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Cervical Intraepithelial Neoplasia – Clinical and Etiological Aspects

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1. Introduction

Cervical cancer is one of the most common cancers among women worldwide. According to the most recent data, an estimated 466,000 new cases of cervical cancer occur among women worldwide each year, the vast majority of them in developing countries[1]. Overall global mortality rate is 60%, with large differences between industrialized countries and low-income countries. Of the 231,000 women who are deceased from cervical cancer annually, approximately 80 percent are from developing countries, where cervical cancer is the most common cause of cancer morbidity among women [2].

Organized screening programmes for cervical cancer using cervical cytology, papsmears, have been shown to be effective in decreasing mortality and incidence, and gynecological mass-screening has reduced the present incidence by more than half since the 1960s and 1970s in many high income countries [3-5].

Organized cervical cancer screening was implemented in Sweden in the mid 60s and since then a significant decrease in cervical cancer has been observed. Annually around 700,000 papsmears are taken in Sweden, with a total population of 9,000,000. Approximately 600,000 are taken within the mass-screening screening program, while the remaining is taken as part other gynaecological examinations. The overall incidence of cervical cancer declined by 67% over a 40-year period, from 20 Cases per 100 000 women (world standard rate) in 1965 to 6.6 per 100 000 women in 2005 [6]. During the last decade, however, the incidence has stabilized [7, 8]. Cancer of the ovaries, on the other hand, is decreasing by 1% to 2% annually based on data for the last 20 years. The increase of HPV infections are one cause that explain the present stability in the incidence of cervical cancer.

2. Background

Hippocrates in 450 b.c. was the first to describe cervical cancer as cancer of the uterus and added that it was a disease so destructive that it should better be left uncured than treated.

Aretaeus of Cappodocia 130-200 a.c. and in particular Aetius of Amida in 600 a.c. described uterine cancer as superficial and deep ulcers that would eventually infiltrate the uterus. He also described another type of cancer, which did not present with ulcers, but rather as a tumor in the uterus. In 1793, Mathew Baillie observed that cervical cancer did not cause enlargement of the uterus, but rather continuous ulceration of the uterus until the whole organ is destroyed [9].

In the early 19th, Rigoni-Stern, an Italian physician, examined the death records of the city of Verona between the years of 1760 and 1839. He noted that cancer of the cervix was common among married ladies and widows, but less among Jewish women, rare in unmarried ladies, and absent in nuns. This was the first report that incriminated reproductive and sexual events in the genesis of cervical neoplasms. Later, Rigoni-Stern found that the disease was very common among prostitutes, and cervical cancer thus became the poor and socially deprived women's disease [10]. von Scazoni, a German physician, reported in 1861 that female sexual activity outside marriage and sex not directed towards reproduction was the cause of cervical cancer development. Accordingly, woman who developed cervical cancer aroused suspicions of having engaged in 'too much sex' or having committed 'self-pollution'.

During the late 19th century, deaths due to cervical cancer in South Carolina, US, were observed to be much higher in black women, and socio-economic status was regarded as one of the risk factors. In 1895, John Clark examined 20 cases of cervical cancer treated by hysterectomy, Clark found that the disease in 15 hysterectomy specimens had extended past the margins of resection and he described the cervical tumor as 'peculiar cauliflower excrescence'.

Virchow stated in 1855 that 'every cell is derived from a cell' (*cellula a cellula*) and that human disease processes were essentially a disease of the cells. Virchow is considered the protagonist of the concept of *Zellular pathologie*, or pathology based on the study of cells. Virchows work can be considered a fore-runner to cervical cytological screening.

Background to cervical cytology screening

The papsmear (vaginal cytology) was developed by George Papanicolaou in the 1920s. Later, G. Papanicolou and the gynecologist Herbert Traut, in 1941 published the first major article on the use of vaginal smears for the diagnosis of cancer of the uterus. Soon thereafter, the papsmear (named after Papanicolou), was born and it is still one of the most sensitive, simple and effective cancer screening tests.

