COLPOSCOPY

IN DIAGNOSIS AND TREATMENT
OF CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN)

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IN DIAGNOSTIEK EN THERAPIE
VAN CERVICALE INTRAEPITHELIALE NEOPLASIE (CIN)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. A.H.G. RINNOOY KAN
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
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To Susan
Paula
Mylène
and to the memory of Han

To Ella
Floor
and Bas

CONTENTS

Chapter 1

GENERAL INTRODUCTION AND AIMS OF THE STUDY

J.A. Wijnen and Wouter M. Huisman

The accessibility of the uterine cervix, the propensity for cells to exfoliate from precancerous lesions, the evidence from pathologic studies of a spectrum of histologic changes from mild epithelial atypias through premalignant lesions to frank malignancy and the apparently prolonged natural history, provide the potential for detection of premalignant cervical lesions or early malignant disease and control of cervical cancer by population screening (43, 169, 246, 305, 306, 509).

The principle behind cervical cytology screening programs is the detection and elimination of cervical intraepithelial neoplasia (CIN) or early invasive cancer in all women at risk of this disease. The management of patients with an abnormal cervical smear remains controversial (15, 302, 306, 512).

To identify the asymptomatic woman with potentially cancerous lesions of the uterine cervix, the adequately performed and interpreted Papanicolaou (PAP) smear of the endo- and ectocervical epithelium has proven its utility as primary screening tool (302,351). Patients eligible for the studies in these theses were selected on the basis of cervical smears suggestive of cervical neoplasia. Although *colposcopy* has been propagated as a screening method to identify suspicious cervices, presently, most gynecologists use this technique to evaluate patients with an abnormal PAP-smear (133). Frequently colposcopy is practiced only as a method to target biopsies toward the most suspicious lesions of the portio whether or not in combination with endocervical curettage (302, 356, 545, 548). In addition, the authors always document a *colposcopic impression* as to the expected highest histopathologic grade of the lesion and/or suspected viral infection. In contrast to many non-colposcopists the authors consider cone biopsy in 1987 in a patient with an abnormal PAP-smear without preceding expert-colposcopy as an obsolete and too agressive primary diagnostic procedure in almost all patients. The first aims of these studies were to investigate in the University Hospital Dijkzigt the predictive contribution to each of the diagnostic steps, i.e. colposcopic impression and histopathologic diagnosis of colposcopically directed biopsies and endocervical curettings in the evaluation of patients with cervical smears that were cytologically suggestive of cervical intraepithelial neoplasia. To exclude with certainty invasive cervical malignancy and to establish in the simplest possible, reliable, patient- and cervix-conserving and cost-effective way the diagnosis CIN in patients with abnormal PAP-smears, a diagnostic decision model was developed. This model is based on colposcopy and aimed at the practical possibilities in gynecologic praxis.

The diagnostic reliability of the colposcopically directed biopsies has to be proven, before management can be based on colposcopy (315). This is usually done

by comparing the biopsy diagnosis with the histopathologic diagnosis of a second surgical specimen (250). Since the targeting of the biopsies is closely related to the capacity of the colposcopist to recognize the most severe lesion, a special study was dedicated to this subject, not only aimed at the reliability of the biopsies, but also at the ways in which the diagnostic results might be improved.

Epidemiologic studies have established a truly venereal origin for cervical squamous cell cancer and its precursors, in which first coitus at young age is the single most important variable and a necessary, although not sufficient condition (194, 200, 330, 489, 597). All other variables such as behavioural, racial and also dietary act through this factor.
In 1962 Van Niekerk (366) described cervical cytologic findings in puerperal patients with megaloblastic anemias due to folic acid deficiency. Whitehead et al in 1973 (585) observed similar cervical changes in several non-anemic women taking oral contraceptive agents (O.C.A.). Although the cytologic changes were not associated with evidence of systemic folate deficiency, they disappeared with oral folate supplementation. The existence of localized folate deficiency in the cervix occuring as a result of O.C.A. use was suggested (284). It has been repeatedly observed that folic acid and vitamin B_{12} deficiency are associated with morphologic changes in cervical smears that are "deceptively similar" to those observed in cervical dysplasia (284, 296, 320).
In a double-blind placebo controlled study in a group of young O.C.A.-users Butterworth et al (95) demonstrated that there was an association between the morphologic features of megaloblastosis and dysplasia - if present - in their cervical smears. These cytologic manifestations improved after 3 months of folic acid supplementation. Although the report on this study was full of trends which were not statistically significant, the folate-treated group did have a significantly less severe degree of dysplasia on biopsy after 3 months than the control patients who did not receive folic acid. The question is: have these authors merely identified a new degree of megaloblastic change which closely resembles dysplasia and is reversible by folate therapy? Or does folic acid actually play a role in cervical neoplasia? The first therapy oriented part of these studies was designed as a randomized placebo controlled trial to verify the possible effects of systemic folic acid supplementation on the cytologic, colposcopic and histopathologic manifestations of cervical intra-epithelial neoplasia.

If invasive cervical cancer is definitively excluded and the diagnosis of CIN with or without viral infection histopathologically confirmed, it has to be decided whether or not the patient has to be treated. The various techniques and their indications will be reviewed. The second therapeutic part of these studies was aimed at the conservative treatment of CIN. After exclusion of invasive cervical malignancy, patients with CIN, grade I, II and III and satisfactory colposcopy were randomized to outpatient laser- or cryotherapy. Side-effects and long-term efficacy were compared.

Summary

AIMS OF THE STUDIES

1. To investigate the diagnostic value of colposcopy under research conditions, in patients with an indication for colposcopy. (Wouter M. Huisman)
2. To investigate the possibility of accurately predicting histopathology by means of the colposcopic impression, when a standard set of diagnostic criteria is used. (Wouter M. Huisman)
3. To investigate the predictive contribution of colposcopy in grading CIN and in the recognition of human papilloma virus (HPV) infections within the standard facilities of the University Hospital Dijkzigt-Rotterdam in the routine cytologic, colposcopic and histopathologic evaluation of selected patients with cervical smears suggestive of cervical intraepithelial neoplasia. (J.A. Wijnen)
4. To investigate in a prospective randomized study the possible effects of systemic folic acid supplementation on the cytologic, colposcopic and histopathologic manifestations of cervical intraepithelial neoplasia. (J.A. Wijnen)
5. To investigate in a prospective randomized study the therapeutic effectiveness and side-effects of laser- or cryotherapy in patients with cervical intraepithelial neoplasia. (J.A. Wijnen).

Chapter 2

MORPHOLOGY OF EPITHELIA OF THE UTERINE CERVIX

Wouter M. Huisman

2.1 Introduction

In the adolescent and the adult cervix, three types of epithelium may be present, each with its own characteristic histopathologic and morphologic appearance (490). These types are:

1. Original epithelium, either squamous or columnar
2. Metaplastic squamous epithelium
3. Atypical epithelium

The current views on a spectrum of change in the cervical epithelium, from metaplasia, through dysplasia to invasive cancer have evolved from the contributions of numerous histopathologists and gynecologists over a period of more than a century. The insights were deepened by the research of epidemiologists, microbiologists, biochemists and electronmicroscopists. Molecular biology and genetics are among the disciplines that have, more recently, contributed information on the epithelial changes in early forms of cervical neoplasia (257). Despite the very large amount of research and a wealth of literature on this subject, some authors feel that it is still not possible to make authoritative statements on the etiology, the natural history and the management of premalignant conditions of the cervix (103).
What can be said is, that of the first type of epithelium, the original columnar epithelium may change into metaplastic epithelium. This change involves an instability in the junctional interface between the original epithelia. The instability has been held responsible for setting the stage for the development of cancer (129). The first two types of epithelium will be discussed in this Chapter. The third type, a result of metaplastic epithelium "somehow" acquiring a neoplastic potential (129, 255, 422), will be discussed in Chapter 3.

2.2 Original squamous epithelium

This epithelium covers most of the ectocervix at birth. It is of a stratified type similar to the epithelium of the vagina. It can be distinguished from the latter by the appearance of the subepithelial papillae, which may be less well developed or even absent (313, 371). The surface of the epithelium is smooth, at the basal side it may be plicated by stromal papillae, from which it is separated by a basement membrane.
The epithelium contains variable amounts of glycogen (474). Microscopically four layers of cells can be seen (151, 483, 493), at times five layers (490).

These layers are:

1. *a basal layer or stratum cylindricum:* a single row of closely packed cylindrical cells, with relatively large nuclei, forming a palisade on the basal membrane.
2. *a parabasal layer or stratum spinosum profundum:* two to three layers of cells, somewhat larger than in the previous layer. The cells are mutually connected by intercellular bridges (desmosomes). Mitotic figures can be found in the basal layer and in the first row of the parabasal cells, under normal conditions. Further from the basal membrane, the cells are no longer dividing.
3. *an intermediate layer or stratum spinosum superficialis:* five to six rows of flattened cells, with glycogen-rich cytoplasm. Under progestagen influence, glycogen intake causes the cytoplasm to swell and the nucleus to be pushed to the side (61).
4. *an intraepithelial layer or condensation zone (186, 490):* a layer of variable thickness and often not recognizable. When present, this layer consists of closely packed polyhedral cells with keratohyaline granules (400).
5. *a superficial layer or stratum corneum:* five to six rows of cells which are flat and elongated,with pycnotic nuclei and large amounts of cytoplasm. These cells are either dead or dying. They represent keratinization and are well developed at times of high estrogen levels. Desmosomes can be seen in electron microscopy, but they are shorter and smaller in number than in the other layers (483).

The cytologist recognises the cells from this epithelium usually in three groups, according to their characteristics and origin, as basal and parabasal cells, intermediate cells and cornified cells (490). The colposcopist sees a translucent structure, through which the stromal vessels appear in shades of pink.

2.3 Original columnar epithelium

This epithelium is the lining of the endocervical canal, but it may extend onto the ectocervix. It consists of a single row of columnar cells, with a round or oval nucleus that is situated in the lower third of the cell when this is laden with mucus (371). Some ciliated, non-secreting columnar cells are seen. Electron microscopy has shown the basement membrane under this epithelium to be of the same structure as the membrane under the original squamous epithelium (483). In the distal part of the cervix, cubic cells have been observed between the columnar cells and the basement membrane. These cells have an oval nucleus and contain variable amounts of cytoplasm, but neither glycogen nor mucus (186). They were called "reserve cells" (100). Under these reserve cells, the basement membrane is at times not recognizable, which might indicate a fluent transition between epithelium and stroma (243). This observation has raised doubt as to whether the basement membrane should be regarded as a discrete anatomical entity (154).
Macroscopically, the columnar epithelium can be seen as a bright red spot in the area of the ostium externum. This is the equivalent of the obsolete term "erosion" (315). When seen through the colposcope, the columnar epithelium

is arranged in two or three folds (rugae) on either lip of the portio. In the endocervical canal, the rugae are present in the form of longitudinal folds, directed obliquely towards the internal ostium (262,490). The pattern of folds resembles the trunk and branches of a tree, which is why it has been termed the "arbor vitae" (262). The smallest subunit of the arbor vitae is the villus, a finger-shaped protrusion, with its own capillary loop, easily recognized in colposcopy. The villi have a typical grape-like appearance.
In cross-sections of the endocervix, the stroma seems to contain "glands", communicating with the surface through "gland openings". Fluhmann painstakingly examined serial sections of the cervix and demonstrated that no such glands exist, but that the tissue with a glandular appearance is in reality part of a system of clefts in the endocervical surface, lined with columnar epithelium (184, 186). These observations were confirmed by others (64, 262).

2.4 Metaplastic squamous epithelium

2.4.1 *General characteristics*

The junction between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix is called the (original) squamo-columnar junction (SCJ). In the young fetus this meeting place is located in the proximity of the external os (188, 408). At birth, the SCJ was found distally to the external os in 71% of cases, with an extension into the vaginal fornices in 3-5% of neonates (408). During the reproductive years, the SCJ can be found in the vaginal fornix in about 4% of cases (131, 289). In postmenopausal women, the SCJ was observed distally of the external os in only 40% of cases (145). From these observations, the topography of the junction seems to vary only with age (221, 374, 479), but it is also related to sexual activity and parity (129).
The shifts in the position of the SCJ that occur during certain periods of life, were considered to be caused by estrogens (185, 347). A raised amount of circulating estrogens induces growth of the uterine cervix. The increase of the volume of the endocervical columnar epithelium is greater than that of the surrounding fibrous tissue of the cervix, thus resulting in an eversion of the epithelium onto the ectocervix. The part of the columnar epithelium exposed to the vaginal environment, undergoes a transformation from original columnar epithelium into a squamous epithelium (129,257,399). The area in which this transformation takes place is called the transformation zone, the process itself is called squamous metaplasia. The term transformation is a confusing one because at cellular level, no transformation takes place from a columnar cell into another mature type of cell (399).
The cause of squamous metaplasia is not clear. Apart from the role of estrogens, another causative agent was thought to partake. Since the first epithelial changes were observed in those areas most directly exposed to the vaginal environment, a vaginal "factor" was postulated (101). Several theories were developed about the true nature of this factor. A low vaginal pH (308,488,490,575), coitus (129,488) and chronic infection (602) were postulated as the stimulus for squamous metaplasia.

As squamous metaplasia is a multifocal process, it is topographically a variable one with islands of unchanged epithelium existing adjacent to sheets of metaplastic epithelium (490). The borders of the transformation zone are capricious lines, caudally represented by the junction with the original squamous epithelium: the original SCJ. The new border between the original columnar epithelium and the metaplastic epithelium, the cranial border of the transformation zone, is called the neo-SCJ. These borders are of importance to the colposcopist. When the transformation zone is not fully visualized, which is generally due to non-visualization of the neo-SJC, the colposcopic examination is considered unsatisfactory (503). This in turn means that additional diagnostic measures must be taken, as far as management of patients with abnormal smears is concerned.

The physiologic process of metaplasia takes place in stages. When undisturbed, the end point is a mature squamous epithelium considered, by some authors, to be invulnerable to carcinogenic stimuli (131). A disturbance may lead to deterioration of the normal process, which is then called atypical metaplasia. Evidence of a deterioration can be found in cytologic smears, it can be seen by means of colposcopy and be confirmed by histopathology (597).

2.4.2 *Colposcopic and histopathologic characteristics of metaplasia*

Several authors have described the colposcopic and histopathologic characteristics of physiologic metaplasia (129, 408, 490):

1. the process takes place in three more or less defined stages, but it can be arrested at any time and in any stage. Progression towards a more mature stage may be resumed at any stage after an arrest. The trend with time is towards an increase in squamous covering of the cervix and a maturing of the squamous epithelium once formed.
2. the process is most active during late fetal life, during adolescence, and during (the first) pregnancy. (During these periods of life there are high concentrations of circulating estrogens).
3. metaplastic areas, directly exposed to the vaginal environment mature more rapidly than areas in the endocervix. The controversial terminology of "real" as opposed to "apparent" transformation zone, used to distinguish these areas, has been labeled obsolete (490). Full maturation may in some circumstances be measured in days or weeks.

The colposcopically visible stages (129) are described in the following text. These observations were confirmed in electron microscopic studies (262, 483).

Stage 1. The tip of the grape-like villus loses its translucency, which is replaced by a glazed appearance. This change is first observed directly adjacent to the original squamous epithelium, on the highest point of the rugae.
Stage 2. The tips of the glazed villi fuse. Islands of columnar epithelium are enclosed in the deeper parts of the crypts.
Stage 3. The villous aspect is entirely lost. It seems as though tongues of tissue have overgrown the rugae. Scattered openings are seen, called fenestrae or, when

they are the connections of the crypts with the surface, called crypt openings or "gland" openings. Where muscus-secreting epithelium has become obstructed, Nabothian follicles have arisen. The initial red color is replaced by a pink color, due to the overlying metaplastic epithelium. Still, in women in the reproductive years this is a darker shade of pink than that of the original squamous epithelium. After the menopause this zone is somewhat paler. The color changes are due to the presence and the selective obliteration respectively, of large vessels in the stroma. The stroma underlying the original squamous epithelium contains relatively small vessels (426).

The ectopy of columnar epithelium was termed, in the early German literature, pseudo-erosio congenita (220,347). A healing process was thought to occur, in which, through a stage of erosio vera, the columnar epithelium was pushed away by the proliferating squamous epithelium. The process was called "Epidermoidalisierung" (348). Illustrations were published in 1910 (347), of the histopathologic stage of a process which nowadays is called squamous metaplasia.

The histopathologic stages of squamous metaplasia are:

1. Reserve cell hyperplasia or reserve cell proliferation (243, 257, 399, 568).
One or more layers of cubic cells appear under the columnar epithelium, where they were also seen under normal conditions, albeit in smaller numbers (section 2.3). These cells may differentiate in a squamous direction (257). According to some authors, the columnar epithelium is lifted from the basement membrane and completely pushed off (9,257). The exact mechanism is unclear, as is the origin of the reserve cells (315), about which four basic theories are mentioned in the literature. The theory that basal cells from the original squamous epithelium migrate along the basement membrane (175), stands by itself, because it is more in line with the older theories on replacement of columnar epithelium and the so-called squamous epithelization (220,257), than with theories on squamous metaplasia. The other three theories postulated an origin of the reserve cells from the columnar epithelium (186), from embryonic nests under the columnar epithelium (100), or from the stroma (427,496). These three theories might be less contradicting than generally assumed, in view of the observation that the basement membrane may at times be absent (section 2.3). In fact, the latter theory (427) is based on this absence.

2. Immature squamous metaplasia.
Histopathologically, this may be seen, sharply demarcated from the normal portio epithelium by a line perpendicular to the surface, for many years after its formation (257).

3. Mature squamous metaplasia.
In the final stage, the classic layers, as described in section 2.2, of a multilayered squamous epithelium can be seen. Morphologically, the newly formed epithelium is indistinguishable from the original squamous epithelium (399). Schneppenheim et al. (479) have shown that the border between original squamous epithelium and metaplastic squamous epithelium is marked by the position of "the last gland". This also marks the location of the original SCJ (91).

Chapter 3

CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN)

Wouter M. Huisman

3.1 Historical review

Some of the data for this review were derived from the publications of Johnson (88), Langley (309) and van Zanten (602).

The existence of a pre-invasive stage in the development of squamous carcinoma of the cervix has been recognized for a long time (309). Sir John Williams, in the Harveian Lecture for 1886, illustrated a symptomless early carcinoma of the uterine cervix. This is considered the first case recorded in the literature of a cervical tumor, of which most must have been an in situ carcinoma (203). In 1900, structural changes in the epithelium at the margins of invasive cancer were described (148). Others established the concept of surface changes preceding the invasive stages (413, 464, 473). The term "carcinoma in situ" was introduced (464), but not generally accepted until after the publication of Brothers in 1932 (75).

Between 1920 and 1950 the efforts of most gynecologists were focused on finding and treating patients with this form of surface carcinoma (318, 370, 404, 601). In this time period, three important contributions were made to the early detection of this condition. In 1925, Hinselmann described the first colposcope, which allowed in vivo inspection of epithelial abnormalities invisible to the naked eye (238). Schiller (474, 475) discovered that squamous cell carcinoma, and also the pre-invasive stages, contained less glycogen than normal squamous epithelium of the cervix. The abnormal areas remained unstained when immersed in Lugol's solution. Based on this observation he developed the Schiller test, which was readily accepted in the Anglo-Saxon countries after Schiller's emigration to Boston, Massachusetts, in 1932. The third contribution was made by Papanicolaou. This investigator found tumor cells in cervical smears of women with carcinoma of the cervix. In 1928 he first reported on this observation (602). In that same year Babes, in Rumania, diagnosed a cervical carcinoma with the aid of cytology. He concluded that this method would be suitable for the detection of pre-invasive lesions (602). However, this conclusion fell into oblivion (120, 296). It took until after the reports of Papanicolaou and Traut (392, 393) for exfoliative cytology to be recognized as the most effective screening method for pre-malignant cervical disease. In 1949, the first population screening program was started in the Canadian province of British Columbia (70, 350, 351, 574).

The attempts to find the link between pre-invasive and invasive disease, led to an increasing number of biopsies being taken from the cervices of younger, asymptomatic women with colposcopically atypical transformation zones or with atypical cells in cytologic smears (257). This resulted in the discovery of "atypical"

lesions which did not meet with the criteria for the diagnosis of carcinoma in situ. The collective name of "dysplasia" was introduced (573), thereby extending the two-stage concept of the evolution of cervical carcinoma into a three-stage concept. The precursor role of both dysplasia and carcinoma in situ, supported by epidemiologic evidence (167, 255,337) was not considered to be of equal importance (206). The duration of the intraepithelial phase was variously estimated to last between six and twenty years (309).
Long-term prospective histopathologic studies were undertaken to determine the progression and regression rates of these conditions (179,292,337,402,405). Progression was far less than expected, but it was found that the taking of biopsies influenced the natural course of the disease (260,439). Thus, investigations based on the use of cytology alone or coupled with colpomicroscopy, were undertaken with the hope of studying the natural history (191,397,440). After such a study, Richart published his views on the natural history of the intraepithelial stages (440) and introduced the terminology of Cervical Intraepithelial Neoplasia (CIN). This descriptive term encompasses the entire pre-invasive spectrum of "atypical" epithelia, from mild dysplasia up to and including carcinoma in situ. The CIN terminology was generally accepted, the conceptual basis has been repeatedly criticized, both on practical (84,90) and on theoretical grounds (16,17,128).
The link between normal epithelium and CIN is still missing. Based on a flood of publications on the possible role of several types of virus in the pathogenesis of squamous cell cervical cancer, the concept of CIN has recently been revised (302). The etiology, however, is not fully understood (270).

3.2 Aspects of etiology and epidemiology of CIN

Data from experimental oncology have shown that there is not necessarily a straight-forward relationship between a causative agent and carcinoma (424). Several steps may be involved. In the two-stage hypothesis (459), the first step is referred to as initiation. Some agent, the initiator, causes a change in the normal metabolism of the cell, as a result of which that cell may fail to exercise its normal function in the organized whole. A one-time contact of short duration suffices for this irreversible change. Initiation causes at least one mutation (49). The cell has acquired the capacity for malignant change, but morphologic evidence of this is not yet to be found (424). The cell can remain in this situation for many years. The second step, referred to as promotion, results from the application of stimuli, many of which are not in themselves carcinogenic (424). This may cover a long period of time. Promotion has been considered a slow, reversible and non-specific change (49). Examples of promotors are podophyllin and fenol fractions of cigaret smoke (65).
More steps are probably needed to lead to a true malignancy (189,190). Once the cell is deranged, morphologic changes may appear, as a result of progression, which means a process of additional irreversible cell changes. The capacity to invade or metastasize is acquired by selection of genetic and epigenetic characteristics, obtained during the process of progression (315). Selection determines by which cell type the tumor will be recognized as a malignancy.
Progression and selection are only possible in a proliferating cell population

(65). They are not the only possible directions in which a promoted cell may go. Regression is also a possibility (189). This raises the question of whether or not there is a "point of no return", or even a range in which reversibility is still possible. In one animal study employing continuous exposure by means of a carcinogen-impregnated thread, only 12 percent of the animals developed cancer when the stimulus was withdrawn at the stage of beginning dysplasia. When the withdrawal was made at the stage of microinvasion, 36 percent of the animals developed invasive cancer and when invasion was already present, withdrawal did not impair tumor growth (579).
The essence derived from the experimental models is, that more than one step is usually needed, not all of a mutagenic nature, before a malignancy may arise (315). Furthermore, the fate of any given lesion is not always predictable in the sense that progression is inevitable (424).

The observations from animal experiments are not simply transferable to human conditions. Cervical carcinoma can be induced in animals, but its spontaneous appearance is exceptional in the animal kingdom (65). This raises the question if the human way of life might be a conditioning factor for the development of cervical cancer.
Epidemiology may provide an answer to this question. The epidemiology of squamous cell carcinoma of the cervix and of its precursors has been described extensively (14, 126, 152, 173, 205, 276, 352, 406, 458). According to many authors, epidemiologically, dysplasia, carcinoma in situ and invasive carcinoma could be considered together as one group (79, 223, 256, 304, 521). This was at variance with the opinion of others, who found that carcinoma in situ and invasive cancer were one group and the dysplasias belonged to another group, because of differences in epidemiologic behaviour (511, 534, 535). The different conclusions may be explained in part by the fact that in the latter studies the dysplasias were not subdivided into grades and compared per grade of severity with matched controls, as they were in the former studies (302).

Epidemiological data derived from reports on incidence and prevalence rates of CIN were found to vary between 0.54% and 6.5% (302). The method of diagnosing and the differences in the composition of the study populations are among the factors that may explain this wide range. Patten (400) demonstrated this by the different figures found for cellular evidence of CIN (0.98%) and histopathologically confirmed CIN (1.2 to 3.2%).
A prevalence of 0.43% and an incidence of 0.066% was reported for carcinoma in situ among women of twenty years and older (71). The peak-incidence was 0.1% in women between 25 and 29 years of age. These figures were confirmed by others (497). With the exception of some reports (50, 127, 351), cytological reports seldomly accounted for the false-negative rates. The prevalence rates that were given, are therefore in reality the prevalence rates of the true-positive tests. The age-specific prevalence and incidence figures show a great variability (73, 99, 351, 400, 497, 574). Kwikkel has recently presented an in-depth discussion on the various sources of bias (302).

Nevertheless, when the more recent results are compared with the figures of a decade earlier, a certain trend can be observed. Van Lent calculated the mean age at detection of women with CIN or invasive cervical carcinoma (315). The figures were derived from the reports of seven different authors. The reports had been published between 1955 and 1969. For dysplasia, carcinoma in situ and invasive carcinoma the mean ages in years were 36.1, 39 and 50.7 respectively. Some ten years later, the mean ages had dropped about 10 to 14 years (14, 152, 466). A similar trend was observed in The Netherlands (32, 62, 67, 137, 278, 286, 375).
In the same space of time, some authors found that the percentage of women under the age of 30, who had CIN, increased from 8.2% to 15.8% (298). Among fourty thousand teenagers, 0.54% had dysplasia and 0.16% had carcinoma in situ (469). Most authors pointed out that changes in sexual behaviour were to be held responsible for this trend (213, 341).

The connection between cervical carcinoma and a coitus-related factor was demonstrated by Gagnon (200), albeit that this was proved by the fact that cervical carcinoma was absent in a study population of 3280 nuns. The author had previously assessed that this type of carcinoma had not been listed as the cause of death of nuns over a period of 20 years. Others found only few cervical carcinomas in nuns (206, 541). The relative freedom of nuns from this kind of malignancy was explained by the absence of heterosexual activity, and the possibility that their protected position, free of socio-economic stress might be a factor, was considered (206).

Rotkin and King concluded on the basis of epidemiological evidence, that an early sexarche was an important risk factor for cervical carcinoma (456), more specifically a sexarche under the age of 17 (458). This was confirmed by others (6, 330, 412), and an early sexarche is at present generally considered to be the most important risk factor, with promiscuity running a good second. Some authors have criticized the focusing on sexarche (406), by pointing out that recent observations among different ethnic groups are more consistent with promiscuity being of much greater importance than the sexarche itself. A low incidence of cervical cancer was found in studies of ethnic groups that were characterized by one-partner relationships, despite an early sexarche (201, 516, 517). When the sexarche was the same, but promiscuity the difference, the incidence was high (377).

Some of the other postulated risk factors like age at first marriage (69, 457, 533) and age at first pregnancy (35, 48, 119), are highly correlated, which is shown by the fact that they disappear as a risk factor when the data are controlled for age at first intercourse (352). Similarly, a low socio-economic status was found to be a risk factor in some studies (124, 208, 304, 570), but when patient populations were carefully matched for all variables, the differences in the prevalence of cervical neoplasia with respect to this factor were no longer statistically significant (69, 164).
Race has long been considered an important factor. Around the turn of the

century, Jews were considered immune to carcinoma in any form. Graham et al. reviewed the literature on this subject (206). These authors found that the report on a sarcoma in a rabbi's daughter marked the beginning of numerous investigations into the relative incidence of carcinoma in the Jew. The incidence of carcinoma, irrespective of site, was subsequently reported as higher, lower or the same as in non-Jews. Of interest is the observation made by the aforementioned authors, that a higher correlation was found in the incidence of all carcinomas (including uterine) between Jews and non-Jews of one city than between Jews of different cities, indicating an environmental factor of some sort in addition to the racial and religious ones. This observation is in line with the results of more recent investigations, from which it was concluded that differences in sexual habits might be of greater importance than racial, religious or geographic differences (307, 315).
According to Miller and Rawls, some variables suspected in the past of being associated with cancer of the cervix, clearly are not when a number of studies is considered (352). These authors included factors such as coital frequency, mean age at menarche, contraceptive practices and non-circumcision of partners in this category.
Marital instability (separation, divorce, widowhood and extramarital sexual activity) has also been associated with an increased risk of cervical cancer (170,275,330,533). However, marital instability is one way of assessing the number of sexual partners an individual may have (173). The number of sexual partners was found to exert effects independent of other sexual factors (223). Therefore, promiscuity might again be the decisive factor that was measured in these studies.

Given the generally accepted fact that cervical neoplasia behaves epidemiologically as a sexually transmitted disease (126,173,315,352,458,574), it is not surprising that the role of the male was extensively studied. Rotkin saw the "female as the host, the male coital partner as the donor, and an intervening factor as his contribution" (458). The nature of the "contribution" is poorly understood (173). The recommendation to use condoms to help control or prevent cervical neoplasia is based on the belief that something is transmitted venereally from the male to the female (173). Positive results have been reported in respect to delaying or even preventing the occurrence of preinvasive lesions of the cervix with the use of barrier contraceptives (345,438,487,567). One author recommended total sexual abstinence as a preventive method, but hastily added that this might not be a popular means of control (440).
Probably, some men are more "carcinogenic" than others (494). The role of circumcision is at present considered negligible, as mentioned previously, and so is the role of smegma as a carcinogen (7,315), although in experimental studies epithelial hyperplasia could be induced (182,425). The current views on the characteristics of the "high risk male" are as follows:

1. *A low socio-economic status.*
This factor, probably not a single epidemiologic factor (302), has been incorporated in the concept of the carcinogenic properties of spermatozoa

(129,428,429). This concept was introduced as a development of the viral concept, when it was shown that contact with sperm produced effects not unlike those of the carcinogenic viruses in the target cell (430). Sperm heads were observed to be ingested by immature metaplastic squamous epithelium and to be incorporated into the nucleus of dividing cells (129). Basic proteins, especially protamine, present in the sperm head, were held responsible for producing changes in the mucoid coat of differentiating cells. These changes were precisely of the type regarded to antedate the neoplastic state (430). Finally, when the sperm of males of the various social classes was compared, protamine-rich sperm was more frequently found in the lower classes (307,516).

2. *A tendency toward cancer of the genital tract.*
Several authors have postulated a strong association of cervical cancer with genital cancer in the male partner, especially penile cancer (209,330).

3. *A history of cervical cancer in other spouses.*
Kessler et al. analyzed the incidence of cervical cancer in the second (or third) wives of males whose first wife had had cervical cancer (274,275), and found that they had a risk of developing cervical cancer 3.5 times higher than a control population (275).

The possible viral etiology of cervical neoplasia

Of the infectious agents, several organisms have had their period in the investigational limelight (173), either as a causal factor or as a covariable (214). An association of Trichomonas vaginalis (192,395), syphilis (124,451) or Chlamydia (471) with epithelial atypia has been reported in the literature. The difficulty with these observations is, that infection with such organisms and cervical neoplasia are both co-variably associated with promiscuity (173).
Today, viruses are the leading contenders for a major role in the induction of cervical neoplasia. In general, the susceptibility to oncogens may be dependent on the immune status of the individual, or else an altered immune response may result in the inability to recognize and destroy neoplastic cells (134,299,302). The individual's antibody status is an important determinant of the risk of recurrence and the clinical severity of the disease in case of viral infection (173). With respect to cervical neoplasia, two groups of viruses have received special attention as potential carcinogens (92,165,214,335). These will be briefly discussed in the following sections. Excellent reviews have recently been published, to which the reader is referred for further details (173,270,352,526).

Herpes viruses

To this group of DNA-viruses belong, among other types mostly seen in the animal world (173), the herpes zoster virus, cytomegalovirus, herpes simplex virus (HSV) and the Epstein-Barr virus. A causal association of the Epstein-Barr virus with Burkitt's lymfoma and with nasopharyngeal carcinoma is almost certain (555). A relationship between herpes zoster virus and malignancy has

as yet not been postulated and only a few scattered reports have suggested such a role for cytomegalovirus (270). The role of herpes simplex virus type 1 (HSV-1) is not entirely clear (4). Herpes simplex virus type 2 (HSV-2) has been the one most directly implicated in the genesis of carcinoma of the cervix (270). HSV-2 infections primarily involve individuals beyond the age of puberty, predominate in sexually active adults of either sex, and are among the venereally transmitted diseases (108,252,359).
Since the first observation by Naib et al. (360) that there was an increased rate of cervical neoplasia in patients with cytologically detectable herpetic cervicitis, and the subsequent discovery of HSV-2 in approximately 95% of herpetic genital infections (358), a number of seroepidemiologic studies have validated this association. The detection of HSV-2 specific antigens and DNA in tumor cells from cervical and vulvar neoplasia (159,225) and the demonstration that rodent cells in vitro could be transformed (417), added additional data.
Attempts to define specific HSV-2 genes or gene products required for the maintenance of the malignant state have been unsuccessful (270). Furthermore, the frequency of antibodies to HSV-2 in cases of cervical neoplasia in various geographic areas has yielded equivocal results in that the prevalence of antibodies has varied from 14% (564) to 100% (357,409,463). In the prospective study of Vonka, no differences were found between the percentages of antibodies to HSV-2 in patients with CIN or invasive carcinoma compared with the women that remained healthy (564). Higher percentages of antibodies have even been found in controls (555).

Human papilloma viruses (HPV)

The human papilloma viruses form a heterogenous group of small DNA-viruses, of which at present more than 30 types have been identified (270). Probably not all of these types are oncogenic (226). The recent techniques to identify these viruses have been reviewed by several authors (173,270,526) and will not be discussed in detail. The emerging trend from a mere flood of publications in the past years is summarized.
HPV types 6 and 11 have been mainly associated with benign condylomata acuminata. In these lesions the DNA content of the cells is usually diploid or polyloid and atypical mitotic figures are rare (435,587). Lesions with these characteristics in general do not progress to more serious stages of disease (196).
HPV types 16, 18 and 31 have been implicated in the genesis of cervical carcinoma, because these types were found mainly in so-called "flat condylomas" (344, 569). These lesions were associated with aneuploidy and atypical mitotic figures, characteristics that were distinctly correlated with progression to higher degrees of CIN and invasive carcinoma (147,196,435).
From these observations a relationship between HPV and CIN is strongly suggested. It is of interest that the frequency of identification of papilloma virus structural protein has been related inversely to the degree of CIN (270,568). This and other data has led to new working hypotheses of cervical oncogenesis (225). The relationship in itself between virus and CIN has stimulated attempts to develop vaccines against HSV and HPV (302).

Recent hypotheses of synergism in etiology

Based on epidemiological evidence a role of HSV as a sole agent in the development of cervical neoplasia is not very likely. The same might be said about HPV (270), although Kreider et al. did observe morphologic transformation of human tissues with HPV under controlled experimental conditions (297). This suggests that indeed HPV can induce morphologic changes compatible with CIN in previously normal tissue.
Zur Hausen proposed a new working hypothesis, in suggesting that HPV represents the promotor factor in proliferating cells, after recurrent infections with HSV have acted as initiating mutagens (225). This author also included smoking as an initiator in his hypothesis. Based on animal experiments, an initiating role for HSV, and sperm as a promotor was postulated by others (181). The findings of Prakash et al. (410), in patients with cervicitis, dysplasia and invasive carcinoma, suggest multiple causes rather than synergism.
Kaufman and Adam concluded as follows: "all that can be said is that there is an association between HPV and HSV infections and lower genital tract neoplasia" (270).

3.3 Aspects of morphogenesis

3.3.1 *Theories*

Invasive carcinomas of the uterine cervix have been grouped in various ways to gain information about their relative malignancy (400). The squamous cell carcinomas were classified on the basis of the predominant cell type into large cell non-keratinizing, keratinizing and small cell cancers (578). Reagan and Patten examined tissue fragments of 200 cervical carcinomas to study the relationship between the surface reaction of the overlying epithelium and the morphologic subclassification (422). In 54 of the 55 keratinizing cancers the epithelium showed dysplasia. Carcinoma in situ was found in the epithelial covering of 87 out of 104 large cell non-keratinizing cancers and in 40 of the 41 small cell cancers. Others, reporting on a group of 35 cervical cancers, classified 17 as non-keratinizing, 10 as keratinizing and 8 as small cell cancers (30). In contrast to the findings of Reagan and Patten, 14 of the 17 non-keratinizing cancers had a surface epithelium described as dysplastic or keratinous metaplastic. The 10 keratinizing cancers seemed to originate from a surface epithelium presenting structural variations from an almost normal pattern to varying degrees of dysplasia. The 8 small cell cancers had associated surface changes characteristic of carcinoma in situ of the small cell type.
While there were in these and other studies (400, 580) no significant differences with regard to the nature of the surface reaction associated with keratinizing and small cell cancers, there was disagreement in regard to the non-keratinizing cancers. Since this form of cervical cancer is the predominant morphologic pattern observed, it is interesting that a surface reaction of origin remains in question (400).

Based on the observation of different endproducts, a consensus would be expected in the literature about the fact that there could be different pathways of origin, and about the fact that the precursors would be in some way related to the endproduct. Nothing seems further from the truth. Some meant that cervical carcinoma may arise from normal squamous epithelium, without precursor stages (30), while others excluded this possibility and postulated an origin exclusively through precursors, from metaplastic epithelium in the transformation zone (129). A sort of compromise was also postulated (84, 397), by stating that the reserve cells from the columnar epithelium and the basal cells from both the original squamous epithelium and the mature metaplastic epithelium (for definitions see Chapter 2), could degenerate in a malignant direction. An origin from the original squamous epithelium was thought to occur in only 5% of cases (82, 107). However, in the investigation of others, the keratinizing type of cancer represented 21.5% of the cases reported on, and for this type an origin from the original squamous epithelium was assumed by the authors (424).

A working hypothesis for carcinogenesis was presented by Patten (400). This is summarized in Figure 3.1.

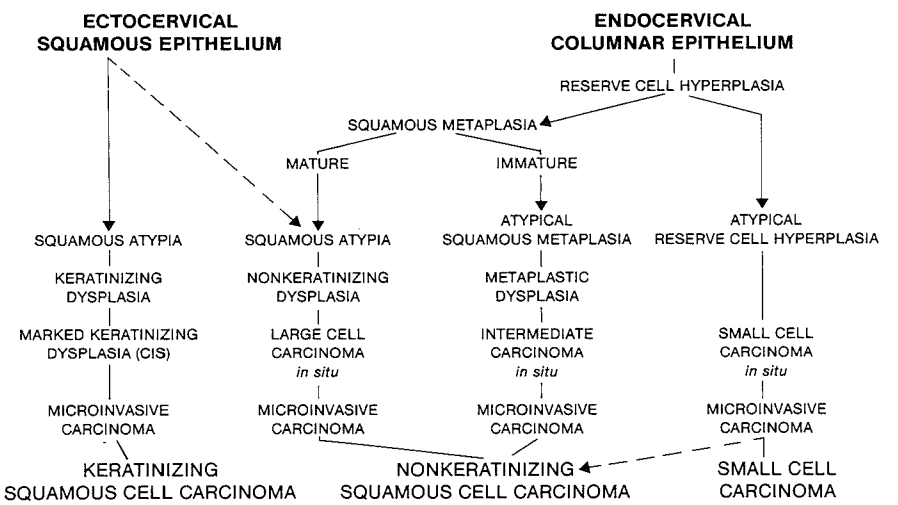

Figure 3.1. Possible pathways of morphogenesis of invasive carcinoma of the uterine cervix. (Adapted from Patten, 1978).

3.3.2 *Morphologic classification of CIN*

The diversity of histologic patterns in abnormal surface reactions of the cervical mucosa has resulted in a complex terminology (398). In 1961 The International Committee on Histological Terminology for Lesions of the Uterine Cervix defined CIS and dysplasia: "only those cases should be classified as carcinoma in situ which, in the absence of invasion, show a surface epithelium in which, throughout its whole thickness, no differentiation takes place. The process may involve the cervical glands without hereby creating a new group.." and: "all other disturbances of differentiation in the squamous epithelial lining, the glands or covering of the surface are to be classified as dysplasia" (586). As the term implies, the group of dysplasias is characterized by disturbances of maturation and depending on the level in the epithelium in which immature cells are present, a subdivision can be made into mild, moderate or severe dysplasia (61,565). The presence of immature cells in higher layers than in normal epithelium is reflected in the morphology of the component cells in the layers closest to the surface: a low nucleus /cytoplasma ratio (N/C ratio) and loss of glycogen production. It is also expressed in the biological potentialities: the presence of mitotic figures and evidence of DNA synthesis (61).

Mild dysplasia
The cells keep their statified pattern and therefore their normal polarity. Maturation and differentiation are not normal, reflected by cells of a smaller size and a larger nucleus. As a result the N/C ratio is raised. The nuclei are hyperchromatic and somewhat irregular in shape. Mitotic figures are seen only in the lower epithelial layers. The cells of the intermediate and superficial layers have abundant and dense cytoplasm, suggesting cell maturation.

Moderate dysplasia
The number of immature cells in the basal layers is increased as a sign of further loss of maturation. The cells are starting to lose their polarity, the nuclei show hyperchromasy and polymorphia more clearly. The N/C ratio is raised further. The rest of the epithelium contains cells with nuclear abnormalities and with abundant dense cytoplasm and has a lower nuclear density.

Severe dysplasia
There is only a slight tendency towards maturation. The stratification is lost. Immature cells are present up to the deeper half of the superficial layer, mitotic figures being seen also in this layer. A moderate amount of cytoplasm is still seen in the cells at the surface, and these cells show a slightly lower nuclear density. When the lesion originates from metaplastic epithelium, the stromal glands may be involved.

Carcinoma in situ
No maturation is observed in any layer of the epithelium. At the surface only immature, dedifferentiated cells are seen, abnormally arranged and with clearly identifiable mitotic figures. Cells, exfoliated from the surface are small and rather monotonous in size and form. They have relatively large nuclei, at times multiple

or multilobed nuclei. The N/C ratio is high. The cytoplasm is basophyllic and lacking in glycogen. These are the characteric cells of CIS as seen in a cytologic smear.

3.4 Natural history of CIN

In many topographic investigations the location of dysplasia and CIS was found to be closely related (82, 107, 255, 257, 337, 414, 422). CIS was either surrounded by areas of dysplasia or located directly adjacent to dysplasia. Some authors found CIS in areas that were previously occupied by dysplasia (256). Another conclusion of most authors was that both dysplasia and CIS were located in the transformation zone in more than 95% of cases. In general, the results of topographic studies do not allow firm conclusions on the natural course of CIN (315). One of the two leading concepts on the natural history of CIN however, was based mainly on observations derived from an extensive topographic study (84).

The age distribution of patients with CIN does suggest a sequential trend (315), as discussed before. Some authors who observed a difference in mean ages of patients with CIS compared with patients with invasive disease, did not find a difference when a comparison was made between patients with dysplasia and CIS (261, 563). This could be used as an argument to illustrate the possibility of invasion directly from a dysplastic lesion, a possibility suggested by many authors (81, 102, 118, 292, 423, 515, 571).

The most frequently cited concept on the natural history of CIN was introduced by Richart, who also introduced the CIN terminology (440). The theme basic to the concept is that cervical neoplasia is a continuum that begins as dysplasia and ends as invasive carcinoma. In this concept, "CIN begins in the squamous epithelium at the squamocolumnar junction, is unifocal and has the appearance of what has been termed mild dysplasia. It is found only in areas of "transformation" on the portio, the limits of the transformation zone define the portio limits of extension of the CIN. The lesion gradually spreads by replacement of contiguous squamous and columnar epithelium, and with the passage of time gradually becomes less differentiated and increases in size. As the lesion becomes larger and the mitotic rate increases, the absolute number of cells, cell divisions, and abnormal genetic constitutions increase as does the size of the population in which natural selection can operate. A clone (or clones) of cells is selected which is capable of moving against the direction of cell flow and penetrating the basement membrane. This clone is generally aneuploid" (440).

The concept was based on several preceding investigations and was tested in prospective studies (33, 34, 441). It was concluded that, once established, a dysplastic lesion would not normally revert to normal and that after about 10 years the majority (66%) of all dysplasias would progress to CIS. The duration of the stage of CIS was estimated to vary between 3 and 10 years (33). The natural history of CIN, at the time of incidence, was considered to be independent of the age of the women when in their reproductive years.

CIN was divided into CIN I, CIN II and CIN III, corresponding to mild dysplasia, moderate dysplasia and severe dysplasia and CIS taken together. The junction

of severe dysplasia and CIS was based on the opinion of the authors (33) that these lesions behave biologically in the same manner and should be managed by equal therapeutic measures.

Coppleson and Brown devised a mathematical model (128) and they concluded that the probability of transition was dependent on age. They calculated a mean transition time of 17 years from CIS into invasive cancer at the age of 25 and of 4 years at the age of 70. The possibility of two different biological processes was considered. It should be noted that Richart and Barron stated that they had insufficient data on women of over 45 years of age to draw conclusions on the rate of progression in this age group (441).

Burghardt proposed another concept on the pathogenesis of cervical carcinoma (82, 84, 90). This author considered the different forms of CIN to be separate lesions, which would not progress from minor forms of dysplasia into CIS, because of their multifocal origin and distribution. Extension of lesions would only be brought about by the apposition of new atypical epithelial fields and not by active spread from one focus. In the majority of cases (95%) a final malignancy originated from atypical squamous metaplasia in the transformation zone, but in about 5% of cases an origin had to be assumed from the original squamous epithelium. The importance of the position of the last gland for a good understanding of the natural history of CIN was repeatedly stressed (84, 87). The argumentation of this author was mainly based on the results of a topographic study (82). From this study it was concluded that lesions of different severity could simultaneously exist on the portio. The localization of the lesion was dependent on its degree of abnormality. CIS was always found more proximal to the external os than dysplasia and the less differentiated forms of dysplasia were invariably located proximally to the well differentiated forms. Dysplasia, in this concept, is a well differentiated form of CIS (91). The fact that dysplasia could revert to normal, was explained by stating that the so-called dysplasia had been but a non-specific reation to exogenic stimuli, e.g. infection. In the recent past, the attitude of discarding infectious dysplasia as insignificant, has markedly changed when the infectious agent was of a viral nature. The reasons for this change in attitude were illustrated in the previous section. The general recommendation is today, to take viral dysplasia as seriously as the non-viral type (435).

A dysplastic lesion may progress, remain in the same state for a longer or shorter period of time, or disappear spontaneously (400, 477, 495). The problem lies in proving this. The percentages of all three possibilities vary greatly in the literature. Taking the group of dysplasias as a whole, progression to CIS was observed in 3 to 70%, regression in 12 to 70% and persistence in 10 to 65% of cases (84, 151, 315, 400). The degree of CIN was found to determine the rates of progression or regression. Mild dysplasia regressed in 70% of cases, moderate dysplasia in 44% and severe dysplasia in 16% of cases (400). Others found progression of severe dysplasia in 50% of cases (151). This is in line with the percentages given for progression of CIS, which varied between 50

and 75% (315). Regression of CIS was seldomly observed by most of the cited authors, but was accepted as more than just a possibility by several others (50,211,338,351).
The tendency to progress depends also on the size of the lesion and on its possible extension into the stromal glands (499).
The progression and regression rates reported in the literature are strongly related to the method that was used to determine the nature and the severity of the lesions (302, 315), to the admission criteria of the study concerned, the duration of the follow-up and probably to the morphologic criteria that were used (362).
The natural course of CIN can be influenced by taking biopsies and by merely taking a cytologic smear (5,292,363,439,440). A minimal trauma may suffice to dislodge the surface epithelium, after which it might seem as if the host had eradicated the lesion (400). A spontaneous disappearance of the more severe lesions is not all that unimaginable. From electron microscopic studies it can be learned, that dedifferentiation also means "loosening". The contact between adjacent cells is loosened by the loss of desmosomes (483), and the contact between epithelium and basement membrane becomes less firm by the loss of so-called pseudopodia of the basal cells (550). This also means that taking repeat cytologic smears from the more severe lesions might have great bearing on the results of subsequent histopathology.

The concepts on the natural history were not only amended for the reasons mentioned above. In 1966 the possibility of two entirely different types of cervical carcinoma was postulated by Ashley, on the basis of epidemiologic data (16, 17). The first type would occur predominantly in younger women and be preceded by pre-invasive stages, which allow its timely detection by cytology or histopathology. The second type, less frequent but more commonly seen in older women, would not be preceded by a pre-invasive stage of any length of time to be diagnosed before the invasive stage. These observations are at variance with those of others, who found a protracted course in women of over 70 years of age (468). However, the conclusions of Ashley were confirmed by others (216,351).
In recent years a great number of reports was published on yet another form of invasive cervical carcinoma, which was termed "rapid-onset carcinoma" (24,165,235,236,237,403). This type of carcinoma was seen almost exclusively in younger women and seemed to occur in such a fast manner, that preinvasive changes escaped any form of interval cytology. The majority of the women had not had any smears at all. When cytology had been obtained, a small number of smears, after revision, had to be considered negative. The relative frequency of this type of carcinoma is not exactly known. The percentage ranges between 0.2 and 15% in the literature, with an extreme of 28% in one report (403). The term rapid-onset carcinoma does not only apply to squamous cervical carcinoma. In fact, the majority of these rapidly developing tumors was adenocarcinoma (602).

3.5 Conclusions

The contemporary insights on the nature of Cervical Intraepithelial Neoplasia have emerged from the contributions of a number of medical disciplines. In view of the steady influx of evidence on different types of squamous cervical carcinoma it is most likely that hypotheses on the various aspects of this disease are in constant need of change. The ultimate goal to clearly understand the etiology and thus be able to take the most appropriate measures to eradicate cervical cancer does not seem nearby. In the meantime, gaps in the knowledge are narrowing, at times to be substituted by other missing links. The incorporation of viral agents as etiologic factors in the concepts on the natural history may prove to be such a substitute. The evidence up till now, is very convincing and matches the epidemiologic postulates of early sexarche and promiscuity being by far the most important risk factors. For want of better insights it is advisable to take the viral forms of CIN as seriously as the non-viral.

In diagnostics, the fact that CIN is a precursor of invasive carcinoma is obviously of great importance. A matter of concern remains the impossibility to predict the fate of a once established lesion. Assessment of the ploidy of the premalignant cells and hybridization are both better ways to provide the answers than routine cytology or histopathology can give, but neither is a standard detection method in general use. The easiest solution to this problem is to treat all lesions, but this clearly means overtreatment in a number of cases and furthermore, in every-day practice exceptions to general rules will always have to be made. The separating line seems to lie between CIN II and CIN III, as far as progression and regression tendencies are concerned. This implies that the distinction between the two is of importance. With the understanding that CIN is probably a heterogeneous group, this should lead to continuous efforts to improve diagnostic capacities, irrespective of the method used, to assist clinical management. Other factors like extent of a lesion, crypt involvement and certainty of adequate follow-up also have to be taken into account.

Prevention should be directed at the venereal characteristics of the disease and at the most likely candidate to start the development of CIN: the young woman who has immature squamous epithelium in her transformation zone. The use of barrier contraceptives might be stimulated, not only to prevent AIDS.
It is a well-known fact that selection of high-risk groups for screening fails as an approach, therefore all sexually active women should be screened. Given the trend towards a lower mean age for CIN, the starting age should be under 35 if one wishes to catch the whole spectrum of precursors.

Chapter 4

COLPOSCOPIC DIAGNOSIS OF CIN

Wouter M. Huisman

Introductory remarks

The diagnosis of CIN includes several steps, the first of which generally involves cytology. If needed, the second step in modern diagnostics implies a colposcopic examination, with the taking of directed biopsies under visual control and, again if needed, the third step may involve further histopathologic tissue sampling e.g. by means of exconization or hysterectomy.
An in-depth discussion of cytology is not within the scope of this thesis. For this, the reader is referred to two recent contributions to the literature, one giving a detailed account of population screening programs (602), the other describing various methods to improve the value of cytology as a diagnostic aid (302).

In this chapter, the outlines of the grading approach we employed, will be given in section 4.4. When discussing the details in chapter 6, section 4.4 will be used for reference, to avoid unnecessary duplication.
For similar pragmatic reasons, section 4.5 will be dedicated to the technical aspects of colposcopy. The adaptations that were made, to improve colpophotography, and the biopsy method that was developed for the purpose of this study, will be included in that section. The developments were made possible under the guidance of J.M. van Meir, gynecologist, in close co-operation with the Departments of Histopathology, Instrument Technology and Photography.

4.1 History of colposcopy

The first colposcope was described by Hinselmann in 1925 (238). A binocular dissection microscope was mounted on a tripod, in combination with a bright light source. The stand allowed movement in all directions and focusing was made possible by a small screw (91). The uterine cervix could be inspected under illumination, at a magnification level between 10.5 and 30 times.

The idea behind this method was based on the popular concept at the time, that cancer arises as a small nodule, originating in the epithelium (133). No such nodules were found on the cervix, but Hinselmann described and systematized a number of cervical lesions that were previously unknown (151), amongst which the alterations now recognized to antedate cervical cancer.

Contrary to Hinselmann's expectations, colposcopy was not established in its country of origin but very slowly and with hesitation (36). Schiller's lugol staining

test (467) at first competed with colposcopy and retarded its adoption (151), but the hesitation on the part of his contemporaries was also due to the fact that the inventor directed his method solely to the detection of cancer and tried to enforce his own terminology (36, 151, 325), especially with regard to the precancerous nature of leukoplakia (239, 240).
After some adaptations were made to improve the visualization of cervical lesions, the technique became suitable for scientific purposes (241). By the time Hinselmann's own students, Mestwerdt and Ganse reported on extensive series of examinations, colposcopy had already received a marked appreciation in South America, especially Brazil and Argentina (36, 151).
In the Anglo-Saxon countries, colposcopy encountered firm resistance, due to the cumbersome and ponderous terminology that went with it (133). Cytology, being simpler and more practical for the early diagnosis of cervical cancer, received widespread acceptance and at the same time almost brought about the collaps of colposcopy (91). However, the competition between cytology and colposcopy became outdated for several reasons. The early exuberance about cytology was progressively tempered by its false-negative rate which has been reported to be as high as 30% (400, 465).
Furthermore, the original classification of Papanicolaou allowed cytologists to limit their responsibility by placing many patterns in the group of indecisive findings, while correlation with surgical histopathology was needed, to assist clinical management (368).

The breakthrough for colposcopy came in the early sixties. By then, several investigators had realized that cytology and colposcopy should be used in combination (27, 80, 227, 317, 364). A new interest was created to close the diagnostic loophole between cytology and histopathologic clarification, the latter until that time mostly depending on blind biopsy or exconization in clinics not employing colposcopy. The increased international communication (36) and the large number of publications in the English language, paved the way for the worldwide acceptance of colposcopy which took place in the past decades (91, 133).

Controversies exist till today and these are rooted in the historic events just described. The English speaking communities have implemented colposcopy mainly for selective use, namely the evaluation of patients with abnormal cytology. The success of colposcopy was thus related to the ability to obtain target biopsies and to prevent diagnostic exconization (157). The European school, using colposcopy as part of a routine gynecologic examination (36, 91), adhered to the original concepts and claimed that the selective use has led to a distorted and one-sided interpretation of the normal and pathologic appearance of the cervix (91). Some of the opposite views have been discussed in the preceding chapter, the implications for the diagnostic approach will be discussed in this chapter.

