectively were. One percent was exclusively AIS and about 2.0% were cancer.

In the study, the specificity at a score of ≥8 was around 70% and the sensitivity was 76% in predicting CIN2+. The specificity and the sensitivity at different scores with CIN3+,

which could be clinically more important, as end point were investigated and this improved the sensitivity (81%), but the specificity became lower (61%). All cases of CIN2+ had a Swede score of ≥5. The three cases of AIS combined with CIN3 were identified by a Swede Score of ≥7 and the non-combined AIS case was identified by a Swede score of ≥9. The four cancer cases were identified by a Swede score of ≥8 and all of them were primarily identified by atypical cytology (CIN3 in three cases and ASCUS in one) rather than by clinical cervical malignancy signs.

In the univariate logistic regression analysis, each of the five variables correlated significantly with the CIN2+ lesions in the following order: vessel patterns, lesion size, margins plus surface, acetowhiteness and iodine staining. For detection of CIN3+ lesions, the variables contributed in the following order: lesion size, vessel patterns, margins plus surface, acetowhiteness and iodine staining.

In a multiple logistic regression analysis, all variables, except iodine staining, were significant independent predictors of CIN2+. Lesion size, margins plus surface and vessel patterns were significant independent predictors of CIN3+.

The ROC curves were similar in predicting CIN2+ and CIN3+ with 0.81 and 0.79, respectively. In a multiple logistic regression analysis, the AUC was calculated for Swede score with CIN2+ and CIN3+ as end-points and compared to prediction of CIN2+ and CIN3+ by different total score. The AUCs for those analyses were 0.82 for CIN 2+ and 0.80 for CIN3+. Thus, the AUC for the total Swede Score (all five variables combined) was only 1% less both for CIN2+ (0.81 vs. 0.82) and CIN3+ (0.79 vs. 0.80).

Comments

This prospective study is to my knowledge the largest study evaluating the use of any colposcopic scoring system in a population of pregnant women with the objective of finding strategies to reduce the need for cervical diagnostic biopsies.

The main findings of the present study were that lesion size, margins plus surface and vessel patterns were the three most important variables to detect HGL. This contrasts with the results found in the study of Swede score among non-pregnant women where acetowhiteness was the most important variable to predict HGL followed by margins and surface, lesion size, vessel pattern and iodine staining (46). As shown by Coppelson et al. (51) there is an increased immature metaplasia during pregnancy. This may be an explanation why acetowhiteness among pregnant women is of less importance in predicting HGL.

The most important issue in diagnostic evaluation of the cervix in pregnant women with atypical cytology is of course to detect cancer (52, 53), since even HGL often may regress post-partum (80). Several studies have shown that about 55% (46-69%) of cervical cancer cases of pregnancy are initially found by an atypical Pap smear that indicates cancer or HGIL lesion (72, 92, 96). In the present study, all cancer cases occurred within the group of women with a total Swede score of ≥8. It is important to identify the group of women with a score of ≥8 since in these cases large biopsies or even conization should primarily be obtained to verify the diagnosis of cancer. However, the increased vascularity of the cervix during pregnancy augments the risk for bleeding at any invasive intervention in this patient population (53, 59-62) and these kinds of interventions should consequently be reduced to a minimum. CIN2+ was also found in the group of women having Swede score points of 5-7. In this group, obtaining single biopsies only may be enough in the diagnostic effort since no cancer was found in this group. However, at a score ≤4 no HGL was found and thus presence of such low Swede score would indicate that a biopsy is not required in a pregnant population. This is clinically valuable information, due to the risk of bleeding associated with multiple or large cervical biopsies in pregnant women. Thus, by using Swede score the number of women needing large biopsies or conization i.e. women having scoring points of ≥8, can be identified and thereby reducing the need for biopsies in the other groups of pregnant women with atypical cervical smear.

In the original study of 297 Swedish non-pregnant women (46) with atypical cervical cytology, the Swede score discriminated LGL and normal findings from HGL/cancer. Furthermore, it identified HGL at a score ≥8 with a specificity of ≥90%. However, the sensitivity was only around 50% (46). In a follow-up study with application of the Swede score on a population in London, 200 women underwent outpatient diagnostic colposcopy/inpatient CIN treatment. In that study, it was shown that a Swede score ≥8 had a specificity of ≥95% in detecting HGL (49). In the present study of pregnant women a specificity of about 70% to find HGL was found at scoring point ≥8 and this lower specificity may be due to the immature dysplasia that gets acetowhite after application of acetic acid and may resemble dysplasia. However, a cut-off point of ≥8 with CIN2+ as end point seems to be most suitable when using Swede Score also in pregnant women.
The lower specificity points out that there is a need to find other methods , as biomarkers, to reduce unnecessary biopsies.
In the present study a Swede score of ≥5 indicated presence of HGL with 100% sensitivity. This proposed HGL cut-off score is identical to that of the original Swede score study of non-pregnant women (46) but a lower cut-off (≥2) was suggested in the UK study (49). This discrepancy may be due to that also inexperienced investigators partici-

pated in the latter study compared to exclusively two experienced colposcopists in the original Swede score study (49) and the present study.

In the present study, an analysis was performed on how Swede score performed for CIN2+ and CIN3+ separately, since only pregnant women with CIN3+ may be recommended biopsy procedure to conclusively exclude invasive cancer, while CIN2 cases can be managed in an expectative manner. Hence, it was evaluated whether CIN3 is a more accurate end-point than CIN2 during pregnancy. Although the sensitivity increased from 76 % (CIN2+) to 81% (CIN3+), the specificity decreased; the number of women who would require biopsies could thus not be reduced. Thus, CIN2+ is adequate as end point among pregnant women as well as in non-pregnant women.

The analysis of AUC, providing data on sensitivity and specificity demonstrated that Swede score performs similarly in pregnant women as in non-pregnant women, with AUC for CIN2+ of 0.81 in this study and 0.87 concerning non-pregnant women (49) and with overlapping confidence intervals. Thus, there is a need for further improvements of scoring systems both in pregnant and non-pregnant women. For pregnant women this could be done by using the three most important variables, lesion size, margins plus surface and vessel patterns, or possibly by incorporating results concerning levels of biomarkers of dysplasia into a scoring system.

In the multiple logistic regression analysis in this study, the AUC was calculated for Swede score with CIN2+ and CIN3+ as end-points and compared to prediction of CIN2+ and CIN3+ by different total score. The AUCs for those analyses were 0.82 for CIN 2+ and 0.80 for CIN3+. Thus, the AUC for the total Swede score (all five variables combined) was only 1% less both for CIN2+ (0.81 vs. 0.82) and CIN3+ (0.79 vs. 0.80). This means that when comparing the AUC for the multiple logistic regression analyses and the total score the differences were negligible.

A weakness of this study is that biopsies were not taken in 5% of the original patient population with atypical cytology. It is however unlikely that HGL was present in significant numbers of these 14 patients, since 12 had normal follow-up cytologies at least two years after delivery.

In conclusion, the study shows that any colposcopic evaluation by a trained colposcopist resulting in a low (≤4) Swede score in a pregnant woman with atypical cytology, indicates that a biopsy is not needed. A Swede score between 5 and 7 should lead to directed biopsy

to exclude CIN2+ and multiple biopsies or LEEP resection/conization are recommended to exclude early invasive cancer if the Swede score is ≥8.

Paper II

Background

Pregnant women in Sweden are recommended, according to national guidelines, to undergo a cervical cytology screening test during the first trimester, if cervical smears have not been obtained during the preceding 2.5 years (95). It is also known that prevalence of atypical cervical cytology is increased among pregnant women compared to non-pregnant populations (64). In Scandinavian countries, the prevalence of atypical cervical cytology during pregnancy has been reported to be around 1.4% (67). An atypical cervical cytology sample should be investigated further to rule out the presence of HGL or invasive cervical cancer. Our recent study on a large cohort of pregnant Swedish women with atypical cervical cytology reported a rate of biopsy verified high-grade lesion of above 50% (Paper I). In a pregnant woman, in comparison to a non-pregnant woman, the invasive diagnostic procedure of a cervical biopsy is more demanding since colposcopic examination of the uterine cervix is a procedure of lower accuracy during pregnancy and that the pregnant cervix has considerably increased vascularity (53).

It has been assumed that any surgical intervention on the pregnant cervix may be related to complications during the remaining pregnancy and delivery. A retrospective analysis of outcome after traditional cervical conization during pregnancy showed a premature delivery rate of about 40% (59, 60). The use of colposcopically directed biopsy has been suggested as a less hazardous means for further investigation in pregnant women with atypical cervical cytology.

The fact that a proportion of dysplastic lesion may spontaneously regress or progress must also be considered in the management of dysplasia during pregnancy. In non-pregnant populations, the rates of regression and progression of HGLs were reported to be around 3% and 14%, respectively (82). The natural course of dysplastic cervical lesions during pregnancy and delivery is poorly understood (74, 80, 83).

In the present retrospective study, a cohort of 251 pregnant women with atypical cytology was followed after the invasive procedure and throughout the pregnancy in order to tinvestigate whether procedures of colposcopically directed punch biopsy, loop-biopsy or LEEP-cone may be related to post surgical or further obstetric complications and also to describe the natural course of dysplastic lesions regarding progression and regression in histology. For comparison of age, parity, gestational length at delivery, mode of delivery and smoking habits data of a control group of about 55 000 women were extracted from the database generated from medical records.

Results

All 251 women were investigated by colposcopy and the results of the directed punch biopsies showed a broad distribution of histological diagnosis in relation to initial atypical cytology. Noteworthy, is that CIN2+ in histology was diagnosed in around 55% of all the women and in the group of those with ASCUS or CIN1 in initial cytology, CIN2+ in histology was found in about 40%. Furthermore, 7% of the women having CIN3 in initial cytology had cancer and nearly 2% of the women in the total study population had cancer in histology during pregnancy.

Sixty-five percent of the entire study population were diagnosed with colposcopically directed biopsy and followed up by this method or LEEP-cones, thus enabling estimation the natural course regarding persistence, regression and progression of the lesions. The meantime from biopsy taken during pregnancy and post-partum follow-up with biopsy or LEEP-cone was around 10 months. The lesions of these women showed persistence, regression and progression in 55 %, 33% and 12%, respectively.

In the subgroup of women where the colposcopic evaluation, regardless of Swede score sum, was judged as showing suspicion of micro- invasive cancer (about 22% of the entire study population) around 84% underwent colposcopically directed loop-biopsies and the remainder LEEP-cones. The rates of persistence, regression and progression were 32%, 67% and 1%, respectively.