Simultaneously, Hans Hinselman and Leitz technicians devised the first working binocular colposcope. In 1925, he published the first paper on colposcopy, and later on in 1933 the book 'Einfurthrung in die Kolposcopi' was published. Colposcopy was developed furthermore in 1925 by Hinselman and Eduardwirth, but routine colposcopic examinations were confined to Germany until the 1960s. In the United States, as early as 1929, Levy described the importance to study the genital tract with some degree of magnification and subsequently Emmert published an article introducing the colposcope to North American physicians. By 1932 the colposcopic technique was used in a few centers. The present form of colposcopy started in 1953 when Bolten introduced the modern colposcope in United States. Initially, it served as a tool to identify women with asymptomatic early invasive

disease. Subsequently, it has also helped physicians identify preinvasive squamous neoplasia of the cervix [11]. At a meeting of the American College of Obstetricians and Gynecologists in Miami in 1964, a group of enthusiastic colposcopists identified the need for a colposcopy society. Thereafter, through the dedicated efforts of many members of the society, various colposcopy courses were initiated. In 1981, Hamou introduced the microhystreoscope for the examination of the cervix and endocervical canal. This provided a panoramic and contact microscopical observation of stained cells in vivo at high magnification.

Treatment: Historical background

The first radical hysterectomy was described by John G. Clark at the Johns Hopkins Hospital, US in 1895. At a pathological examination of 20 cases treated by hysterectomy, Clark found that the disease had extended past the margins of resection in 15 cases. Influenced by the surgical doctrines of William Halsted, he developed an operative technique that is today recognized as the first true radical hysterectomy [12]. The operation was modified and popularized by Ernst Wertheim, whose experience was impressive in magnitude, follow-up, and descriptions of complications associated with the procedure. In 1898, Wertheim introduced abdominal radical hysterectomy with the removal of the adjacent medial portion of parametria and the upper part of the vagina. In 1945, pelvic lymphadenectomy was added to radical abdominal hysterectomy and its gained the name modified Wertheim. [13].

Screening program in Sweden

Cervical cancer screening has been linked to population- and pathological registers in specific counties since the beginning of the 1960s, and all Swedish counties had computerized screening programs in 1993. The screening is free of charge. All women aged 23-50 are invited every third year for gynecological screening. Women between 51-60 years of age are invited at 5 years intervals. The papsmear is taken by midwives and all results are reported to the patient. If the smear shows abnormal findings or CIN, the patient will be referred to a gynecologist for colposcopical examination. HPV DNA-testing is used with triage of ASCUS and CIN1 and in the follow up of CIN2 and CIN3 (see below).

Histopathology

The most common type of the cervical cancer is squamous cell carcinomas and these represent 80% of all cervical cancer, while 15-20% are adenocarcinomas.

Dysplasia is the premalignant squamous cell abnormalities that range from mild, moderate and severe dysplasia, and eventually carcinoma in situ, but this classification has been replaced by cervical intraepithelial neoplasia (CIN). CIN is also used for histological abnormalities that are histopathologically diagnosed in cervical biopsies.

CIN1 (mild dysplasia) is a low grade lesion with atypical cells in the basal, lower third, of the epithelium. Viral cytopathic effects by HPV (koilocytotic atypia) are often present. Another name is low-grade SIL (squamous epithelial lesion).

CIN 2 (moderate dysplasia) is also called high grade lesion HSIL. It refers to moderately atypical cellular changes confined to the basal two-thirds of the epithelium, with preservation of epithelial maturation in the superficial parts of the epithelium.

CIN 3 (severe dysplasia and cancer in situ), also HSIL, refers to severely atypical cellular abnormalities encompassing more than two-thirds or the complete epithelium.

Prognosis

Most dysplasias remain stationary or regress, but some dysplasias progress to carcinoma in situ and subsequently to invasive cancer. The progress of HPV infection/CIN1 to CIN3 is estimated to 10 years, but progression in 1-3 years is not uncommon. Similar estimates are considered for the progress of carcinoma in situ to invasive cancer. The progression rate of CIN 3 to invasive squamous cell cancer has been reported to be 12-30% in different investigations, and might depend on the size of lesion, the age of patient, immunological factors and the characteristics of the study population.

Management of the abnormal papsmear

Management differs between countries, hospital and economical resources. Liquid cytology is increasingly used instead of papsmear, as cells could be spared for HPV diagnosis. A colposcopically directed cervical biopsy undergoes histopathological examination. When the microscopical examination is normal, repeated papsmear shall be performed within 12 months, and when negative, no further controls are required. When the papsmear continuous to be abnormal, a new colopscopical examination is required. When the initial HPV-DNA test was positive, if evaluated, but with no cytological CIN is found, a directed colposcopical biopsy shall be performed and further management depends on the results.