4.2 Procedures of a colposcopic examination

The usual sequence of events in colposcopy is as follows:

1. Visualization of the cervix and vaginal fornices
2. Identification of a possible lesion
3. Tissue sampling
4. Documentation

When colpophotography is employed as part of the documentation, photographs are taken before the step of tissue sampling, for obvious reasons.
The present author feels safe in stating that general agreement on the best possible way to execute each step, can not be found in the international literature. In part, this is related to the event that is to follow colposcopy. A good example to illustrate this, is the role that has been attributed to endocervical curettage (ecc). Some authors considered ecc to be an integral part of a colposcopic examination (548), others could do completely without it (519). Ecc has been advised only in case of unsatisfactory colposcopy (551), or exclusively in case of satisfactory colposcopy (356).
When conservative treatment was part of the management protocol, ecc has been designated as an important factor to reduce the risk of error in outpatient management (502, 547). Although a positive ecc, generally described as a sample containing any degree of CIN, has been considered to contraindicate conservative treatment of CIN by many authors (115,269,389,444,542,548,551), this has been challenged in case of satisfactory colposcopy (39,250,302,455).
When exconization or hysterectomy was to follow colposcopy, the role of ecc has been played down, because it was not considered to contribute to diagnostic accuracy (250,455,519) and the procedure might interfere with proper histopathologic assessment of the endocervial canal in the surgical specimen (110,502).
On the other hand, a positive ecc should be considered as a warning signal for invasive diasease and lead to diagnostic exconization in the absence of clinically overt carcinoma, even if this means cutting through invasive carcinoma in about 5% of cases (315).
In short, there is no opinion on this matter that has not been fervently defended.

4.2.1 *Aids in visualization*

With the patient in the normal lithotomy position, a self retaining speculum is inserted to expose the uterine cervix. The tips of the blades are to be placed well into the vaginal fornices. This provides a good view of the entire ectocervix and it everts the lips of the portio, which allows inspection of the lower part of the endocervical canal (131). The colposcope is rolled or swung in place and under direct observation excess mucus and celldebris is removed with a cotton wool swab.
From this point onward, there are two ways of executing a colposcopic examination, the difference being the method of visualization (91, 263).

I. Classical or extended colposcopy

This method was advocated by the German school, the cervix being rinsed in turn with a 3% acetic acid solution and Schiller's lugol solution (91).

Acetic acid test
The cervix is carefully dabbed by means of a cotton wool swab saturated with acetic acid. Different action mechanisms of acetic acid have been postulated (105,263,544), but there is agreement on the fact that application results in visible changes which allow the distinction between normal and abnormal epithelium. The columnar epithelium takes on a typical grape-like appearance, showing the distribution of the arbor vitae (Chapter 2). Color changes in the epithelia turn the atypical epithelium white and accentuate the surface contour. Since these characteristic changes are main pillars of the colposcopic impression, colposcopic examination has been called incomplete without the acetic acid test (91). The importance of this test has also been stressed by others, who described diagnostic features, based exclusively on their appearance after acetic acid application (131). Ironically, these diagnostic features were originally meant to be studied without acetic acid (287).

The Schiller test
This test involves the immersion of the cervix in Lugol's iodine. It is based on the interaction between iodine and glycogen (474). Normal squamous epithelium contains an abundance of glycogen and stains deep brown. Columnar epithelium and abnormal epithelium, both lacking in glycogen, remain unstained. The unstained area is referred to as Schiller positive or iodine negative (91). These terms have frequently become intermingled (131). Most colposcopists agree that the Schiller test is of doubtful value. It rarely, if ever, reveals areas of abnormality which colposcopy has not already detected (263,291). This statement has been refuted by those who defended the test for its value to delineate a lesion, especially in exconization (105) or in local ablative surgery (91). Schiller already pointed out that the test might not always suffice for diagnostic purposes (475).

II. The saline technique

Since the use of acetic acid or Schiller's iodine hampers the study of the fine angio-architecture of the cervix another technique was developed (287).
Mucus is removed with a cotton wool swab, either dry or soaked in normal saline. When the surface of the cervix dries up during the examination, repeated application of physiologic saline restores translucency. Together with the use of a green filter, this technique allows an accurate study of the subepithelial capillaries (263).

4.2.2 *Identification of a possible lesion*

After the visualization of the epithelia, a search for colposcopic findings (listed

in detail in section 4.3) is begun. These may be located not only on the ectocervix or in the endocervical canal, but also in the vaginal fornices or on the vaginal walls (131). Therefore, it is necessary to rotate the speculum about 90 degrees for a complete inspection. This is particularly important in a search for DES-related lesions and it is a safeguard against overlooking a possibly malignant lesion situated behind the blades of the speculum in its normal position (56).
Unsatisfactory colposcopy may be defined in a narrow (503) or in a broader sense (133), the latter being the definition to which we subscribe. The distinction lies in the fact that the neo-squamocolumnar junction may be in view, but a lesion, e.g. condyloma, may obscure part of the columnar epithelium in the lower endocervical canal from vision, even with the use of an endocervix speculum.
In general, unsatisfactory colposcopy occurs in 10 to 25% of cases (155). Figures as low as 4% (250) or as high as 46% (551) were reported. This figure mainly depends on the age distribution of the study population (315), but it is also related to the usage or non-usage of an endocervix speculum (105).
Non-recognition of unsatisfactory coloposcopy has been designated as one of the two most common mistakes made in colposcopy (the other being non-recognition of atypical vessels compatible with invasive disease) (506).

4.2.3 *Tissue sampling*

The colposcopically directed biopsy has ousted other methods of tissue sampling exept exconization and endocervical curettage (ecc) (502).
Before the resurrection of colposcopy a trend towards simpler and less traumatic diagnostic methods had already set in. Exconization was considered "too much for diagnosis and too little for therapy." (233).

One of the more conservative approaches, based on topographic studies (187,414), was randomly directed four quadrant punch biopsy, performed with the unaided eye (13,29,178,234,327). An average error rate of 22% for CIN III and invasive disease, taken together, was reported with this method (315,502). The method could be improved by taking multiple histopathologic sections from each biopsy (153,485). The error rate was lowered to about 6%, but this still precluded the use of the biopsy diagnosis alone as a basis for therapy (485). A further improvement was found by increasing the number of biopsies (up to 10 per patient) and using the Schiller test as a means of directing the biosies. When a discrepancy was found between cytology and the histopathologic assessment of the biopsies, the latter were simply repeated and an ecc was added (212). The overall accuracy in the detection of CIS and early stromal invasion rose to 96.6%, a figure comparable to that of diagnostic exconization (502).

Another method, now of historic interest only, was Ayre's ring biopsy. This method involved a superficial "exconization" (315). It has been referred to as being simple in concept, but notably complex in execution (151).

When colposcopically directed biopsies were introduced, opponents of the technique drew attention to the possible oversight of an endocervical lesion.

A feeling of insecurity on the part of colposcopists about management without further examination of this area led to the inclusion of endocervical curettage in many protocols (132). As mentioned previously, this marked the onset of discussions up to this day (356). A turning point for the discussions was a report by Townsend et al. (547), which prompted a routine application of ecc by many gynecologists. These authors reported on a series of 76 patients with abnormal cervical smears. Each patient was evaluated by directed biopsies under colposcopic control together with ecc and subsequent exconization. A total of 48 hysterectomies was performed after the exconization. None of the group of 26 patients in whom the endocervical canal was free of neoplastic disease had invasive carcinoma. This led to the first conclusion that, in the presence of a negative endocervical curettage, colposcopically directed biopsies were of sufficient accuracy to establish the diagnosis without the necessity of exconization. In the 50 cases with positive ecc findings (including dysplasia), there were 8 instances of invasive carcinoma (4 of which were microinvasive) not found in the biopsies or curettings but present in the subsquent exconization. The second conclusion was, that even when only moderate or severe dysplasia is found in the endocervical canal, exconization is mandatory to accurately establish the diagnosis. No cytology results were given by the authors. Also, no mention was made of whether colposcopy had been satisfactory or unsatisfactory, which has been considered a more important factor for diagnostic accuracy than the result of the endocervical curettage (315). However, the age distribution might give an indication of this, since the majority of patients with disease in the endocervical canal was a decade older than the majority in the other group (547).
Others have given a more accurate account of the ecc findings (132, 204, 484). Some authors found a classification of endocervical curettings into degrees of atypia to be practical and helpful (204), others claimed that diagnostic exconization was only needed when the curettings contained severe dysplasia (484). An analysis of their results (484) shows that invasive carcinoma was found in one patient with minimal to moderate dysplasia and in one patient with severe dysplasia in the curettage specimen, which contradicts the allegation.
A different view was presented by Dexeus et al. (151). The result of the biopsies was awaited, before a decision was made whether or not ecc would be performed. This means a disengagement of the curettage from the colposcopic examination, which is the point of view to which the present author subscribes. It should be noted, that this is only pertinent to a clinical setting in which conservative treatment has no part, as was the case in our first study. The recommendations of Dexeus et al. were about the same as those given more recently by others (132, 250) leading to a limited diagnostic role of endocervical curettage. The practical implications for our diagnostic study were as follows:

Ecc was to be performed when a significantly more severe lesion was suggested by cytology than found in the biopsies. In case of satisfactory colposcopy this meant a difference of two or more degrees of CIN and in case of unsatisfactory colposcopy one degree of difference would suffice.
Ecc was not to be performed when exconization or hysterectomy was already planned on the basis of the histopathologic classification of the biopsies, a

condition also recommended by those who stressed the importance of an intact endocervical epithelium for histopathologic assessment of the surgical specimen (110, 502).

A modification of the first recommendation was made in our protocol, because we did not always perform exconization in case of unsatisfactory colposcopy, whereas Dexeus et al. advised this in all cases with abnormal cytology. We recommended ecc irrespective of cytology in all patients with CIN II biopsies who entered the follow-up program without further surgery, when colposcopy had been unsatisfactory. The other individual exceptions will be discussed at a later stage.

Many reports have appeared comparing the diagnostic results of colposcopically directed biopsies with those of a subsequent cone biopsy made on the same cervix (37,41,157,250,281,301,302,315,501). In most studies the histopathologic opinion followed the traditional subdivision of lesions into progressive degrees of dysplasia, carcinoma in situ and microinvasive cancer. A good correlation was usually found between the directed biopsy and the opinion ultimately derived from the final examination. The accuracy of the correlation rose when less emphasis was placed on one degree of difference in the lesion sequence (133). Diagnostic accuracy of more than 90% was reported (41,250,281,301). In another study (369), less acceptable figures were found, showing accuracy of diagnosis of directed biopsies to vary in relation to the grade of abnormality: 73% for dysplasia, 77% for CIS and 45% for microinvasive carcinoma (MIC).
The high rate of inaccuracy in the presence of MIC seems, at first glance, to be at variance with most other reports.
This however, touches upon the many problems encountered when in review a comparison is made between the results quoted by different authors. A detailed discussion of these problems has recently been presented (302). For the sake of briefness only a few examples will be discussed at this point.
The main problem is the lack of uniformity in the presentation of results. This means that interpretation from tables presenting the combined results of several authors (315), are based on heterogeneous groups of patients. As an example: 45% of cases of MIC were missed by the above-mentioned authors (369), who did not mention whether or not colposcopy had been satisfactory. An average of 0.9% of missed cases with MIC and 0.3% of missed invasion was quoted from a review (302). However, this figure was based on a calculated rate of missed cases on the total number of 1803 patients, thus not on the prevalence of (micro)invasion in the different series (315). A closer look at one of the included reports (41) shows that the only case of MIC was detected, but 2 out of 3 cases of invasive disease were missed by the directed biopsies, although colposcopy had been satisfactory in all patients.

Attempts to seek diagnostic accuracy in a more strict sense have been repeatedly ridiculed in the literature (129,131,133) on the grounds that "such an attitude fails to acknowledge not only the new concept of intraepithelial neoplasia as

a continuum rather than subsets, but the new approach to management where factors such as the size and distribution of the lesion are more important than overprecision in histology" (133). There is no reason to argue the second part of this statement. As a beginner, the present author would have hesitated to argue the first part, albeit that this thesis is based on almost the opposite view. The statement, however, comes from an author who has claimed that the captaincy of the team should go to the colposcopist (130). This is at variance with the opinion of other well-recognized authorities (91, 105, 502, 581), who meant that the main purpose of colposcopy is to seek accurate correlation with histopathology. Burghardt added to this: "it can only be hoped that other colposcopists will reach the same conclusion by carrying out their own correlative studies" (91).

The generally accepted reliability of colposcopically directed biopsies has caused a dramatic fall in the rates of diagnostic exconization (130, 301, 502). Some authors found, in retrospect, the colposcopic evaluation to have been suitable for management in 85% of their patients (157). Nevertheless, serial step sectioning of a complete cone biopsy specimen is still considered the best diagnostic method (86, 91, 302). An increase in the number of sections from 15 to 80 improved the diagnostic accuracy by 22 per cent (91).
This implied two conditions not generally met with. Firstly, the cone biopsy specimen should be complete. When this means that residual disease should not be found in a subsequent hysterectomy specimen, a review of the literature shows that a residue was found in 9-66% of cases, CIS being found in more than one third of the residual lesions (315). Secondly, serial step sectioning is not performed on a routine basis in most laboratories.
As a consequence, directed biopsies have been claimed to be more accurate than routine examination of the cone biopsy specimen (301, 315). To those who wish to avoid diagnostic exconization at any cost, this is a valid argument, supported by the fact that the diagnostic error of exconization varies between 0.6 and 9 per cent (502). The same argument could be used to define the golden standard of comparison (when the result of both the directed biopsy and the cone biopsy specimen is available) as the highest histopathologic classification found in either specimen (41, 519).

The beginner in colposcopy is frequently urged to do two things: to test his diagnostic realiability by means of exconization (315) and to take multiple biopsies of all suspicious lesions (105, 151, 263). Apart from the practical impossibility to execute the first recommendation in all patients, there is a slight contradiction between the two. When the diagnostic results of the directed biopsies are good, this means that the most abnormal area has been represented in one of them. When enough biopsies are taken, the chance of this happening would be greater as shown previously in case of random biopsies. This does not support the case for colposcopy. It might be more helpful to know where to direct the biopsy to, but given the great confusion on what is a colposcopic finding or a diagnostic criterion, we ofcourse resorted to multiple biopsies.

4.3 Colposcopic findings

During the Second World Congress on Cervical Pathology and Colposcopy in Graz in 1975, a terminology committee formulated a new colposcopic nomenclature, with the aim of reconciling the differing interpretations of various colposcopic appearances and to produce an internationally acceptable terminology. Following is the nomenclature that was termed "international" as opposed to the European (91) version, which indicates at least some criticism. The text will be found to differ slightly from the first report on this (503), but this report was designated premature (91), as some of the definitions had not yet matured.

I. *Normal colposcopic findings*
   - A. Original squamous epithelium
   - B. Columnar epithelium (ectopy)
   - C. Transformation zone

II. *Abnormal colposcopic findings*
   - A. Atypical transformation zone
     1. Mosaic
     2. Punctation
     3. Acetowhite epithelium
     4. Leukoplakia or keratosis
     5. Atypical vessels
   - B. Suspect frank invasive carcinoma

III. *Unsatisfactory colposcopic findings*
(squamo-columnar junction not visible)

IV. *Miscellaneous colposcopic findings*
   - A. Inflammatory changes
   - B. Atrophic changes
   - C. Erosion
   - D. Condyloma
   - E. Papilloma
   - F. Others

The normal colposcopic findings have been discussed in Chapter 2. The term "glandopenings" was accepted as a valid term to describe the surface openings of the clefts in the columnar epithelium.

Only the definitions of those findings, pertinent to the following discussion will be given. Reference indices are given, because definitions differ in the literature. For a more detailed account the reader is referred to the standard textbooks and atlases.

Atypical transformation zone exists when one or more of the following specific appearances is encountered, suggestive of CIN (503) (following the application of acetic acid) (131).

Mosaic: a focal abnormal colposcopic lesion in which the tissue has a mosaic pattern. The fields are separated by reddish borders, the capillaries of the underlying stroma (105).
Punctation: a focal abnormal colposcopic pattern in which the capillaries appear as a stippled pattern (503).
White epithelium: a focal abnormal colposcopic pattern seen after the acetic acid test. The white epithelium is a transient phenomenon which is seen in the area of increased nuclear density (503). It constitutes the basic appearance of the atypical transformation zone (131).
Leukoplakia (keratosis): a focal colposcopic pattern in which hyperkeratosis or parakeratosis is present and which appears as an elevated whitened plaque, identifiable before the application of acetic acid (503). It may also overlie the original squamous epithelium (131).
Atypical vessels: a focal abnormal colposcopic pattern in which the bloodvessels appear not as mosaic, punctation or as delicately branching vessels, but as irregular vessels with abrupt courses appearing as commas, corkscrew capillaries, or spaghetti-like forms (503).
Suspect frank invasive carcinoma: Colposcopically obvious invasive cancer which is not evident on clinical examination (503). This uncommon but clinically important entity is to be distinguished from overt cancer evident on clinical examination (131).

The international nomenclature has its weakness in the concept of the atypical transformation zone. Termed a catchall for findings that cannot be easily classified (272) it made it necessary to regard diagnostic criteria that characterize special lesions as findings (91). Atypical vessels do not constitute a finding per se, but are almost without exception seen in the presence of invasive cancer (91,291). Mosaic and punctation can be seen inside and outside a transformation zone, normal or atypical (131,315). Immature metaplastic epithelium may take on a white appearance after acetic acid application, which may lead to misinterpretation (557).

A different terminology, as suggested by others (36, 91, 530), has relative advantages, but the mere fact that different systems exist may be an indication that none is ideal.

4.4 Assessment of colposcopic findings

A paper on the accuracy of the colposcopic impression or colposcopic diagnosis may contain the following sentence. The colposcopic findings were classified according to the international nomenclature, the findings were graded according to the system described by Coppleson and Reid (1967), using the standard criteria described by Kolstad and Stafl (1977). Given the fact that the criteria for diagnosis and the grading system are not very specific and assuming that a specific colposcopic picture for microinvasive carcinoma does not exist (339), some doubt may be raised when, going back to the standard paper, the colposcopic impression is subsequently divided into mild, moderate and severe dysplasia, carcinoma

in situ, microinvasion and invasion. This means, again, that the interpretation of the results may be hampered by lack of uniformity.

4.4.1 *Diagnostic criteria*

The criteria mostly used in the Anglo-Saxon literature are those described by Kolstad and Stafl (291):

vascular pattern
intercapillary distance
surface contour
color tone and opacity
clarity of demarcation

Burghardt (91) added to these as criteria for differential diagnosis:

response to acetic acid
surface extent (size)
combination of abnormalities
iodine uptake, and
keratinization

Coppleson and Reid (129) based their grading system on four "qualities" of colposcopic lesions:

flat or irregular surface contour
regular or irregular punctation or mosaic pattern
fine or coarse calibre epithelial vessels
whiteness of the epithelium (after acetic acid)

Some authors recently suggested that warty lesions could reliably be distinguished from CIN (431,562), but others could not confirm this (282,460,461). A series of publications on this subject (432,433,434,435) resulted in the presentation of a colposcopic index of five signs, which was correlated with 12 histopathologic signs of HPV infection and 12 features of premalignant change (432). The conclusion that there is a strong relation between warty lesions and CIN is generally accepted. The colposcopic index used, showed overlap between the different categories and was challenged not only for this, but also for the lack of using intercapillary distance as a criterion (507). The regularity of the spacing of the vessels, irrespective of the type seen, was postulated as the most important clue to differential diagnosis (133).
Two other criteria were not mentioned. When "suspicious" vessels, which is not the exact equivalent of atypical vessels, were added to the image of mosaic and punctation, the chance of finding CIN III was 67 per cent (166). Finally, elevated glandopenings (cernes blancs), based on thick aceto-white epithelium, were only found in higher graded lesions (135). This was confirmed by others (151,283) and implementation of this appearance in the classification of CIN was suggested (283).

4.4.2 *Grading of colposcopic findings*

The range of colposcopic findings varies in quality and quantity, which has a profound significance for diagnosis and management. Formal grading of appearances thus suggests itself (129). Several grading systems have been introduced (129, 195, 501). One of these (501), based on a combined colposcopic-cytologic scoring with subdivisions into eight or nine categories, is not easy to unravel. In the grading system of Coppleson and Reid (129) a subdivision was made into appearances which were termed insignificant (Grade I), significant (Grade II) and highly significant (Grade III). The grades were defined as follows (130, 131, 133):

Grade I Flat white epithelium, fine calibre, regularly shaped vessels, absence of atypical vessels, small intercapillary distance. Histopathology: overlap from normal to mild dysplasia.

Grade II Flat, whiter epithelium, dilated calibre, regularly shaped vessels, absence of atypical vessels, usually increased intercapillary distance. Histopathology: mild dysplasia to microinvasive carcinoma (1981: severe dysplasia to CIS).

Grade III Very white epithelium, dilated calibre, irregularly shaped, often coiled, often atypical vessels, increased but variable intercapillary distance and usually irregular surface contour - microexophytic epithelium. Histopathology: CIS and microinvasive carcinoma.

As stated by the authors, this grading system is simple (131). The colposcopic appearances of the different grades have varied through the years, as have the histopathologic interpretations. Some of the vascular patterns of the system of Kolstad and Stafl (291) have been incorporated. Increased capillary distance, however, was implemented but not considered of much value. The authors found this characteristic to be variably developed and not consistent with major grade lesions wherein the intercapillary distance might be quite narrow (133).
This way of grading cannot be called accurate, when correlation with histopathology is sought for. This was not the aim of the designers of this system, as repeatedly stated (130, 131, 133). Interestingly, when used by others, in combination with the diagnostic criteria of Kolstad en Stafl (291), the colposcopic impression was in agreement with the biopsy diagnosis in 89.6 per cent of cases for Grade I-II lesions and in 89 per cent of Grade III lesions (250). Grades I, II and III of the grading system were interpreted as CIN I, II and III respectively, which seems an oversimplification of an already simple system.

The grading system of Kolstad and Stafl was tested by the authors themselves (254, 510). In these studies, accurate prediction of the histopathologic outcome was achieved in 96.5 per cent and 85 per cent respectively. Allowance was made for one degree of severity of CIN and colposcopy was satisfactory.

The rates of diagnostic error for colposcopic impression vary in the literature between 1.5 and 50 per cent (28, 41, 302, 315, 334, 363). The lower error rates were usually found in series of patients with satisfactory colposcopy, the higher

rates when colposcopy was unsatisfactory. In general, allowance was made for one degree of severity of CIN. The problems of comparison of results are largely the same as mentioned previously. They will be discussed in more detail in Chapters 5 and 6.

4.4.3 *Grading approach in the present study*

One of the aims of the present study was to diagnose the different colposcopic lesions as accurately as possible, to predict the histopathologic grade of CIN with greater accuracy than was generally done. There was one scientific reason to attempt this, namely doubt. Doubt that there could be general consensus about the fact that this would not be possible, while on all other issues previously discussed, there were as many different opinions as there were authorities.
It has been stated that it takes 3 to 4 months of training to recognize a lesion, one year to direct the biopsy to the most advanced area of a lesion and several years to predict histopathology from a colposcopic pattern (133). When the present author took over the Colposcopy Clinic he felt it was his responsibility to use the colposcope for the reason it was introduced: to take colposcopically directed biopsies from the most serious part of a lesion. To do so would mean to understand the histopathologic significance of the colposcopic images. The existing grading systems were not considered to suffice for accurate diagnosing in their present form. The criteria were not specific for a diagnosis, although they would permit a certain degree of accuracy when the observer had enough experience (291). Lacking this, rather than devising a new grading system, a different approach was chosen to interpret the diagnostic criteria. The basis for this was found in the advise given by Richart (440). This author stated that the most prudent approach was to first exclude invasive disease in all patients. Carrying this a little further, the next step can be the exclusion of CIN III, then CIN II and finally CIN I.
It was realized that no single colposcopic finding would be present in all lesions. In screening colposcopy the single findings did not exceed the 20 per cent mark (91). Furthermore, no single criterion would be one hundred per cent specific for a diagnosis, although atypical vessels of the branching type were postulated to be just that (287,291). As a group, atypical vessels were seen in benign conditions (0.6%), dysplasia (0.7%), CIS (2.8 to 16.7%), microinvasion (50 to 86%) and invasive disease (96.6 to 100%) by different authors (151,287,291,581).
For this, the diagnostic criteria were arranged in a decision model, similar to a Problem Oriented Patient Simulation. Assuming colposcopy were put into a simulation, decisions would be taken on the basis of a few key criteria (e.g. atypical vessels), supported by additional information ("differential diagnostic criteria" e.g.. distribution of a lesion, surface contour). When in doubt, the differential diagnostic criteria would tip the scale towards a higher or a lower diagnosis, or in this case a higher or lower diagnosis to be excluded.

Table 4.1 shows the classification of the criteria, in the absence of which a histopathologic diagnosis was rejected, starting with the diagnosis of (micro)in-

vasion. It would involve a lengthy discussion to illustrate the exact deliberations that led to the classification of all the criteria concerned. To avoid this, four observations are made.
Firstly, a thorough search of the literature preceded. Characteristics of colposcopic findings were only used as key criteria, when mentioned as specific for a diagnosis. An example: the reluctance of Coppleson et al. to implement intercapillary distance as a criterion was based on the fact that small distances were also seen in major lesions, whereas increased intercapillary distance was specific for CIN III. We used increased intercapillary distance as a criterion and disregarded its counterpart. Secondly, the "system" was to be tested in a prospective study, so the justification would be found or not found by the results of it. Thirdly, since patient simulations have been found to go wrong at times, the grading system of Coppleson was used simultaneously. Fourthly,the entities seen, were carefully documented, to allow an evaluation in more detail than would have been possible when based on the criteria shown in Table 4.1 only.

Table 4.1. Classification of criteria for exclusion of a colposcopic prediction of a histopathologic diagnosis.

Key criterion	Diagnosis
Atypical vessels	(micro)invasion
Irregular mosaic /punctation Strong reaction to acetic acid Coarse vessels of mosaic /punctation Increased intercapillary distance	CIN III or more
Well-demarcated lesion	CIN II or more
White epithelium	CIN I or more
Differential diagnostic criterion	**Diagnosis**
Irregular surface contour Elevated glandopenings Circular distribution of a lesion of >50%	CIN III or more

What is left after the exclusions, is a non-circumscript colposcopic image that may show

fine, regular punctation or mosaic,
some smooth white epithelium (metaplasia),
with rounded glandopenings
and normal, atrophic or inflammation type vessels,

also called the transformation zone.

4.5 Technical aspects and adaptations for research

4.5.1 *Colpophotography*

The Zeiss Opmi I colposcope (Fig. 4.1) is a modified binocular (×12.5) operation microscope, with an objective of f=200 mm, mounted on a movable stand. Standard equipment is an electronic flash, a greenfilter and a magnification changer. The latter permits magnification steps of between ×6 to ×40 without the need to refocus. A magnification of ×16 is most convenient for regular colposcopy (291).

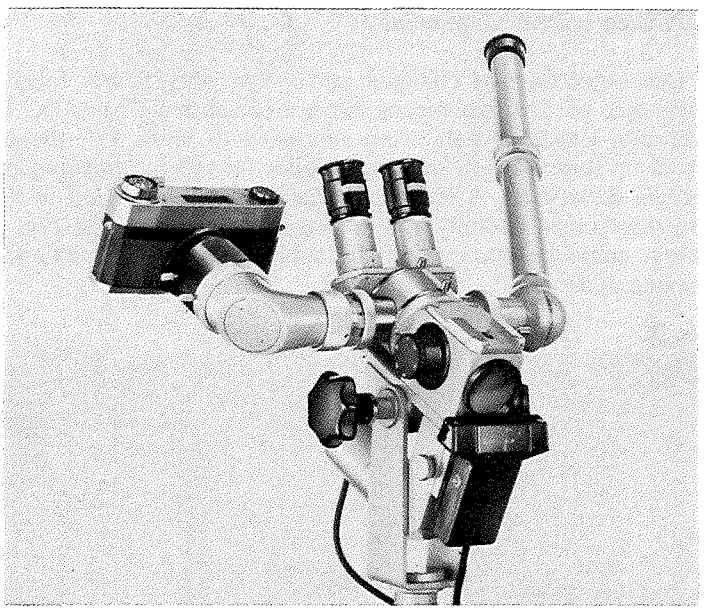

Figure 4.1. Standard model Zeiss Opmi I colposcope with photo adapter and observation tube.

An optic beam splitter allows the attachment of photographic equipment and a monocular observation tube used for teaching. Color slides with sufficient depth of field for normal documentation purposes can be produced on high-speed ektachrome daylight film.
On color photographs however, the red bloodvessels do not show up in enough contrast to the reddish adjacent tissue, to permit a detailed study of the terminal vascular bed. For this, a high resolution photographic system is needed with an ultra-fine-grain film.

The type of black and white film recommended by others (291) was not commercially available in the Netherlands, so we chose Copex Pan Rapid 19 DIN, to be developed for 16 minutes at 24°C in Neofin-Blau developer using

a dilution of 1:20. This film has orthochromatic sensitivity. The red vessels appear darker than normal, which imitates the picture seen through the (red absorbing) greenfilter. The film is also very slow and thus requires intense illumination of the object (291).

The problem of illumination can be solved by intensifying the light source or decreasing the focal length. The latter solution has the added advantage of a larger primary magnification on the film and thus an even greater total power of resolution. This solution was adopted by Koller for his monograph on the vascular patterns in precancerous and cancerous lesions of the uterine cervix (287). It involved several adaptations.

The 200 mm objective was changed into a lens of 125 mm focal length. A working distance of 125 mm means the use of shortened specula. The blades of self-retaining Cusco specula were shortened to 8 cm. For the colposcope to be moved in close enough to focus, Koller moved the opening mechanism of the specula downward. A technically easier way was found in moving the adjustment mechanism laterally and leaving the hinge in place (Fig. 4.2). Also, the speculum could be manipulated by simply touching the extended frame, without having to defocus.

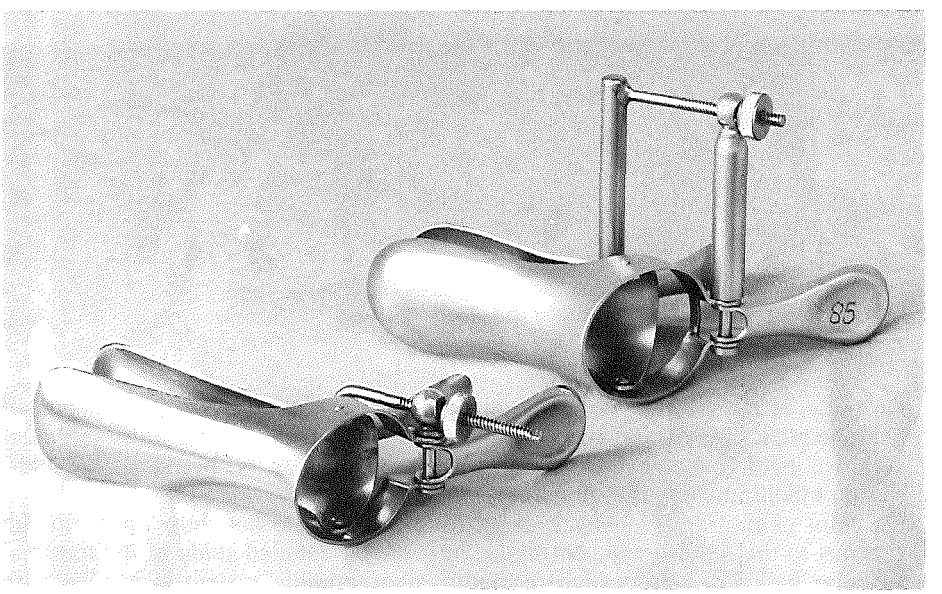

Figure 4.2. Standard self-retaining Cusco speculum (bottom) and modified speculum with adjustment mechanism moved laterally, but hinge in original position (bottom).

The camera was mounted on top of the microscope, to photograph not through but around the optics of the colposcope. This was necessary because the optic beam splitter caught away too much of the reflected light. By deflecting the beam before it reached the splitter, the film received sufficient illumination.
The mounted camera set-up was thought to fit research purposes only, because biopsies could not be taken when the working distance was 125 mm (287). A compensation was found by the interposition of an objective changer. This device contained two lenses, one of 125 mm focal length for photography and one with a focal length of 200 for a convenient working distance. Fig. 4.3 shows the objectiv changer in detail and Fig. 4.4 shows the colposcope with the device in place.

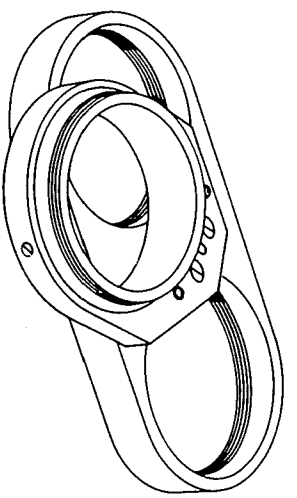

Figure 4.3. Objectiv changer (detail).

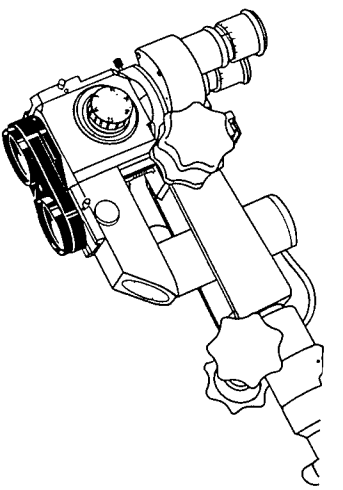

Figure 4.4. Fitted objective changer.

For the objectiv changer to be fitted, the flash chamber and the camera mount had to be placed forward by 3 cm. This led to the interposition of an extra photo-objectiv to avoid getting pictures with rounded off edges on rectangular paper.
A comparison between the optics of the standard photo adapter method and the mounted camera method is shown in Fig. 4.5.

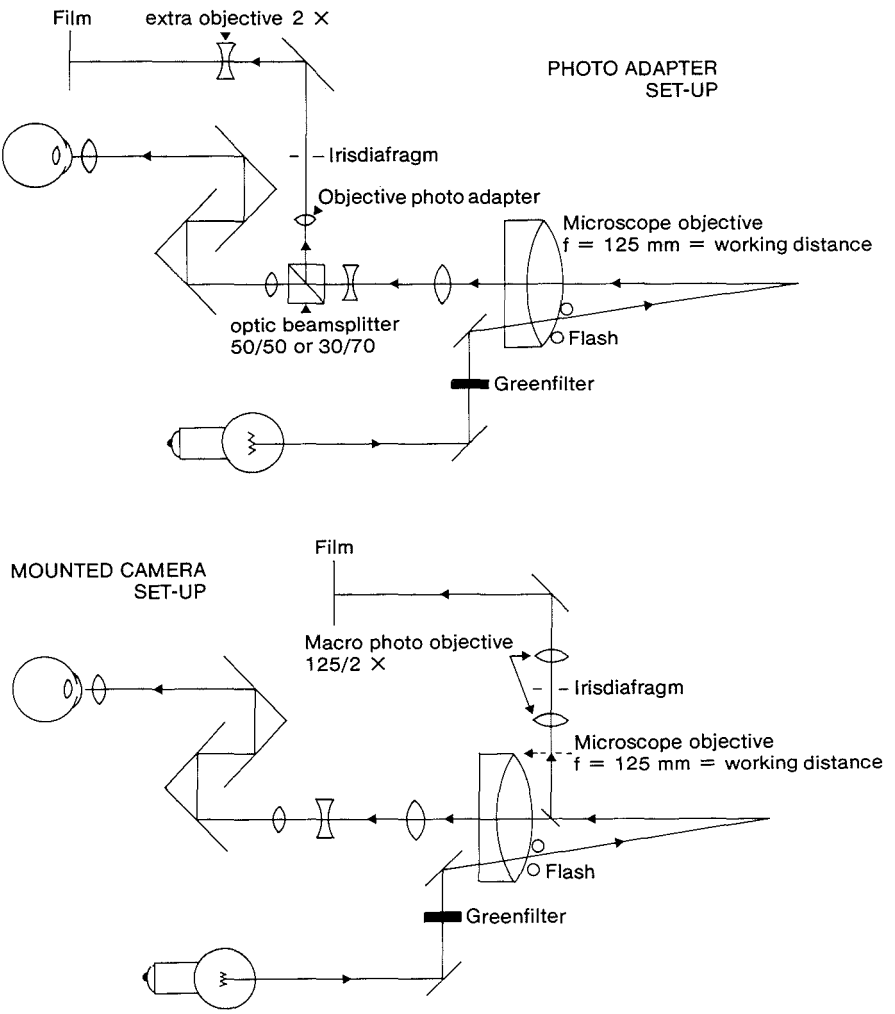

Figure 4.5. Optic beam deflection for photo adapter set-up (top) and mounted camera set-up (bottom).

After the adaptations were completed, the fully equipped colposcope (Fig. 4.6) combined the teaching facilities of the standard model with the research facilities needed for the study. Movements back and forth from photo-distance to working distance were necessary, because of the biopsy technique that was used. When the adjustment knobs on the extension arm of the colposcope were screwed just a little less than tight, the body of the microscope could be moved forward by the head and backward by the knee of the observer. This left the hands free for photography or handling of the swabs and biopsy instruments.

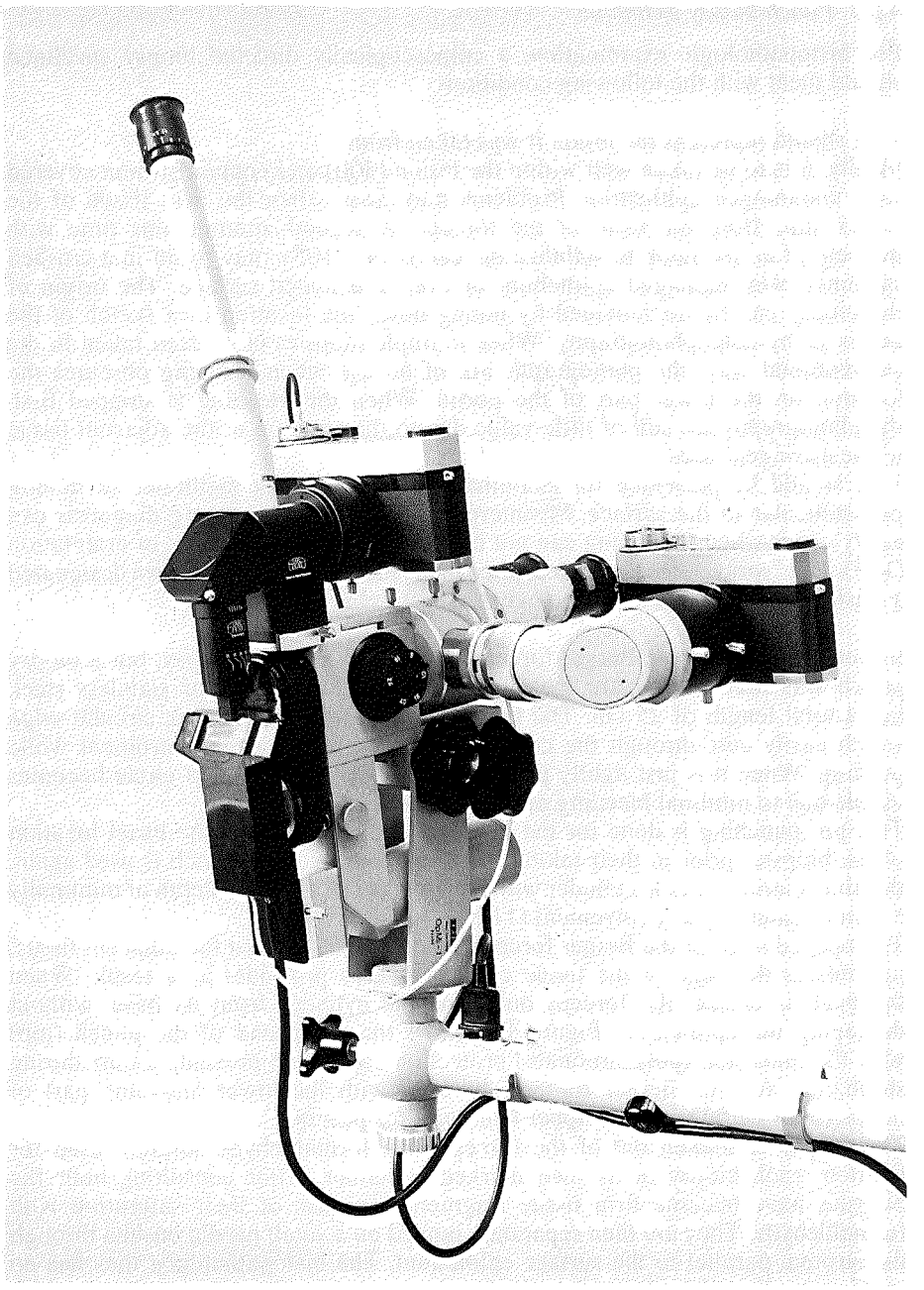

Figure 4.6. Fully equipped colposcope with 2 methods for photography.

4.5.2 *Punch biopsy technique*

For histopathologic examination, a colposcopically directed biopsy specimen should meet with the following conditions.

1. It should represent the lesion it was taken from.
Ideally, it is to be taken well within the lesion (300) and contain stroma covered with undamaged epithelium. Problems may arise when the firm tissue of the cervix slips from the beak of the forceps. A second attempt, this time with the help of an iris hook to stabilize the cervix (91, 105), may result in a crushed specimen with damaged epithelium or even a denuded surface. The origin of the biopsy can be documented by noting down the location in a sketch of the cervix or by colpophotography. When multiple biopsies have been taken in the conventional way, the photographs are of no use when bleeding obscures the location on the lower part of the portio. When the bleeding is stopped first, the photographs are still of little value due to the reaction of the adjacent tissue to the astringent used.
2. It should be presented for examination in a way that facilitates sectioning perpendicular to the surface. Misinterpretation and thus a wrong diagnosis can easily occur when sectioning can not be done in this way, for lack of orientation (105). The "spray carcinoma" described in the literature (476) has been designated a misinterpretation because of tangential sectioning (315).

In our study we used a Berger forceps to actually take the biopsy, but a biopsy punch was used to prepare the site. The punch, made out of stainless steel, has a total length of 25 cm. The lower end is a thin, razorsharp, circular edge which easily cuts through the cervical tissue by rotating the instrument while pushing. When it is just lightly pushed against the epithelium, a circle becomes visible due to minimal bleeding of the capillaries.
This pre-punching is done for the purpose of photographing the exact location of the biopsies prior to their taking. After photography the punch is used again, this time cutting a tissue cylinder with a diameter of 8 mm to a depth of minimally 5 mm to catch crypt involvement (11).
The opened beak of the Berger forceps, when pushed against the adjacent tissue, just misses the edge of the tissue cylinder, which protrudes as a result. When the beak is closed, the forceps only cuts the cylinder from its base, without damaging the epithelium. Figure 4.7 shows the sharp end of the punch (top) with the macrolon (polycarbonate) protection cap which prevents a burr during sterilizing, and the Berger forceps (bottom) with the lower, movable part of the beak for cutting and the upper fixed part for pushing.
The biopsy is shaken out of the forceps in a formaldehyde solution used for fixation, each biopsy in its own marked container. After consulting hour the biopsies have become firm tissue fragments because of their saturation with formaldehyde. They are then separately pinned on a small needle pushed through the stroma, parallel to the surface epithelium. The histopathologist thus has no problem to direct his plane of sectioning.

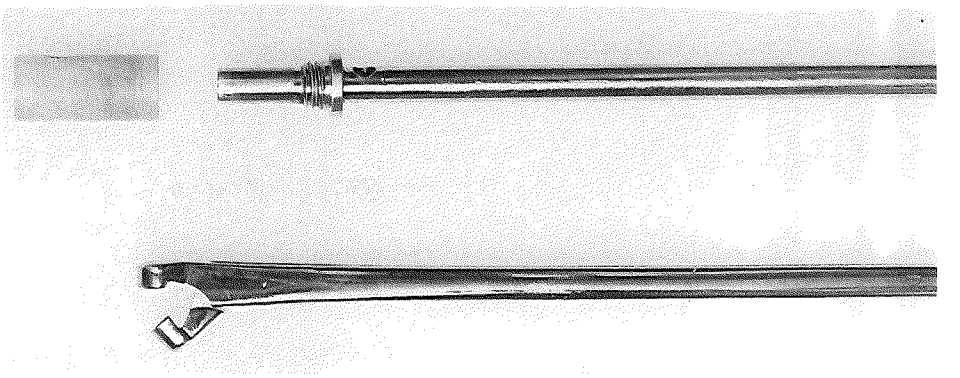

Figure 4.7. Biopsy punch and biopsy forceps used in our study.

The biopsy site is touched with a silver nitrate stick to stop the bleeding. A hydrophylic gauze pad is rolled into a tampon and inserted with the tip against the portio, to be removed after six hours by the patient. Two patients in our series needed stitching after the removal of the tampon because of continued bleeding.

Chapter 5

THE DIAGNOSTIC VALUE OF THE COLPOSCOPIC EXAMINATION

Wouter M. Huisman

5.1 Introduction

In the literature great emphasis is placed upon the fact that sufficient experience with the method of colposcopy should precede the incorporation of this method into clinical and outpatient management (250,263,315). To assess if the diagnostic value of the colposcopic examination was high enough, to introduce a more conservative treatment of CIN, the diagnostic accuracy of colposcopically directed biopsies was evaluated. As stated in Chapter 4, an evaluation limited to the diagnostic reliability of the directed biopsies would not suffice, when multiple biopsies are taken. Furthermore, when colposcopy is used as a selective diagnostic mode, as it was in this investigation, the results of the preceding cytology are to be taken into account for several reasons. Firstly, because it has been postulated that cytology increases the diagnostic accuracy of colposcopy, when both methods are used in combination (317,364), and this has in itself been a decisive factor for the introduction of colposcopy in many countries. Secondly, the result of the cytology report plays an important role in the decision whether or not additional diagnostic measures are to be taken (151). For the purpose of combining the results of cytology and colposcopy, cytology is temporarily termed a diagnostic mode.

Given the confusing terminology, found in the literature, some of the entities used in our evaluation are defined here. The cytologic diagnosis is represented by the highest-graded cytologic classification per patient in the preceding four months. To avoid confusion in regard to the "colposcopic diagnosis", the results of the colposcopic procedure are divided into three categories. The "colposcopic impression" represents the judgment of the colposcopist on the most severe lesion seen, based on the diagnostic criteria mentioned in Chapter 4. The opinion that this judgment is of limited value, since only the histopathologic classification of the biopsies would represent a diagnosis (105), is not generally shared (41,250,263). The highest-graded histopathologic classification of the biopsies taken per patient is the second category, termed the "biopsy diagnosis". The colposcopic impression and the biopsy diagnosis taken together and represented by the highest grading of the two, is considered to be the final result of the colposcopic procedure and is termed, after Benedet et al. (41), the "colposcopic evaluation".
The standard of comparison was based on the available information after a second histopathologic tissue examination. This meant that a selection had to

be made from the original patient population, including only those patients who underwent exconization or hysterectomy after colposcopy. As the standard of comparison, or the "true state of the patient", the final diagnosis per patient was taken. This was defined as the highest histopathologic classification per patient, thus the highest histopathologic grading of any biopsy, the cone biopsy or the hysterectomy specimen in this selected group of patients.
It is recognized that the selection may have an impact on the conclusions to be drawn. However, submitting all the patients to further surgery was not considered ethically justified. Furthermore, the selection bias is mitigated to some extent by the inclusion of patients who had surgery for reasons other than CIN and mainly by the prudent approach that was taken, in which cytology played an important role. The selection is basically non other than recommended in the literature (291) and its nature is considered standard clinical practice in a diagnostic approach. Finally, a patient population referred to a Colposcopy Unit is by definition a highly selected group of patients. Attempts to remedy difficulties in comparing results may introduce new biases (302).
The aims of the present investigation can be summarized as follows:
1. to assess the diagnostic accuracy of colposcopically directed biopsies.
2. to study the possibility of improving the diagnostic accuracy of colposcopically directed biopsies by means of combining the results with those of other diagnostic modes such as cytology and the colposcopic impression.

5.2 Patient selection and methods

Between July 1, 1978 and January 1, 1981, 308 patients had colposcopy and colposcopically directed biopsies. Each patient had previous cytology. The colposcopic examinations were performed by the author, in the manner described in Chapter 4. Special forms were used to fully document all the details and colpophotography was used for additional documentation.
From the original patient population 117 patients met with the inclusion criteria which were:
1. a second histopathologic diagnosis based on the examination of a cone biopsy and /or hysterectomy specimen and
2. a cone biopsy or hysterectomy specimen which would sufficiently allow for localization of a remaining lesion if such was the case after biopsies had been taken.

Failure to meet with the second condition was responsible for the exclusion of 10 patients who had further surgery. At the time of reexamination of the material it was found that 4 cones had been classified as "cervicitis", but were void of surface epithelium and almost all endocervical epithelium. Three specimens, although covered with some surface epithelium contained only a few fragments of endocervical epithelium and could not be considered a "cone" biopsy specimen but with great difficulty. Localization was not possible in the fragmented tissue. In addition to this, the cervix of 3 hysterectomy specimens had not been cut radially, which raised sufficient doubt about the nature of the lesion in between the planes of sectioning to exclude these patients from the evaluation.

In the 117 patients that were selected, the colposcopic examination was satisfactory in 62 cases and unsatisfactory in 55. The ages ranged from 23 to 71 years, with a mean age of 38.6 years. Figure 5.1 indicates the age distribution of the patients and the relative distribution of satisfactory and unsatisfactory colposcopy.

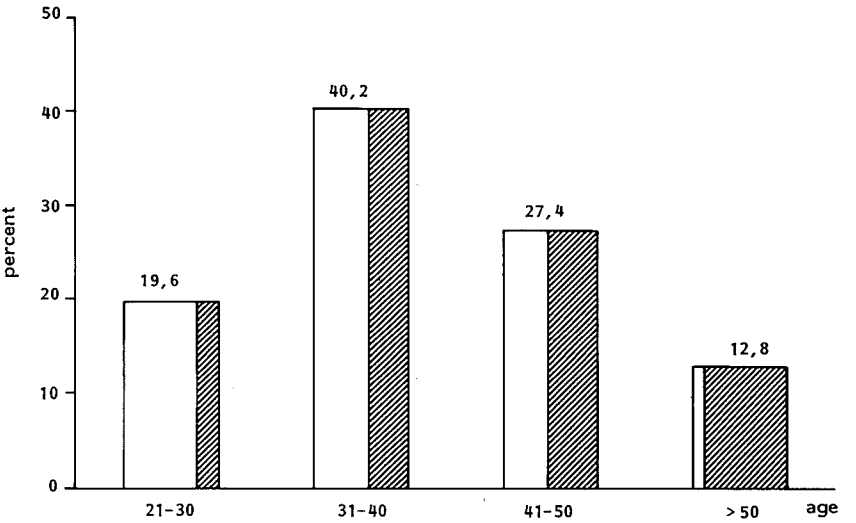

Figure 5.1. Age distribution of 117 patients with a second histopathologic diagnosis and relative distribution of satisfactory and unsatisfactory colposcopy.

In 78% of cases in the lowest age group the entire transformation zone and the lesion could be visualized, which was possible in only 13% of cases in the highest age group. In both groups of patients between 30 and 50 years of age the distribution was almost even.
The indications for colposcopy are shown in Table 5.1.

Table 5.1. Indications for colposcopy (N=117).

Abnormal cytology	89
+ abnormal vaginal bloodloss	16
+ macroscopically suspect cervix	9
Contact bleedings	2
Macroscopically suspect cervix	1
Total (n)	117

The selective use of colposcopy is illustrated by the fact that only 3 patients out of the 117 had normal cytology.

The standard indications for further surgery included the following:
1. a biopsy diagnosis of CIN III or (micro)invasion, 2. a discrepancy between cytology and the biopsy diagnosis. (In cases with satisfactory colposcopy a discrepancy of one degree of dysplasia was accepted, but in cases with unsatisfactory colposcopy any discrepancy was an indication for diagnostic exconization), 3. uncertainty about follow-up. When CIN was present either in cytology or histopathology of the biopsies, the necessity of follow-up was discussed with the patient. Elective surgery by means of a diagnostic exconization might result from such a discussion if the patient could not or would not attend to follow-up.
The actual indications are shown in Table 5.2.

Table 5.2. Indications for surgery in the study population (N=117).

Directed biopsy showing CIN III or (micro)invasion	65
Discrepancy between cytology and histopathology	43
Elective surgery instead of follow-up	4
Prolapsus uteri	1
Metrorrhagia, resistant to conservative therapy	4
Total (n)	117

The indications given are the primary indications. In some patients there was more than one indication, e.g. a CIN III biopsy diagnosis combined with metrorrhagia.

Endocervical curettage was not a part of the colposcopic examination. The referring gynecologist was advised to perform endocervical curettage, when the patient did not have one of the standard indications for further surgery, in case of a discrepancy between cytology and histopathology or when colposcopy had been unsatisfactory. The limited use of endocervical curettage in patients who already had an indication for surgery, was aimed at the preservation of the endocervical epithelium for histopathologic assessment. However, endocervical curettage could also be a part of a diagnostic curettage, which is an outpatient procedure in our department. Furthermore, individual exceptions could be made by the referring gynecologists. The results of the curettages that were performed in the selected group of patients will be discussed separately.

After January 1981 all the slides, including cytology, were reexamined by a gynecopathologist who had no knowledge of the previous classifications. The reevaluation of the histopathology is discussed in detail in Chapter 6. To enable a comparison between the different diagnostic modes, all classifications were divided into 5 groups: negative, CIN I, CIN II, CIN III and invasive disease. The classifications of severe dysplasia and carcinoma in situ were combined into the group of CIN III. The reasons for this were discussed in Chapter 3. Agreement between a judgment or a classification and the standard of comparison was only considered to exist when there was exact agreement. Differences were scored as overdiagnosis or underdiagnosis respectively.

Follow-up depended mainly on repeat cytology, which was started 3 months after surgery, to be repeated at 6 and 12 months postoperatively. After this, the interval was either 6 or 12 months, which depended on the preceding histopathologic diagnosis. Abnormal cytology within the first year was recorded as persistent disease, when cytology became abnormal after the first year a recurrence was recorded. In both instances a colposcopic examination was repeated and directed biopsies were taken for histopathologic confirmation. Adequate follow-up was defined as a follow-up of at least one year and based on a minimum of 2 cytology reports.

5.3 Statistical methods

The clinical data of all patients was stored into a VAX/VMS computer (version V4.3). In selected files from the main data base, statistical data analysis was carried out using the Statistical Package for Social Sciences. When specific tests were used this will be indicated in the text. The diagnostic indices used to estimate error rates are summarized below, as adapted from Kwikkel (302).

Indices of a diagnostic test.

	Disease		
Test result	present	absent	total
positive	a	b	(a+b)
negative	c	d	(c+d)
total	(a+c)	(b+d)	(a+b+c+d)

true positives = a; false positives = b; true negatives = d; false negatives = c
sensitivity: a /(a+c); specificity: d /(d+b);
pre-test probability of a positive result = prevalence: (a+c) /(a+b+c+d);
pre-test probability of a negative result: 1 - prevalence;
predictive value positive (PV+: a /(a+b); predictive value negative (PV-): d /(c+d);

5.4 Results

Of the 117 selected patients, 74 had exconization only, 17 had exconization followed by hysterectomy, and 26 patients had hysterectomy only. Thus, the final histopathologic diagnoses were obtained after examination of 91 cone biopsy and 43 hysterectomy specimens. The diagnoses were subdivided as follows: 6 patients had negative histopathology, 5 had CIN I, 36 had CIN II, 53 had CIN III and 17 patients had invasive disease. In the latter group there were 8 cases with microinvasive carcinoma, which is defined in our clinic as an invasion depth of up to 3 mm measured from the basement membrane. In 3 cases the invasion depth was between 3 and 5 mm and in 6 cases the invasion depth was more than 5 mm.