In the subgroup of women with LGL during pregnancy (about 12%), none of the cases progressed, 59 % regressed to normal and 41% persisted as LGL**.** Among the women with CIN3 and CIN2 histology during pregnancy, the rates of regression, persistence and progression were around 26%, 71%, 2% and 45%, 54% and 0%, respectively.

As mentioned above, the study identified four cases of cervical cancer (three micro-invasive and one invasive cancer of stage IB1) at investigation of only atypical cytology and with no cervical cancer symptoms. Moreover, two more cases of invasive cancer were found after delivery, resulting in a total cancer rate in the material of around 2.4%. In one of these cases, CIN3 was diagnosed in biopsy during pregnancy, but invasive squamo-cellular cancer stage IB1 (grade 1) was diagnosed after conization four months post-partum. In the second case of post-partal identified cancer, the histology of LEEP-cone showed AIS in early pregnancy and further follow-up with cervical smear during pregnancy indicated adenocarcinoma, but no cancer could be seen on colposcopy. Conization was done 10 days after CS (36^{th} week of pregnancy) and invasive adenocarcinoma was diagnosed. In both these cancer cases diagnosed after parturition, the follow-up have been uneventful for 3 and 7 years, respectively.

In this study, one of the aims was to investigate if there were any complications as bleedings, premature birth or miscarriage after colposcopically-directed biopsies or more extensive loop-biopsy/conization during pregnanacy. About 6% women experienced bleeding after the diagnostic procedure, with around 5% after punch biopsy and 11% after colposcopically directed loop-biopsy. None of the nine patients undergoing LEEP-cones experienced postoperative bleeding. None of the women with bleeding after punch biopsy or LEEP-biopsy needed any surgical intervention (diathermy, suture) or blood transfusion treatment. However, topical treatments by silver nitrate or policresulen were used as single treatment in a minority of women. Two women experienced miscarriage; one case in the 12^{th} gestational week (three weeks after biopsy) and the other case in the 22^{nd} gestational week (ten days after biopsy). In the latter case, the fetus had multiple malformations. Thus, the total incidence of miscarriage in the study population was around 1%. One women experienced intrauterine death in the 27^{th} gestational week (ten weeks after punch biopsy) with Listeria monocytogenes infection found as the cause at post mortem examination. Three women chose to terminate their pregnancies by legal abortion.

Data concerning smoking habits three months before pregnancy were available in 96% of the patients in the study group and 44% in the control group. Significantly more women of the study population smoked (30%) as compared to the control group (21%).

Pregnancy lengths were similar between the study population and controls. Comparing mode of delivery no significant differences were registered between the groups, even if five women in the study group underwent CS due to findings of dysplasia or cancer.

Comments

A major finding of the present study was that the colposcopically directed diagnostic procedure of the cervix is safe during pregnancy with seldom (around 6%) occurrence of bleeding complications and all of these being minor. These procedures are also safe concerning pregnancy outcome with the obstetric outcome of the study population being similar to that of the large control population. Furthermore, the progression rate was low with an overall progression rate of 12% and in the subgroup undergoing colposcopically directed loop-biopsies or LEEP-cone the progression rate was as low as 1%.

In the present study, it was found that only a minority (12%) of the dysplastic lesions progressed to a higher grade of dysplasia during pregnancy and after delivery. Compared to other studies this regression rate is in the same range (10%) shown in a study of HGL during pregnancy in Argentinian women (81), but considerably lower than that was found in a Danish cohort of 182 women where the progression rate of CIN in histology after delivery was 28% (67). However, lower progression rate of dysplasia during pregnancy than that was found in this study has been reported. In one study of a US population (85), where the results was entirely based on cytology, and in a Spanish study (86), which to a large extent relied on cytology, progression rates between 3% and 4% were reported. This further indicates that the large differences in reported progression rates of dysplasia during pregnancy may be due to different definitions of this entity. Another explanatory background factor may be related to disparities in HPV exposure or prevalence of different HPV types in population. There may also be differences in screening intervals, coverage of screening in the various populations and the skills and experience of colposcopist, which will influence accuracy of the biopsies. Thus, in the Danish study with low progression rate the colposcopies were carried out by gynecologists with varying experience (67) but in the present study all colposcopies were carried out by two doctors with long experience and specialized interest in colposcopy (Paper I).

Naturally, CIN3 is the grade of cervical dysplasia with the highest risk to progress to cancer as shown among non-pregnant women (120), with a reported progression rate to cancer of about 4% per year (82). It is known that CIN3 lesions diagnosed during pregnancy may regress after delivery (80) and this observation combined with that invasive surgery on the cervix should be avoided, have led to general recommendations that CIN3 lesions during pregnancy can be followed in an expectative manner. In the present study, regression of CIN 3 occurred in 26% of the women with CIN3 and this result was entirely on histological evaluation of biopsies taken as colposcopy directed or LEEP-cones. Previous studies have reported a great variety in regression of CIN3 lesions during pregnancy. A very high (70%) regression rate was reported in a study of 82

pregnant women in USA, with the index histology obtained in the first trimester and with the post-partum histology obtained by biopsies 6 weeks post-partum (80). In a series of 77 German pregnant women, 34% of the CIN 3 lesions regressed (84). Furthermore, a regression rate of CIS as low as 12% was reported in a study from USA of 26 pregnant women (84) and a regression rate of 16.7% in CIN2 and 3 lesions were reported among 30 women in Argentina (81). These large differences are most likely explained by variations in the extent of the biopsies taken during pregnancy, where a large biopsy would increase the likelihood that the lesion would disappear as indicated by the fact that around 65% of these lesions regressed after loop-biopsies in our study**.** Another explanation may be different definitions of regression and different criteria for the diagnosis of CIN3.

In the present study, around 40% of the women with ASCUS at initial cytology showed CIN2+ in histology and this included also one case of microinvasive cancer. Moreover, among the 17 women having CIN1 in initial cytology, 41% showed CIN2+ in histology. These facts stress the importance of prompt colposcopic investigation also when ASCUS or CIN1 in cytology are diagnosed during pregnancy.

In the present study, four cases (1.6%) of invasive cancer were found, with three being microinvasive. In addition, two cases were found after delivery, resulting in a total cancer incidence of 6/251 (2.4%). These incidences appear somewhat lower than reported in a Czech study by Robova et al. (87) where an incidence of 3.6% was reported, with all of them being microinvasive cancer. Two other studies found an incidence similar to that of the present study. Thus, Kaplan et al. found three microinvasive cancers (1.9%) in the follow-up post-partum of their 157 pregnant US women with atypical cytology (68) and Palle and co-workers in Denmark found an incidence of around 1.1% (67). These differences could be due to different protection by screening guidelines and effectiveness.

In the present study, two cases of invasive cancer were found after delivery. In one case CIN3 was shown in biopsies during pregnancy, but conization four months post-partum showed a macroscopic SCC stage of IB1 (grade 1). It is unclear whether the cancer was present during pregnancy since only colposcopically directed punch biopsies were taken but it seems likely since fast progression from CIN3 to a macroscopic tumor seems less probable. The two cancer cases diagnosed post-partum stress the importance of proper follow-up during pregnancy to observe signs of progression. Moreover, they also illustrate the need for diagnostic interventions soon after delivery if there are any suspicions of cancer or progression of dysplasia in cytology and/or in colposcopic appearance at follow-up, which is usually done in the early stages of the third trimester. This view is also supported by the finding of around 13% progression to cancer in pregnant

women with CIN2/3 post- partum in the recent study by Coppolillo et al. (81)**.** Furthermore, these cases illustrate that if there are large and ambiguous colposcopical lesions in early pregnancy restricted loop-biopsies may be considered in order to excise a larger area.

It is well described that conization during pregnancy may lead to complications such as bleeding, miscarriage and premature rupture of membranes (59-62). In the present study, LEEP-cones were performed in only nine women due to suspicion of cancer up to the 22nd week of pregnancy and the 46 loop-biopsies were performed up to the 27th week of pregnancy. It is important to note that none of these women experienced any major peri-operative or obstetric complications. This is in contrast to the results of Robinson and Schaefer who found blood loss needing transfusion, premature labor (61, 62) cervix incompetence and missed abortion after LEEP-cone (62). Among the 46 women who were biopsied with colposcopically directed loop-biopsies in this study five (10.2%) sought medical attention at the gynecological emergency outpatient clinic due to bleeding complaints after surgery. The mildness of these complications is indicated by both the fact that only topical treatment on the bleeding site was needed and that no further obstetrical complications were reported in these patients. Furthermore, there was no increased incidence of premature births in the study group compared to the control group.

Previous studies have shown that punch biopsies can be considered safe also during pregnancy since no major complications seemed to occur (84). This is in accordance with the results of the present study, where only nine (4.6%) of the women that were investigated by punch biopsies experienced bleeding after the procedure.

The incidence of miscarriage in the present study was 0.8%. This is a lower rate than that was found in a study of a large cohort of Swedish women, where the incidence was 12% (121). It does not seem probable that the miscarriages nor the intrauterine death that was reported in the present study were caused by the investigation of atypical cytology since there existed other factors that were more likely to be causative to these outcomes of pregnancies.

Previous studies have shown that cervical dysplasia is more common among smokers compared to non-smokers (122). In our study group, around 30% were smokers and the proportion of smokers in this group was higher compared to the control group (21%), with a similar rate as the general female population in childbearing ages (144). The higher proportion of smokers in this group may have influenced the results since it has been described that cervical dysplasia during pregnancy are less prone to regress among

smokers than non-smokers (79). However, in our study we could not see any statistically significant differences in regression rate in the smokers and non-smokers of the study group as compared to the control group.

In conclusion, conservative management of cervical dysplasia during pregnancy is safe with a high regression rate after delivery, also among cases of HGL. If there is a suspicion of microinvasive cancer colposcopically directed loop-biopsies could safely be used for diagnosis without any major complications in women with large or ambiguous colposcopic lesions at least if performed by experienced colposkopists. Histologic re-evaluation should be performed soon after delivery in all cases with HGL but definite treatment can then be postponed some months, but only after invasive cancer has been excluded.