An alternative strategy has been suggested, i.e. that all women above 35 years of age with cytological ASCUS (atypical cells of undermined significance) or CIN1 shall be examined for detection of HPV-DNA, as this test has a high sensitivity for CIN 2 and CIN3 compared to repeated papsmears.

When biopsy has been taken with directed colposcopy without HPV testing, the approach according to this alternative management will include colposcopy and a new cytological test (PAP) within 6 months in women with ASCUS or CIN1.

See-and-treat

See-and-treat is based on colposcopical findings without previous histopathological grading. This includes an electrosurgical loop excision of the cervical transformation zone (ELECTZ), a superficial cone biopsy, that facilitates the subsequent histological diagnosis and might be the sole treatment of CIN if microscopically radical, thus eliminating the need for a preliminary cervical biopsy and additional patient visits. Requirements for the procedure are an abnormal cervical papsmear and a colposcopical suspicion of CIN. This procedure is not recommended in case of ASCUS and CIN1, as in general there is spontaneous regression of mild lesions. See-and-treat could also be used when the colposcopy findings after an abnormal papsmear are not conclusive.

In CIN2 and CIN3 colposcopically directed examinations including the vagina are essential. When indicated, a biopsy should be taken. If no lesion can be detected, papsmear shall be taken and if it positive, a diagnostic conisation is preferred. When the biopsy shows CIN2 or CIN3 a therapeutic conisation should be performed.

Management of glandular cell atypia or AIS (adenocarcinoma in situ) is similar to that of squamous cell high grade lesions, but should include endometrial biopsy in women above 40 years of age. The conisation should be extended higher in the cervical canal than in squamous cell lesions.

Treatment methods

All methods of treatment of cervical intraepithelial neoplasia are surgical, and might differ with histological findings, extension of lesion, adverse reactions and cost effectiveness [14]the age of the patient, the possibility of pregnancy, as extensive treatment methods can decrease fertility and pregnancy outcome. Treatment methods are ablative or excisions.

Ablative methods

Cryotherapy: By using liquid nitrogen at -270 C° in a closed cylinder with cervical application for 5-10 minutes the abnormal tissue are frozen to temperatures as low as -29, and the tissue will be removed.

Laser ablation: A laser beam is used to evaporate the abnormal tissue with the aid of application of acetic acid and colposcopy in order to visualize the affected area in the cervix.

Excisional

Cold knife, using a scalpel, or laser conization: The affected area and the complete transformation zone are removed with a cone biopsy, where the size depends on the lesion, by traditional surgery.

Loop electrosurgical excision [LEEP, LLETZ]): This is a surgical procedure that uses an electrified wire to remove tissue from the cervix. It is done under local anaesthesia and colposcopy examination.

There were no differences in the effectiveness and outcome between ablative or excisional surgery according to a systematic review of randomized controlled trials in women who underwent treatment of low- and high-grade CIN with cryotherapy, laser ablation, or LEEP 15. Cold knife or laser conization is by many investigators considered as the most effective treatments.

Topical treatment with Acyclovir (nucleotide analogue), cyclooxygenase inhibitors and other pharmaceutical treatments have been suggested and tried. The success rates have been poor.

Vaccination

Prevention by vaccination will probably decrease the incidence of HPV infections, CIN and cervical cancer in the future. The identification of the HPV oncogenes E6 and E7 led to the development of effective vaccines with immunological activation of HPV antibodies, but is at present only directed against HPV 16 and HPV 18. Some cross reactions with other high risk HPV types have, however, been observed against other high risk HPV types [16]. As 13-18 HPV types are considered high-risk, conclusive results will not be available in 10-20 years.