The diagnostic results will be given in the following section. For the sake of completeness the results of the endocervical curettings and the follow-up results will also be presented.

5.4.1 *Diagnostic results in relation to the final diagnosis*

The relationship between the diagnosis per diagnostic mode and the final histopathologic diagnosis is shown in Table 5.3. The results are given for the entire group of 117 patients and for the group with satisfactory and unsatisfactory colposcopy respectively. Results are shown in numbers with percentages in parentheses. The detailed results per mode are shown in Table 5.3 A through 5.3 R in the addendum.

Table 5.3. Relationship between the diagnosis per diagnostic mode and the final histopathologic diagnosis.

		Final diagnosis					
				Colposcopy*			
		Total group					
Diagnostic mode	Prediction per mode	(N=117) n	(%)	TZ+ (N=62) n	(%)	TZ- (N=55) n	(%)
Cytology	exact	65	(56)	35	(56)	30	(55)
	overdiagnosis	32	(27)	19	(31)	13	(24)
	underdiagnosis	20	(17)	8	(13)	12	(22)
Biopsy	exact	101	(86)	60	(97)	41	(75)
	overdiagnosis**	—	—	—	—	—	—
	underdiagnosis	16	(14)	2	(3)	14	(15)
Colposcopic impression	exact	101	(86)	56	(90)	45	(82)
	overdiagnosis	6	(5)	3	(5)	3	(5)
	underdiagnosis	10	(9)	3	(5)	7	(13)
Cytology + biopsy	exact	80	(69)	42	(68)	38	(69)
	overdiagnosis	32	(27)	19	(30)	13	(24)
	underdiagnosis	5	(4)	1	(2)	4	(7)
Colposcopic evaluation	exact	108	(92)	59	(95)	49	(89)
	overdiagnosis	6	(5)	3	(5)	3	(5)
	underdiagnosis	3	(3)	0	(0)	3	(5)
Cytology + colposcopic evaluation	exact	83	(71)	43	(69)	40	(73)
	overdiagnosis	33	(28)	19	(31)	14	(25)
	underdiagnosis	1	(1)	0	(0)	1	(2)

*Colposcopy: TZ+ = satisfactory colposcopy; TZ- = unsatisfactory colposcopy.
**Overdiagnosis not possible by definition.

The highest accuracy in predicting the final diagnosis is found for the directed biopsies in cases with satisfactory colposcopy (97%) and the lowest accuracy for cytology in cases with unsatisfactory colposcopy (55%). A statistically significant difference in diagnostic accuracy is found for the biopsy diagnosis in cases with satisfactory colposcopy compaired to cases with unsatisfactory colposcopy (Fisher test: $p < 0.001$). For the other diagnostic modes or combinations thereof no statistically significant difference in diagnostic accuracy can be demonstrated between satisfactory and unsatisfactory colposcopy.
The differences between percentages of accurate prediction of the final diagnosis, were statistically analysed by means of the McNemar test. The diagnostic accuracy of cytology is significantly increased when cytology is combined with either the biopsy diagnosis or the colposcopic evaluation, but both combinations lower the diagnostic accuracy of the biopsy diagnosis and the colposcopic evaluation respectively ($p < 0.001$).
There is no statistically significant gain in diagnostic accuracy of the biopsy diagnosis when combined with the colposcopic impression ($p = 0.17$). The gain in diagnostic accuracy of the colposcopic impression however, is statistically significant for the same combination into the colposcopic evaluation ($p = 0.02$).

When a similar analysis is made in regard to the percentage of underdiagnosis, each one of the three combined modes has a significantly lower percentage of underdiagnosis than its singular components ($p = 0.02$ for colposcopic impression compared to colposcopic evaluation; in all other instances: $p < 0.001$). Thus, the detection rate is significantly increased when diagnostic modes are combined.

There were 17 patients with (micro)invasive disease. The diagnosis was predicted by the colposcopic impression in 3 out of 3 cases with satisfactory colposcopy, but missed in 4 out of 14 cases with unsatisfactory colposcopy. The colposcopic evaluation missed 1 case with an invasion depth of between 3 and 5 mm below the basement membrane, and this case was not diagnosed by cytology either (Table 5.4). The numbers in parentheses are the cases with a final diagnosis of less than 3 mm invasion depth.

Table 5.4. Number of missed cases with (micro)invasive disease per diagnostic mode.

		Colposcopy	
Diagnostic mode	Total group n=17	Satisfactory n=3	Unsatisfactory n=14
Cytology	11	3 (2)*	8 (3)
Biopsy	4	1 (1)	3 (1)
Colposcopic impression	4	0	4 (2)
Cytology + biopsy	3	1 (1)	2 (1)
Colposcopic evaluation	1	0	1
Cytology + colposcopic evaluation	1	0	1

*see text

For the detection of (micro)invasive disease, the colposcopic evaluation is the most valuable diagnostic mode. The diagnostic indices of this and the other modes are summarized in Table 5.5. The indices are calculated from the diagnostic results given in Tables 5.3 A through 5.3 R (see addendum). In case of cancer detection, the test with a good performance would have a high sensitivity and show a balance between sensitivity and specificity. Given the prevalence of 15% (17/117) of (micro)invasive disease, the predictive value positive (PV+) and negative (PV-) can not be considered useful indices (9).

Table 5.5. Sensitivity and specificity per diagnostic mode, when disease is (micro)invasive carcinoma, in a group of 117 selected patients.

Diagnostic mode	Sensitivity	Specificity
Cytology	35%	97%
Biopsy	76%	100%
Colposcopic impression	76%	99%
Cytology + biopsy	82%	97%
Colposcopic evaluation	94%	99%
Cytology + colposcopic evaluation	94%	96%

5.4.2 *Results of endocervical curettage*

In Table 5.6 the histopathologic classification of 17 endocervical curettages, the indications and the final diagnosis of each of the 17 patients are presented. The first 9 curettings listed were performed in cases with satisfactory colposcopy. When the endocervical curettage was part of a diagnostic curettage, this is listed as "D&C". The other curettings were performed either for reasons of a discrepancy between cytology and the biopsy diagnosis, or on the instigation of the referring gynecologist.

Table 5.6. Results of endocervical curettage (N=17).

Indication	Classification	Final diagnosis
1. D&C	negative	negative
2. D&C	insufficient sample	negative
3. D&C	negative	negative
4. D&C	negative	CIN I
5. D&C	negative	CIN III
6. D&C	negative	CIN III
7. Discrepancy	negative	CIN II
8. Discrepancy	negative	CIN III
9. "Discrepancy"	negative	invasive disease*
10. Discrepancy	insufficient sample	CIN II
11. Discrepancy	negative	negative
12. Discrepancy	negative	CIN I
13. D&C	insufficient sample	CIN III
14. D&C	negative	CIN II
15. against protocol	CIN III	CIN III
16. discrepancy	negative	microinvasion
17. against protocol	CIN III	CIN III

*see text

The curettage marked with an asterix was performed in an exceptional case. This patient was 29 years old. She had two previous cytology reports which were both negative. A small polyp was removed from the outer surface of the portio, at the 1 o'clock position. Colposcopy was performed during the same session at the request of the gynecologist, because of the location of the polyp. Colposcopic impression: focal invasive disease between 11 and 2 o'clock, part of a circular lesion of CIN II and CIN III. Five biopsies, including the polyp. Diagnosis: at 6 o'clock CIN II, at 8 and 10 o'clock CIN III, at least microinvasion at 11 o'clock and carcinomatous polyp. Wedge biopsy: undifferentiated squamous cell carcinoma. Radical hysterectomy: same diagnosis, vasoinvasive disease, lymfglands negative, cutting edges free from disease. After radiation therapy for recurrence, death within first year after operation. Conclusion: rapidly growing undifferentiated squamous cell carcinoma.

The only curettings that were positive were performed against protocol. Biopsies contained CIN III as did the curettage sample, and the final diagnosis was CIN III. All other curettings were negative or produced a sample that was insufficient for diagnosis. The positive samples were found in cases with unsatisfactory colposcopy.

5.4.3 *Follow-up*

Out of 91 exconizations the cone biopsy specimen was incomplete in 17 cases (19%). This occurred in 6 cases with satisfactory colposcopy (lateral edges) and 11 cases with unsatisfactory colposcopy (either the top or the lateral edges). Four of these patients entered follow-up without further surgery. In 11 cases a hysterectomy was performed, showing a residual lesion in 6 specimens. Two patients had re-conization, in 1 case a residue was found. Non of the residual lesions was more severe than the original lesion. Six patients underwent hysterectomy after diagnostic exconization had resulted in a complete cone. No residual lesions were found in these cases.

The follow-up to 3 years after the study period is summarized. Follow-up input into the computer was discontinued after this for analysis of the main data file. Ten patients had inadequate follow-up (8.5%). Of the remaining 107 patients, 9 completed 2 years of follow-up, 9 had completed 3 years, 14 were followed up to 4 years, 35 up to 5 years and 40 up to 6 years at the last computer update. Two of these 107 patients had negative cytology before colposcopy and negative histopathology. The third patient with negative cytology had an invasive carcinoma. Persistent disease, based on cytology, was found in 2 out of 105 cases (1.9%). Of the 4 patients who had no additional treatment after incomplete exconization, one had persistent disease and two had negative cytology for 60 months. The fourth patient had negative cytology for 48 months, before a recurrence was found. Directed biopsies showed CIN II. There were 2 other recurrences, after 33 months and 48 months respectively. One patient had CIN I, the other CIN III. The cone biopsy specimen of the latter patient had been considered "possibly incomplete" at the time of reexamination. (This would have

meant stricter follow-up, but personal circumstances interrupted the follow-up for 15 months). The 3 recurrences were found in 103 patients without persistent disease, thus in 2.9% of cases, when the 9 patients that were lost to follow-up after two years are disregarded.

5.5 Discussion

There is general agreement in the literature about the fact that cytology and colposcopy should be used as complementary methods. This complementary aspect is usually described as to result in an increased diagnostic accuracy of colposcopy (37,317,364,519). There seems to be a mix-up of two entities: diagnostic accuracy and detection rate. The results of this study illustrate the necessity to keep these entities well separated. The diagnostic accuracy of cytology was 56%. This seems a low percentage, but it is in line with the findings of others, when accuracy is defined as exact agreement (302,315). By combining cytology with other diagnostic modes, the accuracy of cytology was increased, but the diagnostic accuracy of the mode it was combined with, was decreased. The detection rate was significantly increased by the combinations when the highest classification was related to the standard of comparison. This effect was mainly due to the number of overdiagnoses of cytology. The high percentage of overdiagnoses was not merely caused by a selection bias. The cytology results that were used were the classifications after reevaluation, which mitigated the effect of the original discrepancies. Furthermore, the possibility of spontaneous regression was compensated by limiting the interval between cytology and colposcopy, from which a cytologic report was included.
The detection rate was highest for the final combination of cytology and colposcopic evaluation and the impact was greatest in unsatisfactory colposcopy. In the subgroup of (micro)invasive disease the complementary aspect of cytology and colposcopy played a smaller role. The sensitivity of the biopsy diagnosis and of the colposcopic impression was raised, and one more patient was detected, but the colposcopic evaluation had the highest combined sensitivity and specificity. Rather than confirming the statements on diagnostic accuracy, our results agree with the observation of Verschoof, that the combination of cytology and colposcopy leads to an almost complete detection of (pre)invasive cervical disease (557).
From the presented information a compensatory effect can be derived in regard to the biopsy diagnosis and the colposcopic impression. This effect was not statistically significant in the total group for the diagnostic accuracy of the biopsy diagnosis, but the percentage of underdiagnosed cases was significantly decreased by the combination with the colposcopic impression into the colposcopic evaluation. In the group of unstisfactory colposcopy both the diagnostic accuracy of the biopsy diagnosis in relation to the final diagnosis and the detection rate were increased. In this group of patients the colposcopic impression could predict the final diagnosis with greater accuracy, and it underestimated the final diagnosis in a smaller number of cases. This means that although part of the endocervical extent of a lesion can be seen, a biopsy can not always be taken from this area. To those who propagate the use of endocervical curettage this would support

their view that a curetting should be performed in all patients with unsatisfactory colposcopy. Those who oppose this view would use the same observation to recommend exconization (315). Our own experiences with endocervical curettage as a diagnostic procedure were limited. In the case in which the diagnosis of invasive disease was missed in the group of unsatisfactory colposcopy, the patient refused endocervical curettage if performed as an outpatient procedure. In the cases in which a curetting was performed, the diagnostic contribution was nil when colposcopy was satisfactory and the contribution in the group of unsatisfactory colposcopy was too limited to omit diagnostic exconization. The exclusion of some patients from this study was partly due to the detrimental effect on the endocervical lining of the cone biopsy specimens, which is not a point against the procedure itself, but against its implementation in a diagnostic protocol.

The percentage of exconizations that resulted in incomplete cone biopsy specimens (19%) is within the range reported in the literature, as discussed previously. This is not meant as a reassurance, for the fact that incomplete cones were also found after satisfactory colposcopy. Combined with the observation that some tissue fragments were not recognized as cones at the reevaluation sessions, the question arises if this operation should be part of the junior residency program. This point was raised by others on similar grounds (315).

The results of the respective parts of the colposcopic examination with regard to the accuracy in predicting the final diagnosis, can not be compared with the results of others but with some difficulty. This is due to factors such as differences in definition of the standard of comparison, differences in the accuracy of agreement between diagnoses and differences in presentation of results. An example was touched upon previously, derived from a review (315), in which directed biopsies missed invasive disease in 1.2% of the total number of patients, in case of satisfactory colposcopy. In the same review, also in case of satisfactory colposcopy, but based on the results of different authors, the colposcopic impression accurately predicted the final diagnosis in 85%, overdiagnosed in 11% and underdiagnosed in 4% of cases. Apart from the fact that comparing percentages in case of invasive disease is of limited value, the conclusion that in the present study the results in case of satisfactory colposcopy were better, does not take the above mentioned differences into account. Therefore, a limited selection is made from the literature, which includes publications with sufficient data to allow for a comparison of results, at least in part, with those of the present study. A second reason for the selection of these reports is that the authors are considered to represent a certain trend or view within the field of colposcopy. A recalculation of results was sometimes necessary e.g. when the biopsy diagnosis was compared with a final diagnosis based on the histopathologic classification of the cone biopsy specimen. In the opinion of the present author the cone biopsy specimen does not represent the true state of the patient at the time of the colposcopic examination, because the taking of biopsies may have changed the lesion or may even have removed it. Such a presentation of results may give evidence of the therapeutic effect of taking directed biopsies (293,440), but it does not, as has been stated (315), prove that the diagnostic reliability of colposcopically directed biopsies exceeds that of exconization.

Benedet et al. published results in cases with satisfactory colposcopy only (41). In this series of 226 patients, exact agreement was found between the biopsy diagnosis and the final diagnosis in 95% of cases, underdiagnosis in 5% of cases. The colposcopic evaluation was in agreement with the final diagnosis in 98% of cases, 2 cases with invasive disease were missed and 1 case was overdiagnosed.
Javaheri and Fejgin performed endocervical curettage in the first 100 patients with satisfactory colposcopy in their series (250). They did not find a single positive curettage sample and thereafter limited this procedure to the patients with unsatisfactory colposcopy. From the total of 930 patients, 268 patients had further surgery after satisfactory colposcopy. The biopsy diagnosis was in exact agreement with the final diagnosis in 95.5% of cases. Underdiagnosis occurred in 4.5% of cases. The final diagnosis was higher than the biopsy diagnosis in 16 patients with unsatisfactory colposcopy. In this large series there were only 32 patients with unsatisfactory colposcopy. The colposcopic impression was not compared with the final diagnosis and the colposcopic evaluation was touched upon but not expressed in figures.
Shingleton et al. performed endocervical curettage in all the patients in their series (484). In 168 patients who underwent exconization or hysterectomy, the biopsy diagnosis was in exact agreement with the final diagnosis in 76% of cases and 24% of cases were underdiagnosed. Six out of 6 cases with microinvasive disease and 2 out of 7 cases with invasive disease were missed on the basis of the biopsy diagnosis alone, but when combined with the result of the endocervical curetting only 3 cases with microinvasive disease were missed. No data were provided on the distribution of satisfactory and unsatisfactory colposcopy.
Swan published the results of a study which was undertaken to evaluate the accuracy of colposcopic examination when performed without the use of endocervical curettage (519). As mentioned by the author, an endocervical speculum was not used either. The definition of the true state and the subdivision of diagnostic modes was similar to that of our study. There were 275 patients with a final diagnosis based on the combination of the biopsy diagnosis and a second histopathologic specimen. In this group, there was exact agreement between the biopsy diagnosis and the final diagnosis in 74.6% of cases. Unfortunately, the distribution of satisfactory and unsatisfactory colposcopy was not given. Furthermore, the results of the other diagnostic modes were calculated for the total study population, including patients without further surgery. The conclusion of the author was that his results were comparable to the results of others who did use endocervical curettage. The reports of Swan and of Javaheri and Fejgin belong to the most frequently cited reports in the recent literature.

5.6 Conclusions

The results of the present investigation into the diagnostic accuracy of colposcopically directed biopsies are comparable to the results of other authors, albeit that the comparison itself is extremely difficult. Nevertheless, it is concluded that clinical management, including outpatient conservative therapy of CIN, can be based on the histopathologic classification of directed biopsies in patients

with satisfactory colposcopy. There will always be a chance of invasive disease being missed. Although not all patients with incomplete cone biopsies had additional treatment, the results of the follow-up would justify the conclusion that no other cases with invasive disease were missed than the ones reported. In patients with satisfactory colposcopy this chance can either be called high or low, depending on the calculation. The risk of missing invasive disease is lower when diagnostic modes are combined.

When colposcopy is satisfactory, no increase in diagnostic accuracy of the biopsy diagnosis is to be expected by combining diagnostic modes. Such an effect is observed when colposcopy is unsatisfactory for the combination of the biopsy diagnosis with the colposcopic impression (colposcopic evaluation).

The (almost) general opinion that the combination of cytology and colposcopy increases the diagnostic accuracy of both methods is not substantiated by the results of the present investigation. Based on our results we conclude that the combination of cytology and colposcopy only increases the diagnostic accuracy of cytology. It decreases the diagnostic accuracy of colposcopy, but the combined use does increase the rate of detection of (pre)invasive cervical lesions.

The results of endocervical curettage had no impact on the diagnostic accuracy of the colposcopically directed biopsies in the selected patients, but no generally valid conclusions can be drawn from our results, since this procedure was only used by us on limited indications.

Chapter 6

THE PREDICTIVE VALUE OF DIAGNOSTIC CRITERIA IN COLPOSCOPY

Wouter M. Huisman

6.1 Introduction

The aim of this study was to investigate the possibility of predicting the histopathologic diagnosis of the colposcopically directed biopsy with greater accuracy than generally reported in the literature. The reasons for this approach were documented in Chapter 4.
The golden standard for this evaluation is the highest histopathologic classification of a set of biopsies, obtained per patient, taken from the visible part of a lesion. This is termed the biopsy diagnosis. It is recognized that this is not the same as the final diagnosis. Especially when colposcopy is unsatisfactory, further diagnostic measures have to be taken to arrive at the final diagnosis. This was demonstrated in the previous chapter.
To make the evaluation possible, several conditions had to be met with. Since multiple biopsies were taken as a safety measure, the clock position of the biopsy site that was considered to be the most severe part of the lesion, was noted down separately from the positions of the other biopsies. The alledged inability of the histopathologist to reproduce a diagnosis, was postulated to preclude accurate diagnosing in colposcopy, when histopathology was accepted as golden standard (131). The implications of the re-evaluation of the biopsy specimens will therefore be discussed in detail.
Given the great confusion in the literature about colposcopic findings and diagnostic criteria, both categories were recorded and will be discussed. When a set of diagnostic criteria was found in the literature, the instructions for use on how to translate this into grading were usually missing. The exception that was mentioned previously, led to a grading system that was not accurate enough for our purposes (see Chapter 4). To solve this problem, the diagnostic process was unraveled. By reviewing the literature, it was found that diagnosing was usually done by a process of addition. Key diagnostic criteria were added together with other factors, termed criteria by some authors and "factors to be taken into account" by others in the construction of a diagnosis (91,129,291). As a generalization, the key criteria were referred to in terms of "are almost exclusively seen" and the other criteria in terms of "may also be seen". To create more favorable conditions for accurate diagnosing, the latter criteria were used in our study either as differential diagnostic criteria or disregarded as criteria but only noted down. The key criteria were used in a process of excluding diagnoses, starting with the assumption that invasive disease might be present in each patient. Working our way down to "normal", the exclusion stopped when the criteria for an intermediate diagnosis were present.

6.2 Results of histopathologic re-evaluation of the biopsy specimens

Between February 1981 and May 1981 the entire cytologic and histopathologic material was re-examined by a gyneco-pathologist, who is also an experienced colposcopist. Part of this was the re-examination of 930 biopsy specimens, obtained from the 308 patients seen in the Colposcopy Clinic during the study period. The slides were collected by the author, the histopathologist did not know the previous classification. The slides were selected on the basis of the name of the patient, in alfabetic order, to prevent the showing of specimens from one category of CIN only. The (new) classification was converted to the patient name and the case number by the author, after the sessions. The results of the re-evaluation are presented with regard to the number of classifications that was altered and with regard to the clinical impact on the golden standard of comparison.

1. The number of single biopsy classifications that was changed, was taken as a measure of the reproducibility of the histopathologic diagnosis. In Table 6.1 the results are specified per group of classifications, ranging from negative histopathology to invasive disease.

Tabel 6.1. Histopathologic re-evaluation of 930 biopsy specimens.

Original diagnosis	Biopsies (n)	Diagnosis changed (n)	(%)
negative	307	14	(4.6)
CIN I	233	30	(12.8)
CIN II	249	22	(8.8)
CIN III	109	6	(5.5)
MIC*	13	1	("8")
INV*	19	0	(0)
Total (n)	930	95	(10.2)

*MIC = microinvasion, invasion up to 3 mm below the basement membrane
** INV = macroinvasive disease

The nature of the changes in classification of the single biopsies is summarized in Table 6.2.

A definite statement was asked, but not always obtained. In 3 instances the original classification had been "CIN I to CIN II". (These biopsies had been recorded as CIN II). In re-examination, the pathologist came up with the same diagnosis and did not change, although he saw these slides (and only these) at least three times, intermingled with others. The biopsies were again recorded as CIN II and are not listed in the table. A lower classification was usually associated with viral infection, either indicated by the pathologist or noticed by the author when the results were converted back to the case number.

Table 6.2. Change in classification of 95 biopsy specimens.

From	To	(n)
negative	CIN I	12
	CIN II	2
CIN I	negative	1
	CIN II	27
	CIN III	2
CIN II	CIN I	12
	CIN III	10
CIN III	CIN II	6
MIC*	CIN II	1
Total (n)		95

*MIC see footnote Table 6.1.

2. The results were also related to the impact on the standard of comparison, the highest histopathologic classification of a set of biopsies, per patient. A classification change did not necessarily mean a change of biopsy diagnosis. An analysis was also made of the impact on the agreement between the altered biopsy diagnosis and the colposcopic impression. As an example: the classification of a biopsy was changed from negative to CIN I. This changed the biopsy diagnosis of this patient. The lesion had been diagnosed as CIN II, so the change had no influence on the agreement with the colposcopic impression, because a discrepancy remained. In another case the classification of one biopsy from a set of three was changed from CIN II to CIN III. The other biopsies had already been classified as CIN III. The colposcopic impression had also been CIN III, albeit that the clock position of the biopsy that was upgraded had not been encircled, so it was thought to have been taken from a less severe part of the lesion. The change of classification "hurt" neither the golden standard nor the agreement thereof with the colposcopic impression, it only hurt the author.

The classification changes of 95 biopsies resulted in a different biopsy diagnosis in 35 patients (11.4% of the total number of cases). In these 35 patients, agreement between the colposcopic impression and the standard of comparison became exact in 23 cases in which a discrepancy had existed before. In 7 cases a former exact agreement changed into a discrepancy. In 5 cases a discrepancy remained. There were 14 cases out of the 35 in which the encircled biopsy was involved.

In retrospect, the altered biopsy diagnoses would not have led to a different patient management in all of the 35 cases. Of the 23 diagnoses that were upgraded, only 6 went from CIN II to CIN III. In 4 cases surgery had been performed because of a discrepancy between cytology and histopathology. One other patient had exconization after satisfactory colposcopy, because of a large lesion. Cytology and colposcopic impression were in agreement (CIN III) and the cone biopsy specimen showed CIN III as well. The sixth patient had presistent CIN II cytology for 17 months, repeat (satisfactory) colposcopy and biopsies showing CIN I.

She had laser treatment in 1981 and negative cytology thereafter for 36 months. Of the 12 diagnoses that changed to a lesser degree of severity, 6 would have been of consequence. The original diagnosis of MIC (Table 6.2) led to diagnostic exconization, which would not have been performed had the biopsy diagnosis been CIN II. The cone biopsy specimen showed CIN II. In 5 cases in which the diagnosis changed from CIN III to CIN II, 2 hysterectomies were performed. One was on request instead of follow-up, the other should be considered overtreatment in the presence of a focal CIN II lesion and satisfactory colposcopy. The remaining two patients had no exconization, as an exception to the protocol. Both were referred from the fertility clinic and they had repeat colposcopy during follow-up. After 24 months one of the two women was treated with cryosurgery, the other had 4 repeat colposcopies in 36 months. After all the biopsies that were taken by that time, no more lesion was found.
In summary, the 6 cases in which a higher grading could have been of consequence, were all accounted for by the time of re-evaluation. Of the 6 cases in which a less severe diagnosis would have influenced the original management, 3 patients were, in retrospect, overtreated and for 3 patients management would not have been different.

6.2.1 *Comment*

In the literature, inter-observer and intra-observer variability rates with regard to the reproducibility of histopathologic diagnoses of CIN vary greatly (125, 295, 484). Ranges of 50 to 90 per cent were reported, depending on the degree of accuracy of agreement between the first and second diagnosis (see also Chapter 7). The results of the re-evaluation of our study material are a mixture of inter- and intra-observer variability. A subdivision would be biased because the majority of the biopsies showing CIN III or more were originally classified by the gyneco-pathologist who did the re-examination, while the lower graded biopsies had for the greater part been originally judged by others. The results give an indication of a greater inter-observer than intra-observer variability and as a whole, compare favorably with the results found in the literature. It has been stated, that one man's dysplasia is another man's carcinoma in situ (244). Part of this problem is solved by the adoption of the CIN terminology (295). A difference compared to the results of others was found for the reproducibility of the different grades of CIN. In our material the least reproducible diagnosis was CIN I, while others reported difficulties mainly in reproducing CIN II diagnoses (295). The observation that the recognition of virus-specific epithelial changes result in lower classifications in a retrospective analysis, is in accordance with the opinion of other authors (54, 57). The limited clinical impact of the changes of biopsy diagnoses combined with the high reproducibility rate of classifications in our material, supports rather than undermines our confidence in accepting histo-pathology as a golden standard.

6.3 Patient selection and methods

Of the 308 patients that were seen in the Colposcopy Clinic between July 1, 1978 and January 1, 1981, there were 6 patients with a purely endocervical lesion of which no representative biopsy specimen could be obtained. These patients were excluded from the present analysis. The age distribution of the remaining 302 patients is shown in Figure 6.1. The mean age was 35.8 years, with a range of 15 to 72 years. Colposcopy was satisfactory in 235 cases (mean age 33.1 years) and unsatisfactory in 67 cases (mean age 45.5 years). Figure 6.1 also depicts the interrelation between age, biopsy diagnosis and visibility of the entire transformation zone.

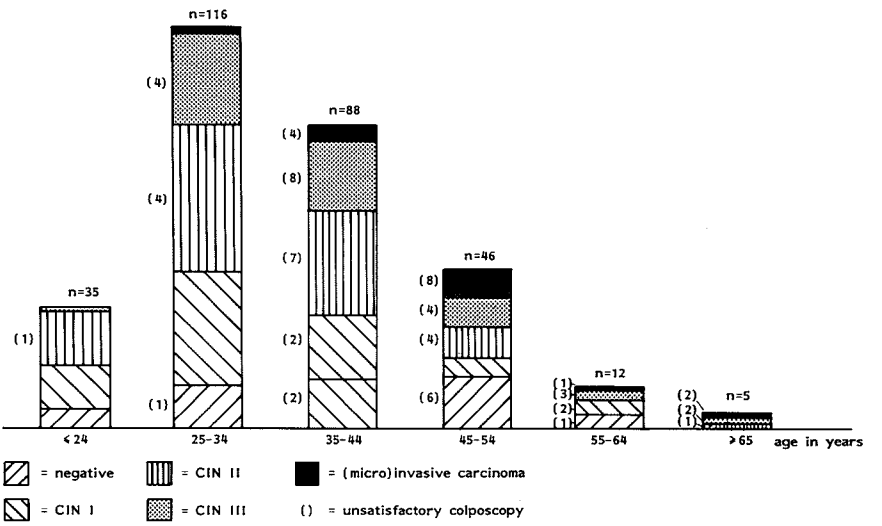

Figure 6.1. Age distribution in relation to biopsy diagnosis and satisfactory or unsatisfactory colposcopy (N = 302).

The indications for colposcopy are summarized in Table 6.3. These are the primary indications. Several patients had combined indications e.g. abnormal cytology in combination with leukoplakia or condyloma. For the patients listed in the category of special indications colposcopic assistance was asked by the referring gynecologist because of unexplained menstrual disorder, bleeding "erosion", possible viral infection and papilloma of the portio respectively.
The vast majority of patients was seen for reasons of abnormal cytology. The number of cytology reports that was available per patient varied from 1 to 8. The cytologic diagnosis of each patient was represented by the highest cytologic classification found in the preceding 4 months. The histopathologic diagnosis was the standard of comparison, defined in the introduction to this chapter.

Table 6.3. Indications for colposcopy in 302 patients.

Indication	(n)	(%)
Abnormal cytology	277	91.7
Contact bleeding	9	3.0
Leukoplakia	4	1.3
Recurrent discharge	3	1.0
Condyloma	2	0.7
DES progenity	2	0.7
Suspect portio	1	0.3
Special indication*	4	1.3
Total	302	100.0

*see text.

The methodology of the colposcopic examination was described in Chapter 4. For visualization the combined saline/acetic acid test was used. A colposcopic examination took 30 minutes, including colpophotography and the taking of biopsies. This is considerably longer than the time usually needed for a routine colposcopy, but the careful documentation that was considered necessary, consumed 10 to 15 of the 30 minutes. Towards the end of the study, a diagnosis was made in less time than before, but documentation of the characteristics of the colposcopic findings still took the same amount of time.
Patients with unsatisfactory colposcopy had endocervical curettage, diagnostic exconization, or both, before entering the follow-up program. None of the endocervical curettings showed more advanced disease than the directed biopsies. Patients with advanced invasive disease were treated by means of radical hysterectomy (Wertheim-Meigs) and/or radiation.

6.4 Evaluation of colposcopy results

The difference between colposcopic findings and diagnostic criteria was discussed in Chapter 4. At this point it is repeated that the colposcopic finding atypical vessels is also a diagnostic criterion, whereas the other findings of the Atypical Transformation Zone are not. In the following text the distribution of colposcopic findings and diagnostic criteria will be discussed. It should be noted that the abbreviation INV (invasion) is used to indicate microinvasive and macroinvasive disease taken together, unless otherwise specified.

6.4.1 *Colposcopic findings*

Table 6.4 shows the relationship between the presence or absence of colposcopic findings of the Atypical Transformation Zone (ATZ) and the biopsy diagnosis. The term "leukoplakia"is preferred in lieu of "keratosis", because a white patch is seen and keratosis is the possible histopathologic diagnosis.

Table 6.4. Presence or absence of colposcopic findings of ATZ and biopsy diagnosis (N = 302).

Finding (+ present - absent)		Biopsy diagnosis						
		negative	CIN I	CIN II	CIN III	INV	Total (n)	(%)
Mosaic	+	6	34	65	45	6	156	51.7
	-	45	40	34	16	11	146	48.3
Punctation	+	18	53	80	56	10	217	71.9
	-	33	21	19	5	7	85	28.1
White epithelium	+	29	74	99	61	15	278	92.1
	-	22	0	0	0	2	24	7.9
Leukoplakia	+	3	2	8	10	4	27	8.9
	-	48	72	91	51	13	275	91.1
Atypical vessels	+	0	0	1	2	16	19	6.3
	-	51	74	98	59	1	283	93.7

The single most present finding was white epithelium (92.1%). Its mere presence would render the finding per se unsuited as an exclusion criterion for two different diagnoses. Therefore, a distinction will have to be made according to the nature of the reaction of the epithelium to the application of acetic acid. Atypical vessels was the least present finding and its relation to the diagnosis is apparent. The high frequency in which most findings were seen is related to the selective use of colposcopy as opposed to screening colposcopy. This does not hold for leukoplakia. This finding was seen in 8.7% of cases, which is about the same frequency as reported in screening colposcopy (28,91). A biopsy was always taken from the area where a white patch was seen. Other specimens usually determined the biopsy diagnosis in the lower groups. In the groups of CIN II and more the leukoplakia specimen usually contained CIN and in one case of microinvasive disease the specimen represented the highest classification. This illustrates the fact that, although not a useful diagnostic criterion, leukoplakia may mask a lesion and a biopsy should always be taken.

6.4.2 *Results of grading*

Two sets of diagnostic criteria were used. The criteria postulated by Coppleson et al (131) were combined into an "ATZ"-score for grading. In this system TTZ signifies the image of a Typical Transformation Zone and ATZ the Atypical Transformation Zone, divided into Grades I, II and III. The criteria used for exclusion were combined into a colposcopic impression, which signifies the prediction of the present author of the histopathologic outcome. The theoretic basis of these two ways of grading was discussed in Chapter 4. The results of the grading by means of the ATZ-score are shown in Table 6.5.

Table 6.5. Relationship between biopsy diagnosis and ATZ-score (N = 302).

Biopsy diagnosis	ATZ-score					
	TTZ	ATZ I	ATZ II	ATZ III	Total (n)	(%)
negative	32	19	0	0	51	16.9
CIN I	2	72	0	0	74	24.5
CIN II	1	86	11	1	99	32.8
CIN III	0	5	53	3	61	20.2
INV	0	0	1	16	17	5.6
Total (n)	35	182	65	20	302	
(%)	11.6	60.3	21.5	6.6		100.0

Strictly speaking, the TTZ is not a part of the grading system. The term signifies that no lesion is seen, which was wrongly diagnosed in 3 out of 35 cases. The Grade I (insignificant) lesions should have ranged from negative histopathology to CIN II. Of the 182 cases in this category, 5 had a biopsy diagnosis of CIN III. The Grade II lesions could range between CIN II and microinvasion, which they did. In the stricter interpretation the lesions should be CIN III, which they were not in 13 out of 65 cases. The lesions in the highly significant category, Grade III were as should be, ranging from CIN III to invasive disease, with the exception of 1 case. It should be noted that the grading as shown was the interpretation of the present author. This meant that all cases in which invasive disease was suspected, were placed in the category of Grade III lesions. The interrelation between the two scoring methods is shown in Table 6.6.

Table 6.6. Comparison between colposcopic impression and ATZ-score (N = 302).

Colposcopic impression	ATZ-score					
	TTZ	ATZ I	ATZ II	ATZ III	Total (n)	(%)
negative	35	12	0	0	47	15.6
CIN I	0	83	0	0	83	27.5
CIN II	0	84	4	0	88	29.1
CIN III	0	3	59	3	65	21.5
INV	0	0	2	17	19	6.3
Total (n)	35	182	65	20	302	
(%)	11.6	60.3	21.5	6.6		100.0

The close relationship between the two methods is clear e.g. in the TTZ-category. The differences are illustrated in Table 6.7, indicating the relationship between the biopsy diagnosis and the colposcopic impression.

Table 6.7. Relationship between biopsy diagnosis and colposcopic impression (N = 302).

Biopsy diagnosis	Colposcopic impression						
	negative	CIN I	CIN II	CIN III	INV	Total (n)	(%)
negative	43	4	4	0	0	51	16.9
CIN I	3	65	6	0	0	74	24.5
CIN II	1	13	75	9	1	99	32.8
CIN III	0	1	3	53	4	61	20.2
INV	0	0	0	3	14	17	5.6
Total (n)	47	83	88	65	19	302	
(%)	15.6	27.5	29.1	21.5	6.3		100.0

The colposcopic impression was in agreement with the biopsy diagnosis in 250 of the 302 cases (82.8%). Microinvasive disease was missed in 3 cases (18% of the cases with invasive disease). Macroinvasive disease was not missed, but it was overdiagnosed in 5 cases. One of these had a biopsy diagnosis of CIN II. The diagnosis was based in this case on the interpretation of the vessels (Table 6.4), which, in retrospect, when the colpophotograph was studied, should not have been called atypical vessels. No explanation was found for the misdiagnosis of 5 cases with a CIN III impression and CIN II biopsies. The other 4 cases were interpreted as CIN III because of the color tone after acetic acid (see below). The discrepancies in the lower categories were mainly due to the interpretations of white epithelium and the demarcation of the lesions. This is further commented on in section 6.6.

6.4.3 *Diagnostic criteria used as an exclusion parameter*

Coilposcopic findings were not always present. Since diagnosing was done by exclusion, the absence of some criteria, which were characteristics of colposcopic findings of the Atypical Transformation Zone, might have raised a problem. The absence of all criteria would simply result in the prediction of negative histopathology. There were 68 cases in which two important colposcopic findings, mosaic and punctation, were absent. The colposcopic impression was invasive disease in 7 of these cases, 9 were judged as CIN III, 8 as CIN II and 9 as CIN I. The remaining 35 cases were thought to be compatible with negative histopathology. The cases with a colposcopic impression other than CIN III were diagnosed on the basis of their own criteria (see below). The diagnosis of CIN III was substantiated by the other criteria of this group, which were intercapillary distance and strong reaction to acetic acid. There were 24 cases without white epithelium, 2 of these had a colposcopic impression of invasive disease, 1 was diagnosed as CIN I (the biopsy diagnosis was negative) and 21 had a prediction of negative histopathology, which was confirmed by the biopsy diagnosis.
The diagnostic indices for each criterion were calculated. The theoretic basis is shown in section 5.3 in the previous chapter. The separation between "disease"

and "non-disease" is not the same for each criterion. Different separating values are therefore used for the various categories. It was considered important to exclude "disease", because this is more in line with the practical situation of arriving at a colposcopic impression. However, the presence of a parameter will be used in conjunction with the presence of "disease" for the calculation of the diagnostic indices, to avoid confusion about positive and negative test results. Suffice it to say, that the sensitivity of a test based on the presence of the criterion in case of "disease", equals the specificity of the absence of the criterion for the exclusion of the same "disease" and the latter was of essence in excluding a diagnosis.

6.4.3.1 Exclusion of invasive disease

Atypical vessels were seen in 19 cases and not seen in 283. The biopsy diagnosis was not always invasive disease, as shown in Table 6.4. There were 8 cases with a biopsy diagnosis of microinvasion and 8 with a biopsy diagnosis of macroinvasion. The one case with CIN II biopsies was mentioned previously, being a case in which the vessels were incorrectly interpreted. There were 2 other cases with atypical vessels in which the colposcopic impression was CIN III, because of the completely flat epithelial surface. The impression was confirmed by the biopsy diagnosis and this situation was expected to occur, since atypical vessels may be present in non-invasive disease (151). However, there was a third category, in which the vessels were labeled as "dubiously atypical". This was not anticipated. Of the 10 cases in which such dubious vessels were seen, 1 had a biopsy diagnosis of microinvasion, 7 of CIN III and 2 of CIN II. The colposcopic impression had been invasive disease in 2 cases, and CIN III in 8 instances. The one patient with microinvasive histopathology was correctly diagnosed, the CIN II histopathology was not. The low prevalence of invasive disease (5.6%) invalidates the use of PV+ and PV- as indices for the performance of atypical vessels as a test for invasive disease.

Diagnostic indices of atypical vessels, when "disease" is (micro)invasive disease: sensitivity 94%; specificity 99.6%.

6.4.3.2 Exclusion of CIN III and invasive disease

Irregular mosaic

As an example, the difference between theory and practice is shown in two crosstabulations of mosaic by colposcopic impression (Table 6.8) and mosaic by biopsy diagnosis (Table 6.9) respectively.
The diagnostic indices of irregular mosaicism and of the other key criteria used for the exclusion of CIN III and invasive disease, are shown in Table 6.13. Using this separating value, the prevalence of "disease" is 25.8% (78 /302) and the pre-test probability of a negative result is 74.2% (100-24.8).

Table 6.8. Irregular or regular mosaic and colposcopic impression (N = 302).

Mosaic	Colposcopic impression					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
irregular	0	0	2	31	5	38
regular	2	37	59	19	1	118
none	45	46	27	15	13	146
Total (n)	47	83	88	65	19	302

Table 6.9. Irregular or regular mosaic and biopsy diagnosis (N = 302).

Mosaic diagnosis	Biopsy diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
irregular	0	0	6	28	4	38
regular	6	34	59	17	2	118
none	45	40	34	16	11	146
Total (n)	51	74	99	61	17	302

Irregular punctation

The overall distribution of punctation is shown in Table 6.4. Irregular punctation was seen exclusively in 44 patients with a colposcopic impression of CIN III or invasive disease. Only one prediction of CIN III was corrected by a biopsy diagnosis of CIN II. The relationship between the characteristics of punctation and the biopsy diagnosis is demonstrated in Table 6.10.

Table 6.10. Irregular or regular punctation and biopsy diagnosis (N = 302).

Punctation diagnosis	Biopsy diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
irregular	0	0	1	34	9	49
regular	18	53	79	22	1	173
none	33	21	19	5	7	85
Total (n)	51	74	99	61	17	302

Strong reaction to acetic acid

The criterion was "strongly white epithelium", seen after the application of acetic acid (Table 6.11).

Table 6.11. Epithelial reaction to acetic acid and biopsy diagnosis (N = 302).

Reaction	Biopsy diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
strongly white	1	3	12	49	11	76
normal white	28	71	87	12	4	202
none	22	0	0	0	2	24
Total (n)	51	74	99	61	17	302

Coarse mosaic and coarse punctation

An irregular mosaic was not always composed of coarse vessels, and an irregular punctate pattern was neither. However, the differences in distribution of the characteristics of the respective findings were minimal, when compared with the biopsy diagnosis. For the sake of briefness, rather than present extra tables, the diaganostic indices are mentioned in Table 6.13.

Increased intercapillary distance

This criterion could be judged in all patients. The results are shown in Table 6.12.

Table 6.12. Intercapillary distance and biopsy diagnosis (N = 302).

Distance	Biopsy diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
increased	3*	0	9	45	16	73
normal	48	74	90	16	1	229
Total (n)	51	74	99	61	17	302

*3 cases with condyloma only.

The calculated diagnostic indices of the key criteria for exclusion of CIN III and invasive disease are summarized in Table 6.13. The interrelationship between the criteria for this definition of disease was separately analyzed (section 6.5).

Table 6.13. Diagnostic indices of exclusion criteria for CIN III and (micro)invasive carcinoma (N = 302; prevalence of "disease": 25.8%).

Criterion	Sensitivity	Specificity	PV+	PV-
Mosaic				
irregular	41%	97.3%	84%	82.6%
coarse	37%	91.5%	60%	80.7%
Punctation				
irregular	55%	99.6%	98%	86.4%
coarse	72%	86.2%	64%	89.8%
Increased intercapillary distance	78%	94.6%	84%	92.6%
Strongly white epithelium	77%	92.8%	79%	92.0%

6.4.3.3 Exclusion of CIN II, CIN III and invasive disease

Demarcation

In 50% of cases a circumscript lesion was seen. The biopsy diagnosis was negative in 4 of these 151 cases. Negative histopathology was predicted in 43 out of 151 cases with a non-demarcated "lesion". The biopsy diagnosis added an extra 2 cases to this category (Table 6.14).

Table 6.14. Demarcation and biopsy diagnosis (N = 320).

Demarcation	Biopsy diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
Well-demarcated	6	26	57	52	10	151
Not demarcated	45	48	42	9	7	151
Total (n)	55	74	99	61	17	302

Diagnostic indices of a well-demarcated lesion, when "disease" is CIN II, CIN III and (micro)invasive carcinoma:
sensitivity 67.2%; specificity 74.4%; PV+ 78.8%, compared with a prevalence of 58.6%; PV- 61.6%, compared with a pre-test probability of a negative result of 41.4%.
When the separating value is placed at CIN I, the sensitivity is 57.7% and the specificity 88.2%. The predictive values however, do not gain by this, because the predictive value of a positive result would be 96%, but has to be compared with a prevalence of 83.1% (251/302), and the predictive value of a negative result would be 29.8%, compared with a pre-test probability negative of 16.9%.

6.4.3.4 Exclusion of CIN I, CIN II, CIN III and invasive diseases

White epithelium

The diagnostic indices of white epithelium can be calculated from Table 6.11. When disease is defined as CIN I or more, the indices of the overall presence of white epithelium are:
sensitivity 99.2%; specificity 43.1%; PV+ 89.6% (prevalence 83.1%); PV- 92% (pre-test probability negative 16.9%). The low specificity of the presence of white epithelium is caused by the high number of false positives: 29 cases, of which only one with strongly white epithelium.
The exclusion of CIN and invasive disease, based on the absence of white epithelium would have a specificity of 99.2% for negative histopathology. That this does not preclude the presence of invasive carcinoma is shown in Tables 6.4 and 6.11. These two cases of invasive disease could not easily be missed, because atypical vessels were present and there was surface ulceration. The necrotic tissue does not stain with either acetic acid or Schiller's solution.

6.4.4 *Differential diagnostic criteria*

Irregular surface contour

An irregular surface countour was used as a differential diagnostic criterion for CIN III and invasive disease. Since it was postulated to be closely related to the reaction to acetic acid (291), and by some authors used only after acetic acid application (131), the results are given in combination with the epithelial reaction to this test (Table 6.15).

Table 6.15. Surface contour and white epithelium related to biopsy diagnosis (N = 302).

Surface / whiteness	Biopsy diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
Irreg* /strong	0	1	2	27	9	39
Flat /strong	1	2	10	22	2	37
Irreg. /normal	3	1	4	3	3	14
Flat /normal	25	70	83	9	1	188
Irreg. /none	0	0	0	0	2	2
Flat /none	22	0	0	0	0	22
Total (n)	51	74	99	61	17	302

*Irreg. = irregular surface contour.

The table indicates the influence of the acetic acid reaction. To illustrate this, the diagnostic indices for both the irregular and the flat surface contour are given, in patients with strongly white epithelium.

Diagnostic indices of an irregular surface contour in patients with strongly white epithelium, when "disease" is CIN III and (micro)invasive carcinoma: sensitivity 46%; specificity 81%, PV+ 92% (prevalence: 25.8%); PV- 81% (pre-test probability negative: 74.2%).
The diagnostic indices of a flat surface contour (which would be compatible with CIN II or less) under similar conditions are: sensitivity 65%; specificity 94.2%; PV+ 65%; PV- 79.6%.

Size of a lesion

The size of a lesion was recorded in relation to its circumferential extent. The results are shown in Table 6.16. The patients with negative histopathology are excluded (51 cases). Of the remaining 251 patients, 194 had satisfactory colposcopy. The number of patients per category with unsatisfactory colposcopy is listed.

Table 6.16. Circumferential extent and biopsy diagnosis (N = 251).

Extent	Biopsy diagnosis				
	CIN I	CIN II	CIN III	INV	Total (n)
< 25%	15	10	0	0	25
25%	9	10	6	0	25
50%	16	17	14	0	47
75%	1	12	3	2	18
100%	33	50	38	15	136
Total (n)	74	99	61	17	251
Unsatisfactory colposcopy:	4	17	21	15	57

It should be noted that the size mentioned stands for the total size of a given lesion. The 17 patients with invasive disease all had a lesion that covered at least 75% of the circumference. The fact that CIN I biopsies were obtained from 33 lesions with a 100% circumferential extent, already indicates a low specificity of this parameter. The diagnostic indices depend on the separating value. For the lesions of 75 to 100%, the sensitivity is 100% when "disease" is (micro)invasive carcinoma only, and the specificity 41.5%. For the separating value of CIN III ("disease" is CIN III and more), sensitivity is 74.3% and specificity is 44.5%, with a PV+ of 37.7% (prevalence 31.1%) and a PV- of 79.4% (pre-test probability negative: 68.9%). Of the lesions with an extent of 50% or less, none showed invasive disease. The focal lesions of less than 25% were all compatible with CIN II or CIN I. There were 18 lesions (only 75% or 100% circumferential extent) with an extention into the vaginal fornices (7.2% of total cases). Three lesions were limited to the endocervix, all three had a colposcopic impression of CIN II and CIN II biopsies. One of these lesions was a small focal lesion and colposcopy was satisfactory in this case.

Elevated glandopenings

Two types of atypical glandopenings were derived from the 5 types mentioned by Kishi et al. (283). Although strongly related to white epithelium, these authors postulated the atypical glandopenings to have their own merit as a diagnostic criterion, also in cases without white epithelium. The elevated and usually oval shaped type (Type III of Kishi et al.) was used as a differential diagnostic criterion for (the presence of) CIN III and invasive disease. Elevated glandopenings were seen in 54 patients. In 134 patients the glandopenings were nicely round and unelevated, in 114 cases no atypical glandopenings were seen. The distribution in our material, in relation to the biopsy diagnosis is shown in Table 6.17.

Table 6.17. Types of glandopenings and biopsy diagnosis (N = 320).

Glandopenings	Biopsy diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
Elevated	2	1	12	31	8	54
Unelevated	11	36	66	20	1	134
Not atypical	38	37	21	10	8	114
Total (n)	51	74	99	61	17	302

Diagnostic indices for elevated atypical glandopenings, when "disease" is CIN III and (micro)invasive carcinoma:
sensitivity 50%; specificity 93.3%; PV+ 72.2% (prevalence: 25.8%); PV- 84.3% (pre-test probability negative: 74.2%).

6.5 Computer analysis of criteria used to separate CIN II from CIN III

The decision to schedule a patient for additional surgery was based on the indications mentioned under Methods in the previous chapter.
Although the biopsy diagnosis was the main determinant, cytology played an important role as well, in retrospect largely due to its tendency to overdiagnose. An assessment was made of what would have happened if the decision to operate were based on the capacity of the colposcopic impression alone to separate "CIN II and less" from "CIN III and more". A retrospective computer analysis was carried out, which encompassed all of the key diagnostic criteria, the differential diagnostic criteria and patient characteristics such as age and satisfactory /unsatisfactory colposcopy. The patient characteristics were included for two reasons. In this selected group of patients, age was found to be strongly related to the chance of having CIN III or more (Fisher-test: $p < 0.001$). Unsatisfactory colposcopy when controlled for age, also represented a greater chance of CIN III or more (Fisher-test: $p = 0.02$). All of these factors were weighed in a multivariate analysis (logistic regression analysis) of the capacity

to predict the chance of CIN III or more. As a result of this analysis, four "criteria" were found to be the most important predictors of CIN III or more:

age
strongly white epithelium
irregular punctation
increased intercapillary distance of mosaic

The weighed probability of the histopathologic outcome CIN III or more can be expressed by the logit of the chance:

$$\text{logit}(p) = \log(p / 1-p)$$

in which p signifies the chance of the patient having CIN III or more, based on the colposcopic impression, construed with the help of the four weighed criteria only. Transferred to the practical situation: the decision to operate would be taken if the chance of the patient having CIN III or more would be high enough. The four predictors were used to estimate the chance per patient. For the presentation of the results of the computer, replacing the colposcopist, the biopsy diagnoses are divided into two groups: CIN II or less and CIN III or more. The probabilities, based on the prediction of the computerized colposcopic prediction, are divided into three categories: one in which the weighed probability of the histopathologic outcome CIN III or more is 10% or less, one in which this would be between 10 and 90% and a third category in which it would be 90% or more. Assuming that a probability of more than 10%, of having CIN III or invasive disease would lead to additional surgery, the results are as shown in Table 6.18.

Table 6.18. Computer prediction of the probability of having CIN III or (micro)invasive carcinoma, compared with the actual histopathologic outcome (N = 302).

Probability of CIN III or more	Biopsy diagnosis		
	CIN II or less	CIN III or more	Total (n)
$p \leqslant 10\%$	180	9	189
$10\% < p < 90\%$	43	27	70
$p \geqslant 90\%$	1	42	43
Total	224	78	302

If the decision to operate would have been based on the colposcopic impression, using the four weighed criteria only, nine of the 189 patients (5%) with a chance of 10% or less of having CIN III or more, would be "underdiagnosed" and not have been operated. This is 12% of the cases with a biopsy diagnosis of CIN III or more. On the other hand, of those who had a chance of 90% or more of having CIN III or more (43 patients), one patient would have been operated, but the biopsy diagnosis would have been CIN II or less. This would have occurred in 0.5% of cases with CIN II or less. Based on the colposcopic

impression that was used in the present study, four patients out of 218 (1.8%) with a prediction of CIN II or less were underdiagnosed (Table 6.7), which is 5% of cases with an actual biopsy diagnosis of CIN III or more. Ten patients with an actual biopsy diagnosis of CIN II were overdiagnosed, which is 4.5% of cases with a biopsy diagnosis of CIN II or less, and 12% of cases with a colposcopic impression of CIN III or more. Thus, the colposcopic impression that was actually used, represented a more prudent approach if management would have been based on it, than when management would have been based on the four weighed criteria. This would also be true when the probabilities are put at the 5% and the 95% level for the decision to operate or not. Underdiagnosis would still have occurred in 6% of cases. The one case that was overdiagnosed had a chance of over 95% of having CIN III or more, so the percentage would be the same. In addition to this, the mathematical model does not give an advice in the cases with a probability of between 10 and 90%, although 27 patients had a biopsy diagnosis of CIN III or more.

6.6 Discussion

The construction of a colposcopic impression as a prediction of the histopathologic outcome of a given lesion may fail in accuracy for several reasons. When the prediction is based on the presence of colposcopic findings and not on the more detailed characteristics of these findings, the result is a "malignancy index" of the findings, as usually reported in screening colposcopy (28,91). Two findings could lead to diagnostic accuracy. One is atypical vessels, which is a diagnostic criterion in its own right. The other finding is white epithelium, which, as demonstrated in our study, is of use to separate the normal from the abnormal, when its absence is used to signify normality.
When the grading depends on the criteria used for the "ATZ-score", diagnostic accuracy is not the main goal. This system was designed to separate insignificant lesions from significant and highly significant lesions. Used in the present study as a backup system, it did exactly that, albeit that a personal interpretation may have played a part in this, as it did when others used this system (250).