Paper III

Background

Cervical cancer is, beside breast cancer, the malignancy which is most commonly diagnosed in association with pregnancy and the reported incidence varies between 12 and 45 per 100 000 pregnancies (90, 92). In countries with established screening programs for cervical cancer, the overall incidence of cervical cancer has gradually decreased (94). Thus, in Sweden, where organized population-based cervical screening was introduced in 1964 with implementation of nationwide screening in 1977, the overall incidence of cervical cancer has been reduced from 20 to 6.6/100 000 women in 1965 and 2007, respectively (93). During the last decades efforts have been made to improve the cervical screening program, with initiation of screening every 3rd year already at 23 years of age, consequent use of colposcopy and directed cervical biopsy in cases having dysplastic lesions, introduction of liquid based cytology and re-invitation of non-responders. National guidelines have been established, recommending that pregnant women, not having undergone screening during the previous 2.5 years before pregnancy, should be tested by a cervical cytological smear at their primary visit to the antenatal care unit.

Surgery has become the primary modality of treatment of FIGO stage I-II cervical cancer, the W-M radical hysterectomy being the traditional procedure and applied subsequent to CS in pregnant women having invasive disease. During the last decade fertility preserving vaginal trachelectomy has been successfully introduced and may be considered post-partum in young women having stage I cancer or stage 2 with size of less than 2 cm (123).

In a previous study of the incidence of cervical cancer during pregnancy in the Western region of Sweden between 1973 and 1992 all these cancer cases were described in detail (92) and that report is an important background material to the results of the present study of the cervical cancer incidence during pregnancy during the following time period. In the light of altered strategies with respect to the regional cervical cytology screening programme as well as of the management of detected cervical dysplasia and cancer during pregnancy, the present study was initiated to compare the results of the last 16 years with those of the previous two decades. The study represents a population-based cohort including all women diagnosed with cervical cancer in conjunction with pregnancy.

The present descriptive study was done at the Sahlgrenska University Hospital, which is the regional unit for treatment of invasive cervical cancer in the Western region of Sweden. The women included in the present study were diagnosed with invasive cervical cancer during pregnancy or within six months after delivery between 1993 and 2008. During the study period the mean number of women of fertile age (20-45 years) was about 285 000. The mean number of annual deliveries was around 19 000. The women were identified from the database on women having invasive cervical cancer or CIN3 at the Regional Cancer Centre Western Sweden Healthcare Region, which administers the registration and follow-up of pre-invasive and invasive diseases in Western Sweden. The medical records and further identification of each single woman were achieved after searching in various registers (Swedish Cancer Register; Swedish Medical Birth Register; West Sweden Regional Cervical Cancer Prevention Quality Register). The medical records women referred to in the text are summarized in appendix. Data on follow-up and survival were not available for one woman who had emigrated one year after treatment.

Results

During the 16 years 1993-2008, cervical cancer was diagnosed in a total of 1361 women in the Western Region of Sweden. In 47 women (3.5%) the diagnosis was made in association with pregnancy. Based on the total number of deliveries during the period, cervical cancer was registered in conjunction with 15.6/100 000 deliveries. The incidence was similar during the first and second 8-year periods of the study (23 and 24 cases, respectively). The mean age of the women was 32 years. The mean parity/pregnancy ratio was 1.4/2.6. Sixteen women were nulliparous.

Sixty-one percent of the women were asymptomatic and 16 reported abnormal vaginal discharge and/or bleeding. Clinically visible tumors were observed in about 19 of the women i.e. as a polyp in five women, one woman in the 2 nd trimester and 3 women post-partum. Another woman had CIN2 in a polyp in the 2nd trimester and was diagnosed with adenosquamous carcinoma post-partum

Cervical cancer was diagnosed in 35 women during follow-up of an atypical cervical cytology smear taken in the 1st trimester or shortly before pregnancy (n=24), and a smaller number of cases were diagnosed in the 2nd trimester (n=5) and at routine postpartum control (n=6). Thus, in about 75% of the women the atypical cervical smear led to the final diagnosis of cancer. The initial atypical cervical cytology represented ASCUS in nine women, CIN1 in two, CIN2 in seven and CIN3 in 17 women. Two women, having CIN3 at cervical smear taken around 3 months before pregnancy, had ASCUS and AIS at follow-up in the 2nd and 5th gestational week, respectively.

There was a fairly wide distribution of cytology results prior to cancer diagnosis among the cases. Thus, ten of the women had ASCUS. Twenty-nine women had an atypical cervical smear in the 1st-2nd trimester. Of them 21 developed stage IA cancer, 5 stage IB1, one stage IB2 and 2 women stage IIA cancer. Among six women, in whom the cervical smear was taken post-partum, four had stage IB1 cancer, one stage IB2 and one stage IA1 cancer.

In all, 22 (about 45%) women had stage IA cancer, with 12 diagnosed in the 1st-2nd trimester and ten during the post-partum period. Stage IB1 cancer was found in 17 women, with the majority (12 cases) found post-partum and fewer found in the 2nd (3 cases) and 3rd (2 cases) trimester. Stage IB2 cancer was diagnosed in six women: one in the 2nd trimester, three in the 3rd trimester and two women post-partum. Two women had stage IIA cancer post-partum.

In comparison with the previous 20 year-period (92), the proportion of stage IA cancer was increased (46.8% vs. 9.9%) whereas the incidence of stage IB and ≥stage IIA cancer was not significantly altered (48.9% vs. 69.7% and 4.3% vs. 21.2%, respectively) although there was a trend to lower stages. The mean age of the women was 32.1 years (range 23-43 years). The mean parity/pregnancy ratio was 1.4/2.6. Sixteen women were nulliparous.

The histology demonstrated SCC in about 77%, adenocarcinoma in about 17% women and adenosquamous carcinoma in about 5%. The proportion of adenocarcinoma was not significantly altered in comparison with previous 20 years period (9.1%) Fifty-two percent of cancer diagnosed in the 3rd trimester and post-partum were grade 3 and 24% were grade 2.

Twenty-four women underwent vaginal delivery at term. Eleven women were followed up because of an atypical cervical smear during pregnancy (6 women ASCUS, 2 women CIN2, 3 women CIN3). Post-partum stage IA1 cancer was diagnosed in six women, IA2 in one, stage IB1 in two and stage IIA cancer in two women. Four women had stage IA cancer in 1st or 2nd trimester and were found not to have worse than CIN3/HGL before vaginal delivery. At conization post-partum histology was benign in one woman and showed CIN3 in two women. Another woman had normal cervical cytology and colposcopy at initial postpartum follow-up of stage IA1 adenocarcinoma diagnosed in the 1st trimester. The cancer recurred after 10 months. In nine other vaginally delivered women cancer was diagnosed post-partum, at follow-up of newly detected cytological atypia (n=5) or visible polyp/tumor (n=4).

Twenty-three women were delivered by CS, 20 of them because of cancer. Of eight women having stage IA1 cancer six women were delivered electively and two by emergency CS preterm. Three women, having CIN3 in the 3rd trimester, underwent CS because of colposcopically suspected cancer, the eventual treatment being postponed until after parturition. In two women with cervical cancer stage IB1 and IB2, the cancer was actually detected in conjunction with delivery, and they underwent W-M radical hysterectomy within two weeks after CS. One woman presented with abnormal vaginal bleeding in the 34th gestational week and stage IB2 cancer was diagnosed. She underwent CS and was radically operated four weeks later. Lymph node metastases were found in all these three women. Six women having cervical cancer of ≥stage IB1 were delivered by CS combined with immediate W-M radical hysterectomy. In the six women who had undergone CS combined with W-M radical operation no lymph node metastasis were found but vascular space invasion was seen in one woman. Among the three women who had the radical operation postponed 1-4 weeks after delivery by CS, lymph node metastases were found in all three women. Three women had undergone CS on other indications and had cancer diagnosed post-partum.

Colposcopical evaluation, preceding punch or cone biopsy, demonstrated HGL/cancer lesion in 13 of 19 women who had stage IA1 cancer. Among five women, evaluated to

have cancer by colposcopy ante-partum, two women had stage IB1 cancer, one woman had stage IA1 and one had stage IB2 cancer.

Eleven women (25%) had a benign cervical smear within 2.5 years before pregnancy. In three of them, stage IA1 cancer was diagnosed in the 2nd trimester and nine of these 12 women had the cancer detected post-partum (stage IA n=2, stage IB n=6, stage IIA n=1).

Twenty-two of the 30 women, where a proper follow-up has been conducted, were considered adequately followed up during pregnancy according to the current regional guidelines. Sixteen of these women had stage 1A cancer and six women IB1 cancer. Out of the eight women not being adequately followed up, five had stage 1A1-2 cancer. One woman had CIN3 12 weeks before gestation, then ASCUS in the 2nd gestational week and benign cytology and colposcopy in gestational week 13. She delivered vaginally and was not followed up until 16 weeks post-partum when stage IIA cancer was diagnosed. Another woman had IB2 cancer detected at delivery, and underwent CS. She had CIN3 in a cytological smear in the 12th and 17th gestational week and was not further followed up during pregnancy but presented with clinically visible tumor in the 40th gestational week. Still another woman had not been adequately screened before pregnancy and was diagnosed by CIN2 in the 12th gestational week. Colposcopically the dysplasia was judged to be HGL and CIN2 in punch biopsy were observed in gestational week 19. At follow-up, which was not carried out until eight weeks after vaginal delivery, adenosquamous carcinoma stage I IA was diagnosed.

The majority of women having elective CS preterm were given corticosteroids to promote fetal lung maturation. In spite of this, six infants developed infant respiratory distress syndrome and needed neonatal care for 1- 8 weeks. Otherwise the outcome of all infants was uneventful.

No pregnancy was terminated because of cervical cancer. Three women, all of them having conization as curative treatment, had another pregnancy after 2, 3 and 4 years, respectively, and were delivered vaginally, one of them preterm.

Six women cases died of disease (mortality rate 12.8%), four within 19 months after delivery. Compared with the study period 1973-1992 the mortality was not significantly reduced (12.8% vs 21.2%). All the other 40 women, being eligible for evaluation, are alive and free of disease (mean follow-up 11.7 years, range 4-19 years).

Comments

Invasive cervical cancer in association with pregnancy is rather rare but is though considered to be one of the most prevalent malignancies during pregnancy (90, 124). Since the detection and treatment of cervical cancer in a pregnant woman may have profound consequences not only for survival of the mother and the child she bears but also for her future fertility, it is of importance to continuously update and revise the principles in the management of pregnancy-associated pre-invasive and invasive cancer. Due to the low incidence of cervical cancer during pregnancy the reported case series are small (74, 91). A strength of the previous study (92) and the present study is the 100% inclusion of a population-based cohort and with long-term follow-up of as many as around 95% of the patients.