Clinical studies have demonstrated that HPV 16 L1 VLP vaccines are well tolerated and generate high level of antibodies against HPV 16. The prophylactic polyvalent vaccine against oncogenic HPVs in young girls prior to the onset of sexual activity is the key for prevention and avoidance of the disease. An early double-blind, randomized study showed that the

vaccine was well tolerated and with high immunological response [17]. In another study 2392 young women 16-23 years of age with negative HPV-16 DNA were included. They were randomly assigned to receive the HPV-16LVLP vaccine or placebo at day 0, and at 2, and 6 months. Forty-one women (3.8 per 100 woman years) in the placebo group acquired HPV-16 infections, including 9 cases of CIN, during follow-up, while no woman who received the HPV-16 vaccine, developed infection or CIN. In one study, HPV types 16, 18, 42, 31, 33, 52 and 35 were in descending order of frequency the most common types in cervical cancer.[18]

3. Risk factors for cervical neoplasia

Among risk factors for CIN, human papillomavirus infection, smoking and sex steroid hormones, in general hormonal contraceptives are the most studied and important. In countries and areas associated with many childbirths, like in developing countries or where the use of contraceptives because of religion, parity is still a major risk factor

Human papillomavirus infections

HPV infections are the most common genital infection worldwide. It is sexually transmitted and mostly clinically silent and self-limiting. Some women remain persistent carriers of the viral infection and become at high risk of progression to CIN and invasive cervical cancer [19].

The lifetime risk of genital HPV infection is approximately 80%. For many HPV infected women (80%), the immune defence will eliminate the infection in general after approximately 12 months. In the remaining cases, progression to cytological abnormalities and CIN is observed in 5% to 10% of persistent HPV infections. After an interval of 7-15 years less than 1% of these infections lead to carcinoma.

Human papillomavirus belongs to the family Papillomaviridae, which includes viruses infecting many different vertebrates. They are strictly species- and organ-specific. HPV are small DNA viruses. The relatively small viral genome of 8000 base pairs are organized in three different regions: the long control region, also called the non-coding region, and the two coding regions, the late (L) and the early (E) regions. The early coding region in human HPV types is divided into E1, E2, E4, E5, E6 and E7. and encodes for the proteins with different regulatory functions [20].

Different HPV types exhibit a type-specific tropism either for squamous epithelium or mucosal sites. Viremia or systemic infections are absent. Despite low or undetectable antibody levels following infection, It is unknown if the HPV type-specific immunity is [21] is lifelong.

The HPV life cycle in the cervix is confined to the epithelium. The border between squamous cell epithelium covering the vagina and glandular epithelium covering the uterus, the transformation zone is the target for HPV invasion. Most HPV-related lesions resolve spontaneously, and progression to cervical neoplasia is a relatively rare event. A key factor in allowing disease to progress is the ability of HPV to evade the immune system and establish a persistent infection. Approximately 50% of infected individuals fail to demonstrate or produce a detectable antibody response to HPV. In those who respond there is no full protection from future HPV infections.

High risk HPV infections

Among more than approximately 150 HPV types, 13 HPV types are considered high-risk and 5 HPV types as moderate-risk for cervical neoplasia. Globally, HPV 16 and HPV 18 are

the predominant oncogenic types, accounting for more than 70% of all cervical infections [18, 22]. Low-risk types are rarely found in cervical neoplasias, but some types, in particular HPV 6 and 11 are associated to benign genital condylomas.

High-risk HPV DNA is according to our own studies, detected in 37% in low-grade lesions (CIN1 and borderline lesions), 89% in high-grade lesions (CIN2 and CIN3), and in 40% of cytological CIN in papsmears, but with no information of histological CIN grade [23]. In invasive cancer HPV DNA are detected in close to 100%. This indicates that while HPV is a necessary factor for cervical cancer, other factors could be responsible for development of CIN.

There are several steps in the pathway from HPV infection to CIN and cervical cancer. The initial viral entry is the target cells of the basal epithelium. HPV DNA integrates into the host genome and the HPV oncogenes E6 and E7 are expressed in some cases of CIN2 and in most cases of CIN3 (carcinoma in situ). The results of integration are cytogenetic instability and uncontrolled cell growth (immortalization). For malignant transformation, CIN3 or invasive cancer, HPV DNA integration is necessary. HPV itself may transform cervical cells from normal into CIN3, but is not sufficient in developing CIN3 into invasive cancer. Co-factors are needed and will be discussed below.

Integration of the HPV genome into the host genome frequently leads to disruption of the E2 gene that regulates the expression of the two major oncogenes, E6 and E7. Protein products of these oncogenes are responsible for transforming and immortalizing cells, which may lead to CIN3 or invasive cervical cancer. The viral oncoproteins E6 and E7 degrade two key tumor suppressors, p53 and retinoblastoma protein, respectively [24]. p53 and retinoblastoma proteins cause cell cycle arrest, allowing for repair of mutant DNA or inducing apoptosis, programmed cell death. Inactivation leads to unscheduled progression through the cell cycle and proliferation, which is required for development and maintenance of malignant cells.