The comparison of our grading results to those of others is hampered by the factors mentioned in previous chapters. At first glance, our results would seem to compare favorably with those of others, but a closer look shows this not to be true for all the categories. This is demonstrated by a comparison with the results mentioned in two reports.
Benedet et al. (41) had an overall exact agreement between the colposcopic impression and the biopsy diagnosis of 72%. In the present study this was almost 83% (see Table 6.7). However, Benedet et al. gave a far better prediction of histopathology in the higher graded lesions than in the lower graded ones: negative histopathology was accurately predicted in 53% of cases, CIN I in 39%, and CIN II in 32%. The figures in the present study were 84%, 88% and 76% respectively. The prediction of CIN III was accurate in the study of Benedet et al. in 93% of cases and (micro)invasion, found in the biopsies, was predicted in 8 out of 8 cases. We found 87% for the prediction of CIN III and correctly

predicted 14 out of 17 of the biopsy diagnoses of (micro)invasion. Benedet et al. further mentioned that their criteria were based on the recommendations of Coppleson et al. and those of Kolstad and Stafl (see Chapter 4).
Javaheri and Fejgin (250) used the same criteria, but took CIN I and CIN II together as one group. When our results are recalculated from Table 6.7 to match this, the comparison shows almost identical percentages. (Our percentages are given in parenthesis). Javaheri and Fejgin had the following results: negative histopathology was accurately predicted in 88% (84%) of cases, CIN I-II in 86% (92%), and CIN III in 96% (87%). Invasion, listed as suspicious invasive, was predicted in 3 out of 3 cases (14 out of 17 in our study), invasion being overdiagnosed in 2 out of 5 cases with a colposcopic impression of invasive disease (5 out of 19 cases in our study).

Of the key diagnostic criteria, atypical vessels performed the best as a "test". Sensitivity and specificity were both high and in balance. The fact that dubious vessels might present a problem, could have been anticipated, considering the warning of Stafl (506), who labeled atypical vessels as the hardest category to interpret. In retrospect, the colpophotograph of the patient with the CIN II biopsy diagnosis and the colposcopic impression of invasion, was interpreted as showing vessels that were out of the ordinary, but lacked irregular shapes and calibre changes. Experience would have prevented this mistake.
The criteria for the exclusion of CIN III or more performed well, one better than the other, but in general with a high enough specificity to be a reliable criterion for exclusion. Irregularity had a higher specificity than coarsness of the vessels in mosaic and punctation, and higher predictive values as well. This is in agreement with the comment of Coppleson et al. (133), who considered irregularity of spacing the most important vascular characteristic. Manifestations of viral disease influenced the interpretation of the punctate pattern, because coarsness of the vessels is compatible with some types of condylomata (see also Wijnen, Chapter 7 in this thesis).
The exclusion of CIN II or more, depending on the demarcation of a lesion, would have had a higher specificity with the separating value at CIN I, but the predictive values would have shown less gain compared with the pre-test probabilities. The results agree with the observations of others who stated that CIN I is not usually demarcated from the surrounding tissue, whereas CIN II is (530).
That the presence of white epithelium is a highly sensitive parameter, came as no surprise. In retrospect, some mistakes were due to the misinterpretation of metaplastic whiteness, which should not have been interpreted as white epithelium for its quick disappearance (315). Of the differential diagnostic criteria, irregular surface contour and elevated atypical glandopenings performed well enough as a test for the category they were meant for, but the interpretation of the surface contour was influenced by the surface changes due to condylomata, which become more white after acetic acid application, depending on the type. The separation between atypical and normal glandopenings was mainly dependent on the acetic acid reaction as well. We did not see the many cases that Kishi et al. (283) reported in their large series, in which atypical glands were judged

without white epithelium being present. These authors added a special subtype of the type IV glandopenings to their original scoring system, type WE to signify type IV seen in white epithelium. In their analysis however, type WE was again included in the overall type IV category, and the figures on prevalence of types V, VI and/or WE in patients with CIN III or microinvasive carcinoma were not significantly different.
The circumferential extent of a lesion has a high sensitivity for the more serious lesions that cover most of the portio but the specificity is low due to the high amount of circular CIN I and CIN II lesions in our series. Another reason for the limited value of this parameter is the interrelation between surface distribution, age and unsatisfactory colposcopy.
An interrelation was also found for other factors, as shown by the results of a limited computer analysis. The computer results are considered a start, not an endpoint. The mathematical model will have to be tested in another study, before definitive conclusions can be drawn. In the present study the "disturbance" of factors such as the counterparts of the key criteria were neglected as much as possible. The provisionary results of the computer analysis have stimulated our belief that further limitations of the number of criteria might be possible. Coarsness of the vessels of mosaic and punctation, postulated as specific criteria for CIN III or more (37) would be one of the first criteria to be stricken from the list.

6.7 Conclusions

In selective colposcopy and under special circumstances, the histopathologic outcome of colposcopically directed biopsies can be predicted with greater accuracy than generally reported in the literature. The reproducibility of histopathologic diagnoses can be high enough to attempt accurate diagnosing.

Chapter 7

RESULTS OF ROUTINE COLPOSCOPIC EVALUATION

J.A. Wijnen

7.1 Introduction

In January 1981, the present author joined the colposcopy clinic of the Erasmus University Hospital Dijkzigt, Department of Gynecology, after a 2 year training experience in a large colposcopy clinic*, which was open one day per week for consultation to patients with abnormal PAP-smears referred by regional gynecologists.
Part of this program was a weekly multidisciplinary "correlation"-session where cytologic, colposcopic and histopathologic results of each patient were presented and visually demonstrated. Frequently there was a striking discrepancy between the expected grade of cervical intraepithelial neoplasia (CIN) in cytology and the final histopathologic grade of CIN, which in many cases appeared to be less severe.
Several reports in the early seventies (41,157,501,542) have documented the advantages and limitations of colposcopy and attempted to establish criteria for its use. Nevertheless, in the early eighties most gynecologists were not familiar with routine colposcopy and there was a tendency to perform a diagnostic cone biopsy when cytology reports suggested a high grade cervical intraepithelial lesion and to skip colposcopy.
This policy resulted in a large number of exconisations, not seldom in young women who after histopathologic examination of the cone specimen appeared to have only minor degrees of CIN.
Based on the correlation experiences, the impression of the present author was, that routine colposcopy in patients with abnormal cervical PAP-smears for a colposcopist not only could serve to direct the biopsy forceps to the most suspicious lesions, it also seemed possible to predict by colposcopic impression the histopathologic grade of CIN at least as accurately as the cytologist could do. This opinion seemed to be supported by figures on the reliability and reproducibility of the other subjective diagnostic modalities used in the diagnosis and grading of cervical intraepithelial neoplasia and cervical viral infections. In reviewing the validity of the PAP-test as primary screening tool in the diagnosis of CIN and invasive cervical carcinoma, Kwikkel (302) reported that false negative rates varying between 0.5 and 45% have been published and false positive rates in a similar variation.
Kern and Zivolich (273) found that pathologists reproduced their own cytologic classification within one category of variance (comparable with grades of CIN) in 94% of cases when slides were reviewed on two separate occasions.
Vooys et al. (566) did not find significant differences in the inter- or intra-observer results in an overall quality control of their cytology reports, but they

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did not report on differences in individual patients. Bellina et al. (39,40) in testing the reproducibility of the histopathologic diagnosis of grades of CIN, found a 50% "inter-pathologist" agreement and in 81% agreement within one grade of variance. Seventy-one percent of the first and second diagnosis of the same pathologist were identical, and 93% of pairs were within one CIN grade. Ringsted et al. (447) reported intra-observer agreement as low as 68% (range 59-74%) and inter-observer agreement as low as 51% (range 42-67%).
These observations seem to make the value of reviewing cytologic or histopathologic slides as to grading CIN by routine microscopic examination, of relative importance.

Another problem in the evaluation of patients with abnormal PAP-smears in 1981 was, and still is, the recognition of the coincidence of human papilloma virus (HPV) infection of the uterine cervix and CIN. Human papilloma virus comprises a heterogeneous group of viruses, of which presently many types and subtypes have been known to induce squamous cell papillomas in various anatomic regions, including the uterine cervix, of humans (523).
At the time this study was performed, it was becoming apparent that HPV infections could have an etiologic role in the evolution of CIN and cervical carcinoma (224,524).
Cytologic criteria for the identification of infection of the uterine cervix with human papilloma virus were described by two teams working independently in Canada (340) and in Finland (415). Before these reports, wart virus infections of the uterine cervix had been thought to be rare (380,416). Using the new diagnostic criteria Meisels et al. (341,343) showed that 70% of lesions originally thought to be low-grade CIN were attributable to the effects of human papilloma virus. They suggested that condyloma may be involved in the initial developmental steps of CIN.
This apparent increase in prevalence, respectively recognition of HPV-infections (54,57,332,492) has been confirmed by colposcopic studies which revealed lesions on the cervix, not visible to the naked eye.
The *colposcopic changes* suggestive of papilloma virus infection were first described by Meisels et al. (341). The typical mature condyloma acuminatum, usually diagnosed with the naked eye as a papillomatous lesion, is colposcopically visualized as a thick white epithelium, sharply demarcated from the surrounding tissue. The surface is irregular with finger-like projections, in each of which a regular capillary loop can be observed after the acetic acid effect has worn out, and this is the most decisive diagnostic feature. These lesions may be located on the cerivix and/or vagina and vulva and are usually multiple. They rarely offer difficulties to the experienced colposcopist but they must be differentiated from an invasive cancer, which shows an irregular surface contour without the typical capillary loops.
In addition to this lesion Meisels et al. (341) reported colposcopic evidence of "early condyloma" and "flat warts" which they considered to be clinically indistinguishable from the lesions of cervical intraepithelial neoplasia. Most of these lesions are neither preceded, nor followed by overt condyloma formation. Colposcopically the early condyloma appears as a white epithelium with an irregular surface, called "asperities" (341,460). Vessels are rarely visible at this

stage, but when they are, the lesion resembles punctation with the red dot located in the center of the asperity. This lesion must always be differentiated from early invasive cancer. The latter shows atypical vessels and contact bleeding. Sometimes, the grape-like structure of columnar epithelium may resemble asperities, especially when inflammation is present.
The most difficult type to identify with the colposcope is the flat condyloma (341). When located in the transformation zone it can not be differentiated from mature metaplasia or CIN. Flat condylomata show an aceto-white epithelium, a flat surface and more or less sharp borders and mosaic-like patterns. When pronounced mosaic and/or punctation are present in the flat white epithelium, CIN is suspected and only a directed biopsy can offer the diagnosis. These lesions also occur outside the transformation zone which makes it easier to identify those flat condylomata.
Histologic criteria for the diagnosis of papilloma virus lesions of the cervix have been described (341,431,432). The flat condyloma or according to Reid (432): "subclinical papilloma virus infection" or "non-condylomatous cervical wart virus infection" (NCWVI) usually can be distinguished histologically by the presence of cells with a striking perinuclear space, which are variously called koilocytotic, halo or balloon cells. These were originally described by Koss and Durfee (292) in a description of cervical koilocytotic atypia. The nucleus is often round and pycnotic; binucleate forms are not uncommon. Usually there is a dense peripheral cytoplasm. One of the most important findings is the frequent occurrence of associated atypia, which, though generally mild, may sometimes be difficult to distinguish from all grades of CIN, including CIS.
The importance of all this is, that these lesions can be mistaken by exfoliative cytology as well as by colposcopy and histopathology for CIN and can therefore lead to inappropriate therapy.

7.2 Objectives of the study

1. The first aim is to determine the diagnostic accuracy of the colposcopic "impression" of the author as to the grade of CIN in the routine evaluation of patients referred with cervical smears suggestive of CIN. This impression will be compared with the histopathologic grade of CIN diagnosed on colposcopically directed biopsies and endocervical curettings as the final "standard"; with the highest suspected grade of CIN in cytology reports of the preceding 6 months, including the cytology repeated at the colposcopy clinic ("maximal-cytology") and with the results of the "repeat-cytology" at first visit to the colposcopy clinic.
2. The second aim is to determine the accuracy of the colposcopic impression of the author in predicting the presence of human papilloma virus infection in these patients. This impression will be compared with the diagnosis of viral infection in colposcopically directed biopsies and endocervical curettings as the final "standard", and with the cytology reports.

7.3 Patients and methods

163 women referred between January 1981 and January 1983 to the outpatient colposcopy clinic of the Department of Gynecologic Oncology of the Erasmus University Hospital Dijkzigt Rotterdam, for the evaluation of cervical smears suggestive of CIN, were selected for this study according to the following criteria:

Inclusion Criteria

1. Suspicion of any grade of CIN in endo- and/or ectocervical cytology smears of the 6 months preceding to referral to the colposcopy clinic.
2. Complete outpatient evaluation by the author, including completion of essential medical history, colposcopy, repeat cytology, documentation of a colposcopic impression of the anticipated histopathologic grade of CIN and/or cervical viral infections; and collection of colposcopically directed tissue specimens for histopathologic examination.

Exclusion Criteria

1. Present pregnancy.
2. Treatment of CIN with any excisional or destructive method in the preceding 6 months.
3. Cytologic evidence of invasive cancer.
4. Colposcopic or clinical suspicion of the presence of an invasive cervical carcinoma at first visit to the colposcopy clinic or absence of any colposcopically suspect finding except cervical viral infection.

Patients under exclusion criteria 3 and 4 were usually not completely evaluated in the outpatient colposcopy clinic and their results could interfere with the first objective of the study. Therefore they are not included.

The cytologically expected most severe grade of CIN and presence of viral infection before referral to the colposcopy clinic are presented in Table 7.3.

Table 7.3. Cytologic grade of CIN and presence of viral infection as reported in the six months preceding to colposcopy.

Cytologic grade of CIN	n	(%)	Viral infection n	(%)
CIN I	25	(15)	3	
CIN II	89	(55)	16	
CIN III	49	(30)	7	
Total	163	(100)	26	(16)

The procedure and techniques used in the evaluation of patients with abnormal cervical smears have been described previously by the author (588).
Briefly: with the patient in lithotomy position the external genitalia and anal area are inspected. A Trelat speculum of appropriate size is inserted in the vagina

Addendum 7.I.

ACADEMISCH ZIEKENHUIS ROTTERDAM DIJKZIGT
ONCOLOGISCHE GYNAECOLOGIE.
KOLPOSKOPIE

geg. pat.

Datum:

Onderzoeker:

Indicatie:

Poliklinisch/klinisch

leeftijd	ras	zw. schap	pariteit	abortus	menarche	sexarche	partner	kinderw.
anticonc.	L.M.	grav./am.						

bijzonderheden

voorgeschiedenis

datum	CYTOLOGIE+LAB.-NR.	KOLPOSKOPIE	HISTOLOGIE+LAB.NR.	THERAPIE

BESCHRIJVING KOLPOSKOPIE

Macrosc. aspect:
Kolposkopie:
SCHETS STATUS LOCALIS

LOCALISATIE AFWIJKING:

- Cytologie
- Proefexcisie(s)
- E.C.C.
- Div.
- Foto

S.C.J.
Bovengrens lesie: zichtbaar/niet zichtbaar
Afmeting lesie:
horizontaal mm.
verticaal mm.

KOLPOSKOPISCHE IMPRESSIE

CORRELATIE CYTOLOGIE KOLPOSCOPIE HISTOLOGIE

BEHANDELING

Event. HISTOLOGIE

Datum:

Volgende controle: maand: , jaar:

and the entire vagina and portio are inspected. The Zeiss Opmi-1 colposcope is installed and focussed (objective f: 300 mm) and under direct vision the portio and vagina are gently cleaned with normal saline on a sponge stick. For comparison separate cytology smears of the endocervix and a pancervical scraping are taken with respectively a saline moistened small cotton-tip applicator and a modified, also moistened, wooden Ayre spatula and send to the hospital cytology laboratory. Following this, the portio and vaginal fornices are gently rinsed with a 3% acetic acid solution and carefully inspected again. Visibility of the entire squamocolumnar junction and lesion(s) are noted. The maximal distances between the borders of the squamocolumnar junction or lesion(s) extending outside this junction were measured in two perpendicular (mostly horizontal and vertical) planes with a calibrated ruler and used for calculation of the surface of the transformation zone ($\pi \times H/2 \times V/2$).
Inspection of the endocervical canal is facilitated by the use of two small moistened cotton-tip applicators or an endocervical speculum (Kogan). According to the standard colposcopic criteria as described in chapter 4.3 (503) colposcopic and if present other findings are noticed and later documented (addendum 7.I). Based on the presence and severity of these standard colposcopic criteria (503) and characteristics of viral infections (341) a colposcopic impression of the expected histopathologic grade of CIN and the presence of a viral infection are documented. Schillers' iodinetest is used in selected cases.
Colposcopically directed punch biopsies to a depth of at least 5 mm are taken from the most suspicious areas with an elongated disposable biopsy punch (Stiefel laboratories) with a diameter of 3 or 4 mm, and separated from the underlying tissue with a Berger forceps.
Tissue samples are placed on a piece of non-absorbant paper with the epithelial surface downward for better orientation, placed in a 10% neutral buffered formaline solution for fixation and sent for histopathologic examination to the gynecologic pathologist of the hospital department of histopathology with standard questions: CIN? Grade? Invasion? Viral infection?
Based on experience, if invasive carcinoma seems to be unlikely and colposcopy is unsatisfactory while a diagnostic cone biosy does not seem to be immediately mandatory, in addition an endocervical curettage with a Kevorkian curette is performed in the same outpatient session without anaesthesia. This is also done in most CIN III lesions when conservative therapy seems to be allowed; and when atypical cylindric epithelium is suspected. The curettings are collected on non-absorbant paper that is immersed in formaline. If (micro-)invasive carcinoma is suspected, only directed biopsies are taken and if these would appear to be negative for invasion, a diagnostic exconization of the entire lesion and transformation zone is considered to be mandatory.
The histopathologic diagnosis and CIN-grade of the most significant lesion of all tissue specimens including the endocervical curettings, will serve as the accepted final "standard" in all cases.

Methods of Analysis

All data from the protocol sheets were coded and prepared for computer analysis (VAX/VMS) and statistics (SPSS-X; Statistical Package for the Social Sciences).

In the present studies (Chapter 7, 9 and 10), grades of CIN were considered as categorical or semiquantitative variables. To test the observed frequencies on statistical significance the χ^2-test was used. In measurement of changes, e.g. CIN measured at different time intervals or before and after treatment, the "law of initial values" has to be taken into account. This is the fact that the initial value will co-determine the maximal possible changes. For qualitative and semi-quantitative variables (e.g. the CIN-grading system) this pitfall can be avoided by applying the χ^2 test per level of the variables at the initial measurement between the two groups. Subsequently all χ^2-values are added and so are the degrees of freedom. The presence of a linear trend per level and over all levels of the initial measurement is then traced.
To estimate the level of pairwise agreement between diagnostic variables as colposcopy, cytology and histopathology, weighted Kappa coefficients with linear agreement weights and standard errors were calculated. The weighted Kappa (Kw) coefficient measures the agreement between each pair of variables, with adjustment for the agreement to be expected by chance. The agreement expected by chance increases, to a certain degree, with the number of observations in a certain category. Therefore the weighted Kappa coefficient is not necessarily high if the majority of observations are situated within one category.
The weighted kappa is calculated as follows:

$$Kw = \frac{Po_w - Pe_w}{1 - Pe_w}$$

Kw = weighted Kappa
Po = weighted observed fraction of agreement
Pe = weighted expected fraction of agreement on a base of independency.

For perfect agreement the value of weighted Kappa = 1.00; the value for agreement by chance equals 0.00. Negative values are obtained when the observed agreement is less than the expected agreement (161,183).

For continuous variables the Mann-Whitney U-test was used to test on statistical significance.

All tests were performed two-sided at a fixed significance level of $\alpha = 0.05$.

The computer- and statistical analyses were performed on advice and under guidance of Dr. H.J. Duivenvoorden.

Results

7.4 Patient characteristics

The characteristics of the patients at referral to the colposcopy clinic are presented in the following paragraphs (7.4.1 to 7.4.4).

7.4.1 *Age*

Table 7.4.1. Age (years)

N =	163
Median*	30.0
Interquartile range**	27-35
Range***	19-60

*Median: the halfway or middle observation of the sample.
**Interquartile range: the first and last 25% points of the ordered sample
***Range: minimum-maximum.

7.4.2 *Marital status*

Table 7.4.2. Marital status.

	n	%
stable relation	96	(59)
single	38	(23)
separated	28	(17)
unknown	1	(1)
Total	163	(100)

7.4.3 The *Obstetrical history* is presented in Table 7.4.3.

Table 7.4.3. Obstetrical history

	Pregnancies		Parity	
Nr.	n	(%)	n	(%)
0	37	(23)	56	(34)
1-2	85	(52)	83	(51)
3-4	31	(19)	16	(10)
⩾5	9	(6)	7	(4)
unknown	1	(1)	1	(1)
Total	163	(100)	163	(100)

7.4.4 *Contraceptive methods* as used in the study-group are represented in Table 7.4.4.

Table 7.4.4. Contraception

	n	(%)
None	42	(26)
Hormonal contraception	67	(39)
IUD	19	(12)
Protectives/pessary	9	(6)
Sterilization	26	(16)
Total	163	(100)

Considering these patient characteristics:
When patients were separated according to satisfactory (n=110) or unsatisfactory (n=53) colposcopy (see 7.6.2), there were statistically no significant differences with regard to

Age	(Mann-Whitney U-test;	$p = ns$)
Marital status	(„ „ „ ;	$p = ns$)
Obstetrical history	(„ „ „ ;	$p = ns$)
and Contraception	(„ „ „ ;	$p = ns$)

When patients were separated according to the presence or absence of viral infection in either cytology, colposcopy, or histopathology (see 7.5 to 7.7), the patients who were diagnosed to have a cervical viral infection (n=82) were significantly younger (median 29 yrs; interquartile range 26-32 yrs) than patients who were not suspect to have a cervical viral infection (n=81); median 32 yrs; interquartile range 28-36 yrs).
(Mann-Whitney U-test $0.001 < p \leqslant 0.01$).

In these viral and non-viral groups there were no significant differences in

- Marital status	(Mann-Whitney U-test;	$p = ns$)
- Obstetrical status	(„ „ „ ;	$p = ns$)
- Contraception	(„ „ „ ;	$p = ns$)

There was no relation between age and cytologic grade of CIN at referral to the colposcopy clinic.

7.5 Results of cytology

When the maximal cytologic grade of CIN in the six months preceding to colposcopy, inclusive the "repeat-cytology" ("maximal-cytology") is compared with the CIN-grade in the endo- and ectocervical smear that was taken at first visit to the colposcopy clinic ("repeat-cytology"), the following results are obtained.

Table 7.5. Results of "maximal cytology" compared with "repeat-cytology".

Maximal cytology	Repeat cytology					
Grade of CIN	Neg.	I	II	III	n	Total (%)
I	2	19			21	(13)
II	2	15	70		87	(53)
III	4	5	20	26	55	(34)
Total	8	39	90	26	163	
(%)	(5)	(24)	(55)	(16)		(100)

Although there is a significant agreement in prediction, ($\chi^2 = 126.9$; d.f. = 9; $p \ll 0.001$; Kw = .51) there is a considerable "overdiagnosis" of the "maximal-cytology", compared with the "repeat-cytology".
As can be expected, there is no "overdiagnosis" by "repeat cytology", which is included in the "maximal-cytology".
When the most severe cytology result in the 6 months preceding to referral to the colposcopy clinic was compared per patient with the "repeat-cytology" taken at the first visit to the colposcopic clinic, there was agreement within the same grade of CIN in 64% of cases, 30% overdiagnosis and 6% underdiagnosis by referral cytology. In 5% of patients there was a difference of two grades of CIN and in 2% of patients a CIN-III smear at referral appeared to be negative in the repeat-cytology. ($\chi^2 = 81.1$; d.f. = 9; $p \ll 0.001$; Kw = .42). There was no significant difference in agreement between referral cytology and "maximal-cytology" versus "repeat-cytology". (Kw = .42 resp. Kw = .51).

7.6 Results of colposcopy

7.6.1 *Colposcopic impression*

In Table 7.6.1 the colposcopically expected grade of CIN and the colposcopic suspicion of the presence of a cervical viral infection (HPV) are presented.

Table 7.6.1. Colposcopic Impression

Grade of CIN	n	(%)	Viral infection suspected n	(%)
Viral infection only	4	(2)	4	
CIN I	52	(32)	23	
CIN II	96	(59)	36	
CIN III	11	(7)	7	
Total	163	(100)	70	(43)

In only 3 patients (1.8%) the classical condyloma acuminatum was present on the cervix; all 3 patients had CIN I-lesions. The other viral manifestations were "subclinical papilloma virus infections".

7.6.2 *Satisfactory Colposcopy*

In this situation there was not any doubt that with the use of the colposcope the entire squamocolumnar junction of the transformation zone as well as all the limits of the lesion(s) could be entirely visualized. In 110 patients (67%) colposcopy was satisfactory and in 53 patients (33%) unsatisfactory.

If only the visibility of the entire squamo-columnar junction was considered, 19% (31 of 163) of all colposcopies were unsatisfactory. If complete visibility of the entire lesion was the only criterium, colposcopy was unsatisfactory in 31.9%.

The percentage of patients with cervical viral infection diagnosed in any of the three modalities i.e. cytology, colposcopy and histopathology together, or separately, was statistically not significantly different in the satisfactory and unsatisfactory colposcopy group: Table 7.6.2.

Table 7.6.2. Suspicion in either cytology, colposcopy and histopathology of viral infection, separated for satisfactory and unsatisfactory colposcopy (n = 163).

Colposcopy	Viral infection present	Viral infection not present	total
satisfactory	56	54	110
unsatisfactory	25	28	53
Total	81	82	163

$\chi^2 = 0.08$ (Yates' correction); d.f. = 1; p = n.s.).

7.6.3 *Surface of the transformation zone*

Table 7.6.3. Surface of the transformation zone (mm^2).

N =	163
Median	282
Interquartile range	175- 490
Range	28-1074

The surface of the transformation zone in patients with unsatisfactory colposcopy was significantly smaller than in patients with satisfactory colposcopy. (Mann-Whitney U-test; $0.01 < p \leqslant 0.02$). If patients were divided according to the presence of viral infection, there were no significant differences in the surface of the transformation zones (Mann-Whitney U-test; p = n.s.).

7.7 Results of histopathology

7.7.1 *Number of biopsies*

In Table 7.7.1 is presented the number of colposcopically directed biopsies taken per patient at the colposcopy clinic.

Table 7.7.1. Number of biopsies

Nr. of biopsies per patient	n	(%)
1	41	(25)
2	86	(53)
3	26	(16)
4	7	(4)
5	2	(1)
7	1	(1)
Total	163	(100)

There were no significant differences in the number of biopsies in patients with satisfactory and unsatisfactory colposcopies, neither in patients with or without suspicion of viral infection (Mann-Whitney U-test; p = n.s.).

7.7.2 *Histopathologic diagnosis*

The maximal grade of CIN and the presence of viral infection at histopathologic examination are presented in Table 7.7.2.

Table 7.7.2. Histopathologic grade of CIN and presence of viral infection.

Histopathologic diagnosis			viral infection	
Grade of CIN	n	(%)	n	(%)
NEG	9	(6)	4	
I	46	(28)	14	
II	81	(50)	21	
III	26	(16)	8	
CARC.	1	(1)	1	
Total	163	(100)	48	(29)

There appeared to be one patient with a histopathologic microinvasive carcinoma which was not identified as such by cytology and colposcopy (see paragraph 7.8 and 7.9).

7.7.3 *Endocervical Curettage* (ECC) performed at the colposcopy clinic

In 63 of 163 patients (38,7%) an ECC was performed for one of the reasons mentioned in the methods section, including all patients with unsatisfactory colposcopy. There were no differences in the percentage of ECCs' performed in relation to the presence or absence of cervical viral infection, in any of the three diagnostic methods.
($\chi^2 = 0.08$ (Yates' correction); d.f. = 1; p = n.s.).
In 4 patients the material was insufficient for histopathologic diagnosis (6,3%).
The histopathologic results of the other 59 ECC's:

Table 7.7.3. Histopathologic results of ECC.

			Viral infection present	
Grade of CIN	n	(%)	n	(%)
NEG	35	(59)	3	
CIN I	14	(24)	1	
CIN II	8	(14)	3	
CIN III	2	(3)		
Total	59	(100)	7	(12)

There was no patient with the histopathologic grade of CIN more advanced in the ECC than in the biopsies. In 17 of 59 patients the results of ECC were equal to those in the biopsies as far as grade of CIN concerned and in the remaining 42 patients less severe. In this selected material, where patients suspect for invasive carcinoma were excluded, ECC did not change the histopathologic grade of CIN.
The relation between satisfactory or unsatisfactory colposcopy and the histopathologic results of the EECs' will be presented in Chapter 7.12.
Sixteen of 48 patients with histopathologic evidence of viral infection had an ECC performed. In 7 of these 16 (44%) signs of a viral infection were present in the ECC. One of these patients had her viral infection diagnosed in the ECC (+CIN I), while histopathology of the colposcopically directed biopsies was negative for viral infection (CIN I). This patient had no signs of viral infection in the cytology (CIN II), but was colposcopically diagnosed as having a viral infection (+CIN II).

Agreement between diagnostic methods

Agreement and differences between cytology, colposcopy and histopathology as far as grade of CIN concerns, in the routine colposcopy clinic evaluation of selected patients with cervical smears suggestive of CIN.

7.8 Agreement with cytology

In Table 7.8.1 the relation is demonstrated between the "maximal" cytologic grade of CIN of each patient and the grade of CIN as determined by colposcopic impression and histopathologic diagnosis. In Table 7.8.3 the same is presented for the "repeat-cytology" at the colposcopy clinic.
In Table 7.8.2 the calculated differences in grades of CIN are demonstrated with the "maximal-cytology" as the standard, and in Table 7.8.4 with the "repeat-cytology" as the standard for each patient.
Although the overall prediction by cytology of the colposcopic grades of CIN was significantly correct, ($\chi^2 = 46.5$; d.f. $= 9$; $p \ll 0.001$) and also of the histopathologic grades ($\chi^2 = 79.4$; d.f. $= 45$; $0.001 < p \leqslant 0.01$),from Table 7.8.1 and 7.8.2, it is obvious that in this series of patients there generally was an overdiagnosis in grade of CIN by the "maximal-cytology" as compared with colposcopy as well as with histopathology, of resp. 50% and 46% (Table 7.8.2).
This is illustrated when the CIN-grades of the three diagnostic methods are tested on agreement with the use of the coefficient "Kappa" (see Methods of Analysis; 7.3).
For the agreement between "maximal-cytology" and colposcopy this will result in:

$$Kw = \frac{80.57\% - 74.41\%}{1 - 74.41\%} = 0.24,$$

which represents a significant, although not really substantial agreement. Kw for "maximal-cytology" and histopathology was 0.28; for "repeat-cytology" and colposcopy Kw = 0.34; and for "repeat-cytology" and histopathology Kw = 0.34; all representing a not very substantial agreement.

Complete agreement of "maximal-cytology" with colposcopy existed in 46% of cases and with histopathology in 49% of cases (Table 7.8.2). For the "repeat-cytology" these percentages were 57% and 55% respectively (Table 7.8.4). In these selected cases there was agreement within 1 grade (over- or underdiagnosis) of CIN between "maximal-cytology" and colposcopy in 95% of cases, and between "maximal-cytology" and histopathology in 90% of cases (Table 7.8.2). For the "repeat-cytology" these percentages were 97% and 93% respectively (Table 7.8.4).
Comparing the Kw-coefficients there were no significant differences between colposcopy and histopathology in their agreement with the cytologic grade of CIN, whether it was "maximal-cytology" or "repeat-cytology".
The overdiagnosis by "maximal-cytology" is illustrated in the 55 cases with cytologically CIN-III lesions. Only 19 of these 55 (35%) were confirmed by histopathology (Table 7.8.1). On the other hand 19 of 26 (73%) of histopathologic CIN-III cases were predicted correctly by "maximal-cytology" (Table 7.8.1).
"Repeat-cytology" at first visit to the colposcopy clinic resulted in correct prediction of histopathologic CIN-III lesions in 12 of 26 (46%) patients (Table 7.8.3). Overdiagnosis will decrease the false-negative rate, but will increase the false-positive rate.
Two cases cytologically predicted as CIN I, and 2 cases predicted as CIN II were on colposcopic impression negative for CIN (Table 7.8.1), but all were

Table 7.8.1. : Agreement between grade of C.I.N. predicted by 'maximal-cytology' with colposcopy and with histopathology (N = 163)

	COLPOSCOPY				'Maximal-cytology'		HISTOPATHOLOGY				
Grade of CIN	**Neg**	**I**	**II**	**III**	**Total n**	**%**	**Neg**	**I**	**II**	**III**	**Carc.**
I	2	**14**	5		21	(13)	5	**10**	4	2	
II	2	33	**51**	1	87	(53)	4	26	**52**	5	
III		5	40	**10**	55	(34)		10	25	**19**	1
Total	4	52	96	11	163		9	46	81	26	1
(row-%)	(2)	(32)	(59)	(7)		(100)	(6)	(28)	(50)	(16)	(1)

Table 7.8.2. : Differences in grade of CIN as predicted by 'maximal-cytology' with colposcopy and with histopathology (N = 163)

COLPOSCOPY					Difference in grade of CIN compared with 'Maximal-cytology'	HISTOPATHOLOGY				
less severe n	**equal** n	**more severe** n	**TOTAL** n	(%)		**less severe** n	**equal** n	**more severe** n	**TOTAL** n	(%)
	75		75	(**46**)	equal		80		80	(**49**)
74		6	80	(**49**)	1 grade	57		10	67	(**41**)
8		0	8	(5)	2 grades	14		2	16	(10)
82	75	6	163		Total	71	80	12	163	
(50)	(**46**)	(4)		(100)	(row-%)	(46)	(**49**)	(5)		(100)

Table 7.8.3. : Agreement between grade of CIN predicted by 'repeat-cytology' with colposcopy and with histopathology (N = 163)

	COLPOSCOPY				Repeat cytology		HISTOPATHOLOGY				
Grade of CIN	**Neg**	**I**	**II**	**III**	**n**	**(%)**	**Neg**	**I**	**II**	**III**	**Carc.**
Neg	**1**	3	3	1	8	(5)	**2**	2	2	2	
I	2	**23**	14		39	(24)	6	**18**	10	5	
II	1	26	**61**	2	90	(55)	1	24	**58**	7	
III			18	**8**	26	(16)		2	11	**12**	1
Total	4	52	96	11	163		9	46	81	26	1
(row-%)	(2)	(32)	(59)	(7)		(100)	(6)	(28)	(50)	(16)	(1)

Table 7.8.4. : Differences in grade of CIN as predicted by 'repeat-cytology' with colposcopy and with histopathology (N = 163)

COLPOSCOPY					Difference in grade of CIN compared with 'repeat-cytology'	HISTOPATHOLOGY				
less severe n	**equal** n	**more severe** n	**TOTAL** n	(%)		**less severe** n	**equal** n	**more severe** n	**TOTAL** n	(%)
	93		93	(**57**)	equal		90		90	(**55**)
46		19	65	(**40**)	1 Grade	41		20	61	(**37**)
1		3	4	(2)	2 Grades	3		7	10	(6)
0		1	1	(1)	3 Grades	0		2	2	(1)
47	93	23	163	(100	Total	44	90	29	163	
(29)	(**57**)	(14)		(100)	(row-%)	(27)	(**55**)	(18)		(100)

suspect of viral infection. Histopathologically, 3 of these 4 patients were indeed negative for CIN (2 had histopathological signs of viral infection) and 1 had a CIN I lesion plus viral infection.
In this group of selected patients the false positive rate of "maximal-cytology" regarding the presence of CIN was compared with colposcopy 4 /163 (2,5%) and with histopathology 9 /163 (5.5%). For the repeat-cytology these percentages were respectively 1,8% and 4,3%.
One histopathologic micro-invasive squamous cell carcinoma was cytologically diagnosed as CIN II. (False negative rate of cytology for carcinoma in this selected series: 1 /163 = 0.6%).

7.9 Agreement with colposcopy

In Table 7.9.1 is demonstrated the relation between the grade of CIN as determined by colposcopic impression,compared with the cytologic and histopathologic grade of CIN of each patient.
In Table 7.9.2 are presented the calculated differences in grades of CIN with colposcopy as standard.
There was a significant agreement between the colposcopic impression of each patient and the grade of CIN determined by "maximal-cytology" ($\chi^2 = 46.5$; d.f. = 9; $p \ll 0.01$; kw = 0.24) as well as by histopathology ($\chi^2 = 80.9$; d.f. = 12; $p \ll 0.01$; kw = 0.35).

Compared with "maximal-cytology", colposcopic impression underdiagnosed and compared with histopathology there was overall a balance between underdiagnosis (25%) and overdiagnosis (19%) (Table 7.9.1 and 7.9.2) by colposcopy. Colposcopy in this series designated histopathologic CIN-III lesions in only 23% (6 of 26 patients) as CIN III. However, within one grade of discrepancy in 81% (21 of 26 patients) the prediction was correct (Table 7.10.5). Complete agreement of colposcopy with "maximal-cytology" was present in 46% of cases and with histopathology in 56% of cases (Table 7.9.2).
In these selected series (n=163) there was overall agreement within 1 grade of CIN between colposcopy and cytology in 95% of cases and between colposcopy and histopathology in 96% of cases (Table 7.9.2).

There were no significant differences between cytology and histopathology in their agreement with the colposcopic grade of CIN when Kw-coefficients were compared.
Of 11 cases, colposcopically diagnosed as CIN III, 6 (55%) were indeed CIN III on histopathology, 5 CIN II (45%), and 1 patient had on histopathology a micro-invasive squamous cell carcinoma (Table 7.9.2). The histopathologic results of the cases who were colposcopically negative for CIN have been reported in paragraph 7.8.
Of the 11 cases of colposcopic CIN III lesions, 10 were cytologically also predicted as a CIN III lesion and 1 as a CIN II lesion (Table 7.9.1).
In these 163 selected patients the false positive rate of colposcopy for the presence of CIN compared with histopathology was 1 /163 = 0.6% and the false negative rate for the presence of carcinoma 1 /163 = 0.6%.

Table 7.9.1. : Agreement between grade of CIN determined by colposcopic impression with 'maximal-cytology' and with histopathology (N = 163)

'MAXIMAL-CYTOLOGY'				COLPOSCOPY		HISTOPATHOLOGY				
Grade of CIN	I	II	III	Total n	(%)	Neg	I	II	III	Carc
Neg	2	2		4	(2)	**3**	1			
I	**14**	33	5	52	(32)	4	**24**	19	5	
II	5	**51**	40	96	(59)	2	21	**58**	15	
III		1	**10**	11	(7)			4	**6**	1
Total	21	87	55	163		9	46	81	26	1
(row-%)	(13)	(53)	(34)		(100)	(6)	(28)	(50)	(16)	(1)

Table 7.9.2. : Differences in grade of CIN determined by colposcopic impression, with 'maximal-cytology' and histopathology (N = 163)

'MAXIMAL-CYTOLOGY'					Difference in	HISTOPATHOLOGY				
less severe	**equal**	**more severe**	**TOTAL**		Grade of CIN compared with	**less severe**	**equal**	**more severe**	**TOTAL**	
n	n	n	n	(%)	colposcopy	n	n	n	n	(%)
	75		75	(**46**)	equal		91		91	(**56**)
6		74	80	(**49**)	1 Grade	29		36	65	(**40**)
0		8	8	(5)	2 Grades	2		5	7	(4)
6	75	82	163		Total	31	91	41	163	
(4)	(**46**)	(50)		(100)	(row-%)	(19)	(**56**)	(25)		(100)

7.10. Agreement with histopathology as the final "standard"

In Table 7.10.1 and 7.10.3 the grade of CIN is demonstrated as determined by histopathologic diagnosis, which is used as the final "standard" for diagnosis in this study, and compared with "maximal-cytologic", "repeat-cytologic" and colposcopic grades of CIN of each patient.
In Tables 7.10.2 and 7.10.4 the calculated differences in grades of CIN with histopathology as final "standard" are presented.

As has been demonstrated in the previous paragraphs, no overall significant differences in agreement between the histopathologic grades of CIN and the grades of CIN determined by cytology and colposcopy could be demonstrated.

Complete agreement of "maximal-cytology" and histopathology in grades of CIN was present in 49% and of colposcopy and histopathology in 56% (Table 7.10.2). For the "repeat-cytology" this percentage was 55% (Table 7.10.4).
In these 163 selected patients there was agreement within 1 grade of CIN between "maximal-cytology" and histopathology in 90% of cases (Table 7.10.2), between "repeat-cytology" and histopathology in 92% (Table 7.10.4) and between colposcopy and histopathology in 96% of cases (Table 7.10.2).

Table 7.10.1. : Agreement between histopathologic grade of CIN as final 'standard' with 'maximal-cytology' and with colposcopy (N = 163)

	'MAXIMAL-CYTOLOGY'			HISTO-PATHOLOGY		COLPOSCOPY			
Grade of CIN	**I**	**II**	**III**	**n**	**(%)**	**Neg**	**I**	**II**	**III**
Neg	5	4		9	(6)	**3**	4	2	
I	**10**	26	10	46	(28)	1	**24**	21	
II	4	**52**	25	81	(50)		19	**58**	4
III	2	5	**19**	26	(16)		5	15	**6**
Carc.			1	1	(1)				1
Total	21	87	55	163		4	52	96	11
(row-%)	(13)	(53)	(34)		(100)	(2)	(32)	(59)	(7)

Table 7.10.2. : Differences in grade of CIN between histopathology as the final 'standard', with 'maximal-cytology' and with colposcopy (N = 163)

'MAXIMAL-CYTOLOGY'					Difference in Grade of CIN compared with histo-pathology	COLPOSCOPY				
less severe n	**equal** n	**more severe** n	**TOTAL** n	(%)		**less severe** n	**equal** n	**more severe** n	**TOTAL** n	(%)
	80		80	(**49**)	equal		91		91	(**56**)
10		57	67	(**41**)	1 Grade	36		29	65	(**40**)
2		14	16	(10)	2 Grades	5		2	7	(4)
12	80	71	163		Total	41	91	31	163	
(7)	(**49**)	(44)		(100)	(row-%)	(25	(**56**)	(19)		(100)

Table 7.10.3. : Agreement between histopathologic grade of CIN as final 'standard' with 'repeat-cytology' and with colposcopy (N = 163)

	REPEAT-CYTOLOGY				HISTOPATHOLOGY		COLPOSCOPY			
Grade of CIN	**Neg**	**I**	**II**	**III**	**n**	**(%)**	**Neg**	**I**	**II**	**III**
Neg	**2**	6	1		9	(6)	**3**	4	2	
I	2	**18**	24	2	46	(28)	1	**24**	21	
II	2	10	**58**	11	81	(50)		19	**58**	4
III	2	5	7	**12**	26	(16)		5	15	**6**
Carc.				1	1	(1)				1
Total	8	39	90	26	163		4	52	96	11
(row-%)	(5)	(24)	(55)	(16)		(100)	(2)	(32)	(59)	(7)

Table 7.10.4 : Differences in grade of CIN between histopathology as the final 'standard' with 'repeat-cytology' and with colposcopy (N = 163)

REPEAT-CYTOLOGY					Difference in Grade of CIN compared with histo-pathology	COLPOSCOPY				
less severe n	**equal** n	**more severe** n	**TOTAL** n	(%)		**less severe** n	**equal** n	**more severe** n	**TOTAL** n	(%)
	90		90	(**55**)	equal		91		91	(**56**)
20		41	61	(**37**)	1 Grade	36		29	65	(**40**)
7		3	10	(6)	2 Grades	5		2	7	(4)
2		0	2	(1)	3 Grades					
29	90	44	163		Total	41	91	31	163	
(18)	(**55**)	(27)		(100)	(row-%)	(25)	(**56**)	(19)		(100)

In Table 7.10.5 the 26 patients with histopathologic CIN III lesions are presented in relation to the corresponding CIN-grades as determined by "maximal-cytology" as well as "repeat-cytology" and colposcopy.

Although the cytologic prediction of a histopathologic CIN-III lesion within the same grade was significantly better than the colposcopic impression ($\chi^2 = 13.1$; d.f. $= 2$; $0.001 < p \leqslant 0.01$), within one degree of discrepancy the prediction of the histopathologic CIN-III lesions was not significantly different between "maximal"- and "repeat"-cytology and colposcopy ($\chi^2 = 3.3$; d.f. $= 2$; $p =$ n.s.). One patient with a histopathologic diagnosis of micro-invasive squamous cell carcinoma was colposcopically as well as cytologically diagnosed as CIN III. The false-negative rate for carcinoma in this series was 0.6% for cytology as well as colposcopy.

In Table 7.10.6 the 9 patients with negative histopathology are presented in relation to the CIN-grades determined by "maximal"- and "repeat"-cytology and colposcopy.
In this selected series the false positive rates for the presence of CIN in the histopathologic specimens are for "maximal-cytology" 5.5%, for "repeat-cytology" 4.3% and for colposcopy 3.7%.

Table 7.10.5: Results of 'maximal'- and 'repeat-cytology' and colposcopy in patients with histopathologic CIN-III lesions. (N = 26)

	'MAXIMAL' CYTOLOGY			COLPOSCOPY		'REPEAT-CYTOLOGY'			
Grade of CIN	**I**	**II**	**III**	**n**	**%**	**Neg**	**I**	**II**	**III**
I	**1**	3	1	5	(19)		**3**	2	
II	1	**2**	12	15	(57)	1	2	**5**	7
III			**6**	6	(23)	1			**5**
Total	2	5	19	26		2	5	7	12
(row %)	(8)	(19)	(73)		(100)	(8)	(19)	(27)	(46)

Table 7.10.6: Patients negative for CIN on histopathology (N = 9)

	'MAXIMAL-CYTOLOGY'			COLPOSCOPY	'REPEAT-CYTOLOGY'		
Grade of CIN	**Neg**	**I**	**II**	**n**	**Neg**	**I**	**II**
Neg		2	1	3	**1**	2	
I		**3**	1	4	1	**3**	
II			**2**	**2**		1	**1**
Total		5	4	9	2	6	1

7.11 Results of cytology and colposcopy combined in the prediction of the histopathologic grade of CIN

When of each patient the most serious grade of CIN of "maximal- resp. "repeat-cytology" in combination with colposcopy is compared with the final histopathologic grade of CIN (Table 7.11.1 and Table 7.11.2), the predicted CIN-grades of 2 combined diagnostic methods are not significantly different from the results obtained with cytology and colposcopy separately (Table 7.11.3) ($\chi^2 = 56$; d.f. = 12; $p \leqslant 0.001$; Kw = 0.27; resp. $\chi^2 = 74.5$; d.f. = 12; $p \leqslant 0.001$; Kw = 0.35).
With this approach still 6.1% to 11.7% of patients had more severe lesions on histopathology and for histopathologic CIN-III lesions the percentage underdiagnosis with the combination of cytology and colposcopy remained high: 30 to 52% in this series; however, within one grade of discrepancy the percentage underdiagnosis was much lower: 4-11%.
Combination of cytology and colposcopy results did not change the false positive rate, which was 5.5% compared with histopathology.

Table 7.11.2.: Differences in grade of CIN as determined by cytology and colposcopy combined, with the histologic grade of CIN as the final 'standard' (N = 163)

'Maximal-cytology' + colposcopy					Difference in grade of CIN compared with histo-pathology	'Repeat-cytology' + colposcopy				
less severe	equal	more severe	TOTAL			less severe	equal	more severe	TOTAL	
n	n	n	n	(%)		n	n	n	n	(%)
	80		80	(**49**)	equal		91		91	(**56**)
9		59	68	(**42**)	1 Grade	16		49	65	(**40**)
1		14	15	(9)	2 Grades	3		4	8	(4)
10	80	73	163		Total	19	91	53	163	
(6)	(**49**)	(45)		(100)	(row-%)	(12)	(**56**)	(33)		(100)

Table 7.11.1.: Agreement between cytology and colposcopy combined, with the histopathologic grade of CIN, as the final 'standard' (N = 163)

'Maximal-cytology' + colposcopy				Histopathology		'Repeat-cytology' + colposcopy			
Grade of CIN	I	II	III	n	(%)	Neg	I	II	III
Neg	5	4		9	(6)	**1**	6	2	
I	**8**	28	10	46	(28)		**14**	30	2
II	2	**53**	26	81	(50)	5	**63**	13	
III	1	6	**19**	26	(16)		3	10	**13**
Carc.			1	1	(1)				1
Total	16	91	56	163		1	28	105	29
(row-%)	(10)	(56)	(34)		(100)	(1)	(17)	(64)	(18)

Table 7.11. 3. : %-Agreement between 'maximal-' and 'repeat'- cytology, colposcopy, and the combinations of cytology and colposcopy, in grading CIN, with the histopathologic grade of CIN as the final 'standard' (N = 163)

Grade of CIN predicted by:	Agreement within the same grade of CIN	Over-diagnosis	Under-diagnosis	Agreement within one degree of histopathologic grade of CIN	Kw
'Maximal-cytology'	49.1	43.6	7.4	90.2	.28
'Repeat-cytology'	55.2	27.0	17.8	92.6	.34
Colposcopy	55.8	19.0	25.2	95.7	.35
'Maximal-cytology' + colposcopy	49.1	44.8	6.1	90.8	.27
'Repeat-cytology' + colposcopy	55.8	32.5	11.7	95.7	.35

In conclusion of Chapter 7.5 to 7.11:
In evaluating cytology and colposcopy individually as well as in combination, as investigative tools in the routine evaluation of patients with cervical smears suggestive of CIN, the overall degree of accuracy was found to be about the same for each method in predicting the histopathologic grade of CIN in this selected material (Table 7.11.3).
In this series colposcopy more frequently underdiagnosed the histopathologic grade of CIN, especially in CIN-III lesions, than cytology did. However, cytology more frequently overdiagnosed the the definitive histopathologic grade (Table 7.11.3).
In the routine colposcopy clinic-approach, overall colposcopy was better (respectively not worse), than cytology in predicting exactly the final grade of CIN in histopathology, although the differences were not significant and better in predicting the histopathologic grade of CIN within one degree of discrepancy (Table 7.11.3). Combination of cytologic and colposcopic results in this series did not improve the prediction of the final histopathologic grade of CIN compared with colposcopy alone. False positive as well as false negative results were few in this material.

7.12. Satisfactory colposcopy

7.12.1 *The influence of satisfactory or unsatisfactory colposcopy on the correct prediction of the histopathologic grade of CIN*

In Table 7.12.1 for each patient the relation between the colposcopic grade of CIN and the histopathologic grade of CIN is demonstrated, separated according to satisfactory and unsatisfactory colposcopy. In Table 7.12.2 the calculated differences in grade of CIN are presented.
There was significant agreement between colposcopy and histopathology, in the satisfactory colposcopy group ($\chi^2 = 52.3$; d.f. $= 9$; $p \ll 0.001$; Kw $= .35$),

Table 7.12.1.: Agreement between the colposcopic grade of CIN and the histopathologic grade of CIN, separated according to satisfactory and unsatisfactory colposcopy.

Satisfactory colposcopy HISTOPATHOLOGY						Colposcopic impression of grade of CIN	Unsatisfactory colposcopy HISTOPATHOLOGY						
Neg	I	II	III	Total n	(%)		Neg	I	II	III	Carc	Total n	(%)
2				2	(2)	Neg	**1**	1				2	(4)
3	**19**	12	5	39	(35)	I	1	**5**	7			13	(25)
2	13	**39**	7	61	(55)	II		8	**19**	8		35	(66)
		4	**4**	8	(7)	III				**2**	1	3	(6)
7	32	55	16	110		Total	2	14	26	10	1	53	
(6)	(29)	(50)	(15)		(100)	(row-%)	(4)	(26)	(49)	(19)	(2)		(100)

Table 7.12.2.: Differences in grade of CIN as determined by colposcopic impression with the histopathologic grade of CIN, separated according to satisfactory and unsatisfactory colposcopy.

Satisfactory colposcopy HISTOPATHOLOGY					Colposcopic difference in grade of CIN	Unsatisfactory colposcopy HISTOPATHOLOGY				
less severe	equal	more severe	Total n	(%)		less severe	equal	more severe	Total n	(%)
	64		64	(**58**)	equal		27		27	(**51**)
20		19	39	(**35**)	1 Grade	9		17	26	(**49**)
2		5	7	(6)	2 Grades					
22	**64**	24	110		Total	9	**27**	17	53	
(20)	(**58**)	(22)		(100)	(row-%)	(17)	(**51**)	(32)		(100)

as well as in the group with unsatisfactory colposcopy ($\chi^2 = 34.2$; d.f. = 18; $0.01 < p \leq 0.02$; Kw = .34), as far as grade of CIN concerns (Table 7.12.1).
Complete agreement of satisfactory colposcopy with histopathology was present in 58% of cases and of unsatisfactory colposcopy with histopathology in 51% of cases (Table 7.12.2). The histopathologic grade of CIN was more severe than the colposcopic impression in 22% of satisfactory and 32% of unsatisfactory colposcopies.
There were no significant differences between satisfactory colposcopy and unsatisfactory colposcopy in their agreement with the final histopathologic grade of CIN (Table 7.12.2) when the Kw-coefficients were compared.
In these selected 163 patients, in the group with satisfactory colposcopy there was agreement within one degree of discrepancy in 93.6% of cases and in the group with unsatisfactory colposcopy in 100%.
In the group with satisfactory colposcopy in 4 of 16 (25%) patients with histopathologic grade III lesions the colposcopic impression was also grade III, in 7 of 16 (44%) grade II and in 5 of 16 (31%) grade I.
In the group with unsatisfactory colposcopy there were 10 of 53 patients with CIN III lesions and 1 with a micro-invasive squamous cell carcinoma in the histopathology, which colposcopically was diagnosed as a CIN III lesion (Table 7.12.1). Two of 10 histopathologically CIN III patients had a colposcopic impression of CIN III and 8 of 10 of CIN II.

When the relation between the "maximal-cytology" and satisfactory and unsatisfactory colposcopy results with respect to the grade of CIN was studied, there appeared to be a significant agreement in prediction between the CIN grades of cytology and satisfactory colposcopy ($\chi^2 = 31.7$; d.f. = 6; $p \ll 0.001$). However, when colposcopy was unsatisfactory, there was no significant agreement in correct prediction ($\chi^2 = 14.9$; d.f. = 9; p = n.s.). The grades of CIN by cytologic diagnosis being significantly higher than those suspected on the (unsatisfactory) colposcopic impression in these cases. This discrepancy could be attributed to a combination of relative overdiagnosis by cytology of the histopathologic grade of CIN (48%) and a relative underdiagnosis by colposcopy (32%) in these cases of unsatisfactory colposcopy. Complete agreement with histopathology was present in 51% of colposcopies and in 42% of cytologic predictions.

7.12.2 *Satisfactory Colposcopy and ECC*

In section 7.7.3 the results of endocervical curettage were presented. Separation of the results of endocervical curettings (n =63) into patients with satisfactory and unsatisfactory colposcopy are presented in Table 7.12.3. Four curettings in the unsatisfactory colposcopy group were insufficient for histopathologic diagnosis and were excluded (3 × CIN III and 1 × CIN II on definitive histopathology). As can be observed from Table 7.12.3, in 1 of 10 patients with satisfactory colposcopy who were selected to have an ECC performed, the grade of CIN was one CIN-grade more severe in the ECC than the colposcopic impression was and 7 of these 10 patients had CIN demonstrated in the ECC. In the group with unsatisfactory colposcopy there were 5 of 49 patients (10%) who had a one CIN-grade more severe lesion in the ECC than on colposcopy. In 17 of these 49 patients CIN was demonstrated in the ECC (35%).
The only patient with histopathologically a micro-invasive squamous cell carcinoma in this series had a negative ECC. Comparison with the definitive histopathologic grade of CIN (Table 7.12.3) revealed considerably lower grades

Table 7.12.3.: Agreement between the colposcopic grade of CIN and the histopathologic grade of CIN as determined in the endocervical curettings, separated according to satisfactory or unsatisfactory colposcopy. In addition the maximal definitive histopathologic grade of CIN as determined by biopsies and ECC combined.