The reported incidence of cervical cancer during pregnancy varies and local differences in the staging and timing of diagnosis before gestation or after delivery may contribute to this variation. Thus, incidences from 12 to 45 per 100 000 pregnancies have been reported (65, 90, 91, 125). In the present material, the incidence was 15.6 cases per 100.000 deliveries. This difference in incidence was not statistically significant compared to the 11.1/100 000 deliveries during the preceding twenty years (92). In the present study, the stages of cervical cancer were less advanced than in the previous study with almost 50% being of stage IA in the present study as compared to around 10% in the previous study. The higher incidence as well as cancer down-staging may be a result of better diagnostic routines and improvements in cervical cancer screening as women are screened at a younger age and they may also show better compliance in the re-call process, improving the chances to detect cancer at an early stage.

Abnormal vaginal bleeding is often encountered in pregnant women. The cervical screening history should be assessed in pregnant women admitted due to bleeding or abnormal discharge. When infection and/or obstetrical bleeding are excluded, colposcopy combined with cervical smear sampling and/ or biopsy is advisable. This appears of special importance in the 3rd trimester, when timing and mode of delivery might be crucial in case a dysplastic disorder is detected. In the present study, one woman was repeatedly admitted due to bleeding until the diagnosis of cancer in the 31stgestational week. Another woman experienced bleeding throughout pregnancy, interpreted as due to vaginal varicose veins, cervical cancer being diagnosed not until 16 weeks after vaginal delivery. These cases illustrate the importance of considering sources of abnormal bleeding other than those of obstetrical origin.

Seventy percent of the women had an atypical cervical smear leading to detection of cancer, which is a larger proportion than previously reported (72, 92, 126). This observation stresses the importance of taking cervical smear at the initial antenatal care visit as well as at postpartum control in women not being accurately screened. Nevertheless, 13 women, being properly screened within 2.5 years before becoming pregnant, developed cancer. This fact raises the question whether all pregnant women, irrespective of screening history, should have a cervical smear performed in early pregnancy.

It is notable that nine women, having ASCUS and one woman having CIN1 in early pregnancy, developed invasive disease. This observation highlights the importance of prompt follow-up of all cases having low-grade cervical atypia. More than one third of these women were not properly followed-up during pregnancy according to current regional guidelines, which, however, were not introduced until 2009. Probably, a stricter adherence to these guidelines, including repeated colposcopy, cervical smear and biopsy could further decrease the incidence of invasive disease and lead to further down-staging, enabling fertility preserving therapy.

Since the previous study there have been important changes with regard to the management of cervical cancer during pregnancy. For instance, radiotherapy is no longer considered as a primary modality of treatment. Between 1973 and 1992, 11 pregnancies were interrupted by operative procedures or as a consequence of radiation (92), whereas in the present study no termination was done. In the former study, two children died after preterm CS whereas all infants survived in the present one. When cervical cancer is detected in late 2nd trimester, the question may arise, whether the pregnancy should be terminated or not. It seems reasonable to let the pregnancy continue until around the 30th-32nd week in women having ≤IB1 cancer diagnosed around or after the 20th week of gestation (74). In the present study, two cases were delivered by CS around week 30, due to stage 1B cancer. In these cases it was determined to perform CS due to invasive cancer.

In accordance with our current principle, the majority of women having stage IA cancer diagnosed during pregnancy were delivered by elective CS preterm whereas four women were delivered vaginally at term. Irrespective of mode of delivery the course was uneventful for these women. Based on this experience and according to observations by other authors (125) vaginal delivery does not seem to be contraindicated in women having stage 1A cancer. On the other hand, elective preterm delivery by CS will bring forward the follow-up post-partum to achieve definite diagnosis and prevent progress of disease, which is of importance if the lesion was not excised with free margins.

Fifteen of the women having stage I cancer underwent conization post-partum as the eventual treatment. This concept appears to be an adequate therapy even in cases when dysplasia may persist in the postpartum cone specimen. However, in cases where stenosis of the cervical canal does not permit representative cervical sampling or the woman experiences anxiety for recurrence of cancer, hysterectomy appears advisable and was applied in six women in this study. In the present study, conization was performed by CO_2 laser or loop electrosurgical excision as late as the 25th gestational week without any severe complication. As compared with cold-knife (59, 60) conization these modern techniques appear to be associated with reduced risks of obstetrical complications (62, 118).

Colposcopy is the standard diagnostic method to evaluate cervical dysplasia and enables directed punch or cone biopsy sampling, thereby avoiding arbitrary taking of multiple and large biopsies (55). The recently introduced Swede score colposcopic scoring system has been shown to be a promising tool in evaluating atypical cervical cytology in pregnant women (Paper I). In the present study, there was a good concordance between colposcopical judgement of HGL/cancer lesion and histology. Moreover, as shown in this study, an unequivocal colposcopical judgement of cancer may underlie the clinical planning.
We previously discussed the possible risk of spreading of cancer cells during uterine involution starting immediately after delivery (92). Generally, the risk of cancer spreading may primarily concern women having ≥stage IB1 cancer (72). In the present study, lymph node metastases or vascular invasion of cancer cells were found in all women having 1-4 weeks delay between CS and eventual W-M operation whereas no metastasis was observed in women undergoing combined CS and radical operation, even though the tumor was locally advanced. On this basis, it cannot be excluded that an activated lymph drainage post-partum may contribute to spreading of cancer cells and it seems reasonable to recommend CS and concomitant radical W-M operation in women having stage IB-IIA cervical cancer.

To conclude, it should be suggested that all pregnant women should have a cervical smear performed at their initial antenatal visit. Any atypical cervical smear should be evaluated without delay by colposcopy and directed punch- or cone biopsy. When there is HGL, follow-up until around the 30th week is advocated to consider timing and mode of delivery. Women diagnosed with, radically treated and followed-up for stage IA1 cancer may probably be safely delivered vaginally. In women having ≥stage IB cancer concomitant CS and radical W-M operation should be performed when infant survival is

considered reasonable. Conization may be adequate treatment for stage IA1 cancer with the advantage not to compromise fertility.

Paper IV

Background

During the last decades, it has become evident that almost all cases of cervical cancer are due to an initial cervical infection with HPV. The pioneering work in this field was done more than 30 years ago by the German scientist zur Hausen, who later received the Nobel Prize in Physiology and Medicine in 2008 for his groundbreaking work in this field. Studies using sensitive PCR techniques have now established that HPV DNA is present in virtually all (99.7%) of cervical cancer (10) and the role of HPV in transformation of the cervical epithelial cells into cancer cells is at present well established.

HPV is specific to humans and more than 100 different types of HPV exist. After analyzing HPV DNA in cervical cancer specimens from many countries worldwide, 12 genotypes of HPV have been termed high risk HPV (HR-HPV), one termed probable high risk and seven termed possibly high risk. Detailed studies have shown that DNA for HPV 16 and 18 are found in around 70% of cancer cases and the other HR-HPVs are present in almost all other cases of cervical cancer.

Necessary in the HPV-dependent transformation of the infected cervical cells are two viral proteins termed E6 and E7, which generally become overexpressed in the transformed cells. One of the most important roles of E6 is to inactivate the p53 tumor suppressor protein. This protein prevents tumor formation, by initiating pathways that are instrumental in DNA repair, cell cycle arrest and apoptosis. The most prominent effect of E7 is by interaction with the tumor suppressor pRb, resulting in cell proliferation and dysregulated cell death, and differentiation. E7 also stimulates the cell cycle by up-regulating cyclin and their associated kinases.

Recent data suggest that in different parts of the world, the most common HPV-types in cervical cancer may vary. HPV16 and 18 are the most common world-wide, and with a few exceptions, the most common genotypes after HPV16 and 18 are HPV 31, 33, 35, 45, 52 and 58, in varying order. For this reason calculations of sensitivity, specificity, PPV and NPV were performed also for these eight genotypes.

In this study the clinical performance of an in house real-time PCR assay detecting and typing E6/E7 mRNA of all HR-HPV was evaluated.

Results

The study was cross-sectional and included 210 liquid based cytology (LBC) samples from 204 women who were undergoing screening or had been admitted to referral center because of atypical cytology. Histological evaluations from biopsies and/or total excised specimens taken at the same time were available in 155 (74%) of the 210 LBC samples.

It was found that HPV16 was the most prevalent type in the 87 samples that were histologically classified as CIN2+ (52%), followed by HPV18 (21%), HPV31 (20%), HPV33 (15%), HPV52 (14%), HPV39 (7%) and HPV45, HPV51 and HPV56 (6%). The five most common HPV-types in the 28 samples with cancer were in order HPV16, HPV18, HPV33, HPV45 and HPV31.
Concerning HPV mRNA analysis, the genotypes that were found were similar. The most common genotype in CIN2+ samples expressing E6/E7 mRNA was HPV16 (47%), followed by HPV18 (18%), HPV31 (17%), HPV33 (9%), HPV52 (8%) and HPV45 (6%).

In four CIN3+ samples (three cancers), no HR-HPV mRNA could be found. In two of these samples, both cancers, HR-HPV DNA was undetectable, but the samples tested positive for either HPV68 or HPV70 with other methods. Out of 34 samples with benign histology, 71% were HPV-positive and 35% expressed E6/E7 mRNA. However, all these women had a history of dysplasia. When looking at a screening-cohort of 51 women (median age 31) with benign cytology and no histology available, the prevalence of HPV-infection was 43% of which about 10% showed expression of E6/E7 mRNA.
Overall, there was a good agreement between DNA and mRNA testing, and as expected, the DNA analysis had a higher detection rate and identified presence of 140 HPV infections in which no expression of E6/E7 mRNA could be detected.

The sensitivity, specificity, PPV and NPV of detecting CIN3+ or CIN2+ lesions were calculated for analysis of HPV DNA, mRNA and with the commercial PreTect HPV-Proofer. Furthermore, calculations were made for the two HPV DNA and HPV mRNA in house tests when including only the five or eight most common HPV-types in cervical cancer; HPV16, 18, 31, 33, 45 and with addition of HPV35, 52 and 58. When predictions of CIN2+ were tested by HPV DNA test targeting 12 HR-HPV, 8 HR-HPV and 5 HR-HPV the sensitivities were 0.95, 0.92 and 0.86, respectively and the specificities were 0.38, 0.50 and 0.57 respectively. When predictions of CIN2+ were tested by HPV mRNA test targeting 12 HR-HPV, 8 HR-HPV and 5 HR-HPV the sensitivities were 0.91, 0.87 and 0.83, respectively and the corresponding specificities were 0.68, 0.74 and 0.76. The mRNA test targeting 5 HR-HPV was compared to the PreTect HPV-Proofer assay in

prediction of CIN2+ and the sensitivity and the specificity of the latter test were 0.75 and 0.77, respectively.