4. Smoking

Epidemiological studies

During the 1980s, studies started to appear, where the correlation between smoking and cervical neoplasia, independent of sexual risk factors was evaluated. As far back as 1966 it was reported that smoking was twice as common among women with CIN as those without, but it was regarded as a confounder for sexual risk behaviour [25].

A number of studies from 1980 and onwards confirmed that smoking was an risk factor, independent of sexual risk behaviour, for CIN [26]. The first report on smoking and CIN that adjusted for sexual risk behaviour on CIN estimated relative risks for smokers to be above 2.0 in multivariate analyses, and slightly, but not substantially, higher in crude analyses. Thus, the association between smoking and sexual risk behaviour was not very strong [27].

In 1983 followed three studies on CIN, one by us and another two independent studies (Hellberg, Valentin et al. 1983; Lyon, Gardner et al. 1983; Trevathan, Layde et al. 1983). They all confirmed the results of the initial study. We suggested, and later performed, studies on cervical mucus in smokers. There was a slight decrease in odds ratios between crude and multivariate analyses in these studies. Independent odds ratios in all three studies were between 2.0 and 3.0. In addition, our results indicated that passive smoking could play a role. Many confirmatory studies where proper adjustments were made have followed these

four initial studies smoking habits might ever be more important in CIN than in invasive cancer. Thus, there are reports of a higher relative risk with CIN compared to invasive cancer, and smoking. It suggests that smoking is particularly involved in early carcinogenesis and might be a biological co-factor to a progressive HPV infection[28]. In some studies, women above 40 years of age show lower odds ratios for smoking and CIN compared to younger women [29, 30]. The role of the hormonal environment in premenopausal women will be discussed below.

One study [30] also found a strong trend with increased risk by pack-years of smoking and age of starting smoking. A number of early, unadjusted studies were also able to show a dose-response relationship, a prerequisite in most epidemiological studies where exposure is analysed. A number of later studies have confirmed the dose-response relationship for smoking [31]. Some studies on previous smokers have reported substantially decreased risk to acquire CIN after some years of smoking cessation [31-33].

Decreasing odds ratios in multivariate analyses compared to crude analyses are of some concern. There is always a possibility of residual confounding, i.e. variables that was not controlled for. Age at first intercourse and number of lifetime sexual partners may not entirely reflect sexual risk behaviour. Factors such as sex at first date, sex with casual and unknown partners, sex tourism and anal sex all increase the risk for acquiring a sexually transmitted infection. Moreover, the male's sexual risk behaviour is rarely, if ever, controlled for. As in most epidemiological studies they must be confirmed by similar studies *and* additional biological and experimental evidence is necessary to support the results, to be considered conclusive.

An interesting finding in one study was that smoking attributed little to the risk in women with many sexual partners, but was an important risk factor in women who had only had 0-1 sexual partners and also with increasing risks by years of smoking. The relative risk was impressingly 3.7 in women who had smoked for more than 20 years. The results adds to the findings that smoking also has a role independent of HPV, as these women could not be supposed to practising a sexual risk behaviour [34].

In a calculation of attributable risk (PAR) for CIN for a large number of risk factors, smoking (29%) was second to number of sexual partners (57%), while attributable risk for long-term oral contraceptive use was only 8%. [35].

Finally, a number of meta-analyses on the role of smoking in CIN and cervical cancer have been performed [36-39]. All meta-analyses concluded that smoking epidemiologically seemed to be an independent risk factor for cervical cancer. There were some indications that the risk was higher in HPV-infected compared to non-infected women. Calculated pooled odds ratios ranged from 1.95 to 2.17.

Smoking and human papillomavirus infections

It has been suggested to evaluate the importance of smoking only in women with negative HPV status. HPV detection in both CIN III and invasive cervical cancer is approaching 100% why such studies would be hard to conduct. The presence of HPV is lower in low-grade CIN, and searching for a correlation to smoking might give new information. An epidemiological association between HPV infection and smoking has been searched for in numerous studies. In most studies there are significant corrrelations between smoking habits and HPV infection. It has been speculated that smoking-induced impaired immune

defence is behind the correlation. Declining odds ratios after adjustments raise suspicions of residual confounding, i.e. risk behaviours or factors that were not adjusted for. [26].