Satisfactory colposcopy (N = 110) Endocervical curettage Histopathology n = 10						Colposcopic impression of grade of CIN	Unsatisfactory colposcopy (N = 53) Endocervical curettage Histopathology n = 53					
Neg	**I**	**II**	**III**	Total **ECC's**	All **n**		**Neg**	**I**	**II**	**III**	Total **ECC's**	All **n**
					2	**Neg**	**2**				2	2
		1		1	39	I	5	**4**	3		12	13
	4	**1**		5	61	II	22	6	**2**	2	32	35
3		1		4	8	III	3				3	3
3	4	3		10	110	Total	32	10	5	2	49	53
		3	7	10		Definitive histopathology	2	14	25	7 carc. 1	49	

of CIN in the histopathology of the ECC, than in the histopathology of ECC and biopsies combined. The poor agreement between the results of the ECC and the histopathologic "standard" was not significantly different for the satisfactory and unsatisfactory colposcopy group (Kw = -0.10 respectively Kw = -0.01).

In conclusion of Chapter 7.12:
In this selection of 163 patients there were no significant differences demonstrated in histopathologic grade of CIN to whether colposcopy was satisfactory or not. In patients with unsatisfactory colposcopy the histopathologic grades of CIN were predicted better by cytology than by the (unsatisfactory) colposcopy.
In this series, the routine use of ECC in patients with unsatisfactory colposcopy did not have a significant influence on the accuracy of the colposcopic impression of the histopathologic grade of CIN and did not yield any more severe histopathologic diagnosis. However, the finding of CIN in 7 of 10 ECCs' in patients with satisfactory colposcopy but high grade CIN lesions, provides a strong support for the routine use of ECC in these cases, because this might change therapeutic management considerably.

7.13. Cervical human papilloma virus (HPV) infections

7.13.1 *The influence of the presence of signs of cervical human papilloma virus (HPV)-infection in the histopathology upon the colposcopic and cytologic grading of CIN*

In Table 7.13.1 for each patient the relation between the grades of CIN as by colposcopic impression and the final histopathologic grade of CIN, is demonstrated, separated according to the presence of signs of cervical HPV-infection in the histopathologic specimens. In Table 7.13.2 the calculated differences in grade of CIN are presented.
There was significant agreement between colposcopy and the histopathologic grade of CIN, both in the group with histopathologic signs of HPV-infection (χ^2 = 39.6; d.f. = 12; $p \leqslant 0.001$; Kw = .54) and in the group without histopathologic signs of HPV-infection (χ^2 = 35.5; d.f. = 9; $p \ll 0.001$; Kw = .24).
When the Kw-coefficients were compared, the agreement between both groups was not significantly different. Complete agreement with the histopathologic grade of CIN was 56% for both groups and agreement within 1 grade of CIN was 96% for both groups (Table 7.13.2).
The contribution of the endocervical curettage to the diagnosis of viral infection has been presented in Chapter 7.7.3.
When in the same way the influence of the presence of a histopathologic viral infection on cytologic grading was studied, no statistically significant differences could be demonstrated.

In conclusion of Chapter 7.13:
In this study the histopathologic diagnosis of presence or absence of viral infection had no significant influence upon the cytologic or colposcopic grading of CIN.

Table 7.13.1. : Agreement between the colposcopic grade of CIN and the histopathologic grade of CIN, separated according to the histopathologic diagnosis of cervical HPV-infection.

Viral infection demonstrated N = 48							Colposcopy	Viral infection **not** demonstrated N = 115					
Histopathology					Total		Grade of CIN	Histopathology				Total	
Neg	I	II	III	Carc	n	(%)		Neg	I	II	III	n	(%)
2	1				3	(6)	Neg	**1**				1	(1)
2	**9**	4	1		16	(33)	I	2	**15**	15	4	36	(31)
	4	**15**	4		23	(48)	II	2	17	**43**	11	73	(74)
		2	**3**	1	6	(13)	III			2	**3**	5	(4)
4	14	21	8	1	48		Total	5	32	60	18	115	
(8)	(29)	(44)	(17)	(2)		(100)	(row-%)	(4)	(28)	(52)	(16)		(100)

Table 7.13.2. : Difference in grade of CIN as determined by colposcopic impression, with the histopathologic grade of CIN, separated according to the histopathological daignosis of cervical HPV-infection.

Viral infection demonstrated N = 48					Colposcopic difference in grade of CIN	Viral infection **not** demonstrated N = 115				
Histopathology			Total			Histopathology			Total	
less severe	equal	more severe	n	(%)		less severe	equal	more severe	n	(%)
	29		29	(60)	equal		60		60	(52)
8		10	18	(38)	1 Grade	22		27	49	(43)
0		1	1	(2)	2 Grades	2		4	6	(5)
8	29	11	48		Total	24	60	31	115	
(17)	(**60**)	(23)		(100)	(row-%)	(21)	(**52**)	(27)		(100)

7.13.2 *The predictive value of cytology and colposcopy in this study with respect to the histopathologic diagnosis of cervical HPV-infection*

The positive and negative predictive values of a test can be helpful to evaluate the test or method.
The predictive values depend both on test accuracy and on the frequency of disease in the sample of patients tested (248).

Example:

Test-result	Disease		
	present	absent	total
positive	a	b	a+b
negative	c	d	c+d
total	a+c	b+d	a+b+c+d

a = true-positive; b = false-positive; c = false-negative; d = true-negative.

$\frac{a}{a+c}$ = sensitivity

$\frac{d}{b+d}$ = specificity

$\frac{a}{a+b}$ = predictive value positive test result, (PV+)

$\frac{d}{c+d}$ = predictive value negative test result, (PV-)

The predictive value of "maximal-cytology" used as a test in this study to determine the histopathologic presence of cervical HPV-infection. (Compare Table 7.5 and Table 7.7.2).

Table 7.13.3. Predictive values of cytology with respect to the presence of a histopathologic cervical HPV-infection.

	Histopathology		
Cytology	virus +	virus -	Total
virus +	15	11	26
virus -	33	104	137
Total	48	115	163

The predictive value of "maximal-cytology" as to the presence of a cervical HPV-infection diagnosed by histopathology:

$$PV+ = \frac{15}{26} = 0.58$$

The predictive value of cytology as to the absence of a cervical HPV-infection diagnosed by histopathology:

$$PV- = \frac{104}{137} = 0.76$$

The predictive value of colposcopy in this study as test to determine the histopathologic diagnosis of cervical HPV-infection.
(Compare Table 7.6.1 and Table 7.7.2).

Table 7.13.4. Predictive values of colposcopy with respect to the presence of a histopathologic cervical HPV-infection

	Histopathology		
Colposcopy	virus +	virus -	Total
virus +	40	30	70
virus -	8	85	93
Total	48	115	163

The predictive value of colposcopy as to the presence of a cervical HPV-infection diagnosed by histopathology:

$$PV+ = \frac{40}{70} = 0.57$$

The predictive value of a negative colposcopy as to the absence of a cervical HPV-infection diagnosed by histopathology:

$$PV- = \frac{85}{93} = 0.91$$

7.14 Discussion and comment

Selection

The interpretation of the results of this study has to take in account the criteria for selection of the study population. Exclusion of patients referred with abnormal PAP-smears without any suspect cervical finding on colposcopy, and of patients with a high suspicion or evidence of invasive carcinoma on cytology and/or colposcopy, will allow to focus on the first objective of this study e.g. to establish the ability and accuracy of the colposcopist in grading CIN in a routine colposcopy clinic setting, compared with cytology, and with histopathology as the diagnostic "standard".

Several authors (9,41,250,323,501) have reported on the correlation of the colposcopic impression of the lesions seen in patients with abnormal Papanicolaou tests, with the results of colposcopically directed biopsies and/or cone biopsy or hysterectomy specimens. They claimed that an accurate prediction of the underlying histology could be made on the basis of the colposcopic patterns and features that were observed.

Although few preclinical invasive carcinomas were being missed with this technique, several of the patient groups were small and the incidence of these occult or micro-invasive carcinomas was very low, so that there were not many cases to be missed. This limitation is applicable also to the present study, where however the (detected) false negative rate of cytology as well as colposcopy was only 0,6% with respect to histopathologic (micro-)invasive cervical carcinoma.

Another limitation of these studies, including the present one, is the fact that results of a cone biopsy or hysterectomy specimen are not always available for comparison and assessment. When colposcopically directed biopsy diagnoses were compared with definitive histopathology in the cone biopsy or hysterectomy specimen, underdiagnosis by directed biopsies occurred in 0.3 to 3.0% within one degree of discrepancy and overdiagnosis in 6.5% (157, 281, 385, 501, 542, 556, 583).

Considering the selection of the patients and the above mentioned limitations of the present study, the following comments can be made.

Patient characteristics

With respect to the patient characteristics it is remarkable that the influence of *age* on the results of colposcopic examination pertains only to the patients suspect of human papillomavirus (HPV) infection of the cervix, who appeared to be significantly younger than patients without any evidence of viral infection. This age distribution correlates well with that of other series in the literature in that more than 60% of women with HPV-infections detected are under 30 years of age, or were significantly younger than patients with CIN, but without HPV-infections (342,525,528).
From follow-up studies (54,146,528) it became apparent that cervical HPV lesions in their natural history can be fully compatible with the classical CIN-lesions. The only substantial difference between the two is, that the HPV-lesions commonly occur in women more than 10 years younger than those presenting with classical CIN. This has been interpreted to suggest that HPV-infection accelerates the development of CIN in those women (54,76,198,293,362,528).
Many authors have noted the effect of age on the colposcopic examination. Swan (519) found close to 50% unsatisfactory examinations in the postmenopausal age group due to the endocervical location of the squamocolumnar junction in these patients. In reviewing the results of several studies, with percentages of satisfactory colposcopies varying between 3.3 and 45.2% (250,448,455,532,551,583,584), it becomes clear that there is much observer bias, a great disparity in the definition of unsatisfactory colposcopy and a wide range of age groups.

Satisfactory colposcopy

Although in the present study the median age was relatively low (30 years), the percentage of unsatisfactory colposcopy was relatively high: 33% (paragraph 7.6.2) due to the very strict criteria, which were aimed at selection of patients for conservative destructive treatment. There were no age differences demonstrated between patients with satisfactory and unsatisfactory colposcopy in this selected series. The surface of the transformation zone in patients with unsatisfactory colposcopy was however significantly smaller than in the patients with satisfactory colposcopy (paragraph 7.6.3). This can be expected, as the visibility of the entire squamocolumnar junction (SCJ) was one major criterium to consider the colposcopy satisfactory. Apparently in smaller transformation zones it is more likely that the SCJ and/or the lesion(s) will extend up into the endocervical canal, out of vision of the colposcopist. It is noteworthy that in the present series only the influence of the surface of the entire transformation zone was analyzed with respect to grade of CIN and satisfactory colposcopy, and not the direct influence on the extension of the specific CIN-lesion(s) on these parameters.
Usually the limitation of colposcopy lies in its inability to detect lesion in the endocervical canal. In the present series, patients with absence of any colposcopically suspect finding of the cervix, who might have had severe endocervical lesions, were eliminated. This, and the fact that endocervical curettage did not offer more severe lesions than the colposcopically directed biopsies, could offer

an explanation for the fact that there was no significant influence of colposcopy being satisfactory or not, on the accuracy of predicting the histopathologic grade of CIN in the present study. In contrast, e.g. Wetrich (584) observed that when the SCJ was seen, 9% of patients had CIS or worse; when the SCJ was not visualized 21% had these more severe lesions.

Cytology

Between the "maximum-cytology" and the "repeat-cytology" at first visit to the colposcopy clinic (Table 7.5), there was agreement with respect to the same predicted grade of CIN in 71% and within one degree of discrepancy in 93% of cases. When cytology reports from the six months preceding to the first colposcopy clinic visit were compared with the results of the repeat-cytology at the colposcopy clinic, there was agreement within the same grade of CIN in 64% of patients and within one degree of discrepancy in 93% of cases. In only 6% of cases the grade of CIN was more severe in the "repeat-cytology", but in 30% less severe than in the referral cytology (Table 7.3 and Table 7.5). Because of the fact that with few exceptions patients had their last referral smear taken more than 6 weeks before the first colposcopy clinic visit, this might be an expression of the tendency to spontaneous regression of these lesions, but also could be due to observer bias or disparity in the definition of criteria for different CIN grades between cytologists.

HPV-infections

Considered a rarity only a decade ago, HPV-infection was diagnosed in 1-2% of women screened cytologically (343). Even more recently Lörincz et al (322), using restriction enzyme digestion followed by Southern blot hybridization, to analyze deoxyribonucleic acid (DNA) extracted from exfoliated cervical cells for the presence of human papillomavirus sequences, demonstrated in a non-selected population of women undergoing routine cytologic screening in 33 of 204 (16%) HPV-DNA sequences. This percentage was 92% in cytologically mild to moderate dysplastic smears and 11% in samples of cytologically as normal considered cases. The recognition of the presence of HPV-infection in cytologic smears in the present study (16% in the "maximum-cytology" and 8,6% in the "repeat-cytology") was low as compared with the colposcopic impression of HPV-infection (43%; Table 7.6.1) and the histopathologic diagnosis (29%; Table 7.7.3).
Depending on the population, the familiarity of the cytologist, colposcopist and histopathologist with the morphological features of HPV infections, the coincidence with CIN, and the use of special techniques as immunocytochemistry (384,594) and molecular DNA-hybridization, a wide variation of the presence of HPV-characteristics, HPV-structural antigens and HPV-DNA-sequences has been reported. In premalignant and malignant cervical lesions, HPV was demonstrated in up to 90% (72,322,478).
In an attempt to discriminate colposcopically between HPV-infection and CIN, Reid and Scalzi (437) proposed a revised and improved colposcopic index system

for differentiating benign papillomaviral infections from high grade CIN, comprising sharpness of peripheral margins of the cervical lesion, color, vascular atypia and iodine staining of the lesion. The combination of these four signs yielded a high accuracy in forecasting approximate histologic findings with an accuracy of 97% within one degree of discrepancy. Although these results are excellent, in the opinion of the present author recent progresses in the molecular virologic hybridization studies, implicating HPV in the development of cervical cancer and suggesting preferential distribution of low- (HPV 6 and 11) and high- (HPV 16, 18 and 31) risk HPV-types in CIN that tend to correlate with the morphologic appearance (72), seem to be even more promising.
Another effort to determine the accuracy of colposcopy in predicting the presence of papillomavirus on the uterine cervix was made by Walker et al in 1983 (572). In women referred to a colposcopy clinic because of abnormal cervical cytology, they compared the colposcopic findings with those obtained using histological and immunohistochemical techniques. In their series in 29% of 200 patients a diagnosis of papillomavirus infection was made on colposcopy. In 31% of 152 women who had a directed biopsy taken, histological evidence of virus infection was found and in 20% immunohistochemical evidence of papillomavirus antigen. Comparison between the colposcopic results and those obtained by histology suggested, that, whereas it was not possible to make a distinction at colposcopy between lesions due to papillomavirus and those of CIN, it was possible to identify those epithelial abnormalities that are most likely to be associated with a papillomavirus infection. Histologic evidence of papillomavirus infection was found in 83% of biopsies from patients whose lesion was thought at colposcopy to be a warty lesion, but only in 7% of biopsies from patients with no colposcopic evidence of such lesions. In the present study these percentages were 57.2% resp. 8.6% (Table 7.13.4).
In the study by Walker et al (572) however, only three-quarters of all patients had biopsies taken. Combination of results of immunohistochemistry, colposcopy and histology in their report did not improve the predictive accuracy with respect to the presence of HPV-infections.

That HPV-infection of the cervix is not a new entity, but a previously unrecognized finding, has been demonstrated by several authors (54,57,332,492).
For instance, Bernstein et al (54) reviewed cervical biopsy specimens obtained during 1972, with special reference to changes induced by HPV. In 1972 only 0,7% of biopsy-specimens were reported as consistent with HPV-infection. Upon review however 36,5% of these specimens were considered to demonstrate histologic criteria for the diagnosis of HPV infection. This was in the same range as in their cervical biopsies obtained in 1982 (34%).
Since the present study was performed, numerous reports have been published concerning the epidemiologic and oncogenic significance and the identification - whether it was by morphology, colposcopy, DNA-microspectrophotometry, immunocytochemistry or by molecular DNA-hybridization - of HPV in CIN-lesions of the uterine cervix (72, 92, 98, 138, 147, 165, 198, 202, 322, 326, 333, 343, 384, 437, 525, 528, 529, 572, 594).
An extensive discussion of these reports will be too far out of the scope of the present study.

Despite the circumstantial evidence and the fact that follow-up of patients with cervical HPV-infection has shown that these lesions in their natural history can be compatible with the classical CIN-lesions (528), the final proof of HPV as an etiological agent in cervical cancer still remains to be established (138,224,326,526).
At the present, HPV appears to play a major role as a promotor. Neoplastic transformation is probably determined by specific HPV-types, but - in addition - requires initation by some other carcinogenic stimulus e.g. Herpes Simplex Virus type II or cigarette smoking (72).

Referring to the second aim and the results of the present study, it is concluded that in a routine colposcopy clinic setting the cytologic, colposcopic and histopathologic criteria for the diagnosis of cervical HPV infection remain very much subjective. To distinguish at colposcopy between the changes due to HPV infection and those of CIN is indeed very difficult (282,341,572).
There clearly is a need for more objective adjunctive or alternative methods in our routine hospital facilities, to identify more reliably patients with cervical HPV-infections, at high risk to develop invasive cervical carcinoma.

Accuracy in grading CIN

Regarding the first aim of the present study: as has been described in chapter 7.3, analysis of the changes in grade of CIN was performed according to the weighted-Kappa technique. Kappa-w will take into account the influence of expected frequencies on the observed agreement.
The analysis of the diagnostic accuracy of cytology and the colposcopic impression of the author in the routine colposcopy clinic, as investigative tools to predict the histopathologic grade of CIN, did not yield significant differences between both methods (Table 7.11.3), although overall, colposcopy predicted better than cytology. Cytology tended to overdiagnose the histologic grade and the colposcopist tended to call CIN II, what the histopathologist called CIN III lesions in tissue specimens taken from the colposcopically most severe appearing, and as CIN II designated locations.

In 1962 Graham et al (207) pointed out that exfoliative cytology and colposcopy gave comparable results in the diagnosis of cervical dysplasia and carcinoma in situ. Significant decreases in false negative results of cytology were achieved with colposcopy and cytology simultaneously by Navratil et al in 1956 (365) and Stafl et al in 1974 (502).
Several reports in the literature have analysed the predictive accuracy of cytology and colposcopy in predicting the histopathologic diagnosis and grade of CIN. In 1973 Stafl and Mattingly (501) reported in a large series of colposcopically evaluated patients the correlation between the colposcopic impression and histology of directed biospies. When in their report the same patient selection was applied as in the present study, there was complete agreement between the colposcopic impression of the grade of dysplasia or CIS with histology in 309 of 584 (52,9%) of patients and the correlation was "clinically accurate",

that is within one histologic degree of discrepancy (over or under) in 507 of 584 (86,8%) of patients. Histology was less advanced than expected in 9.8% and more advanced in 1.4%.
In a study of Benedet et al (41) the exact agreement between colposcopic appearance versus histology of directed biopsies was - again in the same selection as the present study - 68% (326 of 479 patients) and within one histologic grade 90% (431 of 479 patients).
Allahverdian et al (9) reported 88% agreement within one level of discrepancy (over or under) between cytology as well as colposcopy and histopathology of colposcopically directed biopsies. Compared with histopathologic grade, there were equal percentages of false-positive and false-negative impression by cytology and colposcopy. The percentages of agreement within the same grade of CIN were not mentioned in this study.
In a study by Tovell et al (540), of 254 patients with abnormal Papanicolaou smears, 53,8% of the colposcopic findings agreed precisely with the histopathologic diagnosis. Within one degree of discrepancy with histopathology 77,8% of cytologic reports and 83,5% of colposcopic findings were in agreement with histology.
In 1982, Lozowski et al (323) reported on patients from a dysplasia clinic. Agreement between cytology and histology was present in 40,6% of cases and within one level (over or under) of discrepancy in 85,2%. These percentages were for colposcopy compared with histology 35,8% resp. 84,1%. For cases comparable with the present study, precise agreement with histology was 42% (53 of 125 patients) for cytology and 31% (42 of 135 patients) for colposcopy. Within one histologic degree of discrepancy these percentages were 90% and 88% respectively.
The results in the present study (summarized in Table 7.11.3) compare favourably with most of the above mentioned reports.

If from the patient-series of Huisman (this thesis, chapters 5 and 6) patients were selected according to the same inclusion and exclusion criteria as in the present study, the study-population (n=221) was comparable with respect to age, marital status, obstetrical history and contraceptive methods and was collected from the same hospital population 2 years earlier. In these 221 patients colposcopy was performed according to the approach as described by Huisman in chapter 4.
Colposcopy was satisfactory in 81% and unsatisfactory in 19% of patients. Endocervical curettings were obtained from 16% of patients and viral infections were suspected colposcopically in 16% and histologically in 12% which was much lower than in the present study, most likely due to not recognizing the colposcopic and histopathologic features of cervical HPV-infections in those years. According to the colposcopic approach applied, 77 patients were expected to have a histopathologic CIN I lesion, 83 a CIN II lesion and 61 a CIN III lesion. Endocervical curettings were negative in 86% of 28 evaluable cases and the ECC classification was in no instance higher than the biopsy diagnosis. In contrast to the present study, in the Huisman series all histopathology was reviewed by one gynecologic pathologist.

In this (except of the colposcopic approach and the pathology review) comparable series, there was agreement within the same grade of CIN between "maximal-cytology" and colposcopy in 41% of cases, with 55% overdiagnosis and 4% underdiagnosis by cytology. Within one level of discrepancy (over or under) the agreement was 88%. These percentages were about the same when cytology was compared with histopathology. However, when the results of the colposcopic diagnosis system as described by Huisman were compared with histopathologic grades of CIN, there appeared to be agreement within the same grade of CIN in 83% and within one degree of discrepancy in 98%. This very high percentage of agreement within the same grade of CIN, which is among the highest reported in the literature (250) and far better than the 56% agreement obtained with the presented routine colposcopy clinic evaluation, might be attributed to a strict adherence to this apparently valuable diagnosing system, to the colposcopist, as well as to a close cooperation between the colposcopist and the histopathologist.

Endocervical curettage

It remains controversial whether to perform an endocervical curettage as part of each routine colposcopic examination (210,302,391,583).
In the present study the ECC did not contribute to the histopathologic diagnosis. However, if patients with satisfactory colposcopy and high-grade cervical intraepithelial neoplasia are being considered for destructive treatment, in the opinion of the author, negative endocervical curettings seem to be a "conditio sine qua non", because of the relatively high incidence of positive ECC's found in these patients (Chapter 7.12).
In patients with unsatisfactory colposcopy suspect for high grade CIN, or in case of severe discrepancies, ECC will rarely obviate the need for a diagnostic exconization, because an ECC might miss an invasive carcinoma located under the surface, in the bottom of the endocervical crypts, or produce an unsatisfactory tissue specimen.

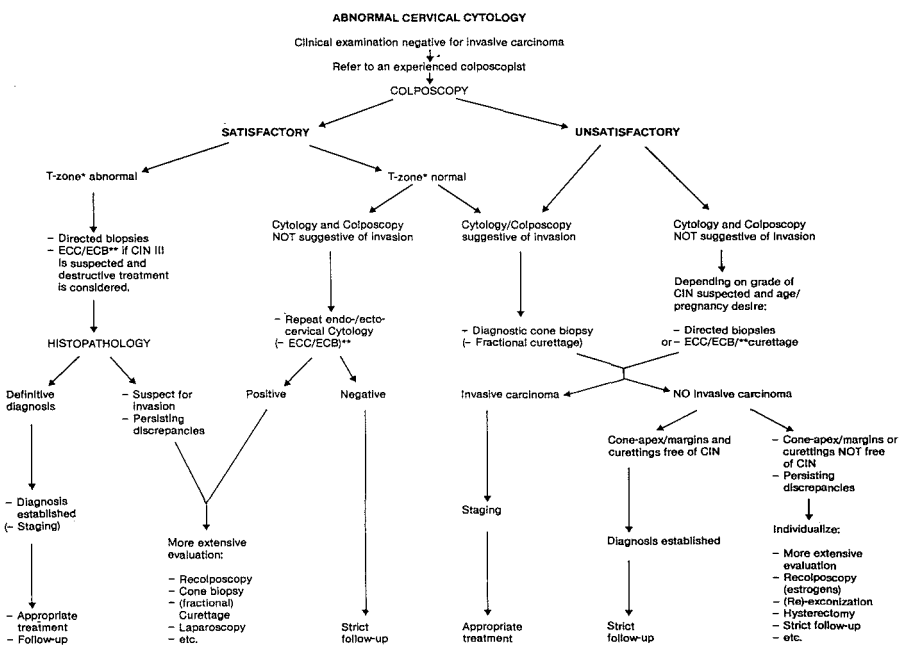
7.15. Conclusion

From a practical point of view, cytology remains unquestionably the most practical method for cervical cancer screening, provided that the entire population at risk is screened with adequate intervals and that all women with cytologic smears suggestive of cervical neoplasia are directed to a diagnostic procedure. The colposcopist can estimate the approximate severity of the lesion and discern the location of the most severe anomaly, but to establish the diagnosis a directed biopsy is always necessary, which is illustrated by the results of the present study.
Pathologists in their part should be able to grade less subjectively the specimens that contain (pre)malignancies and search for methods to be able to provide an objective, highly reproducible diagnosis, which ideally would include a prognostication of the biologic behavior in case of (pre)malignancy.
In the opinion of the present author the main benefit of colposcopy is, that - if colposcopy is satisfactory - the experienced colposcopist, while maintaining

the integrity of the uterus, can identify the presence, location and approximate severity of the most seriously appearing lesion(s) and can tailor the most appropriate treatment plan to the needs of the individual patient and cervical lesion. To become and to remain an expert colposcopist and/or pathologist, will require an extensive, continuous experience which is regularly tested by correlating colposcopic with (histo)pathologic results. To be a good doctor for the patient with CIN, one always has to keep the individual situation in perspective.

Finally, for the routine evaluation of all patients with abnormal cervical cytology, suggestive of (intraepithelial) neoplasia, where clinically invasive carcinoma is not undoubtedly manifest, the following flow-sheet was designed, based on a colposcopic approach. Following these decision lines, it will be possible to exclude with certainty invasive cervical malignancy and to establish in a simple, reliable, patient and cervix conserving and cost-effective way the presence or absence of cervical intraepithelial neoplasia.
When invasive disease is definitively excluded and CIN histopathologically confirmed, a treatment or follow-up plan has to be deviced, tailored to the lesion and the individual patient.

Flow sheet (7.15): Evaluation of patients with abnormal cervical cytology



* T-zone = Transformation Zone ** ECC = Endocervical Curettage; ECB = Endocervical Biopsy.

Chapter 8

TREATMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA

J.A. Wijnen

8.1 The rationale of treating CIN

The rationale of treating CIN is based on the assumption that cervical intraepithelial neoplasia consists of a continuous spectrum of precursor lesions (440), and that the majority of the invasive carcinomas of the uterine cervix will arise from these precursor lesions (397). CIN can manifest itself from mild changes in grade I to undifferentiated epithelium in grade III lesions. It is supposed that progression to invasive squamous cell carcinoma can be interrupted by eradication of these potentially malignant precursor lesions (89,294,440).

Many studies have documented the efficacy of treatment of carcinoma in situ and it is believed that if CIN is treated, this will reduce significantly the mortality rate from cervical cancer (132). Mettlin et al (346) documented in a limited number of patients with CIS that the cancer-specific 10-year survival rate was 96.6% among patients who had not been treated and 99.3% and 98.8% for those who had been treated by exconization, respectively hysterectomy.
However, no prospective randomized studies have been performed to evaluate directly the effect of treatment versus no treatment of these precursor lesions.

Controversy persists about the proportion of CIN-lesions that will progress to malignancy and the timescale over which this will occur. Van Lent (315) and Buckley et al. (78) reviewed the literature. Depending on the diagnostic interventions, severity of the lesions, criteria of progression, and selection of the population, it can be estimated that between 30% and 70% of untreated CIN-lesions will progress, 24 to 70% of CIN I-II lesions might regress and 15 to 44% will persist, when followed conservatively (83,315). Transition from CIN to invasive disease, if this occurs, was estimated to take from 3 to 20 years (309,315,396,421,443,565), although the possibility of direct or much faster malignant transformation from normal cervical epithelium has also been postulated (17,85). Kinlen & Spriggs (279) reported a group of 60 women with positive cervical smears who refused subsequent biopsy or treatment. After a mean interval of 5.2 years regression in cytology was documented in about one third, but in 13 (22%) invasive cervical carcinoma had developed. McIndoe et al. (336) described some 300 women from New Zealand who had evidence of carcinoma in situ and who were not treated. At the end of 10 years 18% and over a 20 years period 30% had developed invasive cervical cancer. This evidence suggests very strongly that CIN-lesions can be premalignant.

The urgency with which CIN-lesions should be treated remains difficult to assess and until recently it was even more difficult to identify the specific lesion that will progress to invasive disease. Spriggs and Boddington (499) indicated that cases with poorly differentiated cells in the cytologic smear more frequently progress. In contrast, Fu et al. (197) found progressive and non-progressive CIN-lesions indistinguishable by morphologic criteria. Boon et al. (63) using a morphometric classification based on the nucleus-cytoplasma-area ratio, discriminated between immature and mature dysplastic cells and consequently made a prediction about the progressive and regressive properties of the lesion. To appreciate the value of this method of discrimination, long-term follow-up results of their patients have to be awaited.

Fu et al. (196) assessed DNA ploidy levels in cervical lesions with a micro-spectrophotometric technique and suggested that up to 80% of CIN III-lesions are aneuploid and have a higher malignant potential than polyploid and diploid lesions. Fujii et al. (199) demonstrated that CIN I and II lesions which appear aneuploid are more likely to progress to CIN III and invasion than the polyploid or diploid CIN I and II lesions. Zur Hausen et al. (226), using DNA/RNA hybridization techniques, have found a difference between low- and high-risk human papilloma viruses (HPV). The latter, types HPV-16 and HPV-18, seemed to be integrated in the host cell DNA. The association of abnormal mitotic figures and aneuploidy with HPV-16 and HPV-18 (196) may allow clinicians to allocate patients with CIN to low-risk and high-risk groups, and so tailor the treatment and follow-up programs to the needs of the individual patient (98). These techniques are still experimental however and not routinely available.

Although CIN has a low morbidity and no mortality, it seems prudent to consider all histologically confirmed CIN-lesions, especially CIN II and III lesions, as potentially malignant and most clinicians, including the author, favour active, but conservative treatment in order to prevent progression to invasive carcinoma. The major exception might be when these CIN lesions are diagnosed in pregnancy. In pregnant women, colposcopic evaluation and punch biopsies can almost always exclude invasive carcinoma and treatment can be postponed until after delivery.

8.2 Survey of methods of treatment for CIN

The treatment of CIN falls into two categories:

EXCISION

This includes simple *hysterectomy* with or without a vaginal cuff and therapeutic *cone biopsy*. The latter is most commonly performed by the so-called cold knife technique and different modifications have been attempted to obtain hemostasis. Recently *laser-excisional (micro-)conization* has been introduced (158,539) and *diathermic stripping*, also called *ablatio cervicis* (105,215,319). This is a modification of treatment by (multiple) *excisional biopsies* (5,293,440,531).
Excisional methods have in common that they provide an adequate tissue sample for histopathologic examination and that they aim to be therapeutic as well as definitively diagnostic.

LOCAL DESTRUCTION

With the upsurge of colposcopic assessment and the rapidly increasing number of young women with CIN (265) there is a definite trend towards more conservative treatment in carefully selected patients with CIN. Local destructive techniques have in common that they aim to eradicate completely the CIN containing tissue by physical destruction.
Presently the most popular techniques are *cryotherapy* or freezing and carbon dioxide *laser vaporization,* which method destroys the lesion in contrast to the laser-excisional techniques mentioned above. Both methods will be discussed later.

Electrocoagulation diathermy
Using intensive heat to destroy the tissue with needle- and ball-electrodes is an effective treatment modality but very painful and requires general anaesthesia (112, 114, 242).

"Cold coagulation"
This involves the application of moderate heat (maximally 100°C) through a Teflon coated heating device (modified Semm Coagulator) to the cervical tissue, to destroy the lesion (162). Initial success rates are comparable to those of cryo- and laser therapy, side effects are few, although a heavy discharge and destruction of healthy tissue surrounding the lesion may occur as with electrocoagulation diathermy. General anaesthesia is not required.

Several excellent reviews concerning treatment of CIN have been published recently (51, 113, 132, 264, 302, 311, 315, 482, 491, 546).
To fit within the scope of this thesis, general principles of treatment and selected treatment methods will be discussed, the other techniques were already briefly summarized.

8.3 General principles of treatment for CIN

As has been mentioned before, CIN originates with few exceptions (64) within the transformation zone (T-zone) and every therapy that removes or destroys the entire T-zone and any potential gland extension to a depth of about 6 mm, will be curative for squamous intra-epithelial lesions and will eliminate the possibility of residual CIN being covered by new squamous epithelium and remaining undetected until it suddenly appears as invasive carcinoma (1, 11, 64, 414, 421).
To prevent over- and undertreatment, no women with abnormal cervical cytology should proceed to treatment for CIN without prior colposcopic assessment (462), unless invasive cervical cancer is clinically unmistakable. The diagnosis should always be based on histopathologic classification and invasive cancer should be definitively excluded. Close co-operation between clinician, cyto- and histopathologist is essential and they must know each others' diagnostic accuracy and the significance of the different terms used (40, 447, 566, this theses, chapter 2-7).

The efficacy of any treatment method is maximized when the exact topography of the lesion is known and this is especially true when the CIN-lesions extend toward the vagina or into the endocervical canal.
Based on the colposcopic findings, size and distribution of the lesion and histopathologic diagnosis, the age of the patient, her wish for future pregnancies and other concurrent ailments or gynecologic symptoms, the gynecologist can make a definitive decision as to whether this particular lesion is suitable for an excisional or destructive treatment method, or can be strictly followed without therapy.

8.4 Excisional methods of treatment for CIN

In the past the most accepted therapies for CIN were radical excisional methods: hysterectomy, with or without a vaginal cuff; cold knife exconization and amputation of the cervix. All have proven to be effective methods for treatment of CIN (88, 132, 311, 315).

8.4.1 *Simple hysterectomy*

Simple hysterectomy produces cure rates better than 96%, and hysterectomy with a cuff off vagina can cure almost 100%. Routine vaginal hysterectomy produced 4 times less recurrences than the routine abdominal approach, because of better visualization of the textent of the lesion (88).
However, hysterectomy is a large price to pay for a lesion that may be very small and which can be removed adequately by other excisional methods.

8.4.2 *Cone biopsy or exconization*

Cone biopsy or exconization (589) is an accepted treatment for CIN and even for micro-invasive cervical carcinoma, provided that the pre-treatment diagnosis is correct and the margins of the cone are free of disease (86, 321).
In the absence of colposcopy a Schiller directed exconization, aimed to be diagnostic as well as therapeutic and to remove all of the endocervical canal, can expect to cure up to 98% of patients with CIN I and II lesions and 93% of CIN III lesions (132, 590). However, quite innocent epithelial alterations other than CIN might stain Schiller negative, while premalignant lesions can stain Schiller positive. A colposcopically directed cone biopsy will give similar cure rates but will allow the size and shape of the cone to be tailored to the needs of the individual patient, and produce a cone that, although radically removing the entire T-zone and lesion, can be much smaller than the non-colposcopically directed one (55). This might lower the complication rates of cold knife exconization which have been reported to be related to the size of the cone removed, and to vary between 3 and 26% (58, 59, 121, 311, 315, 324, 589, 590).
The complications of exconization or amputation of the cervix relate mainly to hemorrhage (varying from 6-71% as reported by Berkus & Daly (52), cervical stenosis (with follow-up, menstrual and fertility-problems) and pregnancy com-

plications (259,311). This created the statement that "cone biopsy is a minor surgical procedure with a major complication rate".

Cone biopsy during pregnancy before colposcopy was routinely practised, produced an unacceptable incidence of complications, including significant operative hemorrhage, perinatal mortality and morbidity (20,68,149,222,314,450). Coppleson (132) has stated that with colposcopically guided evaluation and punch biopsies the cone biopsy rate during pregnancy could be reduced to almost zero. The safety of this management has been confirmed by other authors (42,280,334,501).

The complication rates of hysterectomy are well known (267) and not within the scope of this thesis.

8.4.3 *Carbon dioxide laser excisional conization*

Recently several laser proponents have reported results on carbon dioxide laser excisional (mini-) conizations (23,66,158,247,310,311,539,596) or a combination of laser vaporization of the ectocervical and /or vaginal lesions with central laser excision ('top hat' technique) (22,598). In contrast to local destructive techniques including laser vaporization, colposcopically guided laser exconization or -excision offers the advantage of a suitable specimen for histologic investigation, while being more conservative and fertility preserving than cold knife exconization or amputation of the cervix. This technique can be performed under local anaesthesia as an outpatient procedure. With the use of sutures to secure the descending branches of the uterine arteries and /or injection of a hemostatic agent such as epinephrin in combination with a local anaesthestic into the cervix, the amount of blood loss during the operation could be reduced to a few milliliters. This was significantly less than caused by cold knife exconization (310). Postoperative hemorrhage could also be significantly reduced (66,310). While the morbidity was equal to laser vaporization, Baggish (23) accomplished cure rates better than 97% with laser exconization compared with 91% for pure laser vaporization. As the application of carbon dioxide laser energy creates minimal adjacent tissue necrosis and vaporizes the neoplastic tissue, a lessened repair phase ensues. This may account for the minimal scar formation and rapid healing time. Therefore it seems likely that these laser techniques give promise of reducing cervical stenosis associated with the cold knife procedure, and make follow-up colposcopic visualization of the squamocolumnar junction more reliable.

8.4.4 *Diathermic stripping*

Another conservative non-destructive method that can be diagnostic as well as therapeutic is diathermic stripping, also called *ablatio cervicis* (105,215,319).
In an outpatient setting and with the use of local anaesthesia the colposcopically atypical transformation zone is excised to a depth of 5 mm with a 0.3 mm stainless steelwire (of varying sizes) attached to a standard diathermic apparatus.

This technique appears fast, cheap and safe in experienced hands; morbidity is low; healing is rapid and histopathologic specimens are satisfying. However, cure rates were lower (78% after 1 ablatio and 85% after 2 treatments) (271) than reported with laser techniques performed by laser experts. This might be due to greater precision of laser-surgery with respect to deeper lesion extensions and the possibility to include more of the endocervical canal in laser excision or destruction.

8.4.5 *Excisional biopsies*

Treatment by excisional biopsies (5,293,440,531) achieved a cure rate of maximally 61% in patients with carcinoma in situ. This technique rather illustrates that cases of "spontaneous" regression or therapeutic results of other treatment methods may in fact be based on the effect of the diagnostic biopsies, than that it is an effective treatment for CIN (302).

One common problem in all excisional treatment methods for CIN is the situation where the upper limits of the transformation zone or the CIN-lesion can not be completely visualized with the colposcope, not even with the aid of an endocervical speculum. According to Jordan (264) in these cases a cone biopsy which removes the entire epithelial surface of the endocervical canal is required. However, because CIN frequently does not extend far into the canal (1,64,414) this may be unnecessarily radical. Smaller, shallower, individual tailored cone biopsies could be performed in these patients, supplemented by and endocervical curettage, if it were possible to assess the extent of the endocervical involvement by CIN. This could reduce the morbidity of exconization without compromising the therapeutic efficacy. Soutter et al. (498) and Fenton et al. (174) reported their preliminary experiences with the use of the *Hamou-micro-colpo-hysteroscope* (MCH) (217) to determine the extent of endocervical involvement by CIN in patients who were to undergo cone biopsy of the cervix. There appeared to be good correlation between the preoperative MCH measurements and the endocervical extension of CIN on subsequent histopathologic examination of the cone specimens. Apical residual disease could be prevented using this technique. Colposcopically guided *laser-excision* was also able to produce radical removal of the endocervical CIN extensions, especially if care was taken to remove a cylindrical shaped block of tissue or a truncated cone (8,482). Laser vaporization of the remaining cone-bed to a cylindric shape also appears effective in eliminating potential residual disease in the endocervical crypts above the apex of the cone that was removed by laser excision (247).

Whatever excisional method is chosen, one has to realize that even after complete removal of CIN lesions, invasive cervical squamous as well as adenocarcinomas have been reported: After cone biopsy as definitive treatment up to 0.9% and after hysterectomy up to 2.1% (290,482). Therefore strict long-term cytologic follow-up seems to be indicated.

8.5 Destructive methods of treatment for CIN

8.5.1 *Introduction*

If surgical excision by exconization or hysterectomy give such excellent therapeutic results, why should other methods, some of which have a lower "cure rate", be attempted?
Firstly, most centers now accept that hysterectomy is over treatment for most patients with CIN and there is now a growing belief that even cold knife cone biopsy is over treatment for many (265).
Secondly, in recent years the number of experienced colposcopists has increased and colposcopic assessment of patients with CIN has shown that the lesion is often small and can be easily destroyed by local treatment. Colposcopy has allowed accurate diagnosis of CIN to be made on an outpatient basis in approximately 85-90% of all women with abnormal cytology and grossly normal cervix (142, 157, 301, 501, 542). The histopathologic diagnosis of biopsies taken by an expert colposcopist also appeared to be accurate compared with the definitive histopathologic examination in nearly 98% of cases (157, 301, 373, 386, 501, 542, Huisman, this thesis chapter 5).
Thirdly, and most importantly, is the rapid increase in patients with CIN. Many of these patients are young, often nulliparous women and in this population it is particularly important to preserve fertility.
Finally, rising costst of hospitalization and surgery stimulated interest in an approach that conceivably could be carried out in an ambulatory office-based setting.

Before any destructive method can be considered it is imperative that the following criteria are met:

Local destruction treatment is appropriate only:
1. If colposcopy is considered "satisfactory" by an experienced colposcopist. This means that the entire lesion and squamocolumnar junction are clearly visualized including the upper extension, which usually lies just within the endocervical canal.
2. If the experienced colposcopist has excluded invasive carcinoma colposcopically and by colposcopically directed biopsies and endocervical curettage.
3. If endocervical curettings do not contain abnormal histology.
4. If there is no discrepancy between cytologic results suspect for invasive disease, and colposcopic and histopathologic findings.
5. If the experienced colposcopist performs the destructive method himself.
6. If adequate cytologic and colposcopic follow-up can be guaranteed.

If these criteria to exclude unsuspected invasive cancer cannot be met, in most other cases a diagnostic excisional cone biopsy, aimed at removing the entire lesion, is mandatory. If colposcopy is unsatisfactory, this should be followed by an endocervical curettage, if not previously performed. If discrepancy exists or glandular atypia is present, a fractional curettage and examination under anaesthesia can also be indicated. If these criteria are strictly adhered to, outpatient local destructive techniques can offer a high grade of safety. At the same time diagnostic cold knife exconization rates can be lowered significantly.

8.5.2 *Treatment of CIN by cryocoagulation*

Various techniques of cryotherapy have been used for more than 40 years with variable success in the treatment of benign cervical lesions. Crisp et al. in 1967 (144) were the first to use this approach in the management of CIN. Reviews of the history, technique, mechanisms of action, indications, treatment results and side-effects of cryotherapy in gynecology, especially for CIN, have been presented by Van Lent (315); Charles and Savage (115, 116); Figge and Creasman (180); Kwikkel (302) and Boonstra (64).

This technique involves the freezing of the cervical tissue by the use of a gun-type cryosurgical probe. Adiabatic isotropic expansion of a compressed gas through a small orifice into a lower pressure area produces a substantial lowering of the surface temperature (Joule-Thompson effect). The gases used for cryosurgery have a boiling point in the cryogenic range e.g. carbon dioxide (CO_2), nitrous oxide (N_2O) and nitrogen. The freezing temperature of all these gases is lower than -75°C.

All current techniques involve the contact freezing principle and depend on firm application of one of the interchangeable probes – fitting to the individual portio and lesion – to the surface. Before freezing, a watersoluble gel-lubricant is applied to the probe allowing a better heat transfer, and resulting in a more uniform freezing, as air gaps from a distorted or irregular cervix surface are now eliminated. The refrigerant is applied continuously (44, 514, 546, 549) or through a freeze-thaw-refreeze cycle, which in several studies appeared to be more effective (139, 142, 472). In practice, the length of the freeze was not as important as the size of the iceball around the probe, which has to extend 5 mm beyond the periphery of the probe and the edge of the lesion. This usually takes more than 3 minutes. In an elegant study Boonstra (64) demonstrated that freezing with a freeze-thaw-freeze technique for no longer than until a 5 mm ice-zone around the probe was achieved, resulted in a 21.6% overall morphometric-histopathologic failure rate. Cryosurgery in this study was performed the day before hysterectomy for benign conditions. In his opinion to fully utilize the freezing potential to destroy all selected CIN lesions, a longer freeze-thaw-refreeze time (5-5-5 minutes) seems to be necessary.

In large lesions and transformation zones, adequate destruction may require more than one freeze application with overlap of the iceballs. Once the appropriate patient and lesion is selected and, after exclusion of invasive disease and colposcopic assessment, cryocoagulation is easy to perform in an outpatient setting. No special preoperative preparation of the vagina is needed. There is usually no bleeding and anaesthesia is not required because of the inherent anaesthesia and hemostatic properties of the technique (144). Complications are uncommon. Faintness and mild abdominal cramps may occur during the procedure. A heavy watery discharge, at times profuse, and sometimes blood-stained, occurs for several weeks after treatment but bleeding is rare. Temporary occlusion of the cervical canal by the necrotic "coagulum" has been observed incidently (538). Intercurrent pelvic infection may flare up post-therapy and thus should be treated before therapy is performed. The routine use of antibiotics or antiseptic creams postoperatively is not necessary. Following cryocoagulation, each patient has to be advised against coitus and the use of vaginal tampons

for as long as discharge continues. There seems to be little or no effect on subsequent fertility and labour (47,115,231,388).
Destruction of the lesion is directly related to the size of the probe and the lesion, and the adaptation to the surface of the cervix, and also to the temperature and freezing time. Nitrous oxide is the preferred gas because of fewer equipment problems. The pressure within the gas tank must remain adequate during treatment or freezing will be suboptimal. Data suggest (64,543) that tissue underneath the probe is adequately treated; however, if the lesion extends 4 to 5 mm beyond the probe, even an iceball that distance does not provide adequate treatment. From the studies of Anderson & Hartley (11); Abdul-Karim et al. (1) and Boonstra (64) it could be calculated that if CIN extends into the endocervical clefts, it would be necessary to destroy the tissue up to about 5 mm in depth and to sufficient linear extent in order to eradicate nearly 100% of CIN III lesions. Theoretically, the faster the freeze, the better the destruction of tissue. Five mechanisms have been described to explain the destructive effect of cryotherapy (115):.

1. dehydration and toxic concentration of electrolytes due to removal of water from solution.
2. crystallization with rupture of cell membranes.
3. denaturation of liquid-protein molecules within the cell membranes.
4. thermal shock.
5. vascular stasis.

Cahan (97) and Kashimura (268) studied the histologic changes and reparative processes in the uterine cervix after cryocoagulation and their results have been summarized by Kwikkel (302).

Results of cryotherapy
During the 1970's the efficacy of cryosurgery in the treatment of CIN was extensively studied. Various cure rates have been reported and in the review by Charles and Savage (115) the success rate was noted to range from 27 to 96%. This wide range in results can be caused by many factors: The selection of the patients, the number of patients treated, the experience of the gynecologist, the criteria used to determine a cure and the freezing equipment, technique and time, and the refrigerant. Since that review article several other experiences have been reported, all noting a high success rate in adequately selected patients but using different criteria to determine efficacy (44,77,141,142,251,514). In their extensive review of 770 patients treated by cryocoagulation, Creasman et al. (142) reported persistence of CIN in 10% of cases. Re-treatment on an outpatient basis resulted in eradication of the lesion in even more than 96% of these patients. It was more difficult to destroy CIN III disease and large lesions; although in their series, grade appeared to be more important than size of the lesion, because high grade CIN-lesions were usually larger. Townsend and Richart (549) found their cryosurgery failure rate dependent on the size of the lesion, regardless of the grade of disease. Bryson et al. (77) reported a 7.1% failure rate of primary double freeze cryotherapy of CIN I-III lesions in 422 patients. Outpatient re-treatment of those with persistent disease achieved an overall cure rate of 98%. In their series the type of freeze (a freeze-thaw-freeze cycle) was the only variable to show statistical significance.

The importance of proper selection of patients is illustrated by the registration of more than a hundred cases of invasive carcinoma following cryocoagulation for supposedly benign disease or CIN (372,481,545,547,549). From these reports it appears that incomplete and inadequate pre-treatment evaluation was the disturbing cause of most of these events, or inappropriate treatment of a lesion actually more severe than CIN. It appears that cryosurgery can achieve reasonably good destruction of CIN if a very rigid method of patient selection and treatment is employed. However, only the experienced colposcopist and cryotherapist who uses meticulous technique will achieve success rates better than 90% with initial treatment. Follow-up must be consequently carried out over at least 24 months in order to detect persistence and about 90% of recurrences (180), before patients can be referred for routine cytologic follow-up. After this period the cumulative risk of recurrence of CIN in patients successfully treated with cryocoagulation for CIN, does not seem to be significantly different from a patient in a normal population (442). Factors in favor of cryosurgery are that it can be carried out entirely as an outpatient procedure, the patient acceptability is high, and morbidity is low. It achieves a high "cure rate" with one or two applications. Fertility and pregnancy problems are similar to those of the normal population and the costs of the equipment and treatment are low.

8.5.3 *Treatment of CIN by laser vaporization*

The use of the carbon dioxide laser is now widely accepted as one of the most effective conservative treatment methods for CIN (22,23) being especially suitable for the younger women wishing to retain her reproductive potential (8). The word LASER is an acronym derived from the first letters of the words Light Amplification by Stimulated Emission of (electromagnetic) Radiation (39,598). The laser is a device which converts some form of energy such as heat, light or electricity into radiant energy at a specific wavelength, determined by the type of laser. When the wavelength and radiant energy lie within the visible portion of the electromagnetic spectrum, it is called light.
Not all lasers emit their radiant energy as light, but the radiation emitted by all lasers has three special qualities. It is coherent (all the waves are exactly in phase with each other, in both space and time); it is collimated (the rays are parallel to each other); and it is monochromatic (all the waves have exactly the same wavelength.)

Presently the CO_2 laser is most suitable for use in surgical gynecology. This laser produces its light at a wave length of 10.6 microns. This is in the infrared portion of the spectrum where it is invisible to the naked human eye. When used for the treatment of CIN, the laser is attached to a colposcope and by a system of mirrors and lenses the laser beam is directed to the target. A co-axial helium-neon laser with visible red aiming-beam provides a visual target at the treatment site, so that the procedure can be performed under continuous direct (colposcopic) vision of the laser surgeon.
Unmodified laser light is parallel and contains energy which is however of no practical use unless it is focused by a lens to a "spot". The spot-size is dependent

on the focal length of the lens and is usually 1.5 to 2.0 mm in diameter. At its focal point the laser releases an enormous amount of energy, expressed as the power density (Watts /cm^2). The power density can be controlled by a simple watt-regulator and is inversely proportional to the square of the focal length of the lens. The power density of a system depends on the electric power introduced into the system, the lasing material, the efficiency of the system and the spot size. The usual wattage recorded is 20-30 Watts and this will give a power density at the point of impact of 400-2000 Watts per cm^2.
Tissue at the focal point of the laser beam is destroyed by absorption of the energy in the extracellular and intracellular fluid as well. This will be heated and vaporized; therefore the damage produced by a laser beam is essentially a thermal one. The interaction of laser light with living tissue, surgical oncological aspects, laser surgical standards and safety measures as well as the process of healing after laser induced lesions have been excellently summarized by Kwikkel (302).
The laser beam is easily controlled by a micromanipulator attached to the colposcope and an on-off-exposure time switch, operated with a foot pedal. This will allow the surgeon to control the beam completely under direct vision, and to destroy the target tissue with great precision.
The laser was first used for the treatment of CIN in 1974 (21,38,104,504). As with any destructive technique, laser vaporization is appropriate only in selected patients, i.e. if the criteria described in section 8.5.1 are met.
In most cases laser vaporization for CIN can be performed in an outpatient setting. The patient is placed in the routine lithotomy position. No preoperative preparation of the vagina is needed. A self-containing speculum is inserted into the vagina to expose the cervix and colposcopy is repeated to identify the lesion and the transformation zone. The power density is chosen and the exposure time - which can be varied in steps from 0.1 to 1 second or in a continuous mode - and a smoke evacuating system to remove the vapour plume is connected. The procedure can be performed without anaesthesia, however, most patients will experience some pain or discomfort which is described as a warm feeling, cramps or "needle sticking" and many will appreciate some form of local anaesthesia. A paracervical block or injection of 2 $\times$ 1 ml lidocaine 2% with or without adrenaline at 3 and 9 o'clock positions into the cervix, will be adequate in almost all patients. The key to successful eradication of CIN by laser vaporization is adequate depth of destruction and removal of the entire transformation zone with a 2-3 mm margin of colposcopically normal (squamous) epithelium (266).
Based on experiences obtained by trial and error and - later - on the studies of Anderson and Hartley (11) and Abdul-Karim et al. (1) which were mentioned before, it was concluded that laser vaporization to a depth of at least 5-7 mm extent (266) will be able to eradiate CIN, even if this involves cervical crypts or clefts or glands. This under the condition that preoperative colposcopy was satisfactory and invasive disease excluded.
After some single bursts to test the reaction of the patient, the area to be vaporized is demarcated under colposcopic vision. To prevent blood and/or ooze from obstructing vision and absorbing laser energy, vaporization is started at the most dorsal point of the transformation zone. With rapid, streakwise movements of

the laser beam the entire transformation zone including all lesions is vaporized in a cylindrical shape, including a small margin of normal squamous epithelium and stroma (8). Rapid movements will reduce heat accumulation in the tissue, thus minimizing pain and cramps and thermal damage to the margins of the remaining cervix. The depth of destruction can be measured initially with a calibrated probe, but after gaining more experience, the physician will have no problem to estimate the depth of destruction colposcopically.
The indications and techniques of laser-excision and exconization have been alluded to in section 8.4.3.

Results of laser vaporization
Laser techniques can offer definite advantages despite the initial high costs of equipment. The healing of tissues subjected to laser evaporation is usually far superior to that produced by cryocoagulation and electrocoagulation diathermy (491). The laser produces almost no destruction of normal surrounding tissue. Usually within 8-10 days new immature metaplastic cells cover the entire surface of the lesion and in all cases the treated area will be covered by relatively mature squamous epithelium within 4 weeks. Less postoperative fibrosis and stenosis will occur than with other destructive techniques and the endocervical canal is readily accessible for follow-up examination. There appear to be no complications associated with subsequent pregnancy in laser treated patients. No significant infections have been reported. Laser temperatures sterilize the field and no necrotic tissue will remain as a potential focus for infection.
Patient acceptance of the procedure, if performed under local anaesthesia, is high, although 10% will experience mild to moderate discomfort during the five to fifteen minutes procedure (491).
Blood loss during the procedure is usually not a major problem since hemostasis of smaller vessels is instantaneous. Troublesome bleeding that compromises the speed and efficacy of laser vaporization has reported to amount 10% (21) and will occur particularly in the presence of severe inflammatory changes or in the secretory phase of the menstrual cycle. Severe secondary hemorrhage occurs infrequently (1-1,5%) (21), but minimal bleeding in the postoperative period is not unusual. Application of silver nitrate or Monsels' solution is usually sufficient to produce coagulation. Following laser treatment, patients have to be advised against coitus and the use of vaginal tampons as long as discharge continues.