Comments

This study aimed to evaluate the clinical performance of a real-time PCR assay that detects mRNA transcripts coding for the oncogenic proteins E6 and E7 of 12 high-risk HPV and 2 low-risk, using the same primers and probes as described previously for HPV DNA. For 210 LBC samples with various grades of CIN, there was good agreement between HPV mRNA and HPV DNA results although the detection rate was higher with the DNA assay, as expected.

The assay for mRNA detection, which includes a step that verifies that the mRNA signal is not due to detection of DNA, had a sensitivity of detection of CIN2+ and CIN3+ that was only slightly lower than for DNA-detection (0.91 vs. 0.95 for CIN2+lesions and 0.93 vs. 0.97 for CIN3+lesions, respectively), but the NPV did not decrease compared to the DNA-test. Importantly, the specificity was higher for mRNA than for DNA detection (0.68 vs. 0.38 for CIN2+ lesions and 0.58 vs. 0.32 for CIN3+ lesions).
The high specificity by mRNA testing was illustrated by the finding that in a screening cohort of 51 women (median age 31) with normal cytology (but with no histology available), HPV mRNA was detected in 9.8% and HPV DNA in 43%. The PPV of detection of both CIN2+ and CIN3+ was therefore higher for mRNA-detection compared to DNA-detection (0.67 vs. 0.52 and 0.47 vs. 0.36, respectively), suggesting that mRNA testing may be a useful tool not only in triage, but also in primary screening of cervical dysplasia. It is important to emphasize that not all CIN2+ lesions will progress to cancer, and a hypothetical perfect test identifying only truly precancerous lesions would rate poorly in sensitivity with CIN2+ in histology used as a golden standard, as in this and most other studies.

The five most common genotypes present in CIN2+ and CIN3+ lesions were HPV16, followed by HPV18, 31, 33 and 52 in that order. However, in LBC samples from our patients with cancer, the five most common genotypes were HPV16, 18, 33, 45 and 31. In the present study, evaluation was performed concerning performance of the real-time PCR detection of mRNA for only the eight genotypes mentioned above that are also most commonly observed in cancer. With this limitation, the sensitivity of the assay increased somewhat compared to analysis of five genotypes, but the specificity did not substantially decrease and the PPV remained, suggesting that incorporation these eight HR-HPV types in the test might constitute a good balance between sensitivity and specificity. This was relevant also for HPV DNA testing, since analyzing eight HR-HPVs as compared to all

genotypes resulted in a significant increase in specificity at the expense of only a small loss in sensitivity, however without decreasing the high NPV.
The data of the paper suggests that mRNA-testing with real-time PCR may be a useful tool in diagnostic investigation as well as in primary screening for cervical dysplasia, and there might be an idea to consider which genotypes to include in further investigations to optimize sensitivity and specificity, especially in a post vaccine era when it may be necessary to reconsider HPV testing strategies.
In summary, the results have shed light on the hypothesis that analysis also of HPV-type specific E6/E7 mRNA may be complimentary tests in the future to understand what cervical lesions may progress and thereby be of risk for the patient in terms of cancer development. These types of studies should also be performed on pregnant populations, since the physiology and hormonal milieu of the pregnant cervix greatly differ from the non-pregnant and this may influence the results.

Paper V

Background

It is of special importance to accurately determine the grade and extent of cervical dysplasia during pregnancy, in order to avoid multiple biopsies or extensive excision during the pregnant state. This thesis has investigated colposcopic evaluation and scoring as one means of increasing the accuracy of diagnosis in pregnant women with atypical cytology. Different assays to detect HR-HPV types are commonly used as complimentary tests to cervical cytology. There exist wide variations concerning to what extent such tests are used. In Sweden the recommendations are to test for presence of HR-HPV in women having CIN1 and ASCUS in women ≥35 years and as control after conization. The possible usefulness of these tests in investigation of pregnant populations is uncertain.

HR-HPV can in principle be detected by DNA or expression of mRNA. Concerning HR-HPV detection, the commercial Linear Array assay (Roche) is used by many investigators in the field. The Linear Array HPV genotyping test is a DNA-based genotyping assay detecting 12 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and 25 low-risk HPV genotypes. In the present study the Linear Array was compared to our in house DNA assay which is a real-time PCR test covering the same 12 HR-HPV as the Linear Array and also two LR-HPV (HPV genotype 6, 11)**.** This assay uses primers of the genes in E6/E7 region.

Another way to detect HR-HPV is, as mentioned above, to detect HPV mRNA. One such commercial test, which has been on the market for several years, is the PreTect HPV Proofer test, which detects E6/E7 mRNA of only of the in cervical cancer five most HPV genotypes (16, 18, 31, 33, 45). Another method to detect HR-HPV mRNA is our in house real-time PCR test that targets the same 12 HPV types as our HR-DNA test.

Much work on cervical dysplasia has also been done in order to find protein biomarkers that may be related to the grade of lesion or presence/absence of cancer. Currently developed biomarkers can be grouped into three different groups: 1) markers of increased HPV oncogene expression, such as HPV oncogene mRNA and protein; 2) markers of increased cell proliferation such as $p16^{INK4a}$, Ki-67, MCM2, TOP2a; 3) markers of chromosomal instability, such as a gain of chromosome arm 3 q and HPV integration, (108). In recent years, $p16^{INK4a}$ has been suggested as a potential biomarker for cervical HGL and the usefulness of this in diagnosis of dysplasia in a pregnant population was investigated in this paper. The promotor of the $p16^{INK4a}$ gene is blocked by the pRB in normal cells and consequently the transcription of $p16^{INK4a}$ is inhibited. However, in infected, transformed epithelial cells the oncoprotein coded by the viral E7 gene interacts with the pRb and this induces a premature degradation of the pRB and the cells overexpress $p16^{INK4a}$, which then is evident in proliferating parts of dysplastic epithelium. Overexpression of $p16^{INK4a}$ seems to be related to overexpression of E7 (9, 127).

The patient material of the present study was made up of both non-pregnant and pregnant populations in order to establish whether differences in the usefulness of the different tests exist in variable subpopulations. It should be noted that this study represents a first study, with relatively small experimental groups. The focus of the study was the pregnant population, since few studies exist that have attempted to compare different HPV-related assays in pregnant women with atypical cervical cytology. Five different assays were evaluated in this study; two HPV DNA genotyping tests (Linear Array and an In-house real-time PCR), two HPV mRNA tests (PretectHPVProofer/Norchip and an In-house real-time PCR) and one $p16^{INK4a}$ immunohistochemistry assay.

Results

This cross-sectional study included five different subpopulations, with four of them representing usual study populations; a non-pregnant screening population without atypical cervical cytology, a non-pregnant population referred for further investigation of atypical cervical cytology or treatment for cervical dysplasia, a pregnant screening population without atypical cervical cytology and a pregnant population referred for further investigation of atypical cervical cytology. The population of cervical cancer

patients was used as a positive control population, with predicted high rate of HR-HPV and $p16^{INK4a}$ positivity.

In line with previous studies of non-pregnant populations also pregnant women with normal cytology were HR-HPV DNA positive (42% and 35%, respectively). In booth non-pregnant and pregnant women with atypical cytology HR-HPV DNA positivity was increased two-fold. HR-HPV mRNA positivity in pregnant women with atypical cytology were 54% and 73% with both mRNA tests, and $p16^{INK4a}$ was 84%. In the subgroup of pregnant women with histology verified dysplasia, stepwise logistic regression analysis showed that $p16^{INK4a}$ test and the In house E6/E7 mRNA test in combination was the most suitable test to predict CIN2+.

Interestingly, in two samples from women with advanced cancers HR-HPV DNA was not detected and in three samples from women with advanced cancers HR-HPV mRNA was not detected. However, all the 25 women with cancer tested positive for $p16^{INK4a}$.

Comments

The study represents to my knowledge the first study that has compared presence of HR-HPV DNA and mRNA with different methods in a pregnant population. The results clearly show that neither test can independently be used for further non-invasive detection of HGL. The patient group of special interest of this study is the subgroup of pregnant women with atypical cytology but that also had HGL in histology. The mRNA tests did not detect all of the CIN3 lesions, indicating that the use of these tests may need the additional information of a p16 ^{INK4a}-assay. The stepwise logistic regression analysis showed that the most suitable tests in detecting HGL-lesions among pregnant women were a combination of p16 ^{INK4a}-assay and the In-house E6/E7 mRNA test. Thus, these methods may be one means of selecting those pregnant women who should be further investigated by colposcopy and biopsies and consequently reduce the need for colposcopy and biopsies during pregnancy.

Table 7. History of women having cervical cancer in association with pregnancy

Patient		Signs/ symptom	Colposcopy	Treatment	Histology stage	Mode of delivery	Final treatment	Follow-up
1.	40 w					Vag.		
37 years	+8w	CIN3						
	+16 w	Tumor		Biopsy	Sq.C.^{3}		W-M	18 years
	+18 w				Sq.C.^{3}, IB1			
2.	-4 w	ASCUS						
40 years	6 w			Biopsy	CIN2			
	22 w	ASCUS						
	26 w		Inc.					
	36 w		LGL					
	40 w					Vag.		
	+8 w			Con.	Sq.C.^{2}, IA1			
	+12 w				Benign		Abd. hyst.	15 years
3.*	40 w					Vag.		
35 years	+8 w	Polyp		Biopsy	SqC^{1}, IB1			
	+9 w			Con.	SqC^{3}, IB1			
	+12 w				Benign		W-M	19 years
4.	6 w	CIN3						
37 years	10 w	Bleeding	HGL	Biopsy	Ad.C.^{1}, IA1			
	13 w		HGL	Con.	CIN3			
	40 w					Vag.		
	+8w	Benign	Benign					
	+16 w	Benign	Benign	C.cur	Benign			
	+24 w	ASCUS	HGL					
	+32 w	CIN3	HGL					
	+40 w		HGL	Con.	Ad.C.^{1}, IA1			
	+44 w				Benign		Abd. hyst.	16 years
5.*	38 w					Vag.		
40 years	+8 w	Polyp						
	+12 w			Biopsy	SqC.^{3}			
	+16 w				SqC.^{3}, IB1		W-M	11 years
6.*	40 w					Vag.		
34 years	+12 w	CIN3						
	+24 w	Tumor		Biopsy	Sq.C.^{3}, IB2			
	+28 w				Sq.C.^{3}, IB2		W-M; SOE; Ch.	17 years
7.	10 w	CIN2						
37 years	18 w	Bleeding	LGL	Con.	Sq.C.^{2}, IA1			
	25 w	ASCUS	Benign					
	34 w	Benign						
	40 w					Vag.		
	+10 w	CIN2	Benign		Benign			
	+20 w		Benign	Con.	Benign		Con.	14 years
8.	12 w	Benign						
34 years	37 w	Bleeding						
	38 w	Tumor		Biopsy	SqC^{3}, IB1	Sectio^{C}		
	+2 w				SqC^{3}, IB1, Lnm 6		W-M; Ch.; R.	17 years
9.	34 w	Bleeding,Tumor		Biopsy	SqC^{3}, IB1			
34 years	35 w				Ad.C.^{3}	Sectio^{C}	W-M; Ch.	+ 6 years
10.*	14 w	CIN3						
43 years	20 w		HGL	Con.	SqC.^{1}, IA1			
	29 w	Benign	Benign					
	42 w					Vag.		
	+16 w		LGL	Con.	CIN3			
	+36 w				Benign		Vag. hyst.	7 years
11 *.	-12 w	CIN3						
31 years	2 w	ASCUS						
	13 w	Benign	Benign					
	41 w					Vag.		
	+16 w	ASCUS,Tumor	Cancer	Biopsy	SqC^{3}, IIA			
	+18 w				SqC^{3}, IIA		Ch.; W-M; SOE; R.	17 years
12.	39 w					Vag.		
34 years	+16 w	Bleeding		Biopsy	SqC^{2}, IB2		Ch; R.	+ 18 months