HPV, smoking and cervical neoplasias

More important, also clinically, than a possible association between a cervical HPV infection with normal epithelium and smoking, would be if smoking in addition to HPV is also involved in the transformation from normal to cervical neoplasia. One approach is to study if smoking is more prevalent in HPV infected women with high-grade CIN compared to HPV infected women with low-grade CIN. Indeed, some studies that found that there was a higher (4.4) correlation between smoking and CIN II-III than to CIN I. [40]. Smoking frequency was reported to be increased from 16% in women having no CIN or CIN I, to 41% in those who had CIN III [41]. There are also studies with discrepant results.

Of interest in this review are some studies that investigated the association between CIN and smoking after adjustment for current HPV infection and this was claimed to insignificantly decrease the correlation between CIN II-III and smoking habits. With presence of HPV infection odds ratios (3.0) were unchanged after adjustments which is somewhat surprising. In studies where HPV negative and positive women were analysed separately, odds ratio for HPV negative women was still significantly increased in smokers [42]. Similar results have been reported in other studies. If these results are true carcinoma in situ (CIN III) can develop in smokers, maybe in combinations with other potential carcinogens, even without a current HPV infection.

Experimental studies

As stated above, experimental results must be added to epidemiological findings. The first biological explanation in humans was our finding of nicotine levels that were 40 times higher in cervical mucus, directly collected from the cervical canal with a syringe, compared to serum levels in healthy smokers. In addition, the stable nicotine metabolite cotinine were found in almost four times higher concentrations in mucus than in serum [43]. In a larger study on smoking women with current CIN we could confirm the results. While nicotine and cotinine could not be measured in serum of non-smokers and passive smokers both substances were found in small amounts in cervical mucus. There was a dose-response relationship by smoking intensity and nicotine/cotinine levels in the cervix [44]. In addition, we measured these tobacco constituents in another female genital gland, the follicle fluid of the ovaries. Nicotine and cotinine levels were found to be equal to those in serum [45]. Subsequent studies confirmed the results of the finding of tobacco constituents in the cervix [46, 47]. A problem was that these studies used a cervical flush technique and direct nicotine levels in mucus could not be estimated.

It is unclear whether nicotine and cotinine in itself exert carcinogenic effects. In cell lines derived from human normal, HPV transfected cells nicotine was added to tissue culture plates at concentrations we found in cervical mucus and in higher concentrations. Nicotine enhanced cell proliferation in all three lines [48].

We tried to analyse carcinogenic tobacco products, during the mid-eighties there was not enough sensitive methods to detect tobacco carcinogens, in particular tobacco-specific nitrosamines and polynuclear aromatic hydrocarbons (PAH), i.e. benzpyrenes. During the late 1990s, the presence of the highly carcinogenic, tobacco-specific nitrosamine NNK and

benzo(a)pyrene in smoker's cervical mucus were finally found by our previous collaborating laboratory (Prokopczyk, Cox et al. 1997; Melikian, Sun et al. 1999).

During the 1950s and the 1960s a number of animal studies aimed at transforming normal cervical epithelium to malignant. Coal-tar PAHs, like benzpyrenes was reported to induce invasive squamous cell carcinoma [49], but studies of human cervical cell lines have been difficult to interpret [50, 51].

Smoke condensate – in vitro studies

Smoke condensate has been administered to HPV immortalized cell lines. When two human HPV 16 containing cell lines underwent condensate treatment for each passage up to 26 months, they progressed to malignant tumours with few exceptions. Treatment with smoke condensate, but not without, formed invasive cervical cancer when injected in nude mice [52]. The same research group reported that in a HPV 18 immortalized cell line from the transformation zone, that addition of smoke condensate, but not without, was followed by malignant transformation [53].

Tumor markers – in vivo studies

There are still few cervical tissue studies specifically investigating smoking and the levels of proteins considered to be tumor markers in invasive cancer, and in particular in CIN. We studied 17 tumor markers in CIN and normal epithelium, correlated to smoking habits. Some of the tumor markers showed no or entire expression, but most were possible to evaluate. In normal epithelium there were no correlations to expression of tumor markers. In CIN, the tumor suppressors p53 and FHIT, and the immunologic marker IL-10 were underexpressed, while the proliferation marker Ki-67, and Cox-2, involved in many carcinogenic processed, were overexpressed. Thus, this provides in vivo biological evidence for a direct promoter role of smoking in CIN [54].