Overall cure rates for one or more laser treatments were 63-99% for all grades of CIN and 50-99% for CIN III (582).
Differences in results might be explained by accumulation of experience, more complete vaporization of the lesion and the transformation zone and different definitions of cure. A primary cure rate in the region of 95% after one application in properly selected patients seems to be feasible (8,22,23,266).

8.6 Treatment of CIN: Conclusions

Presently it is not possible for all clinicians to discriminate CIN lesions in a routine fashion with respect to their potential to progress to invasive carcinoma

or to regress to normalcy. For this reason there would seem to be a strong argument for considering all histologically confirmed CIN lesions as potentially malignant and for treating all of them with conservative methods available now. Selection of any treatment method must be based on expert colposcopic assessment and familiarity of the physician with the treatment of choice. However, in addition treatment must be tailored to the lesion as well as to the individual patient.

Whatever therapy has been performed, adequate longterm follow-up remains indicated in all cases. An occasional invasive carcinoma will occur, albeit very uncommon, following whatever method is used (51,266,290).

Chapter 9

FOLACID THERAPY IN CERVICAL INTRAEPITHELIAL NEOPLASIA

J.A. Wijnen

9.1 Introduction

From epidemiologic studies it appears that the etiology of carcinoma of the uterine cervix is related to sexual behavior and many factors referable to the male and female have been incriminated and extensively described (25, 194, 200,315,330,352,436,489,597).
The typical woman at risk to develop invasive squamous cell carcinoma of the uterine cervix had her first intercourse at early age (sexarche) (456,597); had multiple male sexual partners, who in turn had multiple female sexual partners (60,172,277,331); has a low educational and socio-economic level (31,494); and is divorced or lives separated (168).
The association with venereally transmitted disease (infections and/or oncogenic agents) is well established now (173,276,451,526). In particular, herpes genitalis (HSV-2) has been associated with CIN and cancer in a number of studies (536), and both Chlamydia trachomatis and Human Papilloma Virus (HPV) have been associated with CIN (341,471).
However, specific sexually transmitted agent(s) responsible for the disease are not definitively identified yet. The search for such agent(s) directly linked with cervical carcinogenesis has been pursued using serologic analysis (19,26,419) and, more recently, DNA molecular hybridization (147,480).
Information derived from these various sources has led to the understanding of several factors linked with invasive and/or pre-invasive cervical neoplasia. The role of possible other risk factors in the etiology of cervical cancer is still debated.

One of the currently controversial topics in the epidemiology of cervical neoplasia concerns *nutritional factors*. Patients with cervical neoplasia had lower dietary intakes of total vitamin A and beta-carotene and possibly of ascorbic acid as well, than a comparison group (18, 53, 94, 95, 136, 156, 232, 353, 381, 382, 383, 401, 411, 452, 453, 454, 518, 552, 561, 576, 577, 591, 592, 599).
Dietary practices could relate to cancer occurrence in either a causative (natural mutagens and carcinogens) or a preventive manner (antimutagens and anticarcinogens) (10).
Dietary interventions may either reduce the intake of potentially carcinogenic substances, or increase the intake of substances that may inhibit cancer cell initiation or promotion. The identification of interventions that inhibit the later stages of neoplastic transformation is of particular interest to cancer prevention among persons who are currently at high risk of cancer.

In view of the long latent period associated with the appearance of many cancers, including cervical cancer, it seems reasonable to suppose that high risk individuals possess partially transformed cells which are at risk for subsequent transformation into malignant cells, unless retarded or reformed by some late-stage intervention. Thus far the mechanisms of action of apparent carcinogens and anticarcinogens in the diet are not well understood.
Epidemiologic studies of dietary intakes or serum nutrient levels are not specific enough to isolate clearly the most promising agents for dietary intervention and human data on the side effects of long-term administration of potential anticarcinogens are limited. On the other hand, the potential pay-off for cancer reduction or control is great: to benefit from the application of a preventive agent, it is not necessary to understand the mechanism of action.
Histologic, cytochemic, cytogenetic and epidemiologic evidence strongly suggests that consecutive grades of Cervical Intraepithelial Neoplasia CIN I, II, III c.q. mild, moderate and severe dysplasia and carcinoma in situ are part of a stepwise process that proceeds from normal epithelium to invasive cervical cancer (136,440).
Because of the fact that the uterine cervix is easily visualized and the precancerous condition is well-documented, this disease offers unique opportunities for intervention studies. Prospective trials, although not in a large scale, have been performed and documented the effectiveness of population screening programs on cervical cancer in asymptomatic women. There is no longer any doubt that the incidence, morbidity and mortality of invasive cervical cancer can be reduced by well performed screening programs in the entire population at risk (43,169,246,305,306,509). In The Netherlands the number of registrated cancer deaths due to cancer of the uterine cervix has been decreasing in the last decennia from 464 registered cervical cancer deaths in 1969 to approx. 300 in 1985.

Recently, nutritional factors have been reported to be associated with cervical intraepithelial neoplasia (CIN) (452,576). This offers alternatives to perform intervention studies other than cytologic screening of asymptomatic women (561).

Folic Acid has long been recognized as an inhibitor of tumor growth (316,449). Folic acid is an essential vitamin, involved in the synthesis of nucleic acids, in that it acts as a co-enzyme in the transfer of one carbon units and, as such, is vital in the synthesis of both purines and pyrimidines, the nucleic acid building blocks of DNA. It also helps catalyze a number of other important reactions in the cell, including the initiation of protein synthesis and metabolism of carbohydrates. It plays an important role in growth and in maturation of erythrocytes. When ingested orally it is rapidly and completely resorbed by the gastro-intestinal tract. Toxicity caused by allergy or overdose is described but it is extremely rare (109,228,245,445,593).

It has been observed repeatedly that folic acid deficiency is associated with morphologic changes in cervical smears that are similar to those observed in cervical dysplasia (284, 292, 320, 585). Kitay and Wentz (284) described these changes in a group of pregnant women: the cells were smaller, with an increased nuclear/cytoplasmic ratio and cytoplasmic vacuolization. Similar "megaloblastic" changes, including frequent multinuclear cells, have been reported in women

taking combined oral contraceptives. Whitehead et al (585) noted these abnormalities in 19% of 115 women studied; none of them was anemic and only a few had low serum folate levels. Eight of these patients were then treated with folic acid, 10 mg a day, and by day 21 the cervical cytologic changes in all 8 patients had improved.
Referring to these observations, Butterworth et al. (93,95) postulated that prolonged exposure of target tissue to contraceptive steroids might produce localized alterations in folic acid metabolism in such a way as to favor neoplastic transformation. They suggested that correction of such a localized deficiency by providing a daily systemic exogenous supplement of this vitamin, was capable of altering the course of early cervical dysplasia c.q. cervical intraepithelial neoplasia (CIN). In 1980 and definitively in 1982, they reported results from a randomized trial: 78 combination-type oral contraceptive users with mild or moderate dysplasia of the uterine cervix (CIN I or II) diagnosed by cervical cytology smears were enrolled in a prospective study. All were randomized to receive oral supplements of folic acid, 10 mg, or a placebo, ascorbic acid, 10 mg, daily for three months under double blind conditions. All had used oral contraceptive agents (O.C.A.'s) for at least 6 months and continued these while returning monthly for follow-up examinations. Of the 47 (60%) evaluable patients all smears and a biopsy obtained at the end of the trial were classified without knowledge of treatment status, using a scoring system as to the degree of intraepithelial neoplasia. The mean biopsy dysplasia score and final versus initial cytology score were significantly better (paired T-test; $p \leqslant 0.05$) in patients receiving folic acid. Treatment with folic acid was associated with a consistent downward trend (i.e. improvement) of cytology scores.
Their study seems to indicate that either a reversible localized derangement in folate metabolism may sometimes be misdiagnosed as cervical dysplasia, or else such a derangement is an integral component of the dysplastic process that may be arrested or in some cases reversed by folic acid supplementation.

Upon publication of the first results of Butterworth's study in 1980 (93,117), several considerations have motivated the present author to further investigate the possible benefits of intervention with folic acid in cervical intraepithelial neoplasia:

a. The concept of localized nutrient deficiency provides rationale for a folic acid intervention study. It seems doubtful that folic acid deficiency itself can lead to cervical neoplasia. However, suboptimal levels of folate, perhaps even localized deficiencies, influenced in some way by steroid hormones, may reduce the ability of cervical epithelium to repair changes in DNA introduced by other agents, such as oncogenic viruses.
b. The study by Butterworth et al. (93,95) did not include initial biopsies, nor impressions of the colposcopic grade of CIN at subsequent follow-up examination. Among 25 subjects receiving the placebo, there were five cases in which the biopsy was two stages more severe than the most severe cytology score at the outset; four of these were read as carcinoma in situ. In the group of 22 subjects receiving folate, there was only one example of a two-stage difference; namely, a biopsy read as severe after four previous cytology readings of mild dysplasia. Those changes have been looked upon as "progression".

There were no cases of carcinoma in situ among subjects receiving folate. It is conceivable that the cases of CIS diagnosed in the placebo group may well have been present initially in view of the usual time course of progression (441), but underdiagnosed by cytology.

c. The Butterworth-study was restricted to current OCA-users; corresponding results for non-users would be interesting also. Thus far no convincing causal relationship between CIN and OCA's has been demonstrated. The occurrence of CIN in OCA-users seems to be rather related to sexual history than to the OCA's (74, 123, 537, 554, 559).
d. Only 48 of 78 (60%) women enrolled in the study (1976-1982) completed the protocol and were included in the analysis. Thus, serious bias in the reported results seems possible.

Objective of the study

The aim of the study was to assess the effects of oral folate supplementation in the prevention of progression, or the promotion of regression to normalcy in outpatients with cervical smears suggestive of CIN after colposcopic and histopathologic evaluation.

9.2 Patients and Methods

From December 1981 to June 1983 a prospective study was performed, involving 100 consecutive patients with cytologic suspicion of cervical intraepithelial neoplasia (CIN), who met the eligibility criteria and who agreed voluntarily upon verbal informed consent to participate in the study, to assess in a randomized double blind design whether a daily supplementation of oral folic acid for 100 days is capable of altering the course of cervical intraepithelial neoplasia.

9.2.1 *Patient selection*

Candidates for the study were selected from a population of women referred to the outpatient colposcopy clinic of the Department of Gynecologic Oncology of the Erasmus University Hospital-Dijkzigt, for evaluation of cervical smears, suggestive of CIN. As referral cytology diagnosis was documented the most abnormal cervical smear, taken more than 6 weeks and less than 1 year before initial examination at the colposcopy clinic.

INCLUSION CRITERIA

All patients were colposcopically evaluated by the present author.
If according to the referral cytology report(s) and the colposcopic impression cervical intraepithelial neoplasia was confirmed and invasive disease considered unlikely, for practical reasons at this point of time, patients were potentially eligible for enrollment in the study.

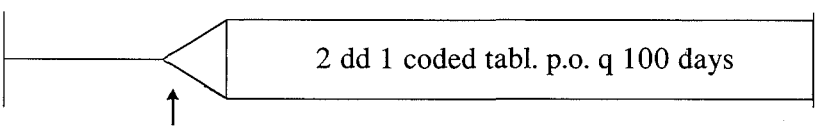
EXCLUSION CRITERIA

1. Age > 60 years.
2. Pregnancy or intended pregnancy within 3 months.
3. History or suspicion of any invasive malignancy.
4. Extension of the lesion toward the vaginal fornices.
5. History of intra-uterine diethyl stilbestrol exposure (DES).
6. Pernicious anemia.
7. Use of anticonvulsants or antimalarial medications.
8. Circumstances that were likely to prevent completion of the trial.
9. Not consenting to participate in the study upon informed consent by the author.

9.2.2 *Informed consent*

The author explained carefully to all candidates before enrollment in the study the non-invasive nature of the cervical lesion, including the risk of progression to cancer if unattended for 3 or 4 months and described the various forms of available therapy. Patients were presented with the option of participating in the study for 100 days with the understanding that they would return for follow-up examination and, when indicated, definitive treatment after 3 months and with the understanding that the experimental treatment might be of no benefit, but otherwise harmless. To all subjects it was explained that they would receive coded tablets for 100 days, either folic acid or a placebo, and that the (author-) physician did not know how the tablets were distributed among the participants.

9.2.3 *Study design*



day 1: randomization
Initial examination
medical history
colposcopy
cytology
histopathology

day 100-110
Final examination
medical history
colposcopy
cytology
histopathology

Those patients consenting to participate received a coded 100 day supply of 200 tablets, each containing 5 mg folic acid (UCB-Nederland) or the placebo. (Contents of the placebo: Lactose 31.6%, Avicel 66.8%, Aerosil std 0.1%, Color certolake, Yellow quinoleine 0.667%, Yellow ferrous oxyde 0.33%, magnesium stearate 0.5%).
They were given instructions to take 1 tablet orally twice a day, in between meals, for 100 days. All tablets were identical in appearance and were prepared

ERASMUS UNIVERSITY HOSPITAL DIJKZIGT
PROJECTFORM FOLACID
Registration nr.
date

PERSONAL DATA
Name
Address
Date of birth

Age	Race	Marital status	Nr. grav.	Nr. parity	Nr. spont. abortions	Nr. ab. a. prov.
Menarche	Sexarche	Nr. sexual partners	Pregnancy desired?	Contra-ception	L.M.P. day 1	

HISTORY
HISTORY CERVICAL PATHOLOGY

	FINAL EXAMINATION	INITIAL EXAMINATION
DATE		
CYTOLOGY		
endocervic	neg./-itis/CIN I/II/III	neg./-itis/CIN I/II/III
ectocervix		
(viral)infection	viral: yes/no; infect. yes/no	viral: yes/no; infect. yes/no
COLPOSCOPY		
* = location		
biopsies		
findings:		
S.C.J.	yes/no/?	yes/no/?
lesion compl. visible	yes/no/?	yes/no/?
T.Z. Hor.	mm	mm
Vert.	mm	mm
Nr. Biopsies		
ECC	yes/no	yes/no
Viral infection	yes/no/?	yes/no/?
COLP. IMPRESSION	neg./itis/CIN I/II/III	neg./-itis/CIN I/II/III
HISTOPATHOLOGY		
ECC/Bx		
Biopsies	neg./itis/ CIN I/II/III	neg./-itis/CIN I/II/III
Viral infection	yes/no/?	yes/no/?
SERUM FOLATE	nmol/l; not done	nmol/l; not done
TABLET INTAKE		
REMARKS		
TREATMENT		no/yes type:

by UCB-Nederland: from 1 to 100 numbered prescription bottles were filled from a list of random numbers and the contents were documented and kept secret by UCB-Nederland. Allocation of patients to the Folacid or the Placebo group was by consecutive supply from 1 to 100 of the numbered bottles by the author. The code was not broken until the end of the study.

9.2.4 *Study requirements*

Prestudy requirements included completion of personal and medical histories, which were documented on the protocol-form (see addendum 9.I).
Colposcopic evaluation was performed as described previously (Chapter 7, this thesis; 588). A colposcopic impression of the grade of CIN and the presence of a viral infection was documented, endo- and ectocervical smears were repeated and colposcopically directed punch biopsies were taken from the most suspicious areas of the portio. If colposcopy was unsatisfactory, i.e. the entire lesion and / or squamocolumnar junction could not completely be visualized or a grade III CIN-lesion was suspected, an endocervical curettage and blind endocervical biopsy were performed. Tissue samples were sent in standard fixative for histopathologic examination by the hospital Pathology Department with standard questions: CIN? Grade? Viral infection? Malignancy?
Pre- and post-study blood samples for serum folate concentration test by the hospital hematologic-biochemical laboratory (radio isotope dilution assay) were proposed to all patients, but not considered to be obligatory for enrollment in the study because of primary emphasis on the effects of supplementation, cytologic, colposcopic and histopathologic evaluation. Participants were then scheduled for the final evaluation at the colposcopy clinic as soon as possible after completion of the 100 tablet days, which visit was usually within 110 days of the tablet start. No gynecologic interventions or medications were planned during the tablet intake period, except in emergencies. At the final study-visit medical history was completed, colposcopy and endo- and ectocervical smears were repeated; final colposcopic impression of the grade of the lesion(s) was documented and biopsies with or without endocervical curettage were repeated under colposcopic guidance. Electively, serum folate concentration was measured again to monitor blood folate levels as well as to assess tablet intake compliance. In addition, asking for and counting of non-ingested pills further helped to monitor compliance. All final cytology and histopathology samples were sent to the pathology laboratory without mentioning of the initial results.

9.2.5 *Evaluation criteria*

Study end points were defined as the findings at the final colposcopy clinic session, scheduled after 100 days.
Assessment of this prospective randomized study will be divided into the following sections:
- Evaluation of the patient characteristics and comparability of the Folacid and Placebo group at enrollment in the study (9.4).

- Evaluation of the cytologic, colposcopic and histopathologic findings at initial examination in both treatment groups (9.5).
- Evaluation of the cytologic, colposcopic and histopathologic results of the final examination in both groups (9.6).
- Lastly, the primary goal of the study will be tested: are there significant differences between the initial and final examination results between both groups (9.7)?

The following standards were defined as to evaluate progression, stabilization or regression of CIN: The difference in grade of CIN between the initial and final evaluation as determined separately by cytology and colposcopy, and as the final "standard" histopathology: the highest grade of CIN in biopsies and endocervical curettings.
PROGRESSION was defined as the presence of CIN in the final evaluation at least one grade more severe than in the initial examination.
STABILIZATION as the presence of the same grade of CIN in both initial and final evaluation.
REGRESSION as the absence of CIN, or CIN at least one grade less severe in the final evaluation than in the initial examination.

9.2.6 *Methods of analysis*

All data from the protocol sheets were coded and prepared for computer-analysis (VAX/VMS) and statistics (SPSS-X; statistical package for the Social Sciences). For a description of the methods of analysis, the interested reader is referred to chapter 7.2.

If there is an effect of folacid supplementation on CIN which will manifest itself within 3 months (93,95), and the spontaneous regression rate to be expected equals .50 (315), to demonstrate a 30% improvement under the conditions of the α-level of the statistical sifnificance ($\alpha = 0.05$) and the β-error $= 0.15$, then a sample size of 50 patients in each treatment group will be needed.
To test for significant differences between quantitative intervals i.e. between the initial and final serum folate concentrations in both groups compared, a multivariate analysis of variance (MANOVA) was used.

RESULTS

9.3 Evaluability

From the randomization center only 98 numbered bottles were received, so that a total of 98 patients were enrolled and all 98 patients were seen for final evaluation.
Twelve of 98 patients enrolled in the study (12.2%) were considered not evaluable and were removed from the analysis. In 8 (8.2%) CIN, expected on the impression of cytology and colposcopy at the initial examination, was not confirmed at the histopathologic evaluation (false positive cytology and colposcopy 8.2%). In 2 (2.0%) patients data of the final evaluation were incomplete with respect to histopathology, and 2 (2.0%) patients admitted at the final evaluation that they had not taken their pills for more than a couple of days. Of the remaining 86, who were considered evaluable patients and who completed the protocol, 43 were in the "Folacid" and 43 in the "Placebo" group.

9.4 Comparability of the study groups: patient characteristics

9.4.1 *AGE at enrollment in the study distinguished by treatment groups*

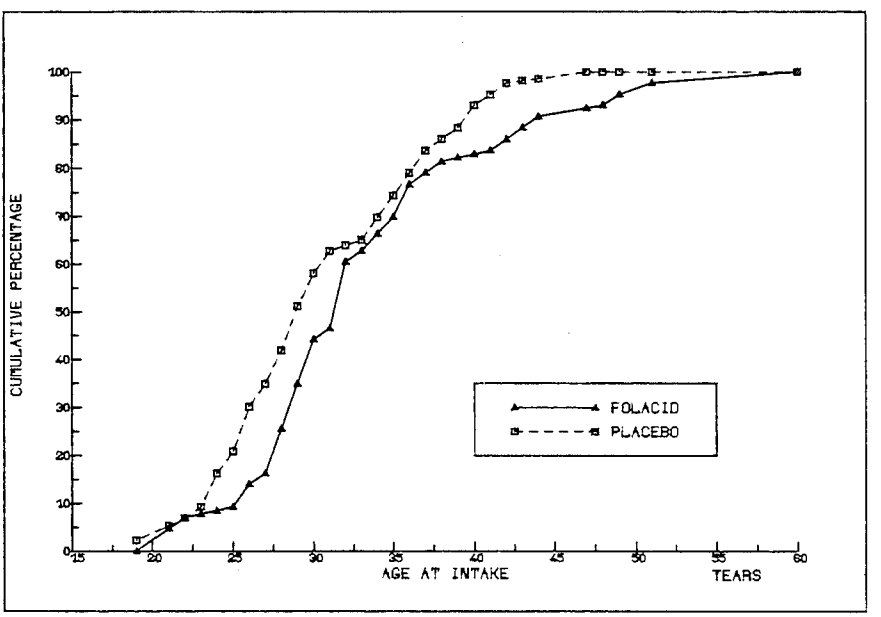


Figure 9.4.1. Age (years) at initial examination.

AGE (years)	FOLACID	PLACEBO
n =	43	43
median*	32	29
interquartile range**	28-36	26-36
range***	21-60	19-47

*Median: i.e. the halfway or middle observation in the sample.
**Interquartile range: i.e. the first and last 25% points of the ordered sampled.
***Range = minimum-maximum.
There are no significant differences (Mann-Whitney U-test; p = n.s.).

9.4.2 *RACE, distinguished by treatment groups*

Table 9.4.2. Race

RACE	Folacid n=43 %	Placebo n=43 %	Total %	n
Caucasian	81.4	81.4	81.4	70
Negroid	14.0	14.0	14.0	12
Mongoloid	2.3	2.3	2.3	2
Mediterranean	2.3	2.3	2.3	2
Total			100	86

There are no differences ($\chi^2 = 0$; d.f. = 3; p = n.s.).

9.4.3 *MARITAL STATUS, distinguished by treatment groups*

Table 9.4.3. Marital status

MARITAL STATUS	Folacid n=43 %	Placebo n=43 %	Total %	n
Stable relation	53.5	55.8	54.7	47
Single	18.6	30.2	24.4	21
Separated	27.9	14.0	20.9	18
Total			100	86

There are no significant differences ($\chi^2 = 3.2$; d.f. = 2; p = n.s.).

9.4.4 *PREGNANCIES, distinguished by treatment groups*

Table 9.4.4. Number of pregnancies per patient

PREGNANCIES (frequencies)	Folacid n=43 %	Placebo n=43 %	Total %	n
0	23.3	25.6	24.4	21
1-2	53.5	46.4	50.0	43
3-4	20.9	14.0	17.5	15
⩾5	2.3	14.0	8.1	7
Totall			100	86

There are no significant differences ($\chi^2 = 8.2$; d.f. = 7; p = n.s.).

9.4.5 *PARITY, distinguished by treatment groups*

Table 9.4.5. Parity

PARITY (frequencies)	Folacid n=43 %	Placebo n=43 %	Total %	n
0	44.2	32.5	38.4	33
1-2	34.8	48.8	41.9	36
3-4	18.7	9.3	13.9	12
⩾5	2.3	9.3	5.8	5
Total			100	86

There are no significant differences ($\chi^2 = 11.0$; d.f. = 7; p = n.s.).

9.4.6. *ABORTIONS, spontaneous, distinguished by treatment groups*

Table 9.4.6. Spontaneous abortions

ABORTIONS (spont.) (frequencies)	Folacid n=43 %	Placebo n=43 %	Total %	n
0	83.7	86.0	84.9	73
1	16.4	4.7	10.5	9
⩾2	0.0	9.3	4.6	4
Total			100	86

There are no significant differences ($\chi^2 = 6.8$; d.f. = 3; p = n.s.).

9.4.7. *ABORTIONS, arte provocatus, distinguished by treatment groups*

Table 9.4.7. Abortions arte provocatus

ABORTIONS (arte prov..) (frequencies)	Folacid n=43 %	Placebo n=43 %	Total %	n
0	79.1	76.7	77.9	67
1	18.6	16.3	17.4	15
⩾2	2.3	7.0	4.7	4
Total			100	86

There are no significant differences ($\chi^2 = 1.1$; d.f. = 2; p = n.s.).

9.4.8 *MENARCHE, age (years) at first menstrual period, distinguished by treatment groups*

Table 9.4.8. Menarche.

MENARCHE (years)	Folacid	Placebo
n =	42	43
median	13	13
interquartile range	11-14	11.5-14
range	10-17	9 -18

There are no significant differences. One patient could not recal her age at first period (Mann-Whitney U-test; p = n.s.).

9.4.9. *SEXARCHE, age (years) at first coitus, distinguished by treatment groups*

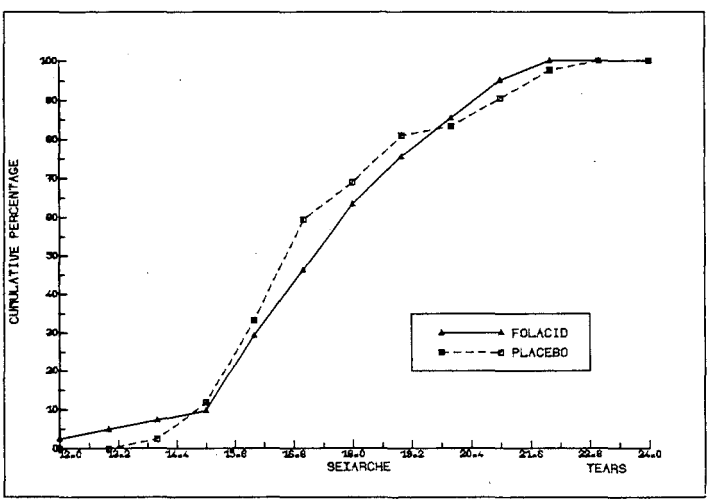


Figure 9.4.9. Sexarche.

SEXARCHE	Folacid	Placebo
n =	41	42
median	18	17
interquartile range	16-19.5	16-19
range	12-22	14-23

There are no significant differences (Mann-Whitney U-test; p = n.s.).
Three patients refused to mention their sexarche.

9.4.10. *MALE SEXUAL PARTNERS, distinguished by treatment groups*

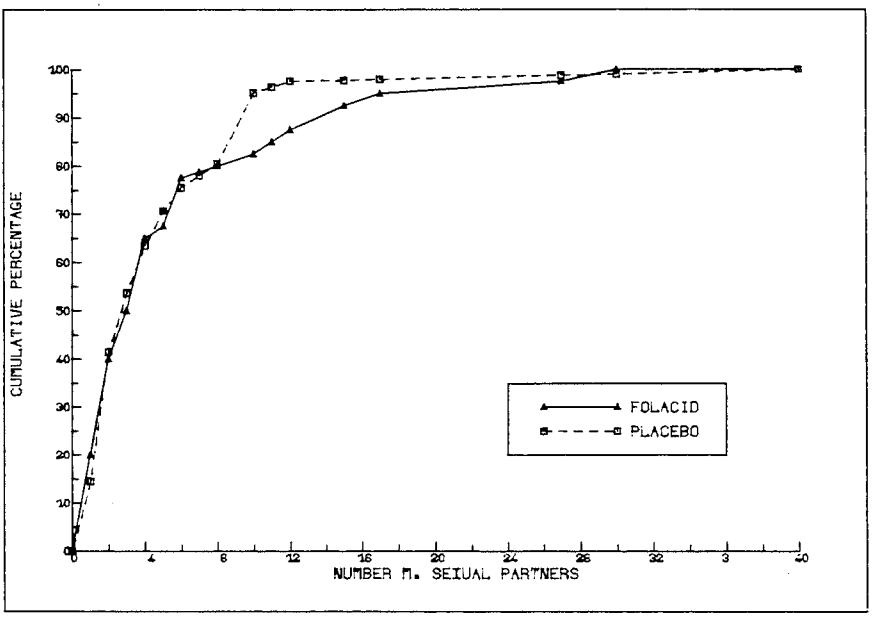


Figure 9.4.10. Number of male sexual partners

MALE SEXUAL PARTNERS	Folacid	Placebo
n =	40	41
median	3.5	3.0
interquartile range	2-6	2-6.5
range	1-30	1-40

There are no significant differences (χ^2 = 15.9; d.f. = 15; p = n.s.; Mann-Whitney U-test; p = n.s.). Five patients refused to mention the number of their sexual partners.

9.4.11 *PREGNANCY DESIRE in the future, distinguished by treatment groups*

Table 9.4.11. Future pregnancy desire

PREGNANCY DESIRE	Folacid n=43 %	Placebo n=43 %	Total %	n
Yes	51.2	65.1	58.1	50
No	44.2	32.6	38.4	33
Not known	4.7	2.3	3.5	3
Total			100	86

There are no significant differences ($\chi^2 = 1.8$; d.f. = 2; p = n.s.).

9.4.12. *CONTRACEPTION, distinguished by treatment groups*

Table 9.4.12. Contraceptive methods at initial examination

CONTRACEPTION	Folacid n=43 %	Placebo n=43 %	Total %	n
None	30.2	27.9	29.1	25
Hormonal	30.2	48.8	39.6	34
I.U.D.	16.3	7.0	11.6	10
Protectives/pessary	2.3	7.0	4.7	4
Sterilization	21.0	9.3	15.0	13
Total			100	86

There are no significant differences ($\chi^2 = 7.0$; d.f. = 4; p = n.s.).

9.4.13. *CYTOLOGY, predicted grade of CIN at referral to the colposcopy clinic, distinguished by treatment groups*

Table 9.4.13. Referral cytology: grades of CIN

REFERRAL CYTOLOGY Grade of CIN	Folacid n=43 %	Placebo n=43 %	Total %	n
I	14.0	23.3	18.6	16
II	58.1	48.8	53.5	46
III	27.9	27.9	27.9	24
Total			100	86

There are no significant differences ($\chi^2 = 1.3$; d.f. = 2; p = n.s.).

In summary (chapter 9.4): there are no significant differences with respect to the documented patient-characteristics between both treatment groups. The predicted grades of CIN in the referral cytology reports appeared to be not significantly different in both groups.

9.5 Evaluation criteria: Initial examination

9.5.1 *SERUM FOLATE ACID CONCENTRATION at enrollment in the study, distinguished by treatment groups*

65 of 86 patients (75.6%) agreed at initial examination to have a serum sample drawn for a serum folic acid concentration test (Radio-isotope dilution assay).

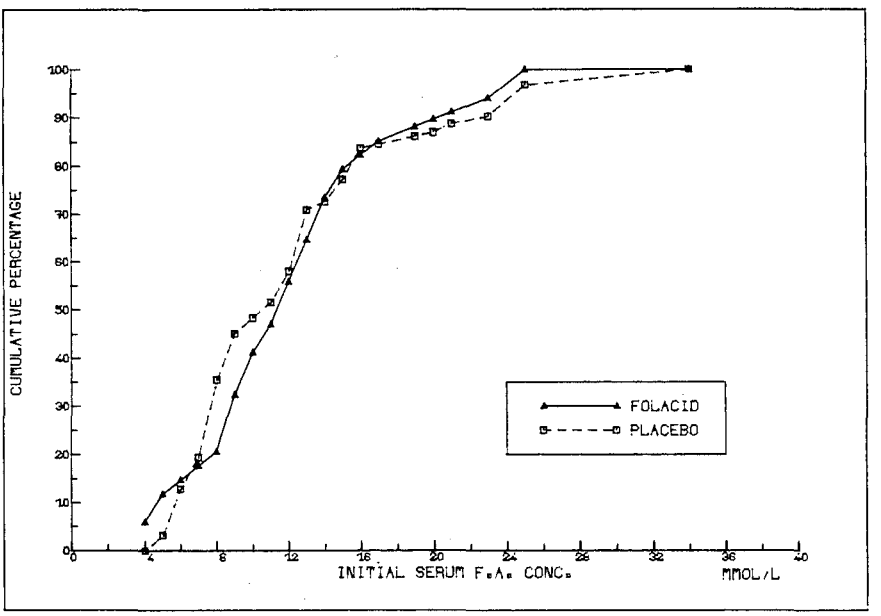


Figure 9.5.1. Initial serum folate acid (F.A.) concentration (normal range 7.1-23.0 nmol/l).

SERUM FOLATE (nmol/l) at initial examination	Folacid	Placebo
n =	34	31
median	12.0	11.0
interquartile range	9-15	8-15
range	4-25	5-34

There are no significant differences (Mann-Whitney U-test; p = n.s.).

9.5.2 *COLPOSCOPIC FINDINGS at initial examination, distinguished by treatment groups*

Table 9.5.2. Colposcopic impression at initial examination

COLPOSCOPIC IMPRESSION Grade of CIN	Folacid n=43 %	Placebo n=43 %	Total %	n
Leucoplakia	2.3		1.2	1
CIN I	41.9	41.9	41.9	36
CIN II	51.2	55.8	53.5	46
CIN III	4.7	2.3	3.5	3
Total			100	86

There are no significant differences ($\chi^2 = 1.4$ (Yates correction); d.f. = 3; p = n.s.).

9.5.3 *SQUAMO COLUMNAR JUNCTION (SCJ), colposcopically visualized at the initial examination, distinguished by treatment groups*

Table 9.5.3. Visualization of the SCJ at the initial colposcopy.

SQUAMO-COLUMNAR JUNCTION Initial examination	Folacid n=43 %	Placebo n=43 %	Total %	n
entirely visualized	72.1	62.8	67.4	58
not entirely visualized	27.9	37.2	32.6	28
Total			100	86

There are no significant differences ($\chi^2 = 0.5$ (Yates correction); d.f. = 1; p = n.s.).

9.5.4 *UPPER MARGINS OF THE LESION(S), colposcopically visualized at the initial examination, distinguished by treatment groups*

Table 9.5.4.1. Visualization of the upper margins of the lesions at the initial colposcopy

UPPER MARGIN LESION(S) VISUALIZED (initial examination)	Folacid n=43 %	Placebo n=43 %	Total %	n
Yes	67.4	62.8	65.1	56
No	21.0	27.9	24.4	21
Questionable	11.6	9.3	10.5	9
Total			100	86

There are no significant differences ($\chi^2 = 0.6$; d.f. = 2; p = n.s.).

Only when both the SCJ and the upper margins of the lesion(s) were colposcopically completely visualized, colposcopy was considered to be satisfactory.

Table 9.5.4.2. Satisfactory colposcopy at initial examination

COLPOSCOPY (initial exam.)	Folacid n=43 %	Placebo n=43 %	Total %	n
Satisfactory	67.4	60.4	64.0	55
Unsatisfactory	32.6	39.6	36.0	31
Total			100	86

There are no significant differences ($\chi^2 = 0.01$ (Yates correction); d.f. = 1; p = n.s.).

9.5.5 *ENDOCERVICAL CURETTAGE at initial examination, distinguished by treatment groups*

To make invasive disease beyond the colposcopically visible part of the endocervical canal very unlikely, in all patients with unsatisfactory colposcopy, e.g. squamo-columnar junction and upper margin(s) of the lesion(s) not entirely visualized, and also in most cases in which a grade III CIN-lesion was suspected, an endocervical curettage usually including a blind endocervical biopsy was performed at the initial evaluation, after taking endo- and ectocervical smears and colposcopically directed biopsies.

Table 9.5.5. Endocervical curettage at initial examination, distinguished by treatment groups

ENDOCERVICAL CURETTAGE initial examination	Folacid n=43 %	Placebo n=43 %	Total %	n
Yes	53.5	44.2	48.8	42
No	46.5	55.8	51.2	44
Total			100	86

There are no significant differences ($\chi^2 = 0.4$; d.f. = 1; p = n.s.).

9.5.6 *SURFACE OF THE TRANSFORMATION ZONE at initial examination, distinguished by treatment groups*

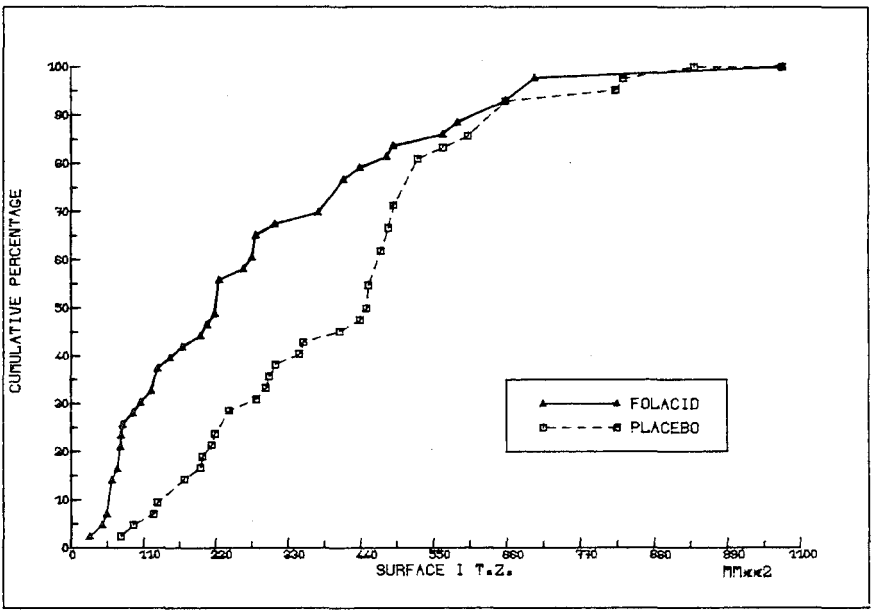


Figure 9.5.6. Surface of the transformation zone (T.Z.) at initial examination.

SURFACE T.Z. (mm^2) initial examination	Folacid	Placebo
n=	43	43
median	226	450
interquartile range	78- 414	235.5-527
range	28-1074	75 -942

There appears to be a significant difference; the surface of the transformation zones in the placebo group being significantly larger than in the folacid group (Mann-Whitney U-test; $0.001 < p \leqslant 0.01$).

9.5.7 *Suspicion of cervical VIRAL INFECTION on COLPOSCOPIC IMPRESSION at initial examination, distinguished by treatment groups*

Table 9.5.7. Colposcopic suspicion of cervical viral infection at initial examination

COLPOSCOPIC IMPRESSION initial exam. Grade of CIN	Folacid n=43 % viral infection	Placebo n=43 % viral infection	Total % viral infection	(n)
Leukoplakia	2.3		1.2	(1/1)
CIN I	20.9	16.3	18.6	(16/36)
CIN II	16.3	20.9	18.6	(16/46)
CIN III	4.7		2.3	(2/3)
Total	44.2	37.2	40.7	(35/86)

There are no significant differences ($\chi^2 = 3.2$; d.f. = 3; p = n.s.).

9.5.8. *Results of REPEAT-CYTOLOGY of endo- and ectocervix at initial examination, distinguished by treatment groups*

Table 9.5.8. Results of repeat-cytology at initial examination

CYTOLOGIC GRADE OF CIN initial exam.	Folacid n=43 %	Placebo n=43 %	Total %	n
Cervicitis only	4.7	9.3	7.0	6
CIN I	23.3	34.9	29.1	25
CIN II	58.0	46.5	52.3	45
CIN III	14.0	9.3	11.6	10
Total			100	86

There are no significant differences ($\chi^2 = 2.6$; d.f. = 3; p = n.s.).

At the initial examination, in only 6 patients of the Folacid and in 1 patient of the Placebo group, there was suspicion of viral infection on repeat cytology. This difference was not significant ($\chi^2 = 10.8$; d.f. = 3; p = n.s.).
For both the Folacid as well as the Placebo group, there was significant agreement between the cytologically expected grade of CIN in the cytology reports at referral to the colposcopy clinic and in the repeat-cytology at initial examination.
(For the Folacid-group: $\chi^2 = 19.1$; d.f. = 6; $0.001 < p \leqslant 0.01$;
for the Placebo-group: $\chi^2 = 16.6$; d.f. = 6; $0.01 < p \leqslant 0.02$;

Between referral- and repeat cytology there was complete agreement within the same grade of CIN in 63% and 54% for resp. the Folacid- and Placebo-

group; "overdiagnosis" by referral cytology in 30% resp. 37%; and "underdiagnosis" by referral cytology in 7% and 9% compared with repeat cytology at initial examination.

9.5.9. *Results of HISTOPATHOLOGIC SPECIMENS taken at the initial examination, distinguished by treatment groups*

Table 9.5.9. Number of colposcopically directed cervical biopsies taken at the initial examination

BIOPSIES taken at initial exam. (frequencies)	Folacid n=43 %	Placebo n=43 %	Total %	n
1	39.5	34.9	37.2	32
2	46.5	53.5	50.0	43
3	14.0	7.0	10.5	9
4		4.7	2.3	2
Total			100	86

There are no significant differences ($\chi^2 = 3.3$; d.f. = 3; p = n.s.).

There was no case in which the grade of CIN in the endocervical curettings or blind endocervical biopsy was more severe than in the under colposcopic guidance taken punch biopsies from the ectocervix or the part of the endocervical canal that could be visualized. There also were no cases in which the ECC was positive for CIN, while the colposcopically directed biopsies were negative. Of 42 endocervical curettings taken in 86 patients at the initial evaluation (48.8%), 24 were negative and 18 (42.9%) positive for CIN: 12 × CIN I, 4 × CIN II and 2 × CIN III. In 5 of 42 ECC's (11.9%) there were signs of viral infection. There were no significant differences in CIN-grades in the ECC's between the Folacid and Placebo group ($\chi^2 = 4.5$; d.f. = 3; p = n.s.).

9.5.10. *The highest HISTOPATHOLOGIC GRADE OF CIN in biopsies and ECC taken at initial examination, distinguished by treatment groups*

Table 9.5.10. Histopathologic grade of CIN at initial examination.

MAXIMAL HISTOPATHOLOGIC GRADE OF CIN at initial exam.	Folacid n=43 %	Placebo n=43 %	Total %	n
CIN I	27.9	48.8	38.4	33
CIN II	41.9	39.5	40.7	35
CIN III	30.2	11.6	20.9	18
Total			100	86

The Folacid group appears to contain significantly more patients with higher grades of CIN ($\chi^2 = 6.0$; d.f. = 2; $0.02 < p \leqslant 0.05$).

9.5.11. *HISTOPATHOLOGIC DIAGNOSIS of cervical VIRAL INFECTION at initial examination, distinguished by treatment groups*

Table 9.5.11. Histopathological diagnosis of cervical viral infection at initial examination.

HISTOPATHOLOGIC DIAGNOSIS initial exam. Grade of CIN	Folacid n=43 % viral infection	Placebo n=43 % viral infection	Total % viral infection	(n)
CIN I	9.3	16.3	12.7	(11/33)
CIN II	7.0	4.7	11.6	(5/35)
CIN III	7.0	4.7	11.6	(5/18)
Total	23.3	25.6	24.4	(21/86)

There are no significant differences ($\chi^2 = 1.8$; d.f. = 2; p = n.s.).

Over all there was significantly more suspicion of viral infection at colposcopic (40.7%) than at histopathologic initial examination (24.4%) ($\chi^2 = 9.65$ (Yates' correction); d.f. = 1; $0.001 < p \leqslant 0.01$), however, this difference was not significant for both treatment groups.
Folacid: $\chi^2 = 3.33$ (Yates correction); d.f. = 1; p = n.s.
Placebo: $\chi^2 = 0.09$ (Yates correction); d.f. = 1; p = n.s.

9.5.12. *Summary of the initial examination*

The analysis of the results of the initial examination does not show significant differences with respect to serum folate concentrations, satisfactory colposcopies,

Table 9.5.12. : Agreement of Cytology and Colposcopy with the histopathologic grade of CIN at the initial examination.

Histopathologic grade of CIN predicted by	% Agreement within the same grade of CIN	% Over-diagnosis	% Under-diagnosis	Significance testing (X^2-test) with histopathology
Referral-cytology				
Folacid	40	37	23	p = n.s.
Placebo	40	44	16	p = n.s.
Repeat-cytology				
Folacid	35	28	37	p = n.s.
Placebo	58	23	19	0.001 < p < 0.01
Colposcopy				
Folacid	35	16	49	p = n.s.
Placebo	60	21	19	0.001 < p < 0.01

number of biopsies and endocervical curettings, expected grades of CIN as determined by cytology and colposcopy, and colposcopic and histopathologic suspicion of viral cervical infections between both treatment groups.
However, the surfaces of the transformation zones in the Placebo group were significantly larger than in the Folacid group, and the histopathologic grades of CIN of the Folacid group were more severe than of the Placebo group.
Taking the histopathologic grade of CIN as the final "standard" the agreement of cytology and colposcopy with this "standard" is presented in Table 9.5.12.

9.6 Evaluation criteria: Final examination

Of the 86 evaluable patients, 85 had their final examination at the colposcopy clinic. One patient insisted secondarily to have her CIN III lesion surgically removed. On day 92 of the tablet period a cone biopsy and endocervical curettage were performed. Because she had taken her pills in the preceeding period, she was considered to be evaluable. Thus, all 86 evaluable patients had specimens taken for histopathology. 85 patients had final colposcopy and in 83 patients representative endo- and ectocervical smears were taken.

9.6.1. *SERUM FOLATE ACID CONCENTRATION at the final examination, distinguished by treatment groups*

Sixty one out of 86 (70.9%) patients had blood samples drawn for serum folate acid concentration test at final examination. 30 patients of the Folacid group and 22 of the Placebo group had serum folate acid concentration determined at initial as well as final examination.

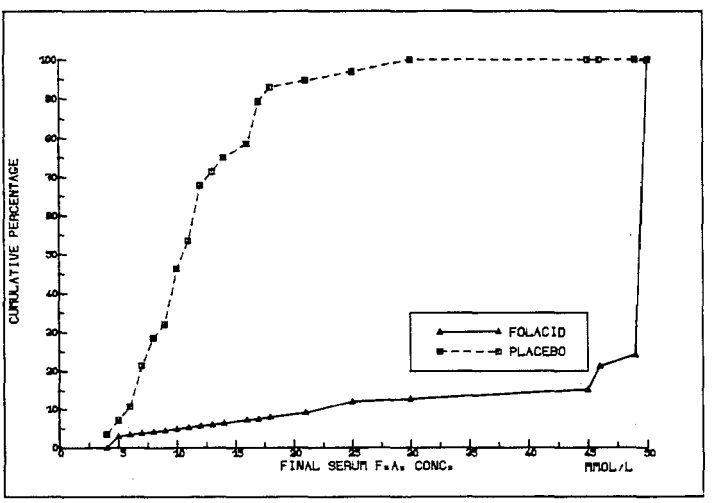


Figure 9.6.1. Final serum folate acid (F.A.) concentration (normal range 7.1-23.0 nmol/l).

SERUM FOLATE (nmol/l) at final examination	Folacid	Placebo
n =	33	28
median	⩾50	11
interquartile range	49.5-⩾50	8-15.5
range	5-⩾50	4-30

There is a highly significant difference (Mann-Whitney U-test; $p \ll 0.001$).

9.6.2. *COLPOSCOPIC FINDINGS at final examination, distinguished by treatment groups*

Table 9.6.2. Colposcopic impression at final examination.

COLPOSCOPIC IMPRESSION final exam. Grade of CIN	Folacid n=42 %	Placebo n=43 %	Total %	n
Neg. for CIN	16.6	11.6	14.1	12
CIN I	28.6	48.8	38.8	33
CIN II	52.4	32.6	42.4	36
CIN III	2.4	7.0	4.7	4
Total			100	85

There are no significant differences ($\chi^2 = 5.6$; d.f. = 3; p = n.s.).

9.6.3. *SQUAMO-COLUMNAR JUNCTION (SCJ), colposcopically visualized at the final examination, distinguished by treatment groups*

Table 9.6.3.1. Visualization of the SCJ at the final colposcopy.

SQUAMO-COLUMNAR JUNCTION final examination	Folacid n=42 %	Placebo n=43 %	Total %	n
entirely visualized	78.6	86.0	82.4	70
not entirely visualized	21.4	14.0	17.6	15
Total			100	86

There are no significant differences ($\chi^2 = 1.8$; d.f. = 1; p = n.s.).

9.6.4. *UPPER MARGINS of the lesion(s), colposcopically visualized at the final examination, distinguished by treatment groups*

Table 9.6.4.1. Visualization of the upper margins at the final colposcopy

UPPER MARGIN LESION(S) VISUALIZED final examination	Folacid n=42 %	Placebo n=43 %	Total %	n
Yes	78.6	83.7	81.2	69
No	16.7	14.0	15.3	13
Questionable	4.7	2.3	3.5	3
Total			100	85

There are no significant differences ($\chi^2 = 1.5$; d.f. = 2; p = n.s.).

When both the SCJ and the upper margins of the lesions were completely visualized, colposcopy was considered to be satisfactory.

Table 9.6.4.2. Satisfactory colposcopy at the final examination.

COLPOSCOPY final examination	Folacid n=42 %	Placebo n=43 %	Total %	n
Satisfactory	78.6	83.7	81.2	69
Unsatisfactory	21.4	16.3	18.8	16
Total			100	85

There are no significant differences ($\chi^2 = 0.1$ (Yates correction); d.f. = 1; p = n.s.).

9.6.5. *ENDOCERVICAL CURETTAGE at the final examination, distinguished by treatment groups*

Table 9.6.5. Endocervical curettage at the final examination.

ENDOCERVICAL CURETTAGE final examination	Folacid n=43 %	Placebo n=43 %	Total %	n
Yes	23.3	9.3	16.3	14
No	76.7	90.7	83.7	72
Total			100	86

There are no significant differences ($\chi^2 = 2.1$ (Yates correction); d.f. = 1; p = n.s.).

9.6.6 *SURFACE OF THE TRANSFORMATION ZONE at the final examination, distinguished by treatment groups*

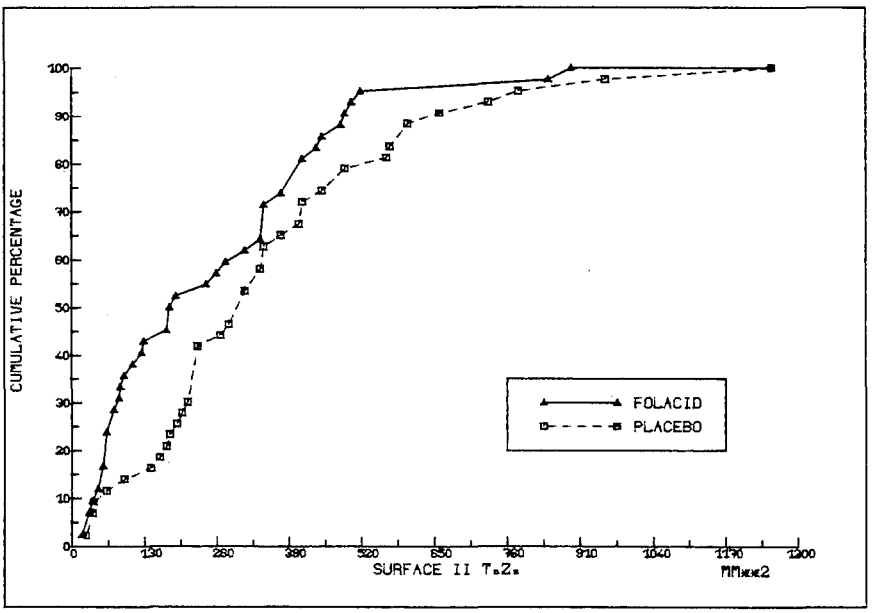


Figure 9.6.6. Surface of the transformation zone (T.Z.) at the final examination.

SURFACE T.Z. (mm^2) final examination	Folacid	Placebo
n=	42	43
median	180.5	310.0
interquartile range	71.75-414	180- 490
range	18-895	25-1253

The surface of the transformation zones in the Placebo group was no longer significantly larger than in the Folacid group (Mann-Whitney U-test; p = n.s.).

9.6.7. *Suspicion of cervical VIRAL INFECTION on COLPOSCOPIC IMPRESSION at the final examination*

Table 9.6.7. Colposcopic suspicion of cervical viral infection at the final examination.

COLPOSCOPIC IMPRESSION	Folacid n=42	Placebo n=43	Total	
final exam. Grade of CIN	% viral infection	% viral infection	% viral infection	(n)
Neg. for CIN	7.1	2.3	4.7	(4/12)
CIN I	9.5	25.6	17.6	(15/33)
CIN II	16.7	11.6	14.1	(12/36)
CIN III	2.4	4.7	3.5	(3/4)
Total	35.7	44.2	40.0	(34/85)

There are no significant differences ($\chi^2 = 4.5$; d.f. = 3; p = n.s.).

9.6.8 *Results of CYTOLOGY SMEARS of endo- and ectocervix at the final examination, distinguished by treatment groups*

Table 9.6.8. Results of cytology at the final examination.

CYTOLOGIC Grade of CIN final exam.	Folacid n=41 %	Placebo n=42 %	Total %	n
Neg. for CIN	48.8	38.1	43.4	36
CIN I	22.0	28.5	25.3	21
CIN II	19.5	16.7	18.1	15
CIN III	9.7	16.7	13.2	11
Total			100	83

There are no significant differences ($\chi^2 = 1.7$; d.f. = 3; p = n.s.).

83 of 86 evaluable patients had representative endo- and ectocervical cytology smears taken and in only 1 patient, there was suspicion of viral infection in the final cytology smears.

9.6.9. *Results of HISTOPATHOLOGIC SPECIMENS taken at the final examination, distinguished by treatment groups*

At the final evaluation, again there was no case in which the grade of CIN in the endocervical curettings or blind endocervical biopsy was more severe than in the biopsies taken under colposcopic guidance. There also were no cases in which the ECC was positive for CIN, while the colposcopically directed biopsies were negative. Of 14 ECC's performed at the final examination, 7 were diagnosed

as negative, 5 as CIN I and 2 as CIN II. In 3 of the 14 ECC's signs of viral infection were demonstrated.
There were no significant differences between the Folacid and Placebo groups ($\chi^2 = 2.2$; d.f. = 2; p = n.s.).
At initial and final examination together, 52 of 86 patients (60.5%) had at least 1 ECC performed and 4 (4.7%) had endocervical curettings taken both at the initial and final examination.

Table 9.6.9. Number of colposcopically directed cervical biopsies taken at the final examination.

BIOPSIES taken at final exam. (frequencies)	Folacid n=43 %	Placebo n=43 %	Total %	n
1	62.8	67.4	65.1	56
2	32.6	27.9	30.2	26
3	2.3	4.7	3.5	3
cone biopsy	2.3	—	1.2	1
Total			100	86

There are no significant differences ($\chi^2 = 4.4$; d.f. = 3; p = n.s.).

9.6.10 *The highest HISTOPATHOLOGIC GRADE OF CIN in biopsies and ECC taken at the final examination, distinguished by treatment groups*

Table 9.6.10. Histopathologic grade of CIN at the final examination.

MAXIMAL HISTOPATHOLOGIC Grade of CIN at final examination	Folacid n=43 %	Placebo n=43 %	Total %	n
Neg. for CIN	48.8	34.9	41.9	36
CIN I	23.3	32.6	27.9	24
CIN II	16.3	23.3	19.8	17
CIN III	11.6	9.3	10.5	9
Total			100	86

There are no significant differences ($\chi^2 = 2.3$; d.f. = 3; p = n.s.).

9.6.11. *HISTOPATHOLOGIC DIAGNOSIS of cervical VIRAL INFECTION at the final examination, distinguished by treatment groups*

Table 9.6.11. Histopathologic diagnosis of cervical viral infection at the final examination.