13.	12 w	CIN3						
31 years	17 w	CIN3	LGL	Biopsy	CIN3			
	40 w	Tumor		Biopsy	SqC[2], IB2	Sectio[C]		
	+1 w				Adsq.C.[3], IB2; Lnm 1		W-M; R.; Ch.	┼ 16 months
14.	16 w	Polyp		Biopsy	Ad.C.[1], IB1			
32 years	22 w		Inc.	Con.	Ad.C.[1], IB1			
	25 w			Con.	Ad.C.[1], IB1			
	33 w				Benign	Sectio[C]	W-M	16 years
15.	40 w					Vag.		
33 years	+12 w	ASCUS						
	+24 w		Inc.	Biopsy	CIN3			
	+ 28 w			Con.	Sq.C.[2], IA1			
	+32 w	Benign						
	+48 w				Benign		Abd. hyst.	1 years
16.	6 w	ASCUS						
40 years	9 w	Benign						
	10 w	CIN3	Benign					
	16 w		LGL	Biopsy	CIN3			
	25 w	Benign	LGL					
	30 w	CIN3						
	39 w					Vag.		
	+4 w		HGL	Biopsy	Sq.C.[1], IA1			
	+6 w		HGL	Con.	CIN2		Con.	18 years
17.	24 w	Bleeding						
34 years	32 w	Tumor		Biopsy	Sq.C.[3], IB2			
	33 w				Sq.C.[3], IB2	Sectio[C]	W-M; Ch.	┼ 19 months
18.*	16 w	ASCUS						
40 years	6 w	Benign	Benign					
	17 w	CIN3	HGL	Biopsy	CIN3			
	31 w	CIN3	HGL					
	40 w					Vag.		
	+16 w		Inc.	Con.	Sq.C.[1], IB1			
	+18 w				Sq.C.[1], IB1		W-M	7 years
19.	38 w					Vag.		
39 years	+8 w	CIN3						
	+ 16 w		Biopsy		Sq.C.[2], IB1			
	+24 w				Sq.C.[2], IB1		W-M	9 years
20.	12 w	ASCUS						
30 years	23 w	CIN3						
	25 w	CIN3	HGL	Biopsy	CIN3			
	28 w		HGL					
	40 w					Vag.		
	+ 16 w		LGL	Con.	Sq.C., IA1		Con.	16 years
21.	39 w					Vag.		
34 years	+12 w	CIN1						
	+28 w	Tumor	Cancer	Biopsy	Sq.C.[2], IB1			
	+32 w				Sq.C.[2], IB1, Lnm 1		W-M; Ch.	13 years
22.	38 w					Vag.		
36 years	+ 16w	Polyp,Bleeding		Biopsy	Ad.C.[1]			
	+24 w				Ad.C.[1], IB1		W-M	10 years
23.*	37 w					Sectio		
25 years	+16 w	Bleeding,Tumor		Biopsy	Sq.C.[3]			
	+24 w				Sq.C.[3], IB1, Lnm 3		W-M; Ch.; R.	11 years
24.	12 w	CIN2						
27 years	19 w	CIN2, polyp	HGL	Biopsy	CIN2			
	42 w					Vag.		
	+8w	CIN3						
	+12 w		Inc.	Biopsy	Adsq.C.[3]			
	+13 w	Tumor		Con.	Adsq.C.[3], IIA			
	+15 w				Adsq.C.[3], IIA, Lnm 2		W-M; Ch.; R.	┼ 44 months
25.	25 w	Bleeding						
30 years	26 w	Tumor		Biopsy	Sq.C[2].			
	31 w				Sq.C[2]., IB1; v. inv.	Sectio[C]	W-M; Ch.; R.	15 years
26.	11 w	CIN1						
30 years	13 w		HGL	Biopsy	Sq.C., IA1			
	15 w		HGL	Con.	Sq.C., IA1			
	21 w	Benign	Benign					
	32 w					Sectio[C]		
	+8 w		Benign	Con.	CIN1		Con.	14 years

27.	12 w	CIN3						
29 years	14 w			Biopsy	CIN3			
	19 w		HGL	Con.	Sq.C., IA1			
	25 w	ASCUS	Benign					
	32 w					Sectio[C]		
	+2 w		LGL	Con.	CIN1		Con.	15 years
28.	16 w	CIN3						
29 years	21 w		HGL					
	22 w			Con.	Sq.C., IA1			
	25 w			Con.	CIN3			
	41 w					Vag.		
	+8 w	Benign					Con.	15 years
29.	11 w	CIN3						
33 years	17 w		Cancer	Con.	Sq.C., IA1			
	30 w	CIN3	LGL					
	36 w					Sectio[C]		
	+2 w		LGL	Con.	CIN3		Con.	7 years
30.	12 w	ASCUS						
31 years	18 w	Benign	HGL					
	36 w					Sectio		
	+20 w	CIN3	Benign	Con.	Ad.C.[1], IA1		Con.	10 years
31.	-4 w	CIN3						
28 years	2 w		HGL	Con.	CIN3			
	23 w	CIN3	HGL	Biopsy	CIN3			
	32 w		HGL					
	41 w					Vag.		
	+3 w			Biopsy	Sq.C., IA1			
	+6 w		HGL	Con.	CIN3		Con.	13 years
32.	12 w	CIN2						
26 years	16 w		HGL	Con.	Sq.C., IA1			
	25 w	Benign	LGL					
	33 w	Benign	Benign					
	36 w					Sectio[C]		
	+8 w		Benign	Con.	CIN1		Con.	16 years
33.*	-4 w	CIN2,Bleeding						
29 years	4 w	Benign	HGL	Biopsy	AIS; CIN1			
	9 w		LGL	Con.	Ad.C., IA1			
	26 w					Sectio[C]		
	+7 w	ASCUS	Benign	Con.	CIN2		Con.	10 years
34.	8 w	CIN3,Bleeding						
31 years	13 w	CIN3						
	18 w	Tumor	Cancer	Con.	Sq.C.[2], IB1			
	30 w				Sq.C.[2], IB1	Sectio[C]	W-M	9 years
35.	8 w	CIN2						
30 years	18 w		Inc.	Biopsy	CIN1			
	39 w					Vag.		
	+16 w	CIN3		Biopsy	Sq.C., IA1			
	+20 w		HGL	Con.	Sq.C., IA2			
	+28 w				Benign		Trach.	8 years
36.	10 w	CIN2,Bleeding						
30 years	15 w		HGL	Biopsy	CIN3			
	32 w	CIN3	Cancer					
	35 w					Sectio[C]		
	+2 w		HGL	Con.	Sq.C.[3], IB1, v. Inv.			
	+6 w				Sq.C.[3], IB1		W-M	8 years
37.	11 w	ASCUS						
29 years	20 w	Benign	Benign			Vag.		
	38 w							
	+16 w	AIS						
	+24 w		Cancer	Con.	Ad.C., IB1; Sq.C., IA1			
	+48 w				Benign		W-M	9 years
38.	8 w	CIN2						
26 years	15 w		HGL	Biopsy	CIN3			
	19 w		HGL	Con.	Sq.C., IA1			
	26 w	Benign	LGL					
	33 w					Sectio[C]		
	+5 w		Benign	Con.	CIN3			
	+16 w	Benign	Inc.				Con.	11 years

39.	11 w	ASCUS						
26 years	20 w	ASCUS	Inc.					
	23 w		LGL	Biopsy	Sq.C., IA1			
	29 w					Sectio[C]		
	+3 w		Benign	Con.	CIN3			
	+28 w			Con.	Benign		Con.	12 years
40.*	-6w	CIN3						
34 years	5 w	AIS	LGL					
	12 w	AIS	LGL	Con.	AIS			
	20 w	AIS	Inc.					
	26 w	AIS	Inc.					
	30 w	ASCUS	Inc.					
	36 w					Sectio[C]		
	+3 w			Con.	Ad.C.[1], IB1			
	+11 w				Ad.C.[1], IB1		W-M	3 years
41.*	40 w					Vag.		
26 years	+16 w	ASCUS						
	+28 w	CIN3						
	+32 w	Bleeding		Con.	Sq.C.[3]			
	+36 w				Sq.C.[3], IB1		W-M	11 years
42.	13 w	CIN3						
29 years	18 w	CIN3	HGL	Con.	CIN3			
	28 w	CIN3	LGL					
	39 w					Vag.		
	+16 w		HGL	Con.	Sq.C.[1], IA1		Con.	7 years
43.*	14 w	CIN3						
26 years	25 w	CIN3	Inc.	Biopsy	CIN2			
	39 w					Vag.		
	+16 w		LGL	Con.	Sq.C., IA1			
	+20 w		Benign	Con.	CIN1		Con.	9 years
44.	12 w	CIN3						
31 years	18 w		HGL	Con.	Sq.C., IA1			
	27 w	ASCUS						
	36 w					Sectio[C]		
	+2 w		HGL	Con.	Sq.C., IA1		Con.	3 years
45.	8 w	CIN3						
29 years	28 w	CIN3,Bleeding	Cancer					
	36 w					Sectio[c]		
	+2 w		HGL	Con.	Sq.C., IA1			
	+16 w		LGL	Con.	Benign		Con.	5 years
46.	29 w	Bleeding						
27 years	34 w	Tumor	Cancer	Biopsy	Sq.C.[3], IB2			
	35 w					Sectio[C]		
	+4 w				Sq.C.[3], IB2, Lnm1		W-M; Ch.; R.	
	+36 w			Biopsy	Sq.C.[3]			
	+37 w						Exenteration	4 years
47.	22 w	Bleeding						
23 years	23 w	Tumor		Biopsy	Ad.sq.C.[3], IB2			
	29 w				Ad.sq.C.[3], IB2, v.inv.	Sectio[C]	W-M; CH.;R	┼ 14 months