In another study of women with normal histology, or CIN I to CIN III, smoking was significantly associated to CIN, while they could not confirm our results of smoking and Ki-67 expression. Odds ratios were, however, 2.0-4.2 depending on smoking intensity which indicates that the study did not have enough power. Trend for number of cigarettes per day was of borderline significance [55].

We also studied expression of 14 tumor markers and correlation to smoking in invasive cervical cancer tissue. Many of these markers were also included in the study of CIN. Interestingly, only decreased p53 and increased Cox-2 expression were significantly correlated to smoking both in CIN and cancer. In cancer, also decreased expression of the tumor suppressor LRIG1, and increased expressions of the angiogenesis protein VEGF correlated to smoking, in contrast to CIN [54, 56, 57]. This indicates that the biological roles of smoking might not be entirely similar in CIN and invasive cancer.

Sex steroids – hormonal contraceptives

Multiparity is a classic risk factor for CIN and cancer, but might be a confounder for high-risk sexual behavior, in particular before the introduction of commonly used modern contraceptives, but that is still not the situation in many parts of the world. It still gave an indication that reproductive factors were involved in the etiology of CIN. Most of the major studies restricting the analysis to HPV-positive women, also report an increased risk for

cervical neoplasia with increasing parity. It has been suggested that the increased exposure of the cervical transformation zone, where cervical neoplasia is initiated, after pregnancy might facilitate HPV infection [58]. In that case, the hormonal influence would only be secondary.

Oral contraceptive (OC) use early emerged as an epidemiological risk factor for cervical neoplasia, but only in the early 1980s, studies with adjustment for other risk factors, i.e. sexual risk behavior and smoking, appeared [27, 59]. It might be expected that women with oral contraceptive use are more sexually active than women without. Sexual abstinence, marriages or other characteristics will decrease the necessity of contraceptive use. Parity could be lower among oral contraceptive users and could introduce a negative bias. Smoking habits might correlate to sexual risk behavior, and detection bias due to frequent papsmear evaluations, must be taken into account. Thus, there are numerous pitfalls in epidemiological studies.

Oral contraceptives increase serum levels of sex steroid hormones. In epidemiological studies it is not possible, to confirm the theory of sex steroid hormones as causal co-factors to HPV in the transition of normal epithelium to CIN and invasive cancer. When the first epidemiological studies with adjustment for sexual risk behavior were published, that also included smoking habits [59], it became clear that OC use, but only long-term use, i.e. more than 4-5 years, was independently correlated to cervical neoplasia, irrespective of sexual risk behavior and smoking [27, 59]. Odds ratios were in general moderate, 1.5-2.0, but significant. Discrepant results were found in some subsequent studies, but in those cases commonly a tendency of a correlation was observed.

OC use must be investigated for a possible correlation to HPV infection, to exclude that OCs are merely bystanders to HPV. If OC use is a risk factor independent of HPV infection, it strengthens the evidence that OCs are true biological co-factors. Several studies have investigated an eventual correlation to HPV infection and adjusted the results for sexual risk behavior [23, 60-62]. These studies found independent correlations between OC use and cervical neoplasia. Interestingly, we found that use of high dose OCs, but not low dose OCs, was significantly associated with HPV infection [60]. Available studies does not indicate that any hormonal contraceptive influence prognosis in CIN or invasive cancer.

Immunity

Sex steroid hormones exert effects on immune responses. Overall, progesterone is associated with tumor suppression, allowing for immunological escape for HPV infected cells. Estradiol seems to be associated with an increased immune defense. During pregnancy, the natural killer cell activity is suppressed, indicating a decreased immunological response. The clinical role of such findings is unclear.

In an early study, progesterone and glucocorticoid response elements were identified in the long region of several types of the HPV genome, and administration of progestins increased expression of the oncogenes E6 and E7, considered crucial in cell transformation [63] . In another study on HPV positive cell lines, progesterone treatment enhanced the colony formation, while no effect was observed on HPV negative cell lines [64]. These studies on human cell lines support the notion that progesterone is the major sex steroid co-factor in cervical cancer. It was, however, also reported that estrogen treatment stimulated HPV 16 transcripts in another cell line, while progesterone did not [65]. Finally, p53 expression was increased in HPV cervical

cancer cell lines after treatment with high doses of estradiol, a favorable effect, but not with low or medium doses, a possible favorable effect in tumor suppression [66].