HISTOPATHOLOGIC DIAGNOSIS final examination Grade of CIN	Folacid n=43 % viral infection	Placebo n=43 % viral infection	Total % viral infection	(n)
Neg. for CIN	4.7	11.6	8.1	(7/36)
CIN I	9.3	16.3	12.8	(11/24)
CIN II	4.7	16.3	10.5	(9/17)
CIN III	2.3	—	1.2	(1/ 9)
Total	20.9	44.3	32.6	(28/86)

There are no significant differences ($\chi^2 = 2.6$; d.f. = 3; p = n.s.).

9.6.12. *Summary of the final examination*

The analysis of the results of the final examination does not reveal significant differences with respect to the number of evaluable patients, satisfactory colposcopies, biopsies and endocervical curettings. The expected grades of CIN and presence of viral infections as determined by cytology, colposcopy and histopathology were also not significantly different between both groups. However, at the final evaluation the serum folate concentrations in the Folacid group appeared to be significantly higher than in the Placebo group. The surfaces of the transformation zones in the Placebo group were at the final examination no longer significantly larger than of the Folacid group.
If the histopathologic grade of CIN was taken as the "standard", the following performance of correct prediction by cytology and colposcopy was obtained at the final examination:

Table 9.6.12. : Agreement of Cytology and Colposcopy with the histopathologic grade of CIN at the final examination.

Histopathologic grade of CIN predicted by	% Agreement within the same grade of CIN	% Over-diagnosis	% Under-diagnosis	Significance testing (X^2-test) with histopathology
Cytology				
Folacid	59	20	22	$p < 0.001$
Placebo	52	24	24	$0.01 < p < 0.02$
Colposcopy				
Folacid	36	47	17	p = n.s.
Placebo	56	30	14	$p << 0.001$

9.7 Assessment of the therapeutic effect of Folacid

To evaluate the possible therapeutic effect of 100 days folic acid supplementation in cervical intra-epithelial neoplasia, the differences between the results of the initial and the final examination are analyzed for both treatment groups.

9.7.1 *Differences in SERUM FOLIC ACID CONCENTRATIONS between initial and final examination, distinguished by treatment groups*

In the Folacid group there was a significant increase in serum folate concentration, while in the Placebo group there was no significant difference between initial and final examination.
From multivariate analysis of variance (MANOVA), of initial and final examination combined, there appeared to be a significant interaction effect of treatment groups and repeated measurements: the Folacid group demonstrating an enormous increase in serum folate concentration, in sharp contrast to the Placebo group, which remained stable. This indirectly seems to confirm - as did the count of the remaining tablets - the general patient compliance, at least in the Folacid group, with respect to the ingestion of the tablets.
This finding is interpreted as to support the assumption that Folacid intake in the present study was adequate and that the results obtained by comparing the final and initial examination will be representative for the Folacid, respectively the Placebo effect (Figure 9.5.1 and 9.6.1).

9.7.2 *Differences in grade of CIN determined by COLPOSCOPIC IMPRESSION between initial and final examination, distinguished by treatment groups*

Table 9.7.2.1. : Comparison per patient of Colposcopic grade of CIN at initial and final examination for both treatment groups.

FOLACID N = 42						Colposcopic impression	PLACEBO N = 43					
Colposcopic impression Final examination						Initial examination	Colposcopic impression Final examination					
Neg	I	II	II	Total n	%	ade of CIN	Neg	I	II	III	Total n	%
		1		1	(2)	Neg.						
4	**8**	5		17	(41)	I	2	**12**	3	1	18	(42)
3	4	**15**		22	(52)	II	3	9	**11**	1	24	(56)
		1	**1**	2	(5)	III				**1**	1	(2)
7	12	22	1	42		Total	5	21	14	3	43	
(17)	(29)	(52)	(2)		(100)	(row-%)	(12)	(49)	(32)	(7)		(100)

There was a significant agreement in grade of CIN between the initial and final colposcopy in the individual patients of the Folacid group ($\chi^2 = 28.0$; d.f. $= 9$; $p \leqslant 0.001$) as well as of the Placebo group ($\chi^2 = 18.2$; d.f. $= 6$; $0.001 < p \leqslant 0.01$).

Table 9.7.2.2. : Differences in grade of CIN determined by Colposcopy at initial and final examination, distinguished by treatment groups.

FOLACID N = 42					Colposcopic impression	PLACEBO N = 43				
Colposcopy at final examination					Differences in grade of CIN between initial and final examination	Colposcopy at final examination				
less severe	equal	more severe	Total n	Total (%)		less severe	equal	more severe	Total n	Total (%)
	24		24	(57)	equal		24		24	(56)
9		5	14	(33)	1 Grade	11		4	15	(35)
3		1	4	(10)	2 Grades	3		1	4	(9)
12	24	6	42		Total	14	24	5	43	
(29)	(57)	(14)		(100)	(row-%)	(32)	(56)	(12)		(100)

When the differences in grade of CIN in both groups combined were tested (χ^2-test for trend) there was no significant difference in the over all trend of changes in the colposcopic grade of CIN between both study-groups (Table 9.7.2.2) (χ^2 (trend) = 7.15; d.f. = 7; p = n.s.).

In Table 9.7.2.3 is summarized the "natural" history of the CIN-lesions in this study (Placebo) and the course under folate acid suppletion (Folacid), in the 100-110 days between initial and final examination.

Table 9.7.2.3. : 'Natural' history of the CIN-lesions between initial and final examination, distinguished by treatment groups.

'Natural' history determined by:	n	% Stabilization	% Regression	% Progression	Significance testing (X^2-test) between initial and final examination
Cytology					
Folacid	41	22	68	10	p = n.s.
Placebo	42	38	45	17	p = n.s.
Colposcopy					
Folacid	42	57	29	14	$p < 0.001$
Placebo	43	56	32	12	$0.001 < p < 0.01$
Histopathology					
Folacid	43	21	65	14	p = n.s.
Placebo	43	37	49	14	p = n.s.

There are no significant differences recognizable between both groups with respect to the changes in grade of CIN, determined per patient by colposcopy at final and initial examination ($\chi^2 = 0.23$; d.f. = 2; p = n.s.).

9.7.3 *DIFFERENCES in grade of CIN predicted by CYTOLOGY at initial and final examination, distinguished by treatment groups*

Table 9.7.3.1. : Comparison per patient of the cytologic prediction of the grade of CIN at initial and final examination for both treatment groups.

FOLACID N = 41						Cytologic prediction Initial examination	PLACEBO N = 42					
Cytologic prediction Final examination				Total			Cytologic prediction Final examination				Total	
Neg.	I	II	III	n	(%)	Grade of CIN	Neg.	I	II	III	n	(%)
2				2	(5)	Neg	**1**	1	1	1	4	(9.5)
6	**1**	1		8	(19)	I	5	**7**	1	1	14	(33)
9	8	**5**	3	25	(61)	II	9	4	**5**	2	20	(48)
3		2	**1**	6	(15)	III	1			**3**	4	(9.5)
20	9	8	4	41		Total	16	12	7	7	42	
(49)	(22)	(9)	(10)		(100)	(%)	(38)	(28)	(17)	(17)		(100)

Table 9.7.3.2. : Differences in grade of CIN predicted by cytology at initial and final examination, distinguished by treatment groups.

FOLACID N = 41					Cytologic prediction Differences in grade of CIN between initial and final examination	PLACEBO N = 42				
Cytologic prediction at final examination			Total			Cytologic prediction at final examination			Total	
less severe	equal	more severe	n	(%)		less severe	equal	more severe	n	(%)
	9		9	(22)	eqyal		16		16	(38)
16		4	20	(49)	1 Grade	9		4	13	(31)
9			9	(21)	2 Grades	9		2	11	(26)
3			3	(8)	3 Grades	1		1	2	(5)
28	9	4	41		Total	19	16	7	42	
(68)	(22)	(10)		(100)	(%)	(45)	(38)	(17)		(100)

Statistically there was no significant relationship between the cytologic grades of CIN of the patients at initial and final examination in the Folacid group ($\chi^2 = 8.9$; d.f. = 9; p = n.s.), nor in the Placebo group ($\chi^2 = 16.6$; d.f. = 9; p = n.s.).
When the differences in grade of CIN in both study-groups combined (Table 9.7.3.1) were tested (χ^2-test for trend), there was no significant difference in trend of changes in cytologic grade of CIN between both groups (χ^2 (trend) = 12; d.f. = 11; p = n.s.).

In Table 9.7.3.1 and 9.7.2.3 however, can be recognized that there is a tendency of cytology to regress more and to progress less in the Folacid than in the Placebo group when cytology results of final and initial examination are compared. Nevertheless, this tendency, tested again on the figures of Table 9.7.2.3, did not reach significancy ($\chi^2 = 4.5$; d.f. = 2; p = n.s.).

9.7.4 *DIFFERENCES in HISTOPATHOLOGIC grade of CIN between initial and final examination, distinguished by treatment groups*

Table 9.7.4.1.: Comparison per patient of the histopathologic grade of CIN at initial and final examination for both treatment groups.

FOLACID N = 43						Histo-pathology	PLACEBO N = 43					
Histopathology Final examination						Initial examination	Histopathology Final examination					
Neg	I	II	III	Total n	(%)	Grade of CIN	Neg	I	II	III	Total n	(%)
4	**5**	3		12	(28)	I	8	**9**	4		21	(49)
10	3	**2**	3	18	(42)	II	6	4	**5**	2	17	(39)
7	2	2	**2**	13	(30)	III	1	1	1	**2**	5	(12)
21	10	7	5	43		Total	15	14	10	4	43	
(49)	(23)	(16)	(12)		(100)	(%)	(35)	(33)	(23)	(9)		(100)

Table 9.7.4.2.: Differences in histopathologic grade of CIN at initial and final examination, distinguished by treatment groups.

FOLACID N = 43					Histo-pathology	PLACEBO N = 43				
Histopathology Final examination					Differences in grade of CIN between initial and final examination	Histopathology Final examination				
less severe	equal	more severe	Total n	(%)		less severe	equal	more severe	Total n	(%)
	9		9	(21)	equal		16		16	(37)
9		6	15	(35)	1 Grade	13		6	19	(44)
12			12	(28)	2 Grades	7			7	(16)
7			7	(16)	3 Grades	1			1	(2)
28	9	6	43		Total	21	16	6	43	
(65)	(21)	(14)		(100)	(%)	(49)	(37)	(14)		(100)

Statistically there was no significant relationship between the histopathologic grade of CIN of the patients at initial and final examination in the Folacid group ($\chi^2 = 6.1$; d.f. = 6; p = n.s.), nor in the Placebo group ($\chi^2 = 9.3$; d.f. = 6; p = n.s.).

When the differences in grade of CIN in both study-groups combined were tested (χ^2-test for trend), there was no significant difference in trend of changes in histopathologic grade of CIN between both groups (χ^2 (trend) = 4.8; d.f. = 8; p = n.s.).

In Table 9.7.4.1 and 9.7.4.2 however, can be recognized that there is a tendency of histopathology - which is not significant - to a higher percentage of regression in the Folacid than in the Placebo group ($\chi^2 = 3.0$; d.f. = 2; p = n.s.).

9.8 Evaluation of the therapeutic effect of Folacid with respect to hormonal contraception

In Table 9.4.12 was demonstrated that 13 (30.2%) of the patients in the Folacid group and 21 (48.8%) of the Placebo group were using hormonal oral contraceptive agents (O.C.A.'s) at the time of this study, all in the form of low-dose combination pills.
In order to analyze whether there was a significant difference in the outcome of patients who were taking birth controll pills, a subdivision was made with respect to the use of OCA's (OCA+) or not (OCA-) within the Folacid and Placebo group.
In the same manner as was done in chapter 9.7, the cross tables of these subgroups were analysed.
Per patient subsequently the colposcopic, cytologic and histopathologic differences in grade of CIN of the initial and final examination were compared and, for both study-groups combined, tested on significance (χ^2-test for trend).
The results, summarized in terms of stabilization, regression and progression of the CIN lesions are presented in Table 9.8.1.

Table 9.8.1.: Influence of hormonal oral contraceptive agents (OCA) on the therapeutic effect of Folacid supplementation in CIN
OAC + : OCA's used during the study period
OAC − : no OCA's used during the study period

Differences in grade of CIN between final and initial examination determined by	n	n	% Stabilization		% Regression		% Progression		Significance testing (X^2-test)	
	OAC +	**OAC −**	**OAC +**	**OAC +**	**OAC +**	**OAC −**	**OAC +**	**OAC −**	**OAC +**	**OAC −**
Cytology										
Folacid	13	28	38	14	54	75	8	11	p = n.s.	p = n.s.
Placebo	21	21	57	19	33	57	10	24	0.001<p<0.01	p = n.s.
Colposcopy										
Folacid	13	29	46	62	31	28	23	10	p = n.s.	0.001<p<0.01
Placebo	21	22	52	59	38	27	10	14	p = n.s.	p < 0.001
Histopathology										
Folacid	13	30	38	13	54	70	8	17	p = n.s.	p = n.s.
Placebo	21	22	43	32	43	54	14	14	p = n.s.	p = n.s.

χ^2-test for trend did not demonstrate significant differences in changes in grades of CIN within the Folacid and Placebo group when patients were subdivided according to the use of OCA's or no OCA's.

For cytology: OCA+: $\chi^2 = 6.4$; d.f. = 6; p = n.s.
OCA-: $\chi^2 = 7.5$; d.f. = 7; p = n.s.
For colposcopy: OCA+: $\chi^2 = 8.7$; d.f. = 6; p = n.s.
OCA-: $\chi^2 = 8.8$; d.f. = 5; p = n.s.
For histopathology: OCA+: $\chi^2 = 4.5$; d.f. = 7; p = n.s.
OCA-: $\chi^2 = 5.9$; d.f. = 8; p = n.s.

Again, the tendency in the Folacid group to more regression and less progression than in the Placebo group is recognizable when differences between final and initial examination in cytology and histopathology are analysed. However, this tendency is not depending on the use of oral contraceptive agents: it is apparent in the OCA+ as well as in the OCA- patients.

At the initial examination 10 of 14 OCA-users in the Folacid group and 12 of 21 OCA-users in the Placebo group had serum folate concentrations that were statistically not different from the serum concentrations of the non-OCA-users in the same group
(Folacid group: $\chi^2 = 16.7$; d.f. = 17; p = n.s.
Placebo group: $\chi^2 = 10.5$; d.f. = 13; p = n.s.).
At the final examination 91% of OCA-users and 86% of non-OCA-users in the Folacid group, who had serum samples taken, appeared to have serum folate concentrations $\geqslant$ 45 mmol/l. In the Placebo group 100% of OCA-users and 100% of non-OCA-users who had serum samples taken at final examination appeared to have serum folate concentrations $\leqslant$ 30 mmol/l.
There were no significant differences in serum folate concentrations in OCA-users and non-users within the Folacid ($\chi^2 = 6$; d.f. = 6; p = n.s.) or Placebo group ($\chi^2 = 16.3$; d.f. = 13; p = n.s.).

9.9 Other possible influences on the evaluation of the effectiveness of Folacid supplementation in the treatment of CIN

9.9.1 *Satisfactory colposcopy*

In this study there appeared to be no influence of colposcopy being satisfactory or not in colposcopic and histopathologic grading of CIN, nor in the Folacid, nor in the Placebo group. It was interesting that overall in the final examination there were significantly more satisfactory colposcopies than in the initial examination ($\chi^2 = 5.7$ (Yates' correction); d.f. = 1; $0.02 < p \leqslant 0.05$). This might be related to the repeat effects after ECC at the initial examination.

9.9.2 *Cervical viral infections*

In this study patients were evaluated twice with an interval of 100-110 days. In the analyses of the possible influence of cervical human papilloma virus (HPV) infections on the evaluation of the effectiveness of Folacid in the treatment of CIN, there appeared to be some striking inconsistencies with respect to this diagnosis in the same patient when initial and final examination were compared. None of the 11 patients who were suspect for cervical viral infection in the referral or initially repeated cytology smears, had this suspicion reproduced in the final cytology smears.
Of all 86 patients, 44 were at least in one examination (37 at initial and 34 at final examination) colposcopically suspect for cervical HPV infection, and 26 of these 44 (59%) in both the initial and the final examination.
In 37 of 86 patients was at least in one examination (21 at initial and 28 at

final examination) the histopathologic diagnosis "cervical viral infection" reported, however in only 10 of these 37 (27%) in both the initial and the final examination.
The inconsistencies were equally divided between the Folacid and the Placebo group.
When predictive values of colposcopy as to the histopathologic diagnosis of the presence or absence of cervical HPV-infection are calculated for initial and final examinations, all values are between 0.71 and 0.76. However, they pertain to a substantial number of different patients when compared for initial and final examination.
It is unlikely that cervical HPV-infections will (dis)appear so rapidly especially when occurring in combination with CIN (526). Because of the inconsistencies in the diagnosis of cervical HPV-infection by cytology, histopathology and in a lesser degree, colposcopy, which were already indicated in chapter 7, no further analysis of the possible influence of the presence of cervical HPV-infection on the therapeutic effect of Folacid on CIN was performed.

9.10 Discussion and comment

It has been recognized that there are many risk factors related to sexual activity that can affect the uterine cervix and increase a woman's chances of developing cervical intraepithelial neoplasia and invasive carcinoma. The cervix however may also be at risk from other unknown carcinogenic factors including dietary factors such as folic acid deficiency.
Localized alterations in the metabolism of this essential vitamin - as might be produced by prolonged exposure to contraceptive steroids - could result in not identified changes in the cervical tissue that cause the area to be more vulnerable to carcinogenesis (95). Sexual activity in addition to this nutritional factor may increase the risk of CIN, while high levels of this vitamin may act to prevent the phenotypic expression.

The present study was designed to assess in a prospective, randomized, double-blind and placebo-controlled fashion the possible beneficial effects of 100 days folic acid supplementation on the course of cervical intraepithelial neoplasia, and was performed in two highly comparable groups of patients.
There appeared indeed to be a trend of patients receiving Folacid to manifest more regression in CIN than patients randomized to the Placebo. However, the differences in improvement with respect to the grade of CIN were not significant between both groups, in any of the parameters measured.
The initial histopathology of patients in the Folacid group comprised more high grade CIN-lesions; in the statistical assessment of the differences in changes, this was compensated for.
When patients using oral contraceptive agents of both groups were analyzed separately and compared with patients not using OCA's, there also were no differences between the Folacid and Placebo group.
In other words, the findings of Butterworth et al. (95) could not be confirmed by the current study, neither by histopathology, nor by cytology or colposcopy.

Butterworth's postulation that "a localized derangement in folate metabolism is an integral component of the dysplastic process that may be arrested or in some cases reversed by oral folic acid suppletion", has not been confirmed by the present study. The current results rather seem to indicate that Butterworth et al. have identified a new degree of reversible megaloblastic change which may sometimes be misdiagnosed as cervical dysplasia in patients with folic acid deficiency of pregnancy (284) or in OCA-users (585).

In the present study, which comprises a small population, but is well controlled, it is demonstrated that Folacid suppletion for 100 days of patients with CIN will result in high serum folate concentrations, but will not influence the biologic behavior of CIN significantly different than a placebo.
The relatively high regression rate between initial and final examination in both groups may be spontaneous, which is possible (315), but not very likely (443,510), or is rather hastened by taking cervical biopsies and performing ECC's at initial as well as final examination. Residual CIN may be destroyed by the inflammatory response to cervical biopsy so that part of the regression rates may in fact have been effected by the biopsies (5,444). This might also be a factor in the inconsistencies in the diagnosis of cervical HPV infection between initial and final examination. The clinical course of this infection and the factors influencing it, are poorly understood.

In the study by Butterworth et al. (95) the mean final cytology score in the patients receiving folate was significantly better than the mean initial cytology score of this group, in contrast to the placebo group. The consistent downward trend (improvement i.e. regression of cytology) in the score of the folate group however, was not significantly different from the score of the placebo group at the final examination in their study.

There are a number of possible explanations for the negative findings in the current study:
The first has already been alluded to: Folacid indeed has no measurable effect on patients with CIN with normal folate levels. In this respect it is noteworthy that in the Butterworths' study patients were generally from a low-income class, with possible dietary insufficiencies. The storage-capacity for folic acid and its derivatives in the human body is limited. When intake of folate is insufficient a deficiency can rapidly occur. In the study by Butterworth et al. the initial plasma and red blood cell concentrations of all OCA-users who had folate levels determined before treatment with folic acid, were indicative of folate deficiency. At the end of the study the plasma folate levels were 8-fold increased and the red cell folate levels, which are generally regarded as a more reliable measure of tissue stores than serum or plasma, 4-fold. In their placebo group most OCA-users also were folate deficient and remained so during the study.
In the current study no patient in the Folacid, nor in the Placebo group who had serum levels determined, was folate deficient at the initial examination, regardless of OCA-use or not. This difference between the Butterworth et al. study and the present one might provide an explanation for the differences in final results.

A second explanation for the negative findings of the current study is the possibility that if the study was continued for a longer time, the placebo-rate would have decreased and the effect of treatment might then become significant. To assess this item was not part of the present protocol. However, in the patients who were being followed without treatment after the final examination of the current study, the general trend to regression did not seem to be significantly changed when analysed retrospectively and this trend did not seem to be different in both groups after cessation of the tablet period.
The thirth explanation could be the relatively small number of patients in both treatment groups and the 14% non-evaluable patients. However the size of the treatment group seemed to be adequate to detect a clinically important difference when this was present and the bias possibly introduced by removing 7 non-evaluable patients from each treatment group of 50, seems to be acceptable.
Finally, the lack of significant differences in the observed changes of CIN might be in the choice of the methods and criteria used to evaluate clinical improvement. In the current study, the author not only used cytology for the assessment of differences in clinical improvement of CIN as Butterworth et al. did, but also colposcopy and histopathology. If there was an effect of folacid that could not be defined by cytology but by one of the other diagnostic methods, it would have surfaced uring the data anlaysis.

In conclusion: in the present study no significant beneficial effect of 100 days Folacid supplementation could be demonstrated on the course of cervical intraepithelial neoplasia.
Further studies are needed regarding the etiology and basic nature of cervical intraepithelial neoplasia including nutritional approaches to its management.

Chapter 10

CRYOCOAGULATION VERSUS LASER VAPORIZATION IN THE TREATMENT OF CIN

A prospective, randomized study with long-term follow-up

J.A. Wijnen

10.1 Introduction

Since the introduction of colposcopy in the evaluation of patients with abnormal cervical cytology, many clinicians have turned to outpatient treatment with conservative techniques to manage cervical intraepithelial neoplasia (CIN) in patients in whom invasive cervical cancer has been ruled out (549; Chapter 8, this thesis).

In the Department of Gynecology of the Erasmus University Hospital Dijkzigt-Rotterdam, until 1981 when the present author joined the colposcopy clinic, the standard management of patients with CIN was either cold-knife cone biopsy, in CIN III and inconclusive cervical lesions, or cryotherapy in selected outpatients with CIN II lesions after colposcopic triage. Patients with low grade CIN-lesions were generally followed by cytology and colposcopy without any treatment, unless progression occurred.

The rapidly increasing number of young women with CIN, the accumulating amount of patients to be followed conservatively, the limited availability of experienced colposcopists and the risks of patients with CIN being followed without therapy to be lost to follow-up, forced the author to change the treatment policy.

Because of the fact that it was not possible to predict which lesions would be progressive or not, all patients with documented persistent or progressive CIN lesions who had satisfactory colposcopy, while invasive carcinoma seemed to be excluded, were proposed to be treated with outpatient cervix-conserving treatment methods.

In January 1981, the therapeutic armament of the department of gynecologic oncology was extended with a CO_2-laser. At that time, no randomized comparative studies concerning cryo- or laser-therapy in patients with CIN had been published, although it had been claimed that laser vaporization of CIN yielded better treatment results and faster healing than cryotherapy and that after laser therapy the squamocolumnar junction was positioned appropriately at the external os.

After gaining adequate experience with both methods the present author decided to design such a randomized study.

The objectives of the study:

To compare in a prospective randomized design the therapeutic efficacy, complications and side effects of outpatient cryocoagulation or laser vaporization in patients with CIN grade I, II or III.

10.2 Patients and methods

10.2.1 *Patient selection*

Candidates for this study were selected from a population of women with CIN, who were being followed in the outpatient colposcopy clinic of the Department of Gynecologic Oncology.
In June 1981 the first of the planned 100 patients who met the eligibility criteria and who agreed to participate upon verbal informed consent by the present author, was entered into study, and in March 1983 the study was closed.

INCLUSION CRITERIA

1. Cervical intraepithelial neoplasia of any size and grade, as diagnosed by the hospital histopathologists on colposcopically directed cervical biopsies.
2. Satisfactory colposcopy; i.e. the entire squamocolumnar junction (SCJ) and all margins of the lesion(s) including the endocervical extension were clearly visualized by the present author.
3. No high grade CIN-lesions in endocervical curettings when these were considered to be necessary to exclude occult lesions with greater certainty.
4. No discrepancies between cytologic or colposcopic results, suspect for invasive disease, and histopathology.
5. Evaluation and treatment performed by the present author.
6. Promise of the patient to keep the follow-up appointments.

EXCLUSION CRITERIA

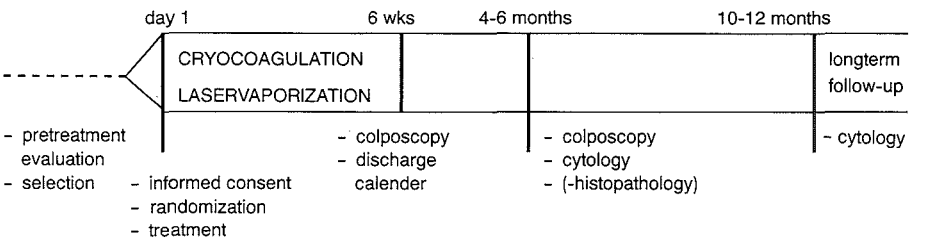
1. Absence of CIN.
2. Suspicion or presence of malignancy.
3. Extension of the CIN-lesion toward the vagina or into the endocervical canal, outside the direct vision of the colposcopist.
4. Concurrent gynecologic ailments requiring a different therapeutic approach.
5. Intra-uterine DES exposure.
6. Not consenting with the randomization or follow-up schedule.
7. Age $>$ 55 years.
8. Present pregnancy.

10.2.2 *Pre-treatment requirements and study design*

Before treatment all patients were evaluated in a separate colposcopy clinic

session by the present author. As was described in chapter 7.3 (addendum 7.I) evaluation included completion of personal and medical history, which comprised the maximal grade of CIN diagnosed in the preceding 12 months (referral diagnosis), colposcopy with documentation of the colposcopic impression and other findings, including the extension of the lesion, repeat endo- and ectocervical cytology and gynecologic examination. Colposcopically directed biopsies were taken and, if indicated, endocervical curettage and blind biopsy were performed to exclude with even more certainty lesions beyond the visible part of the endocervical canal. When the histopathologic diagnosis confirmed the presence of CIN and the absence of invasive disease, 100 consecutive patients who met the eligibility criteria and who agreed to participate in the study upon informed consent, were randomly allocated to cryo- or lasertherapy.

Study design



10.2.3 *Techniques*

All patients were treated in the outpatient clinic by the present author in one treatment session. After colposcopic re-assessment with the use of a 3% acetic acid solution to demarcate the lesion(s), the horizontal and vertical dimensions of the transformation zone were measured with a calibrated ruler and Schillers' iodine solution was used for better visualization of the surface to be destroyed during treatment.

Cryocoagulation (chapter 8.5.2) was performed with the use of a Spembly-TC10 cryosurgical system with nitrous oxide as the refrigerant in all cases. This equipment produces a probe tip temperature of -70 to -80°C depending on the size of the (interchangeable) probe tip and the lesion. A probe tip was always chosen to fit the best to the individual lesion and cervix. To guarantee an optimal freeze, a minimal pressure of 50 kg /cm^2 in the nitrous oxide tank was maintained. Before freezing an adequate amount of a water-soluble lubricant was applied on the probe, permitting a better heat-transfer and resulting in a more uniform freezing.

Freezing was performed until the iceball around the probe extended 5 mm beyond the periphery of the probe and was subsequently continued for another 2 minutes, usually resulting in an application time of 5 minutes or more.

Colposcopically the freeze lesion was then verified to extend at least 5 mm beyond the visual periphery of the lesion or the transformation zone, whichever was most peripherally located. In larger transformation zones this required

multiple freeze applications, not infrequently with different probe tips and overlap of the iceballs, however all performed within the same treatment session. Finally, the horizontal and vertical dimensions of the entire frozen surface were measured. For cryocoagulation no anaesthesia was necessary.

Laser-vaporization (chapter 8.5.3) was performed using a Biophysique FLF-25 CO_2-laser system in a continuous mode, attached to a Zeiss Opmi-1 microscope with a focal distance of 400 mm. In all patients destruction of the entire transformation zone to a depth of at least 6 mm (11) including the endocervical extensions of the lesion(s) was attempted.

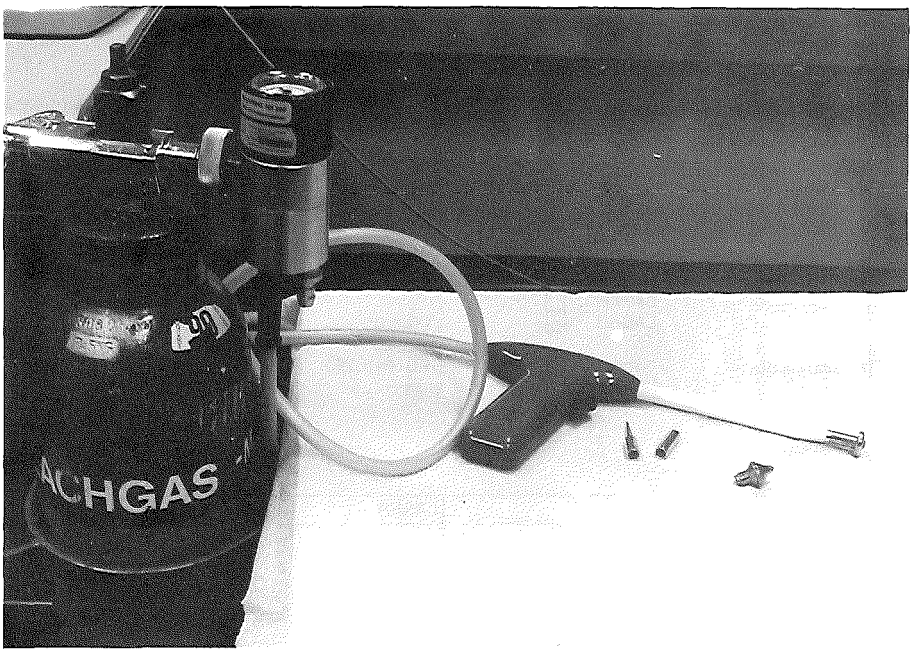
The techniques were described in section 8.5.3. Finally the horizontal and vertical and cranio-caudal dimensions of the destroyed area were measured.
Initially all patients were treated without anaesthesia, but in the later part of the study the choice of local anaesthesia was offered (paracervical block with 20 ml lidocaine solution (5 mg /ml, usually with epinephrine 1:100.000) injected at the 2, 4, 8 and 10 o'clock positions).
All patient treatment data and complications were documented on a "Treatment Form" (addendum 10.I).

10.2.4 *Evaluation and follow-up*

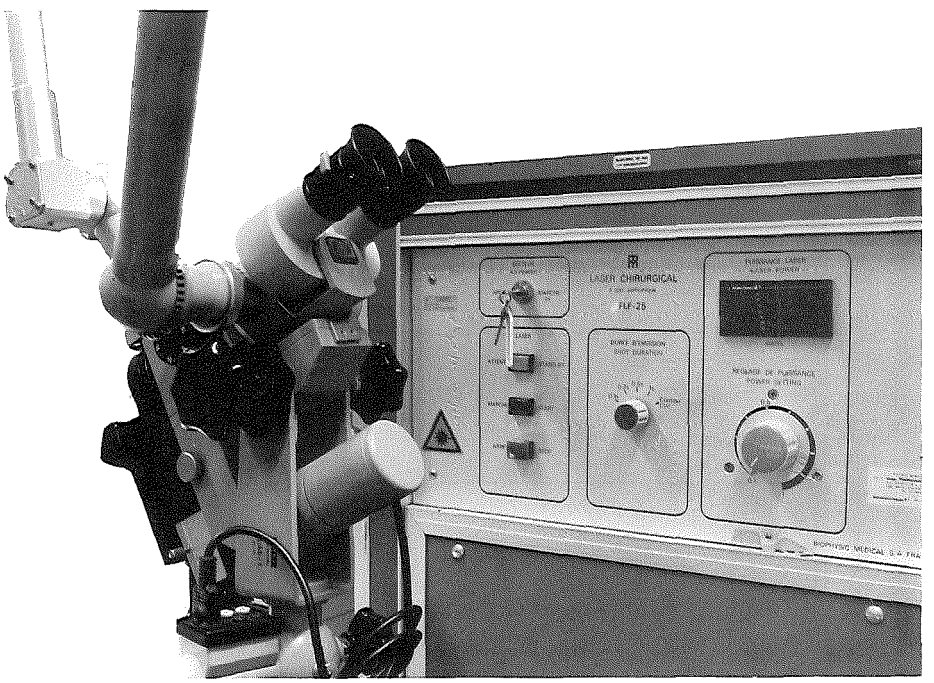
All patients were advised against coitus as long as discharge continued.
Patients were supplied with a calendercard and were asked to keep a daily notice of vaginal discharge, blood loss and menstrual periods, as well as to document other inconveniences and complications. Discharge was subjectively graded by the individual patient as slight (1 point), moderate (2 points) or severe (3 points) and if there was in addition fresh blood loss, except in the normal menstrual period, 1 extra point per day was added to this "discharge score".
Following treatment patients were scheduled for follow-up visits at 6 weeks to document side effects; and at 4-6 months and 10-12 months to repeat colposcopy and endo- and ectocervical smears, and when indicated by cytology, any area suggestive of CIN was subjected to further punch biopsy. If after one year no abnormalities were noted, follow-up with routine endo- and ectocervical smears were performed at least once a year in the general gynecology clinic or at the office of the family doctor.

EVALUATION CRITERIA

Treatment was considered unsuccessful if endo- and /or ectocervical cytology continued to suggest CIN on at least two occasions between 4 and 12 months after treatment, or if in a histopathologic specimen obtained within the first year the diagnosis CIN was made.(*Residual* or *persistent disease: treatment failure*). If after negative follow-up in the first year cytology or histopathology demonstrated cervical neoplasia, treatment was regarded as successful and patients were considered to have *recurrent disease.* Persistent CIN and recurrent CIN combined was designated as *"total-failure"*.



Spembly-TC 10 cryosurgical system with probe tip I (see Table 10.8.2) attached.



Instrument panel of Biophysic FLF-25 CO_2 surgical laser attached (top-left) to a Zeiss Opmi-1 microscope.

UNIVERSITY HOSPITAL ROTTERDAM DIJKZIGT

Dept. Gynecologic Oncology

TREATMENT FORM
CRYLA-PROTOCOL
J.A. Wijnen

Date:

PATIENT DATA

Indication
Randomization — Nr. ☐ CRYO ☐ LASER ☐

ANESTHESIA: none ☐ local ☐ sedation ☐

L.M.P.:

LESION: size Hor mm Vert mm
DESTRUCTED area Hor mm Vert mm

REMARKS:

CRYO		LASER	
Probe(s)		Power/mode	
		Output	Watt
Nr. of applications	~~min~~	Approx. power density	W/cm^2
Tank pressure		Depth of destruction	mm
Duration	min	Duration	min
freeze/thaw/freeze			

COMPLICATIONS — No ☐ Yes ☐

- Haemorrhage (ml) Direct >24 hrs >2 weeks
  therapy
- Pain: none intermitt.
  mild continuous
  moderate
  severe
- Procedure discont'd:
- Infection/Fever:
- Hospitalization:
- Other:

DISCHARGE — calender returned yes/no
days score: remarks

FOLLOW-UP		6 wks	4-6 mnth	10-12 mnth	
date:					
SCJ:					
CS:					
Residual disease:					
Recurrence:					

10.2.5 *Methods of analysis*

All data from the protocol sheets were coded and prepared for computer analysis (VAX /VMS) and statistics (SPSS-X; Statistical Package for the Social Sciences). Statistical methods have been discussed in chapter 7.
The χ^2-test was used to test a difference in overall success rates between cryocoagulation and laser vaporization. The χ^2-test for trend was used to test for differences in persistent or recurrent disease between both treatment groups.

Results

All 50 patients randomized to cryocoagulation (CRYO) and all 50 patients randomized to laser vaporization (LASER) were considered suitable for evaluation.

COMPARABILITY OF THE STUDY GROUPS

10.3 Patient characteristics

10.3.1 *AGE at randomization, distinguished by treatment groups*

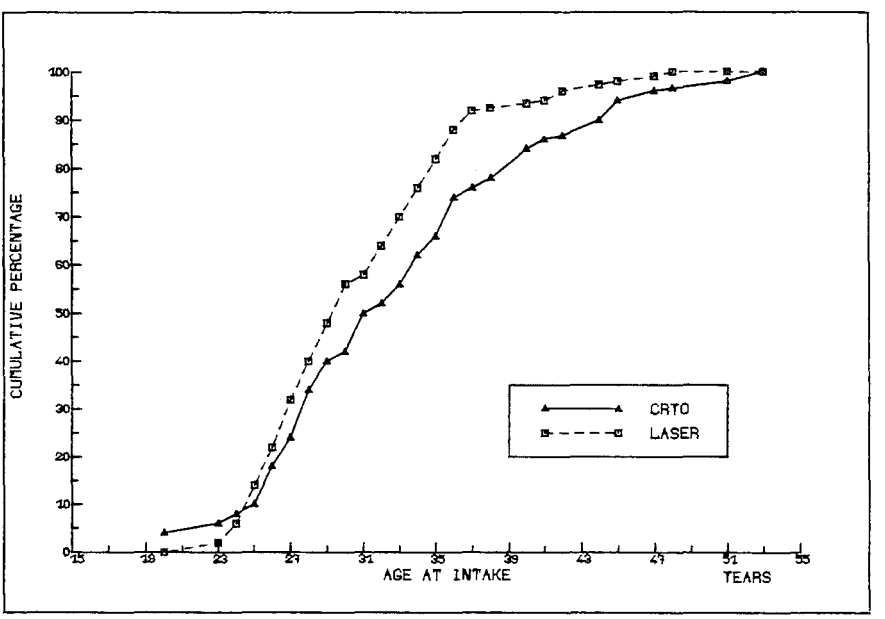


Figure 10.3.1: Age.

AGE (years)	CRYO n=50	LASER n=50
median	31.5	30
interquartile range	27.75-38.0	27-34.25
range	20-35	23-48

There are no significant differences (Mann-Whitney U-test; p=n.s.).

10.3.2 *RACE, distinguished by treatment groups*

Table 10.3.2: Race

RACE	CRYO n=50	LASER n=50
Caucasian	43	47
Negroid	7	3

There are no significant differences ($\chi^2 = 1.0$ (Yates correction); d.f. = 1; p = n.s.).

10.3.3 *MARITAL STATUS, distinguished by treatment groups*

Table 10.3.3. Marital status

MARITAL STATUS	CRYO n=50	LASER n=50
Stable relation	26	35
Single	11	9
Separated	12	6
Unknown	1	0

There are no significant differences ($\chi^2 = 3.5$; d.f. = 3; p = n.s.).

10.3.4 *OBSTETRICAL HISTORY, distinguished by treatment groups*

Table 10.3.4.1. Number of pregnancies

PREGNANCIES (number)	CRYO n=50	LASER n=50
0	5	12
1-2	29	28
3-4	12	9
⩾5	4	1

There are no significant differences ($\chi^2 = 9.0$; d.f. = 8; p = n.s.).

There were also no significant differences in number of spontaneous abortion and abortion arte provocatus between the treatment groups.

Table 10.3.4.2. Parity

PARITY (number)	CRYO n=50	LASER n=50
0	9	17
1-2	28	30
3-4	10	2
⩾5	3	1

There are no significant differences ($\chi^2 = 12.9$; d.f. = 8; p = n.s.).

10.3.5 *MENARCHE, age (years) at first menstrual period, distinguished by treatment groups*

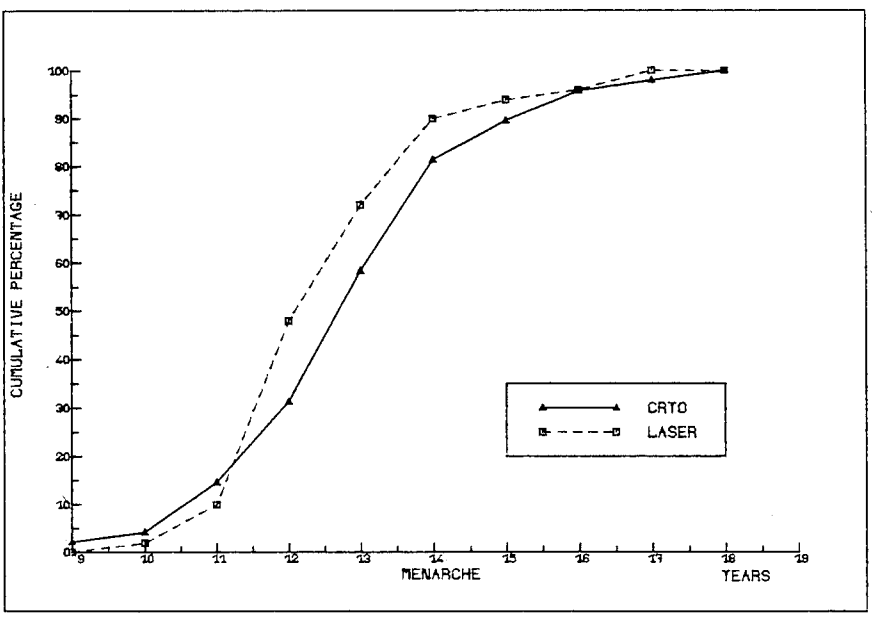


Figure 10.3.5. Cumulative percentage distribution of menarche

MENARCHE (years)	CRYO n=48	LASER n=50
median	13	13
interquartile range	12-14	12-14
range	9-18	10-17

2 patients could not recall their menarche. There are no significant differences (Mann-Whitney U-test; p = n.s.).

10.3.6 *SEXARCHE, age (years) at first coitus, distinguished by treatment groups*

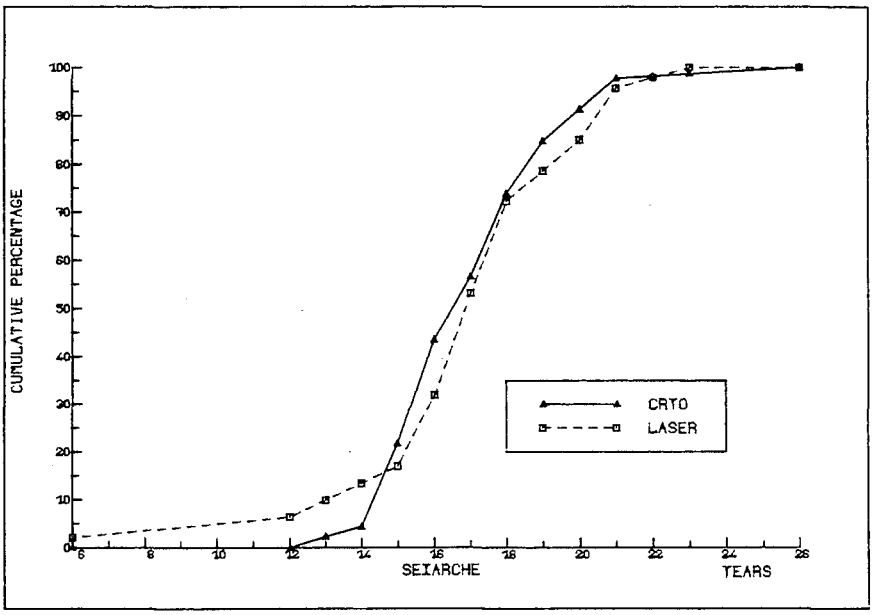


Figure 10.3.6. Cumulative percentage distribution of sexarche

SEXARCHE (years)	CRYO n=46	LASER n=46
median	17	17
interquartile range	16-19	16-19
range	13-26	6-23

7 patients did not mention their sexarche. There are no significant differences (Mann-Whitney U-test; p = n.s.).

10.3.7. *MALE SEXUAL PARTNERS, distinguished by treatment groups*

Nr. MALE SEX. PARTNERS	CRYO n=46	LASER n=49
median	4	4
interquartile range	2-5.5	2-7.75
range	1-⩾100	1-⩾100

5 patients did not mention the number of male sexual partners. There are no significant differences (Mann-Whitney U-test; p = n.s.).

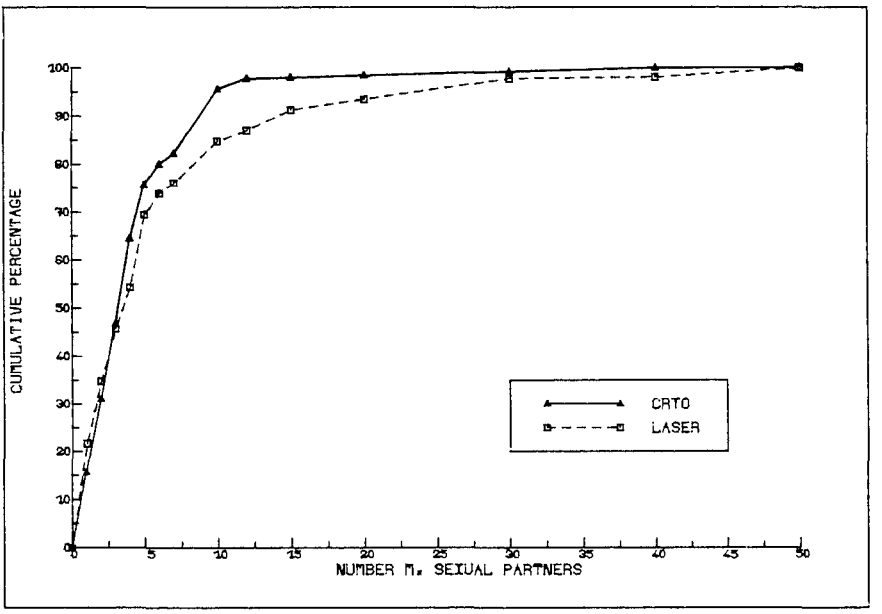


Figure 10.3.7. Number of male sexual partners

10.3.8 *PREGNANCY DESIRE in the future, distinguished by treatment groups*

Table 10.3.8. Future pregnancy desire

PREGNANCY DESIRE	CRYO n=50	LASER n=50
yes	28	34
no	22	15
unknown	0	1

There are no significant differences. ($\chi^2 = 2.9$; d.f. = 2; p = n.s.).

10.3.9. *CONTRACEPTION, distinguished by treatment groups.*

Table 10.3.9. Contraceptive methods

CONTRACEPTION	CRYO n=50	LASER n=50
None	13	6
Hormonal	18	24
IUD	6	4
Pessary/protectives	2	5
Sterilization	11	11

There are no significant differences ($\chi^2 = 7.4$; d.f. = 4; p = n.s.).

10.3.10 *REFERRAL DIAGNOSIS, before evaluation at the colposcopy clinic by the present author, distinguished by treatment groups*

Table 10.3.10. Referral diagnosis

REFERRAL DIAGNOSIS	CRYO		LASER	
GRADE OF CIN	Total sample n	(viral infection) (n)	Total sample n	(viral infection) (n)
I	1		2	
II	27	(6)	26	(5)
III	22	(5)	22	(2)
Total	50	(11)	50	(7)

There are no significant differences ($\chi^2 = 1.3$; d.f. = 2; p = n.s.).

10.3.11 *DURATION of KNOWLEDGE of ABNORMAL CYTOLOGY or established presence of CIN before treatment, distinguished by treatment groups*

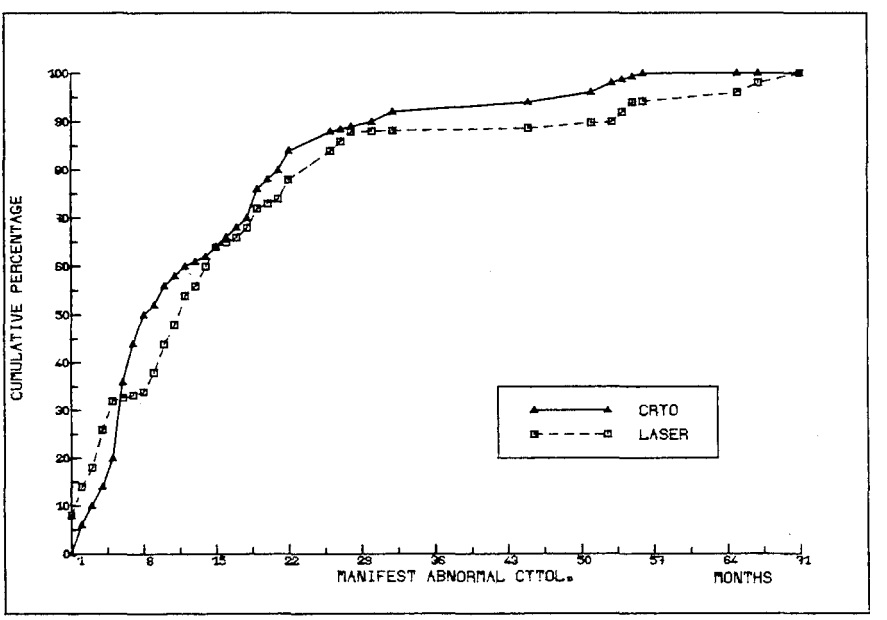


Figure 10.3.11. Duration in months of suspicion of CIN before treatment

DURATION before treatment	CRYO n=50	LASER n=50
Median	8.5	12.0
Interquartile range	6-19.25	4-22
Range	2-56	1-71

There are no significant differences (Mann-Whitney U-test; p = n.s.).

In summary (chapter 10.3):
There are no significant differences between both treatment groups with respect to the documented patient characteristics, the referral diagnosis and the duration of abnormal cytology.

10.4 Pre-treatment evaluation: Colposcopy

10.4.1 *COLPOSCOPIC IMPRESSION, distinguished by treatment groups*

Table 10.4.1. Colposcopic impression

COLPOSCOPIC IMPRESSION	CRYO		LASER	
GRADE OF CIN	Total sample n	(viral infection) (n)	Total sample n	(viral infection) (n)
I	9	(5)	10	(3)
II	37	(15)	32	(11)
III	4	(3)	8	(3)
Total	50	(23)	50	(17)

There are no significant differences ($\chi^2 = 1.7$; d.f. = 2; p = n.s.).

10.4.2 *SURFACE of the TRANSFORMATION ZONE (T-zone), distinguished by treatment groups*

TRANSFORMATION ZONE (mm^2)	CRYO n=50	LASER n=50
Median	235	328
Interquartile range	173.5 -415.75	191.75-571.0
Range	37-824	37-962

There are no significant differences (Mann-Whitney U-test; p = n.s.).

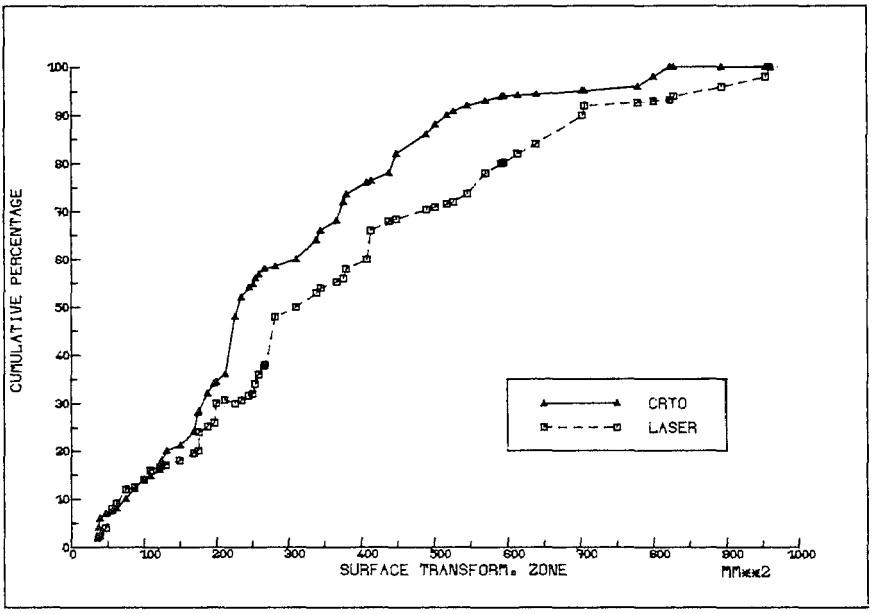


Figure 10.4.2. Surface of the transformation zone (mm^2)

10.4.3 *LOCATION OF THE LESION(S), distinguished by treatment groups*

Table 10.4.3. Location of the lesion(s)

LOCATION LESION(S)	CRYO n=50	LASER n=50
Ectocervix only	30	41
Endocervical extension	20	9

As was mentioned in the selection criteria (10.2.1): all patients had satisfactory colposcopy. However, significantly more patients with lesions extending into the endocervical canal, although within the direct vision of the colposcopist, appeared to be randomized to the CRYO group. ($\chi^2 = 4.9$ (Yates correction); d.f. = 1; $0.02 < p \leqslant 0.05$).

When the surface of the transformation zone (T-zone) was related to the endocervical extension of the lesion(s), the surface of the T-zone in patients in the CRYO group with lesions extending into the endocervical canal appeared to be significantly smaller than the T-zone in patients with lesions on the ectocervix only (Mann-Whitney U-test; $0.01 < p \leqslant 0.02$). This was not true for the LASER group, but in these patients endocervical extension was less frequent.

10.4.4 *DISTRIBUTION of the CIN LESIONS at colposcopy over the cervical-surface, distinguished by treatment groups*

Table 10.4.4. Distribution of the CIN-lesions

CIN-LESIONS colposcopically present in	CRYO n=50	LASER n=50
1 Quadrant	11	12
2 Quadrants	22	19
3 Quadrants	11	10
4 Quadrants	6	9

There are no significant differences ($\chi^2 = 0.9$; d.f. = 3; p = n.s.).

10.5 Pretreatment evaluation: Cytology

Results of REPEAT-CYTOLOGY at pretreatment evaluation, distinguished by treatment groups

Table 10.5. Results of repeat cytology

REPEAT CYTOLOGY	CRYO		LASER	
GRADE OF CIN	Total sample n	(viral infection) (n)	Total sample n	(viral infection) (n)
I	11		8	
II	28	(2)	32	(2)
III	11	(1)	10	(1)
Total	50	(3)	50	(3)

There are no significant differences ($\chi^2 = 0.8$; d.f. = 2; p = n.s.).

When the cytologic grades of CIN of the referral cytology (Table 10.3.10) were compared with the grade of CIN determined by repeat cytology, there was a considerable percentage of "overdiagnosis" in grade of CIN in the referral cytology (Table 10.7.1), however between both treatment groups, these differences were not significant (χ^2 (trend) = 6.8; d.f. = 5; p = n.s.).

10.6 Pretreatment evaluation: Histopathology

10.6.1 *Number of BIOPSIES taken under colposcopic guidance, distinguished by treatment groups*

Table 10.6.1. Number of biopsies

Number of BIOPSIES	CRYO n=50	LASER n=50
1	1	1
2	23	24
3	14	14
4	8	5
5	4	6

There are no significant differences ($\chi^2 = 1.8$; d.f. = 4; p = n.s.).

10.6.2 *ENDOCERVICAL CURETTAGE (ECC), distinguished by treatment groups*

In most patients suspected of high grade CIN lesions or when a lesion extended into the endocervical canal, an ECC was performed and, usually, a blind endocervical biopsy (ECB) was taken to exclude occult malignant disease with more certainty, although colposcopy was satisfactory in all patients.
In total 20 patients of the CRYO group and 18 of the LASER group had an ECC performed; this difference is not significant ($\chi^2 = 0.04$ (Yates correction); d.f. = 1; p = n.s.).