Age indicated below each patient number. * indicates cervical cytology smear taken within 2.5 years before pregnancy. w=gestational week. - and + indicate weeks before and after pregnancy, respectively. Colposcopy: Inc.=incomplete; LGL=low -grade lesion; HGL=high-grade lesion. Cytology: AIS=adenocarcinoma in situ; ASCUS=atypical squamous cells of undetermined significance; CIN (cervical intraepithelial neoplasia) 1, 2, 3. Carcinoma: Ad.C.=adenocarcinoma; Adsq.C. =adenosquamous carcinoma; Sq.C.=squamous cell carcinoma. Grade: [1]=grade 1; [2]=grade 2; [3]=grade 3. FIGO stages: IA 1-2, IB1-2, IIA indicated. Lnm=lymph node metastasis (number of nodes indicated). v. inv.=vascular invasion. Operative procedures: Con.=conization; Abd. hyst.=abdominal hysterectomy; Vag. hyst.=vaginal hysterectomy; W-M=Wertheim Meigs radical hysterectomy; Trach. =trachelectomy + laparoscopic pelvic lymphadenectomy; SOE=salpingo-oophorectomy. Vag.=vaginal delivery; [C] indicates cesarean section due to cancer. Adjuvant treatments: Ch.=chemotherapy; R.=radiotherapy. ┼=dead of disease.

Discussion

In the Discussion section I have chosen to discuss some specific results of the thesis, which merit discussion in a broader context. Other results are discussed to some extent in Results and Comments section and/or in the Papers.

Cervical cancer prevention screening in pregnancy

Cervical screening with Pap-smear was introduced in Sweden in 1964 and since then the overall incidence of cervical cancer has declined by 67%, from 20 per 100 000 women 1965 to 6.6 cases per 100 000 women 2005 (93). In this context it should be mentioned that prevalence among pregnant women in Paper III was found to be 15.6 cancer cases/ 100 000 deliveries. During the last decades efforts have been made to improve the cervical screening program, with initiation of screening every 3rd year at 23 years of age, with education of midwives in taking accurate smear samples, introduction of LBC and re-invitation of non-responders.

In Paper III of the present study, 66% of the women with pregnancy associated cervical cancer had not followed the recommended screening intervals. It has been shown from a national audit in Sweden that non-compliance to the recommended screening intervals is one major reason for cervical cancer morbidity (128). In that study it was shown that the risk for cervical cancer morbidity was significantly increased among women who had not undergone a cervical smear compared to women who had undergone cervical smear sampling at recommended intervals. The risk was increased among those who had neglected cytology smears and was in correlation to FIGO staging with an OR of 1.70 for FIGO-stage IA, 2.10 for FIGO-stage IB and as much as 4.82 for ≥FIGO-stage II. Furthermore, it was shown that an atypical cervical smear in screening was associated with an increased risk with OR of 7.55 for cervical cancer compared to screened cases with normal smears. Moreover, the risk was increased (OR of 1.89) for women with atypical cervical smear that was not followed by biopsy compared to women with atypical cervical smear who were biopsied. This stresses the importance of adequate further investigation of any atypical cervical smear during pregnancy with colposcopically directed biopsies being one approach for advancing the diagnostics. In the study by Andrae and co-workers, it was also shown that unscreened women <30 years of age had an OR for cervical cancer of 2.30 compared to women in the same age who were screened (128). These facts point towards the importance of revising the screening history of every pregnant woman who visits the antenatal care unit and to perform cervical smear sampling when indicated. Several authors emphasize that pregnancy is an opportunity to detect atypical smears and to educate about the importance adhering to cervical cancer screening programs (72-74).

Still, there are cases of cervical dysplasia that are not detected by screening before or during pregnancy and that have developed to cancer. This is exemplified by the 11 (23%) of the 47 cancer cases of Paper III who had undergone proper screening but still developed cancer. The accuracy of cervical smear tests varies and ranges of sensitivity between 30-87% and a specificity of 86-100% were reported in a meta-analysis by Nanada and co-workers (29). However, the studies included in that meta-analysis were performed in different countries with variation of methodological quality and frequency of histological abnormalities, which may explain the wide range in sensitivity and specificity. LBC was recently introduced in Sweden and will also be used as the method in the screening programme in Western Sweden. This new method of processing the cervical smear was used in Paper IV and V of the present thesis. One advantage with LBC, is that it enables analysis of biomarkers in combination to cytological sampling. Strander and co-workers showed in a study of a Swedish screening population that the numbers of inadequate samples were reduced and that 40% more HGL were identified (129) when LBC was compared with usage of conventional cervical smear.

The uterine cervix of a pregnant woman has an increased vascularity (53) and is prone to bleed. This may entail difficulties to obtain adequate cervical samples in pregnant women and midwives may refrain from taking proper cervical samples. The LBC technique may probably reduce the number of inadequate samples among pregnant women due to bleeding, since the samples are processed and most of the blood can be removed prior to preparing the cervical smear samples. As mentioned above, one of the advantages with LBC is that biomarkers can be tested in the sample. A number of biomarkers have been tested as possibly tools to improve screening in detecting CIN2+ (110, 111) and some were evaluated in the present thesis (Paper IV and V). Tests of HPV DNA alone tend to have a high sensitivity but a low specificity. Tests of HPV mRNA tests and immunocytochemistry of $p16^{INK4a}$, indicating a transforming infection in the epithelial cells usually, have a superior specificity but a lower sensitivity compared to the HPV DNA test. In this thesis (Paper IV and V) a new real-time PCR assay detecting E6/E7 mRNA of 12 HR-HPV genotypes was tested in LBC samples of pregnant and non-pregnant women. The results indicate that this test may be a useful tool in screening and especially in investigating of atypical cervical smears, but this should be tested in larger populations, including both pregnant and non-pregnant subgroups. Even though improvements are needed in screening for both pregnant and non-pregnant women, a large Swedish study on a cohort study of 1230 cervical cancer cases of screening ages showed, that there is a higher proportion of women that are considered cured from the disease (five year survival) among screen detected cancers comparing to cancer cases found by

symptom (130). This difference is most likely due to that in general the cancers were of lower stages among those who had been screened accurately (130).

Previous Swedish guidelines concerning cytology screening (131) recommended that pregnant women and women post-partum should have cervical smear performed according to the screening intervals and that additional samples should not be taken in connection with pregnancy. Furthermore, it was recommended that women should not undergo sampling for cervical smear after the 15th week of pregnancy and not within eight weeks after delivery. When the study by Norström and co-workers about cervical cancer in pregnancy (92) and the study in Paper III were performed these guidelines were still in use. The guidelines were later revised and current national guidelines in Sweden, valid since 2010, (95) recommend that pregnant women that have not been subjected to screening during the period of 2.5 years before pregnancy, should be tested by a cervical cytological smear at their primary visit to the antenatal care unit. According to European guidelines for clinical management of atypical cervical cytology (132) Cytopathology 2009) sampling for cervical smears can be postponed for women with negative screening history unless the last smear was more than five years ago. However, if a previous smear was atypical and the woman became pregnant, follow–up should not be delayed. Thus, there is a significant difference concerning screening interval between current guidelines for pregnant women in Sweden and the European guidelines. The results in the present thesis (Paper III) suggest that the stricter Swedish guidelines are more appropriate, at least in Swedish setting, to avoid cervical cancer in conjunction with pregnancy.

In previous studies, it has been reported that cervical cancer in pregnancy is detected by cytological cervical smear in 46-69% (72, 92, 96). In this thesis (Paper III), cancer was detected at follow-up after atypical cervical smear in 35 out of the 47 (74.5%) cancer cases identified between 1993 and 2008. Among these women the cervical cytological smear was taken in the 1st trimester or shortly before pregnancy or in 2nd trimester in 29 out of the 47 women (62%). Furthermore, in this thesis (Paper III) and in the study of Sood et al. (72) it was shown that advanced stages were most frequent among women diagnosed post-partum. Moreover, Sood et al. reported that the survival rate of women diagnosed post-partum was significantly lower compared to a non-pregnant control group matched on age, histology, treatment and time of treatment in relation to time of diagnosis (72). The finding that cancers are more advanced post-partum may be explained by the delay of cancer diagnosis. The results of all the studies mentioned above including and also Paper III illustrate that cytological cervical smears should be taken in early pregnancy and should not be delayed until after delivery. This is of great importance since the detection of stage IA cancer in early pregnancy can be treated by conization only

so that fertility can be preserved whereas most cancers detected post-partum need radical treatment (Paper III).

As in most other studies on cervical cancer in pregnancy, the cohort of women with cancer in connection with pregnancy in this thesis (Paper III) is a small subgroup of all pregnant women. Nevertheless, the results stresses the importance of the fact that cervical cancer prevention screening should be a natural part of antenatal care for every woman and that revision of screening history should be done as well as sampling of cervical smear at the first visit at the antenatal care unit.

In this thesis (Paper III) the screening history, including screening interval, was studied in each cancer case and it was found that 25% of the women had a benign cervical smear within the recommended 2.5 years prior to pregnancy and that the majority of cases were diagnosed post-partum. In a future revision of the current Swedish guidelines it should be considered as an improvement of the cervical cancer prevention screening if not all pregnant women within the screening ages (≥23 years) should have a cytological cervical smear performed at the first visit to the antenatal care unit, since it is known that cervical smear has a poor sensitivity and cervical cancer detected post-partum are more advanced. Perhaps the use of additional biomarkers such as HPV mRNA E6/E7 (Paper IV and V) or $p16^{INK4a}$ (Paper V) could improve the specificity without reduction of sensitivity so that pregnant women with CIN2+ can be identified, but how this should be performed among pregnant women compared to non-pregnant women should be evaluated further.