Serum levels of progesterone and estradiol

The idea of studying cervical neoplasms by correlating clinical variables to serum hormone levels is attracting as it reflects physiologic conditions. We performed two studies on premenopausal women with cancer. Outcome in the first experimental study was S-phase fraction in tissue, a marker of proliferation. High serum progesterone, but not estradiol, levels directly correlated to a high S-phase fraction and after adjustment for eight variables, only serum progesterone and smoking emerged significantly correlated to proliferation [67].

In a clinical study, mortality was studied and adjustment was made for a number of variables, e.g. clinical cancer stage, the most important variable for prognosis. Premenopausal women, who eventually died from cervical cancer, with high serum estradiol showed increased survival-months, compared to those with low serum estradiol, while women with high progesterone levels showed decreased survival-months than those with low levels. A estradiol-progesterone ratio was constructed and the combination of high estradiol and low progesterone correlated significantly to a longer survival, and vice verse [68].

We performed two studies, one clinical and one laboratory, where the above criteria were taken care of. In both studies, all women were analysed together, and pre- and postmenopausal women were also analysed separately. Analyses in the former group showed no differences regarding the variables included, and results were presented only for the premenopausal group. Outcome was S-phase fraction, i.e. the percentage of dividing cells in the cancer tissue that is a marker of proliferation and cancer growth. Close to all tumors where serum progesterone was high, had a high S-phase fraction. There were no correlations to serum estradiol levels and after adjustment for eight variables, only serum progesterone and smoking emerged significantly correlated to proliferation. This supports the theory that progestins are promoters of cancer growth and correlated to poor prognosis [67].

In a clinical study, women with high serum estradiol, but who eventually died from the disease, however, showed increased survival-months, compared to those with low serum estradiol, but this was nonsignificant. Women with high progesterone levels, on the other hand, showed decreased survival-months than those with low levels. A estradiol-progesterone ratio was constructed and the combination of high estradiol and low progesterone increased the prognosis prediction [68].

To find evidence that estradiol or progesterone correlates to CIN grade a study where both cases and controls were HPV positive was conducted. None of the hormones measured did correlate to lesions more or equal to CIN2 compared to low-grade CIN. Serum levels of sex steroids thus have not proved to be useful in distinguishing low- and high-grade lesions [69].

We evaluated tumor marker expression and serum progesterone levels in CIN, and found a significantly higher expression of Cox-2, low retinoblastoma protein (tumor suppressor) and low p16 (tumor suppressor) expression with high progesterone levels, the former were independent of CIN grade. No correlations between serum estradiol and tumor marker expressions were found. It could be concluded that progesterone levels CIN are associated with a negative tumor marker pattern, as was the case in oral contraceptive users [70]. In summary, available results indicate that oral contraceptives and high serum progesterone levels exert unfavorable effects in CIN, both epidemiologically and in laboratory studies, while the role of estrogens are unclear [70].

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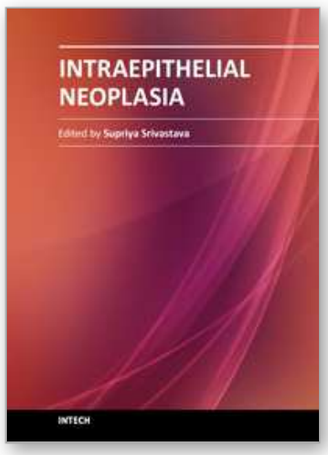

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The book "Intraepithelial neoplasia" is till date the most comprehensive book dedicated entirely to preinvasive lesions of the human body. Created and published with an aim of helping clinicians to not only diagnose but also understand the etiopathogenesis of the precursor lesions, the book also attempts to identify its molecular and genetic mechanisms. All of the chapters contain a considerable amount of new information, with an updated bibliographical list as well as the latest WHO classification of intraepithelial lesions that has been included wherever needed. The text has been updated according to the latest technical advances.This book can be described as concise, informative, logical and useful at all levels discussing thoroughly the invaluable role of molecular diagnostics and genetic mechanisms of the intraepithelial lesions. To make the materials easily digestive, the book is illustrated with colorful images.

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