Investigation of atypical cervical smear in pregnancy

Management of cervical dysplasia in pregnancy differs between countries and also between various institutions and clinics within one country. Moreover, the guidelines are not always well defined. The shortcomings concerning management of cytology. screening during pregnancy may be related to that local guidelines are derived from data of non-pregnant women, expert opinions, anecdotal experience or retrospective, often small studies. Thus, there is a need to establish more proper guidelines based on large prospective studies, such as Paper I as important background data.

The message of the test result of atypical cytology may be a highly distressing condition for the woman, especially during pregnancy (133, 134). Freeman-Wang and co-workers showed that the anxiety in women attending a colposcopy clinic for either investigation or treatment was greater than in women before major surgery and in women with an abnormal serum alpha-fetoprotein result (135). Anxiety and fear of cervical dysplasia are most likely remarkable especially among pregnant women. Thus, in this group of women

with atypical cervical smear, regardless of grade, it is very important to give adequate information about the condition. This should be done by an experienced gynecologist with special knowledge concerning cervical dysplasia and its natural course during and after pregnancy.

In this thesis (Paper I and Paper II), there was only a minority of women with cervical dysplasia having cancer diagnosed during pregnancy or after delivery (2.4%) and in Paper II 33% of all the lesions regressed. Persistence and progression were found in 55% and 12%, respectively. Among CIN2 lesions and CIN3 lesions the regression rates were 45.5% and 26.2%, respectively (Paper II). This can be compared to the reported higher regression rates of CIN2 and CIN3 of 68% and 70, respectively (80). Of the 251 women in Paper II, 55% had CIN2+ or AIS in histology and it would have been desirable to select those women needing further investigation as all grades of CIN lesions, especially LGL often regress in histology after delivery (67, 68, 70). Furthermore, it is among the CIN2+ lesions that cancers are found. Thus, in this group it is necessary to investigate further with colposcopy and biopsies to rule out cancers.

The use of LBC sampling instead of conventional smears also allows testing of different biomarkers that can be quantified. The most established biomarkers for cervical dysplasia and cancer are HPV DNA, with these tests having a high sensitivity in detecting CIN2+ lesions but a low specificity (110, 111). During recent years, several new biomarkers for cervical cancer screening have been studied, such as $p16^{INK4a}$, which is a surrogate marker of deregulated HPV oncogene expression (108). Another biomarker, HPV mRNA E6/E7, indicates an advanced stage of the cell-transformation (108). In studies comparing tests of HPV E6/E7 mRNA with those of HPV DNA it is shown that HPV mRNA E6/E7 tests have a higher specificity than HPV DNA tests. To my knowledge HPV mRNA tests and $p16^{INK4a}$ have not been evaluated in pregnant women prior to this thesis. The study in Paper V is a pilot study consisting of pregnant women with and without atypical cervical smears, non-pregnant women with and without atypical smears and women with cervical cancer. Two types of HPV DNA tests, two types of HPV mRNA tests and $p16^{INK4a}$ immunocytochemistry were used. The aim of this study was to evaluate how these tests performed in predicting CIN2+ in histology in order to select those pregnant women who will need further investigation by colposcopy and biopsy. A multiple logistic regression analysis was performed and it was found that $p16^{INK4a}$ immunochemistry and an In house HPV mRNA E6/E7 test were the most suitable tests to detect CIN2+. Previous studies on non-pregnant populations have also shown that $p16^{INK4a}$ performs well in predicting CIN2+ in screening and is regarded as a tool to detect CIN2+ (117).

The study in Paper IV is a small study, but the In-house HPV mRNA E6/E7 test may be a useful tool in predicting CIN2+lesions in a screening setting. However, it must be tested in larger study populations.

The results of the small Paper V study indicate that $p16^{INK4a}$ immunochemistry and the In house mRNA E6/E7 test may be useful tools and they should be tested in a prospective study among pregnant women to find out if CIN2+ in histology in this group can be safely detected. In such a prospective study, the Swede score (Paper I) should also be tested to find out which method would be the best to identify women with CIN2+ in histology and to reduce the need for biopsies in this patient group. In the future, other new biomarkers may be evaluated to improve the accuracy in finding CIN2+ in histology among pregnant women. The recently introduced dual stain test, a combination of $p16^{INK4a}$ and the protein Ki-67 that is strongly expressed in CIN lesions, may improve the detection of CIN2+ in histology even more. This concept has shown to increase the specificity dramatically compared to PCR-based HPV DNA testing in LBC (136). Another potential screening tool in the future may be immunohistochemical staining for matrix metalloproteinase-2 (MMP-2) and its tissue inhibitor (TIMP-2), which are important regulators of cancer invasions and metastasis (137).

It has been discussed in an US study if there is a need to further investigate ASCUS and L-SIL cytology during pregnancy because it is seldom encountered that women with these cytological diagnoses have CIN2+ in histology (78). In this thesis, showed in Paper I, one of the cancer cases had ASCUS in initial cytology and in Paper III it showed that among the women diagnosed with cancer in conjunction with pregnancy, eight women had ASCUS in initial cytology during pregnancy and two women had ASCUS post-partum. Furthermore, one case had CIN1 in initial cytology during pregnancy and one case had CIN1 in cytology post-partum. These results stress, in contrast to the US study, the importance of prompt further investigation with colposcopy and, if needed, biopsies in pregnant women with ASCUS and CIN1 in cytology. However, in Paper II more than half of the women with ASCUS and CIN1 had LGL in histology and if a biomarker safely could exclude the risk of CIN2+ the investigation by biopsies could be deferred to the post-partum period.

Colposcopy in pregnancy is regarded to be difficult due to the changes of the cervix (53) and should be performed by an expert colposcopist with knowledge of the specific changes on the cervix during pregnancy (53-55, 75). Fader and co-workers reported that the colposcopic impression correlated well to histology in biopsies and the post-partum colposcopic findings when colposcopy was performed by expert colpo-scopists (79). In that study just 7.4% of the women were biopsied and only women having CIN3

in biopsy or a colposcopic impression of CIN3, around 10%, were followed up during pregnancy with colposcopy and repeated cytology. Post-partum follow-up data was available in just 57% of the women and only one of those women developed microinvasive cancer. Fader and co-workers conclude that biopsies are not needed unless cancer is suspected. However, others have shown that even experienced colposcopists may overestimate cervical lesions in pregnancy as well as underestimate (54, 55, 76) in evaluation of colposcopic findings and that cervical cancer may be overlooked (56-58, 75). Colposcopic indices used at examination among non-pregnant women have shown to improve the accuracy to detect women with HGL and may be beneficial to select women that should undergo further investigation with biopsy or conization. Thus, the need for invasive interventions can be reduced (43, 46). However, the most established colposcopic scoring system is the Reid´s index, including four parameters (acetowhiteness, vessel pattern, margins and surface and iodine staining) and this scoring system is further developed by Strander and co-workers by adding a fifth parameter of lesion size (46). The latter colposcopic scoring system, the Swede score, has been found to reduce the need for biopsies in about 17% of non-pregnant women of fertile age. In this thesis (Paper I) the Swede score was evaluated in pregnant women in order to investigate if this scoring system could identify women with cancer and HGL, which should undergo multiple biopsies or conization. All four cancer cases were identified at a score of ≥8 and this cut-off score could be useful in selecting those women who need to be investigated by multiple biopsies or conization to rule out cancer. Thus, Swede score seems to be a means of investigating pregnant women with atypical cytology. However, around 55% of the study population in Paper I who had a score of ≥8 and there is a need to improve the accuracy of the scoring system to detect women with HGL with suspicion of being cancer. In pregnancy, immature metaplastic squamous epithelium develops on the ectocervix and a new TZ is created (51). The normal metaplasia in pregnancy makes the colposcopic lesions more difficult to evaluate since it may resemble CIN after application of acetic acid (52). Furthermore, it is well-known that immature metaplasia is false positive of Schiller´s test (33). In this thesis (Paper I) the outcome of the multiple logistic regression analysis showed that the three most important parameters to predict CIN2+ and CIN3+ were lesion size, margins plus surface and vessel pattern. A new scoring system adapted to pregnant women may consist of these three parameters, but this must be evaluated in a prospective study.

In Paper II it was shown that just a minor proportion of the women experienced bleedings after punch biopsy and loop biopsy. However, these bleedings needed none or just topical

treatment. Furthermore, none of the women who underwent LEEP-conization experienced bleeding and the miscarriages were probably not caused by the investigation procedure. This is in contrast to what Robinson and Schaefer (61, 62) showed with a complication rate around 25% when LEEP-conizations were performed. However, as mentioned above, cervical cancer may be overlooked (56-58, 75) and the risk for microinvasive cancer in a CIN3 lesion increase with the size of the lesion (42). To acquire adequate biopsies to rule out cancer in large lesions, loop biopsies could be a substitute to conization as diagnostic intervention and thus maybe decrease the risk for severe bleedings. Loop-biopsies are shallower than LEEP-cones but the depth of the biopsy is most often enough for histological verification or exclusion of a microinvasive cancer. In the light of the recent published meta-analysis by Underwood and co-workers (138) that questions the reported accuracy of colposcopic directed punch biopsies, excisional loop-biopsies probably should be considered more often. My results indicate that the fear of complications due this procedure is exaggerated. Several studies, however small, support this view at least when performed before early second trimester (139, 140).

Follow-up and investigation of symptoms

The strategies for follow-up of women having cervical dysplasia during pregnancy varies with repeated colposcopy and cervical smear (73) or one colposcopic investigation in the majority of cases (79). In Paper II all cases, with biopsy verified CIN2+ were colposcopically reevaluated around the 28th-32nd week and this concept has shown to be safe. However, one case having stage IB cancer post-partum may have been overlooked during pregnancy (56-58, 75). This fact points to the importance of proper follow-up by cervical smear, colposcopy and if needed punch biopsies and/or conization of all women with cervical dysplasia during pregnancy within 3-4 months after delivery. In the future biomarkers might also be used after delivery as a means to identify those women who really need further investigation with colposcopy and biopsy. Biomarkers could also be a tool to identify those women with regression of lesions who may need more frequent surveillance. This is illustrated by Louvanto and co-workers who followed a cohort of women with cervical smear and HPV DNA-tests from 36th week of pregnancy and up to six years after delivery. In this study it was shown that cervical HR HPV at baseline and type specific HR HPV persistence was significantly associated with progression events (141).

In the study in Paper III clinical visible tumors were seen in 19 of the 47 diagnosed with cancer in conjunction to pregnancy, five being polyps and furthermore, 34% with bleeding or abnormal discharge. These facts emphasize the importance of revising the screening history and carrying out an examination by colposcopy when bleeding of obstetrical origin is ruled out.

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