Table 10.6.2. Results of ECC's (n=38).

ECC GRADE OF CIN	CRYO n=50	LASER n=50
NEG/not done	46	44
I	2	4
II	2	2

There are no significant differences ($\chi^2 = 0.7$; d.f. = 2; p = n.s.).

There was no case in which the grade of CIN in the ECC or ECB was more severe than the grade of colposcopically guided biopsies. There was also no patient with negative biopsies and a positive ECC. For the 10 patients with CIN grade I and II in the endocervical curettings, this was not considered to be a contraindication to local destructive treatment because colposcopy was clearly satisfactory and follow-up seemed to be guaranteed.

10.6.3 *MAXIMAL GRADE of CIN in the HISTOPATHOLOGY specimens and presence of HPV-infection, distinguished by treatment groups*

Table 10.6.3. Histopathologic grade of CIN and diagnosis of cervical HPV-infection

HISTOPATHOLOGY	CRYO		LASER	
GRADE OF CIN	Total sample n	(viral infection) (n)	Total sample n	(viral infection) (n)
I	7	(4)	9	(3)
II	33	(13)	30	(12)
III	10	(3)	11	(4)
Total	50	(20)	50	(19)

There are no significant differences ($\chi^2 = 0.4$; d.f. = 2; p = n.s.).

When the histopathologic grade of CIN of each patient was compared with the surface of the T-zone (Figure 10.4.2), there was no significant relationship between the size of the T-zone and the severity of the lesion in both groups.
(CRYO: ($\chi^2 = 14.5$; d.f. = 18; p = n.s.)
(LASER: ($\chi^2 = 25.2$; d.f. = 18; p = n.s.).
When the histopathologic grade of CIN of each patient was compared with the extension of the lesion over the four quadrants of the ectocervix (Table 10.4.4), the indication that more severe lesions were more extensively distributed over the ectocervix was not significant for either group.

10.7 Pretreatment evalution: Agreement between diagnostic methods

10.7.1. In Table 10.7.1 the *AGREEMENT IN GRADING CIN* between pairs of diagnostic methods used in the pretreatment evaluation is summarized.

In Table 10.7.1:
*(1) Refers to the first mentioned diagnostic method and (2) to the second method.
With the exception of repeat cytology and histology in the CRYO group, there is no significant discrepancy in the accuracy of grading CIN when 2 diagnostic methods are compared pairwise for the same patient in this selected population.
When the differences in grades of CIN between 2 diagnostic methods were tested, comparing both treatment groups (χ^2-test for trend), there were no significant differences between the CRYO and the LASER group.

	$\chi^2 =$	d.f. =	p =
Repeat- vs referral cytology	6.8	5	n.s.
Repeat-cytology vs colposcopy	5.0	4	n.s.
Repeat-cytology vs histopathology	8.9	7	n.s.
Colposcopy vs histopathology	4.7	4	n.s.

Table 10.7.1. : Agreement between diagnostic methods in grading CIN for individual patients, distinguished by treatment groups.

Agreement between (1) and (2)	% Agreement within the same grade of CIN	% Overdiagnosis (1) vs (2)	% Underdiagnosis (1) vs (2)	Significance testing (X^2-test)
Repeat-cytology and Referral-cytology				
Cryo	60	2	38	$0.001 < p \leq 0.01$
Laser	64	2	34	$0.001 < p \leq 0.01$
Repeat-cytology and Colposcopy				
Cryo	58	26	16	$0.02 < p \leq 0.05$
Laser	72	18	10	$p << 0.001$
Repeat-cytology and Histopathology				
Cryo	52	22	26	p = n.s.
Laser	74	12	14	$p << 0.001$
Colposcopy and Histopathology				
Cryo	76	4	20	$p << 0.001$
Laser	64	14	22	$0.001 < p \leq 0.01$

Agreement in grade of CIN within 1 degree of discrepancy (over or under) in this series of selected patients was 100% for colposcopy and histopathology and 96% and 98% for cytology and histopathology for respectively the CRYO and the LASER group.

10.7.2 *AGREEMENT in prediction of the presence of cervical HPV-INFECTION between colposcopy and histopathology*

When the histopathologic diagnosis of cervical viral infection was taken as the "standard", the following predictive values of a colposcopic impression suspect for cervical HPV-infection (PV+) and of a colposcopic impression not suspect for cervical HPV-infection (PV-) were calculated.

The predictive value of colposcopy as to the presence of a cervical viral infection diagnosed by histopathology,

$$PV+ = \frac{32}{33} = 0.97$$

The predictive value of a negative colposcopy as to the absence of a cervical viral infection diagnosed by histopathology,

$$PV- = \frac{59}{67} = 0.88$$

Table 10.7.2. Predictive value of colposcopy in the detection of cervical HPV-infections with the histopathologic diagnosis as "standard".

	HISTOPATHOLOGY		
COLPOS-COPY	HPV+	HPV-	Total
HPV+	32	1	33
HPV-	8	59	67
Total	40	60	100

The predictive values were not significantly different for patients in the CRYO and LASER group separately:

CRYO

$$PV+ = \frac{19}{20} = 0.95$$

$$PV- = \frac{26}{30} = 0.87$$

LASER

$$PV+ = \frac{13}{13} = 1.0$$

$$PV- = \frac{33}{37} = 0.89$$

In summary (chapter 10.5 to 10.7):
With the exception of more endocervical extension of lesions in the CRYO group, there were no significant differences with respect to colposcopic, cytologic and histopathologic findings and presence of viral infections between both treatment groups.

10.8 Treatment procedures, distinguished by treatment groups

10.8.1 *Anaesthesia*

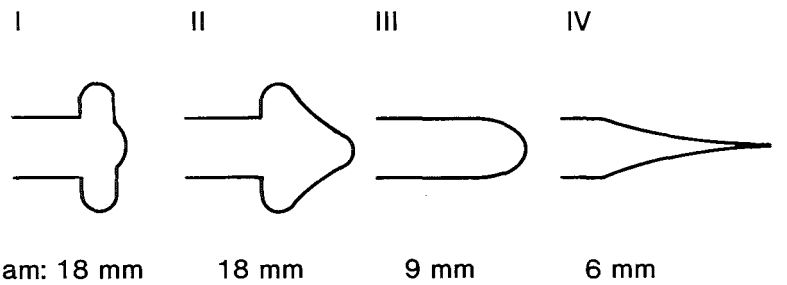
In the CRYO group no patient received (local) anaesthesia or sedatives. In the second part of the study 15 patients randomized to LASER therapy had local anaesthesia (paracervical block).

10.8.2 *Cryo-probe tips and laser mode/power*

Four interchangable cryo-probe tips were available.

10.8.2 CRYO PROBE TIPS

Four interchangable cryo probe tips were available.



To adequately freeze the entire transformation zone and to include all colposcopically visible lesions, the following (combination of) probes was used.

Table 10.8.2. Cryo probes.

Probes used	Patients (n=50)
I	15
II	11
III	1
IV	3
I + II	6
I + IV	6
II + III	3
II + IV	5

LASER patients were all treated with the CO_2-laser in a continuous mode, with standard spot size and usually maximal power (ca. 20 Watts), resulting in power densities varying from 500-1000 Watts /cm^2.

10.8.3 *Number of cryo-applications and depth of laser-vaporization*

Table 10.8.3. Number of cryo-applications

Cryo-applications	Patients (n=50)
1	23
2	22
3	4
4	1

Depth of Laser-vaporization

Table 10.8.3. Depth of destruction by CO_2-laser

Depth (mm)	Nr. of Patients (n=50)	
5	8	Depth
6	8	mean : 7.94 mm
7	5	median: 8 mm
8	11	range : 5-12 mm
9	1	
10	13	
11	1	
12	3	

10.8.4. *Surface of destruction*

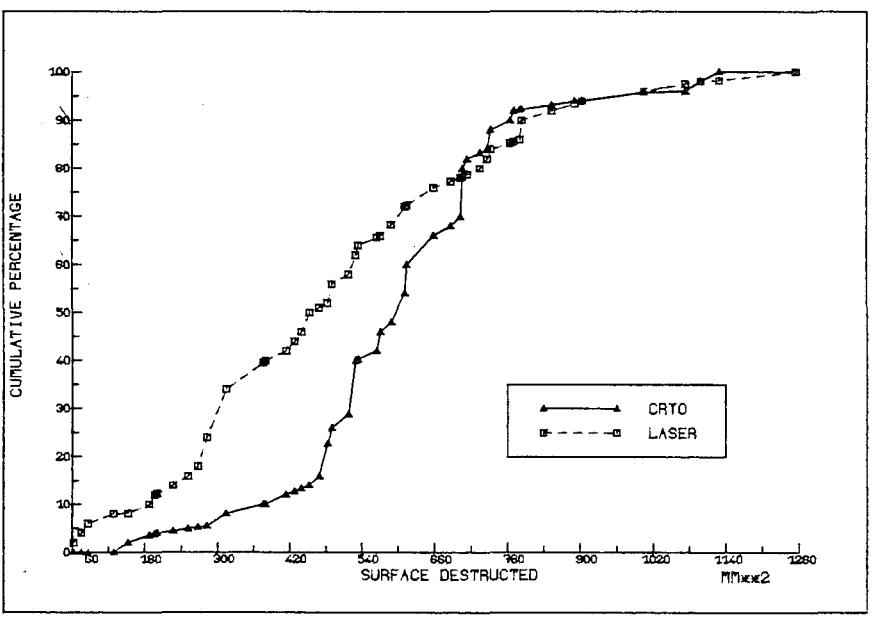


Figure 10.8.4. Surface of the cervix that was destroyed by either cryocoagulation or laser vaporization (mm^2).

SURFACE DESTROYED (mm^2)	CRYO n=50	LASER n=50
Median	612	467.5
Interquartile range	490-706	306-670
Range	153-1130	62-1256

The surface destroyed by CRYO coagulation was significantly larger than the surface destroyed by LASER vaporization (Mann-Whitney U-test; $0.001 < p \leqslant 0.01$).

10.8.5 *Duration of treatment*

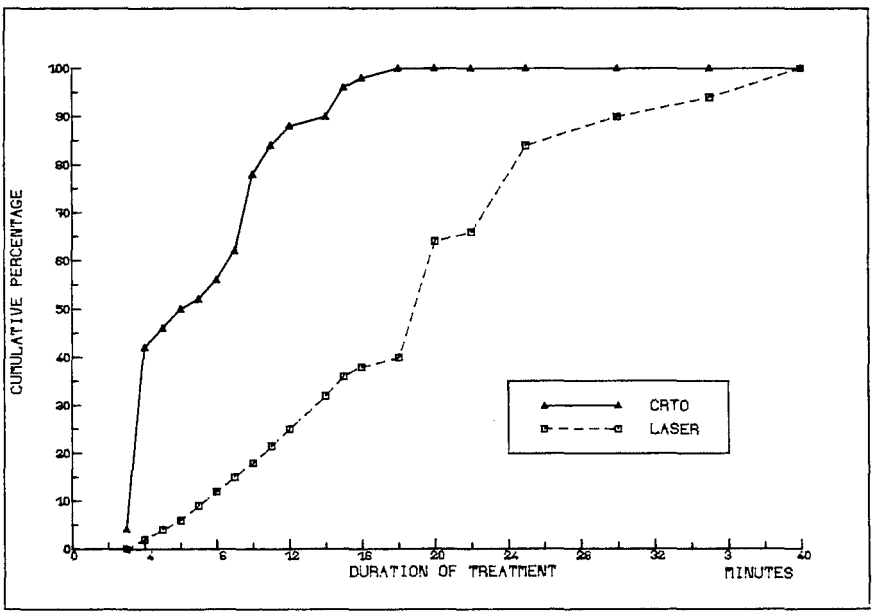


Figure 10.8.5. Duration of treatment (minutes)

DURATION of TREATMENT (min)	CRYO n=50	LASER n=50
Median	6.5	20.0
Interquartile range	4-10	15-25
Range	3-18	4-40

This difference in duration of treatment is significant (Mann-Whitney U-test; $p \leqslant 0.001$).

10.9 Treatment complications and side effects, distinguished by treatment groups

10.9.1 *Direct complications and side effects*

Table 10.9.1.1. Hemorrhage

HEMORRHAGE	CRYO n=50	LASER n=50
None	49	11
Slight	1	19
Moderate	0	10
Severe	0	10

In 2 LASER patients electrocoagulation was necessary for adequate hemostasis. This difference in bleeding during treatment was highly significant ($\chi^2 = 50.3$; d.f. $= 4$; $p \leqslant 0.001$).

Table 10.9.1.2. Pain sensations

PAIN	CRYO n=50	LASER n=50
None	29	18
Light intermittent	12	14
continuous	6	3
Moderate intermittent	1	13
continuous	2	1
Severe	0	1

This difference in the experience of pain sensations was significant ($\chi^2 = 15.8$; d.f. $= 5$; $0.001 < p \leqslant 0.01$).

Pain in the CRYO group was usually described as "light cramps" or "(pre)-menstrual pains". In the LASER group 15 patients had a paracervical block and 12 of these patients did not mention any pain sensation, 3 of them experienced light cramps. LASER patients without anaesthesia had varying pain sensations described as "cramps", "labor pains" or "burning feelings". In 1 patient treatment was prematurely abandoned because of subjectively intractible pains. Treatment interruptions because of side-effects were significant in the LASER-group:

Table 10.9.1.1. Treatment interruptions because of direct treatment complications or side effects.

TREATMENT INTERRUPTIONS	CRYO n=50	LASER n=50
None	50	28
Temporarily	0	21
Definitively	0	1

These differences were significant ($\chi^2 = 29.3$; d.f. = 3; $p \leqslant 0.001$).

10.9.2 *Postoperative complications and side effects*

10.9.2.1 Early postoperative complications

Within the first 14 post treatment days 1 CRYO patient (2%) developed fever above 38°C. She was known to have a history of PID with bilateral salpingectomy. She had no localized pelvic symptoms and the fever resolved spontaneously on day 7.
4 LASER patients (8%) had postoperative vaginal blood loss which required treatment in the outpatient clinic. In three coagulation with silver-nitrate sticks, reassurance and a small vaginal tampon for several hours was sufficient for hemostasis. In one patient electrocoagulation and 1 hemostatic suture was necessary to control the bleeding. In none of these patients a blood transfusion was required.
One additional LASER patient complained of nausea and lower abdominal pain on the first post treatment day, with her symptoms resolving spontaneously one day later.
The differences in frequencies of early postoperative complications between both treatment groups were statistically not significant, although highly relevant ($\chi^2 = 4.8$; d.f. = 4; p = n.s.).

10.9.2.2 Late postoperative complications (2-12 weeks)

In the LASER group no patient mentioned late postoperative complications.
In the CRYO group 3 patients complained of dysmenorrhoea, due to partial stenosis of the cervical canal and 1 patient of amenorrhoea, due to complete stenosis of the endocervical canal. All 4 patients were treated in the outpatient clinic by cervical sounding and dilatation under local anaesthesia (paracervical block), which resolved these problems in all but one patient, who after 3 years had a hysterectomy performed because of recurrent stenosis and dysmenorrhoea. In addition, 1 CRYO patient had a persistently bleeding ectropion, while cytology and colposcopy were not suspect; she was re-treated with infrared coagulation.

Six patients (12%) in the CRYO and 5 patients (10%) in the LASER group experienced postoperative complications. This difference was statistically not significant ($\chi^2 = 0$ (Yates correction); d.f. = 1; p = n.s.).

10.9.3 *Postoperative discharge*

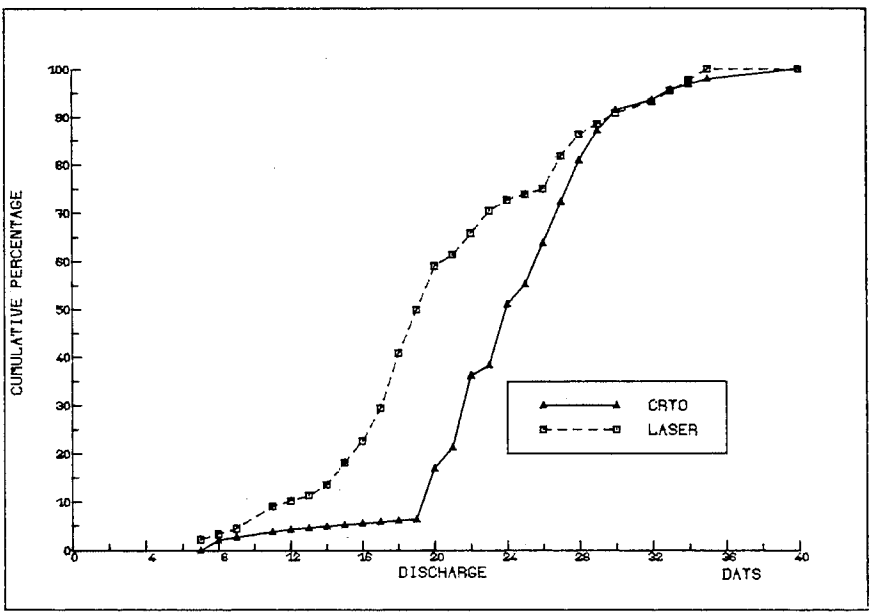


Figure 10.9.3.1. Duration of postoperative discharge (days).

DISCHARGE (days)	CRYO n=47	LASER n=44
Median	24	19.5
Interquartile range	22-28	17-26.75
Range	8-40	7-35

The CRYO patients who kept the discharge record (47) had significantly more days of post treatment discharge than the LASER patients who kept their discharge record (44) (Mann-Whitney U-test; $p \leqslant 0.001$).

DISCHARGE (score)	CRYO n=46	LASER n=44
Median	50	43
Interquartile range	39.5-60.75	32.25-54.25
Range	11-99	14-92

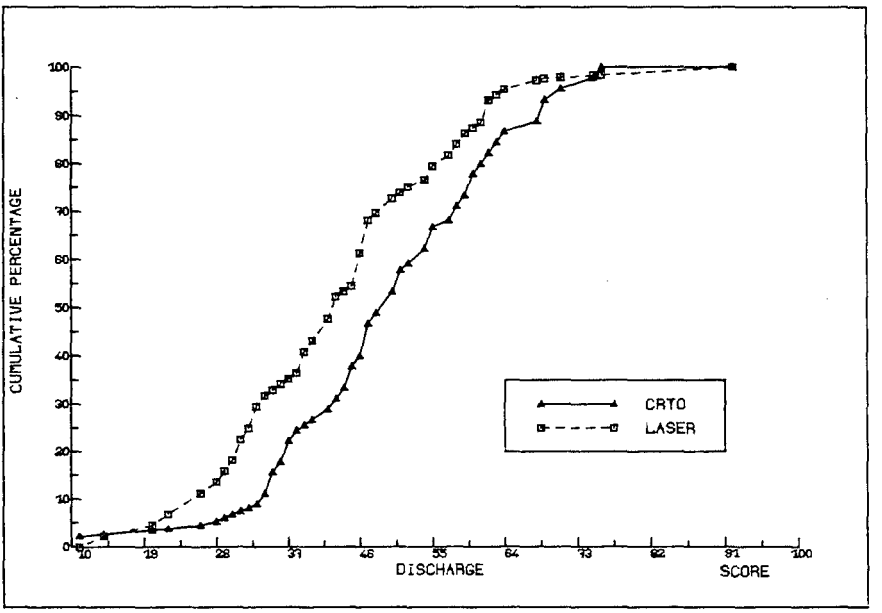


Figure 10.9.3.2. Postoperative discharge score

For 1 CRYO patient who kept her discharge calender it was not possible to interpret the notations and to calculate the score.
The discharge score in the CRYO group was significantly higher than in the LASER group (Mann-Whitney U-test; $0.01 < p \leqslant 0.02$).

10.10 Treatment results

No patient was lost to follow-up in the first year after treatment and thanks to close co-operation with the family doctors, 96 patients could be followed for between 32 and 66 months.

10.10.1 *Persistent disease, distinguished by treatment groups*

Fourty eight CRYO-patients and 47 LASER-patients returned for at least 2 follow-up visits between 3 and 12 months after treatment.
One LASER patient had a hysterectomy performed for benign conditions after 6 months; cervical pathology appeared to be negative for CIN. Another LASER patient moved abroad after 2 negative follow-up smears in the first 7 months after treatment. Three patients (2 CRYO- and 1 LASER-) had only one follow-up smear between 8 and 12 months after treatment, which were all negative for CIN. Therefore all patients were considered suitable for analysis of persistent disease and treatment failure.

Colposcopy, when performed shortly after treatment, was found to be difficult to interpret. Biopsies were taken only if endo- or ectocervical cytology was abnormal.
Cytology of the first follow-up smear (3-6 months after treatment) was positive in 6 patients of the CRYO group (2 CIN I and 4 CIN II) and in 9 patients of the LASER group (2 CIN I and 7 CIN II). Colposcopically there was suspicion of CIN in 1 of these CRYO- and in 4 of these LASER patients.
Directed biopsies were negative in all 6 CRYO-patients, but positive for CIN in 5 of the 9 LASER patients.
In the six CRYO-patients with abnormal cytology, 2 also had abnormal cytology in the second follow-up smear, the other 4 became negative and remained so in further follow-up. Seven of the 9 LASER patients with abnormal first follow-up smears had abnormal cytology again at second follow-up, the other 2 reverted to normal.
In the CRYO-group there was no patient with a negative smear at 3-6 months who had a positive smear at 8-12 months. In the LASER-group there was only 1 patient with a negative first follow-up smear who had CIN I in the second. The cytology in this patient reverted to normal spontaneously in succeeding smears.
When colposcopy between 8 and 12 months was considered to be representative for a stabilized situation after treatment with respect to the visibility of the squamocolumnar junction, the following results were obtained: in the CRYO-group in 21 of 47 patients (45%) and in the LASER group in 37 of 48 patients (77%) the SCJ could be visualized. This difference was significant ($\chi^2 = 9.2$ (Yates correction); d.f. = 1; $0.001 < p \leqslant 0.01$).
According to the definition of *treatment failure*, 2 patients (4%) of the CRYO-group and 7 (14%) of the LASER group had persistent disease. These 9 patients were considered as treatment failures and eliminated for evaluation of recurrent disease. This difference in persistence was statistically not significant, although practically important ($\chi^2 = 1.95$ (Yates correction); d.f. = 1; p = n.s.).

Table 10.10.1.1. Persistent disease c.q. treatment failure

PERSISTENCE determined by	CRYO n=50		LASER n=50	
	persistence n	success rate (%)	persistence n	success rate (%)
Cytology	2	(96)	7	(86)
Colposcopy	1	(98)	4	(92)
Histopathology	0	(100)	5	(90)

In Table 10.10.1.2 several important pretreatment characteristics and relevant data with respect to patients with persistent and recurrent disease are summarized. All patients with persistent disease (9) had histopathologic CIN II or III lesions before treatment, and 4 in combination with cervical HPV-infection. Only 2 patients had an ECC performed, however both ECC's contained CIN II on

Table 10.10.1.2. : Patients with persistent and recurrent disease; Summary.

	Pretreatment pathology grade of CIN	Surface T-zone mm^2	Quadrants affected by CIN	Depth laser destruction	Diagnosis; months after treatment	Cytology grade of CIN	Pathology grade of CIN	Retreatment (months)		Maximal follow-up (months)
Persistence										
Cryo	II	39	2	–	5	I	II	Cone Biopsy	(35)	65
	II V+	226	3	–	4	I	Neg	–		38
Laser	II V+	703	2	5	3	II	II V+	Laser	(16)	39
	II	282	2	5	4	II	II V+	Cryo	(34)	64
	III	571	3	6	5	III	III-microinv.	Cone biopsy	(9)	46
	II V+	615	4	7	3	I	Neg	–		47
	III	955	4	10	5	II	I	Laser	(13)	50
	II V+	706	4	5	4	II	II V+	Cone biopsy	(23)	54
	II	762	4	5	4	II	II	Cone biopsy	(14)	49
Recurrence										
Cryo	III V+	100	3	–	14	I	Neg	–		33
	I V+	367	3	–	17	II	II V+	–		44
	II	596	3	–	43	I	I	–		62
	III	502	4	–	27	II	III-microinv.	Hysterectomy	(31)	43
Laser	I V+	75	4	5	32	I	Neg	–		45
	II	37	1	8	47	I V+	Neg V+	Cryo	(48)	61
	III V+	593	3	10	33	I	I	Hysterectomy	(33)	32

V+: Cervical Human Papilloma Virus infection suspected.

histopathology. The distribution of the CIN lesions on the cervix was significantly more extensive in patients with persistent disease ($\chi^2 = 8.2$; d.f. = 3; $0.02 < p \leqslant 0.05$).
There also were significantly more bleeding-problems ($\chi^2 = 9.6$; d.f. = 4; $0.02 < p \leqslant 0.05$) in patients with persistence of CIN.
It is remarkable (Table 10.10.1.2) that patients with persistent disease, especially in the LASER-group, had T-zones, significantly larger than the non-persistent patients in this group (Mann-Whitney U-test; $0.02 < p \leqslant 0.05$. In addition, in five out of seven patients depth of destruction was 5 or 6 millimeters, while the mean depth for the non-persistent patients was 8.1 mm (χ^2-test; p = n.s.) and in 4 treatment had to be interrupted because of bleeding and/or moderate pain. Two of the LASER patients with persistent disease had local anaesthesia, however both had CIN-lesions in all 4 quadrants of the ectocervix.

There seems to be a connection between large T-zones, extensively distributed lesions, more bleeding and pain and treatment interruptions during treatment, leading to relatively superficial vaporization which in turn results in a higher chance of persistent disease in the LASER group.
Six of the seven LASER-patients with persistent disease had to be re-treated and are being followed without evidence of disease. Three had a cone biopsy with complete removal of residual disease, after 9, 14 and 23 months respectively; 2 were retreated with laser vaporization after 13 and 16 months respectively and 1 patient after 3 years with cryocoagulation because of a persistent CIN II lesion and viral infection.
In one of the cone specimens an extensive CIN III lesion was present with suspicion of micro-invasive, squamous cell carcinoma in one more caudally located cervical crypt, 9 months after LASER-treatment.
In one CRYO-patient with persistent disease on cytology, histopathology was negative and cytology reverted to normal without any other intervention. The other CRYO patient had a therapeutic cone biopsy performed 35 months after CRYO-coagulation.

10.10.2 *Recurrent disease*

Four CRYO-patients and 3 LASER-patients had recurrent abnormal cytology after 14 to 47 months from primary treatment. Six of them originally had CIN lesions in 3 or 4 quadrants of the ectocervix, and 4 had a cervical HPV-infection before treatment.
Two of these 7 had pretreatment (negative) ECC's performed and 6 seemed to be treated adequately; 1 LASER-patient had her small T-zone vaporized to a depth of 5 mm.
Histopathology was positive in four (Table 10.10.1.2). One CRYO-patient had recurrence detected after 27 months and in the hysterectomy specimen a CIN III lesion suspect for micro-invasive squamous cell carcinoma was diagnosed. When the total study population was considered, patients with recurrent disease had earlier sexarche ($\chi^2 = 32.8$; d.f. = 13; $0.001 < p \leqslant 0.01$) and more severe

referral cytology ($\chi^2 = 23.3$; d.f. = 3; $p \leqslant 0.001$) and surfaces of the T-zones and destroyed areas were larger (Mann-Whitney U-test; $0.02 < p \leqslant 0.05$). After exclusion of patients with persistent disease, these differences were less prominent.

In Table 10.10.1.2 follow-up data of patients with persistent or recurrent disease have been revised up to January 1, 1987. Many patients with a 2 year negative follow-up have been referred to their family doctor for annual endo- and ectocervical smears up to 5 years after treatment and then once every 3 years. Patients were strongly advised to have follow-up smears taken and family doctors were asked to refer the patient again when cytology became abnormal. Follow-up cytology reports could be traced of 96 patients up to December 1986, resulting in a follow-up varying between 32 and 66 months.

Overall, to obtain or to regain normal cytology results in the follow-up after CRYO-or LASER-treatment for CIN, thus far 2 patients of the CRYO-group (4%) but 8 patients of the LASER-group (16%) have needed re-treatment in some fashion. This difference, although important, was statistically not significant ($\chi^2 = 2.8$ (Yates correction); d.f. = 1; p = n.s.). Patients who needed re-treatment had significantly more severe referral cytology ($\chi^2 = 9.4$; d.f. = 3; $0.02 < p \leqslant 0.05$), histopathology of endocervical curettings was more severe ($\chi^2 = 7.9$; d.f. = 2; $0.01 < p \leqslant 0.02$), treatment had to be interrupted more frequently ($\chi^2 = 21.9$; d.f. = 3; $p \leqslant 0.001$), and in the CRYO patients the surface of the transformation zone was larger (Mann-Whitney U-test: $0.02 < p \leqslant 0.05$).

When patients with persistent and recurrent disease combined were compared with patients with negative follow-up, there were significant differences with respect to (more extensive) distribution of the lesions in the failure-group ($\chi^2 = 9.0$; d.f. = 3; $0.02 < p \leqslant 0.05$), more frequent treatment interruptions ($\chi^2 = 18.4$; d.f. = 3; $p \leqslant 0.001$) and destroyed surface (Mann-Whitney U-test $0.02 < p \leqslant 0.05$).

Summarizing the results of this randomized study between two highly comparable groups of 50 patients each, it can be concluded that treatment results of *cryocoagulation* were better, although not significantly, than of *laser vaporization.* According to the definition of persistent disease, treatment was successful in 96% of CRYO- and in 86% of LASER-patients. After follow-up varying between 32 and 66 months 2 patients of the CRYO-group and 8 patients of the LASER-group have needed additional treatment for persistent or recurrent disease ("Cure-rates" for CRYO-coagulation 96% and for LASER vaporization 84%).
In the LASER-group, there was one patient with persistent disease who appeared to have a CIN III lesion with minimal invasive squamous cell carcinoma in a cone specimen removed 9 months after treatment, and in the CRYO-group one patient with recurrent disease had the same finding in a hysterectomy specimen 31 months after treatment. Before treatment both patients had a relatively large, as CIN III diagnosed lesion; ECC was not performed.
In the present study persistent or recurrent disease was not significantly related to age or any other specific patient characteristic, duration of CIN before

treatment, endocervical extension of the lesion and presence of cervical HPV-infections. However, there was a tendency to increasing failure rates in more severe CIN-lesions for both groups (Table 10.10.2), and there were less ECC's performed (25%) in the persistent or recurrent CIN-group compared with the patients in the group with negative follow-up (40% ECC's).

Table 10.10.2. Persistent and recurrent disease related to the pretreatment histopathologic grade of CIN (row-%).

Pretreatment histopathologic grade of CIN	CRYO n=50	persistence (%)	recurrence (%)	"total failure" (%)	re-treatment (%)
I	7	0 (0)	1 (14)	1 (14)	0 (0)
II	33	2 (6)	1. (3)	3 (9)	1 (3)
III	10	0 (0)	2 (20)	2 (20)	1 (10)
	LASER n=50				
I	9	0 (0)	1 (11)	1 (11)	0 (0)
II	30	5 (7)	1 (4)	6 (20)	5 (17)
III	11	2 (8)	1 (9)	3 (27)	3 (27)

Patients with persistent or recurrent disease appeared to have significantly more lesions distributed over 3 or 4 quadrants of the ectocervix, (12 of 16 = 75%) than patients with negative follow-up (24 of 84 = 29%) ($\chi^2 = 7.18$; d.f. = 1; $0.001 < p \leqslant 0.01$).
In the LASER-group, patients with persistent disease had significantly larger transformation zones than the other LASER-patients and destruction in these treatment failures was more superficial than in the LASER-patients with negative follow-up. Overall, LASER-patients had slightly larger lesions than CRYO-patients, the latter having more endocervical extensions.
In the present study, complications and side effects during treatment were significantly more frequent in the LASER group, mainly due to troublesome hemorrhage and pain, necessitating treatment interruptions, which were the main cause of the significantly longer duration of the laser treatments. Early and late postoperative complications were equal in both groups; post-treatment healing after LASER was faster, resulting in significantly less severe discharge, which was also of more limited duration. Another advantage of LASER was that 1 year after treatment the squamocolumnar junction was significantly more frequent within the vision of the colposcopist than after CRYOcoagulation.

10.11 Discussion

In chapter 8.5 indications, techniques, results and side effects of cryocoagulation and laser vaporization have been discussed separately.
The results of the present study compare well with other publications. Wetchler

(582) reported from collected series successful treatment with single cryotherapy for all grades of CIN in 89% of cases and with single laser treatment in 86% of cases. However, results of different studies are usually difficult to compare. Kwikkel et al. (302) summarized the many possible sources of variation which may result in a biased comparison when in retrospect success rates in published reports on the treatment of CIN are reviewed. His conclusion was, that a randomized study will be the method of choice to compare efficacy and side effects of laser- and cryotherapy.
In the present randomized study, both treatment groups appeared to be controlled for age and other possible relevant patient characteristics.
In addition, there were no significant differences between both groups with respect to grade of CIN, size of the transformation zone, distribution of the CIN lesions over the ectocervix and findings in endocervical curettings, all of these parameters that are known to influence treatment results (177,549). Moreover a strict and clinically realistic definition of persistent and recurrent disease was practiced and long-term follow-up was achieved in 96% of patients.
In the literature several publications have been found comparing laser and cryosurgery for CIN, however, only few fulfill the criteria of a controlled study:

Table 10.11. Review of treatment results of cryocoagulation and CO_2 laser vaporization for CIN.

First author	Type of study	CRYO n	(success-%)	LASER n	(success-%)
Wright (1981)	retrospective	152	(86)	131	(97)
Townsend (1983)	alternate	100	(93)	100	(89)
Wetchler (1984)	collected series	4549	(89)	2707	(86)
Jobson (1984)	prospective "trial study"	39	(90)	42	(90)
Kwikkel (1985)	prospective	50	(86)	51	(71)
Helmerhorst (1985)	randomized	81	(88)	84	(79)
Ferenczy (1985)	alternate	147	(91)	147	(96)
Present study	prospective randomized	50	(96)	50	(86)

Wright and Davies (595) used historical controls in a retrospective study and suggested a definite advantage for laser, especially for CIN III lesions: CRYO 25% failures; LASER 7.7%.
Townsend and Richart (549) prospectively alternated patient assignment to cryotherapy and laser and did not find significant differences; for CIN III the failure percentages were respectively 10% and 13%.
The study by Ferenczy (177) was not randomized; however, patients were matched according to grade, size and distribution of CIN and alternately treated. Only patients with negative ECC's were treated with either single freeze outpatient cryotherapy or CO_2 laser vaporization in the operating room. All laser patients had local anaesthesia and destruction was performed up to 5 mm deep into

the cervical stroma and 5 mm beyond the lesional margins. CIN measuring less than 30 mm in diameter without extension into the endocervix had similar low failure rates, regardless of histologic grade or treatment technique (CRYO 5% failure after 1 year; LASER 4%). For lesions larger than 30 mm, CO_2 laser produced better results (CRYO 38% failures after 1 year; LASER 8%) and cryo-failures were increased fivefold from laser failures for CIN-lesions that extended 5 mm or less into the endocervical canal. Overall complication rate for CRYO was 0.6% and for LASER 7.4%. Although carefully written, there are some inconsistencies in the numbers of patients who failed therapy in his report.
The design of the studies by Jobson and Homesley (253) and by Kwikkel and Helmerhorst (230, 302), both reporting on what appears to be the same series of patients which was more extended in the second report, seems to fit best with the present study.
Jobson and Homesley (253) reported a 10% overall failure rate at four months and this remained stable through the first year of follow-up. There was no difference in failure rate with respect to lesion size, number of cervical quadrants involved or histologic grade of CIN. Complications were few.
In the Kwikkel and Helmerhorst series (230,302), the original success rates were relatively low, but it was stated that this was due to the strict selection criteria for patients and the specific definition of treatment failure. From the second report it is not clear if the improved results of the laser group (Table 10.11) are attributable to a more extensive experience in laser surgery, to a change in the criteria of treatment success, or to a difference in the lesion-size or selection of the additional patients. In their first report, success rates were significantly related to the size of the colposcopically visible lesion, which, overall however appeared to be larger in the laser group. In both reports success rate was not related to the grade of CIN: in the second report the relation with lesion size was not mentioned.
It is remarkable that in the first report in the results it is stated that "gradually the use of analgesics has been introduced, which serves also to shorten treatment time", while in the second report concerning the same patients it is stated that "cryo- and lasertherapy have been performed without analgesia or anaesthesia".
In the present study, with a composition of patients with respect to grade of CIN comparable to the Kwikkel-Helmerhorst-study, the possible therapeutic effect of biopsies taken at the pre-treatment evaluation was not awaited, as it was in their series. However, it is supposed that this therapeutic effect (5) would be comparable for both treatment groups.
For LASER-patients in the present study there was also a relation between a large size of the T-zone to be destroyed and a higher chance of persistent disease. For both treatment groups there was a tendency toward more failures in high grade and more extensively distributed cervical intraepithelial neoplasia.

Recently Boonsta (64) performed a study on cone biopsy specimens of patients surgically treated for CIN III and reviewed the literature with respect to linear extent, topography and depth of cervical crypt involvement in CIN III. Unfortunately in his material the selection of the patients and the colposcopic findings or results of preoperative ECC's were not explicite. In his CIN III population, if patients with favourably located CIN III lesions were treated with destructive

methods, the results of his study could be seriously biased. His conclusions were that CIN III lesions in his material could vary between 1 and 18 mm in linear extent (mean 7 mm), that the deepest crypt involvement was 4.5 mm, and that crypt involvement was more extensive in patients over the age of 50 and in multiparous women. In his material most lesions were located endocervically, from 8 to 13 mm from the most caudal point of the portio and 95% of all CIN III lesions were within 21 mm endocervical extension from this reference point. In younger patients linear extent was smaller and the lesions were situated nearer to the ectocervix. Nabothian cysts also appeared to be involved with CIN III in several cases.
If one considers locally destructive or even excisional treatment for high grade CIN-lesions, one has to take into account these data, which seem to be supported by the cryotherapy results of Savage et al (470): in patients with cervical gland involvement there was a failure rate of 27% (17 of 63 patients) after cryosurgery. The degree of severity of CIN in patients who failed therapy was not a significant factor.

It seems logical to assume that when an area of CIN is totally removed, regardless of the technique or instrument, the therapeutic results will be similar. There are no data to suggest that there is a different response between the various grades of CIN to different treatment methods used to remove the entire lesion (549).
If, as in the present randomised study, there is a difference in therapeutic results between therapeutic methods to destroy CIN it is reasonable to suppose that this difference must be attributed to incomplete removal of the CIN-areas.

With the techniques applied in the present study, apparently the aim of destruction was attained in 96% of the CRYO-coagulation patients. Although in 4 patients small cervical biopsies were taken because of abnormal cytology at follow-up between 4-6 months. However, this was also done in 9 LASER-patients. This supports the supposition that pre-treatment colposcopic and histopathologic assessment of these patients has been adequate and lesions were within the destructive capacity of the cryosurgical techniques that were used. Considering the results, there seems to be no reason to change our cryotechnique to a routine freeze-thaw-freeze fashion in each patient, if the selection and treatment policy applied in this study is being followed.
LASER vaporization seems to have the potential to destroy CIN more extensively than CRYOcoagulation can do. Nevertheless, in the present study LASER-results were worse than those of CRYOtherapy. Although the surface of the T-zone of the LASER-group was overall slightly, but not significantly, larger than that of the CRYO-patients, this does not seem to be the definitive explanation for the higher persistence rate, because in all other aspects patients of both treatment groups were completely comparable and patients in the CRYO-group had even more endocervical extensions of CIN.
It seems that in the present study, due to direct treatment complications (hemorrhage), troublesome to the physician, and due to side effects (pain), poorly tolerable for some patients, it has not been possible to completely utilize the

potential of the laser-technique in patients with large lesions, which frequently happened to contain extensively distributed, high grade CIN-lesions.
Presently, the author performs, when indicated, LASER-treatment for CIN under local anaesthesia, frequently in combination with a local hemostatic agent. In this fashion, starting from the dorsal point of the transformation zone to prevent blood or ooze from obstructing vision, in nearly all cases a painless, much faster and more effective vaporization or excision to an adequate depth and endocervical extension can be accomplished.

The one patient in the LASER-group who turned out to have a minimal invasive squamous cell carcinoma in one of the caudally located cervical crypts underwent a cone biopsy 9 months after LASER vaporization for an extended CIN III lesion with persistent disease. She had not undergone pre-treatment endocervical curettage because colposcopy was considered to be satisfactory. It would seem prudent to always perform an endocervical curettage in all patients with more severe grades of CIN, even if colposcopy is thought to be satisfactory, when destructive treatment is being considered, and to exclude all patients with positive endocervical curettings from locally destructive treatment. This opinion is in contrast to that of Kwikkel et al. (302) and of Bellina et al. (40) who have stated that a positive ECC with satisfactory colposcopy most often indicates inadvertently taken ectocervical fragments.

Complications and side effects of the two outpatient treatment methods seem to be acceptable provided that LASER vaporization of large and deep tissue volumes is being performed under local anaesthesia. Generally, complications necessitating medical or surgical treatment, particularly for bleeding, are more frequent after laser than after cryotherapy. Although healing of the cervical epithelium is faster after laser therapy, healing time is not considered to influence significantly either the treatment results or the general well-being of the patients.
Following LASER vaporization a significantly larger number of patients (77%) had a colposcopically visible squamocolumnar junction than following cryotherapy (45%). Although this will allow a more reliable follow-up with cytology and colposcopy, it has been suggested that everted columnar epithelium may just serve as a focus for new metaplasia, possibly leading to the development of new intraepithelial neoplasia (258).

The long-term efficacy of both cryo and laser therapy can be evaluated only by observing long-term recurrence rates (442). In the present series the number of patients is relatively small and the follow-up is still much to short for generalization, however, it is thusfar the longest and most complete of the published controlled studies.
Figge and Creasman (180), Ferenczy (177) and Helmerhorst et al. (230) reported that most recurrences developed during the first two post-treatment years. Long-term follow-up results of the present series do not confirm this. Five of the seven "cytologic" recurrences (comprising 7.7% of the patients without residual or persistent disease) were detected between 27 and 47 months after treatment and 4 of these 5 were confirmed by histopathology (4.4%) (Table 10.10.2).

There were no significant differences between the LASER- and the CRYO-group, although the only recurrent patient in the CRYO group who needed retreatment, appeared to have a minimal invasive cervical squamous cell carcinoma, in an endocervical crypt, 31 months after primary treatment. It has been suggested that after cryotherapy cervical intraepithelial neoplasia may be buried within endocervical glands with normal surface epithelium and that it may later become invasive carcinoma (504).

Finally, in view of the present data and the results of other published comparative studies it can be stated that cryocoagulation as well as laser vaporization can be highly effective in treating CIN, provided that the pre-treatment diagnostic triage procedures are strictly adhered to, treatment is performed appropriately and to an adequate extent and duration and a long-term follow-up is guaranteed. The use of a CO_2-laser has the additional advantage that in large and deeply located lesions an excisional conization can be executed under local anaesthesia (23, 158, 539) or a combination of laser excision and -vaporization may be performed (23). Cryocoagulation however, is less costly and easier to learn than laser surgery.
Size, location and distribution of the cervical intraepithelial lesions remain the most important factors that influence treatment results. In the choice of the most cost-effective treatment method for a given lesion and patient, expert colposcopy is mandatory.

Chapter 11

EPILOGUE

Wouter M. Huisman and J.A. Wijnen

The cause of cervical cancer remains unknown. New types were recognized in the past decades, types no one knew existed or could detect in time to prevent serious consequences. As long as the characteristics of these various types of cancer remain unknown, the real women at risk are also unknown. It is possible, and in theory even likely, that the exchange of views one finds in the literature, deals mainly with the most prevalent type of cervical neoplasia. It is as much likely, that definite statements are biased by the inclusion of less prevalent or hitherto unknown types of cervical cancer in the study material. For the clinician this means that he will be faced with ever changing concepts, that label yesterday's truth tomorrow's uncertainty and challenge. This does not rid him of his task to decide on patient management today.

The prevention of the most prevalent type of cervical cancer benefits from screening, but it should benefit as much from good information, based on what we know about the risk factors. The young woman, who has immature metaplastic epithelium in her transformation zone should not delay her sexarche or her first pregnancy until a safer and therefore later date, just for fear of getting cancer. She should be provided with the right kind of information on how to limit her risks e.g. by the use of barrier contraceptives and /or the avoidance of promiscuity in those periods of her life in which the risk is highest. Given the great attention, currently devoted to the prevention of AIDS, now is the time to combine information campaigns, because the lifestyles of the risk groups show great similarities.

All of the main pillars of diagnosis and management of CIN have been criticized through the years.
Cytology for its high false-negative rate and originally for the possibility it presented to classify too many apperances as uncertain. It is still the easiest screening method and reevalution of data from population screening programs has shown the mortality rates of cervical cancer to have dropped as a result of it, at least in some countries.
The screening role of cytology is its major strength, which should be directed at more than just the women who are thought to be at risk of developing cervical cancer. Attempts to limit screening to this category have failed in the past, if only for the fact that the woman most likely to develop cervical cancer is the least likely to attend a screening program.
Still, cytology determines for the greater part, who is the patient to be seen by the colposcopist. Those who have a false-negative smear would need an extra indication for colposcopy. Since the false-negative rate may be high, two

negative cytology reports are needed for complete reassurance, provided there are no clinical symptoms such as contact bleeding or macroscopic suspicion of disease. In these cases colposcopy should be performed even when cytology is negative. The cytologic diagnosis of the patients, referred for colposcopy is frequently an overdiagnosis, not reflecting the true state of the patient. Although the phenomenon itself leads to a higher detection rate, it is also responsible for a great deal of unnecessary anxiety on the part of the patient. The high rate of overdiagnosis may tempt the clinician to do one of two things: he may take the cytologic diagnosis seriously and perform additional surgery when a discrepancy between cytology and histopathology remains unexplained after directed biopsies, or he may, when an overdiagnosis occurs too frequently, be tempted to take the cytologic diagnosis not too seriously and omit extra steps to exclude disease. In situ DNA hybridization techniques may be of considerable help to restore the clinicians faith in the cytologic classification, especially if the report would contain a statement on the presence or absence of types of virus such as HPV types 16, 18 or 31. The presence of these types of virus would give warning of a possible progression to carcinoma.
Histopathology has been criticized for its lack of reproducible diagnoses. This has provoked some authorities in the field of colposcopy to claim the captaincy of the team. The part of our material that was reevaluated does not support this view. It is obvious that through the years, both the histopathologist and the colposcopist became more keen on recognizing virus-specific epithelial changes. As colposcopists, we were convinced that we could see more manifestations of viral disease than we received back in histopathologic diagnoses, but the histopathologist is evidently dependent on the specimen we provide him with. In the earlier part of our colposcopy, the attention was mainly focused on the detection of CIN and virus related appearances were noted down, but not always biopsied if the only visible manifestation was e.g. a surface epithelium with asperities. When a warty lesion was seen, a biopsy was always taken because of the difficulty of separating a viral from a CIN lesion. The continuous feedback between histopathologist and colposcopist has increased the diagnostic rate of viral disease of both. The incorporation of the new techniques for virus identification will also increase the value of the histopathologic diagnosis in terms of prognosis of a lesion, making this less dependent on morphologic criteria only.

Colposcopy has gained an important place in our clinic as an aid in the diagnosis of premalignant cervical lesions. The two theses not only show the results obtained in diagnosis and treatment of CIN, they are also an account of the history of colposcopy within our clinic and a justification of the present role of this technique. Colposcopy was criticized from the start for its allowance for personal interpretations. The fact that it is almost impossible to compare results in the international literature, without taking at least a dozen restrictive factors into account, seems to support this criticism, but that does not make it true. In the practical situation there are enough ways to circumvent this. The most important way is to raise doubt about the certainties that are reported and vividly copied, in the literature. This was our basis for diagnosing as accurately as possible both in the diagnostic and the therapeutic studies. The results did not prove

us wrong. Circumstances prevented a full integration of both diagnostic approaches, since the results reported on in Chapters 5 and 6 had not yet been analyzed at the onset of the studies mentioned in Chapters 7, 9 and 10.
The capacity of colposcopy to assist in conservative treatment of CIN has been adequately shown. The results are not only important because they have led to lower exconization rates. Contrary to the view of many, that this is the greatest gain of colposcopy, exconization still has its place in our clinic, both for diagnostic and for therapeutic reasons, albeit a more limited one than before colposcopy. A greater gain, derived from our experiences with conservative treatment is the fact that there is a choice where formerly there was none.
What this choice will be, depends on several circumstances. Today, no patient is scheduled for additional surgery or outpatient treatment without a full evaluation by colposcopy, colposcopically directed biopsies and/or endocervical curettage. The combination of colposcopy and histopathology decides on further management. Unsatisfactory colposcopy precludes local destructive treatment, even if the endocervix curetting is negative. The presence of CIN in an endocervical curettage, even if colposcopy is satisfactory, is also considered to contraindicate local destructive treatment.
Systemic treatment with folic acid has not been enforced, because the allegation that compensation of a possible focal deficiency might be a treatment modality for CIN, was not substantiated by the results of our study. A larger, epidemiologic study might lead to other results, especially if patients with a systemic folic acid deficiency would be included.
The choice between local outpatient treatment by laser vaporization or cryocoagulation is optional, when the results of these methods are compared. This is not to say that the effects of both methods are the same. Laser treatment is felt to be less damaging to the cervical tissue surrounding a lesion, than cryocoagulation, but more residual lesions are seen when the vaporization has to be stopped because of a pain reaction from the patient or when local hemorrhage interferes. A combination of local anaesthesia and a haemostatic agent would solve this problem. For routine use, cryocoagulation is less costly and as effective as laser therapy, provided the freezing is adequate, both with regard to the duration and the extent.
The fact that both treatment modalities may result in the transformation zone still being visible, which is not as often the case after exconization with sutures to cover the defect, raises the interesting question if this is indeed an advantage of local treatment. Aside from the obvious advantages of not having to be admitted to the hospital, not needing general anaesthesia and not being exposed to the complications and sequelae of exconization, a patient receiving local treatment may have one disadvantage. If columnar epithelium is again exposed to the vaginal environment, the process of metaplasia may start all over again. Who is to say that this patient would not need life-long follow-up?

SUMMARY

In Chapter 1 general aspects of etiology, diagnosis and treatment of cervical neoplasia are introduced. The objectives of the studies which were designed and performed independently and successively by the authors, are summarized. All studies are focused on colposcopy in diagnosis or treatment of cervical neoplasia.

In Chapter 2 the morphology of the cervical epithelia is discussed. A survey is presented of the causes and consequences of the process of sqamous metaplasia and of the colposcopic and histopathologic stages.

In Chapter 3 the development of the present concepts of Cervical Intraepithelial Neoplasia (CIN) is discussed. The historic contributions to the early detection of this condition by means of colposcopy, lugol-test and exfoliative cytology are highlighted. Aspects of etiology and epidemiology and theories on morphogenesis and natural history of CIN are presented. It is concluded that the evidence of viral agents playing a role in the etiology of CIN is convincing and matches the epidemiologic postulates of early sexarche and promiscuity being by far the most important risk factors. Prevention should therefore be directed at the venereal characteristics of CIN. Improvement of diagnostic capacities, irrespective of the method used, is advocated for optimalization of clinical management.

In Chapter 4 the history of colposcopy, the procedures of a colposcopic examination and the assessment of colposcopic findings are described. A critical review of the literature is presented. The colposcopic grading approach and the biopsy method used in the studies, documented in Chapters 5 and 6, is introduced.

In Chapter 5 the results of a study on the diagnostic accuracy of colposcopically directed biopsies are presented. The study population consisted of 117 patients of whom a biopsy diagnosis and a second histopathologic diagnosis based on the examination of a cone biopsy and/or hysterectomy specimen was available. Each diagnostic mode, including cytology which was for the purpose of this study considered as such, and a combination of diagnostic modes, was compared with the final diagnosis, which was defined per patient as the highest histopathologic classification found in any tissue sample.
It is concluded that, provided colposcopy is satisfactory, clinical management of CIN, including outpatient conservative therapy, can be based on the histopathologic classification of colposcopically directed biopsies.
The generally accepted opinion that a combination of cytology and colposcopy

increases the diagnostic accuracy of both methods is not substantiated by the results of the present study.
Not the diagnostic accuracy, but the rate of detection of (pre)invasive cervical lesions benefits from combinations of diagnostic modes.

In Chapter 6 the results are presented of an investigation into the predictive value of colposcopic diagnostic criteria. A colposcopic impression, based on the grading approach presented in Chapter 4 served as colposcopic diagnosis to be compared with the histopathologic biopsy diagnosis. The value of the individual criteria, used in a process of exclusion of diagnoses is discussed.
With emphasis on vascular characteristics of colposcopic findings and on the reaction of the epithelia to acetic acid, a high percentage of accurate prediction of the histopathologic outcome of the biopsies was accomplished. The analysis of the grading approach is preceded by an account of the re-evaluation of the biopsy specimens.
In only 10,2% of 930 biopsy specimens the histopathologic classification was changed, resulting in a different final diagnosis in 35 out of 302 patients (11,4%). In retrospect, this would have had an impact on therapeutic management in only 3 patients.

In Chapter 7 the procedures and techniques applied after January 1, 1981 at the Colposcopy Clinic in the routine evaluation of patients with abnormal cervical cytology are described. For this routine evaluation, the accuracy of cytology as well as the colposcopic impression in predicting the histopathologic grade of CIN and the presence of cervical human papilloma virus (HPV) infection was assessed.
The conclusions are that the colposcopist estimated the severity of a CIN lesion at least as reliably as the cytologist and that the colposcopist was capable of taking biopsies from the most suspicious areas, although he tended to have called CIN II, what the histopathologist designated as CIN III.
Endocervical curettings and blind endocervical biopsies did not alter the histopathologic grade of CIN of any case but were helpful in tailoring the most appropriate treatment to the needs of the individual patient and cervical lesion.
In the present series the cytologist hardly ever mentioned a suspicion of cervical HPV infection, the colposcopist did so in 43% and the histopathologist in 29% of the 163 cases.
For the routine evaluation of patients with cervical smears suggestive of CIN, a flow-sheet is proposed, based on a colposcopic approach, to exclude or diagnose invasive cervical carcinoma and to establish the presence or absence of CIN, as conservingly as possible.

In Chapter 8 the rationale, general principles and methods of treatment for CIN are described and discussed.
Emphasis is placed on relevant aspects of cryocoagulation and laser surgery for CIN in view of a prospective randomized study (Chapter 10).

In Chapter 9 the design and results of a nutritional intervention study on CIN are described. In a prospective, double-blind, placebo controlled setting, the effects on CIN of 100 days of oral folate supplementation were assessed.
In this study no significant beneficial effect of folic acid on the prevention of progression or the promotion of regression to normalcy in outpatients with cytologic, colposcopic and histopathologic signs of CIN could be demonstrated. This was irrespective of the suspicion of a cervical human papilloma virus infection or the use of oral contraceptive agents.

In Chapter 10 the design and results of a prospective randomized study comparing the therapeutic efficacy, complications and side effects of outpatient cryocoagulation or laser vaporization for CIN are presented. Patients were selected after a complete colposcopic triage procedure. In the present study the results of cryocoagulation were better and complications and side effects during treatment were less than those of laser vaporization. However, the differences, although important, were statistically not significant.
Treatment results of laser surgery for CIN seemed to improve when adequate local anaesthesia was used. For every-day praxis both outpatient methods can offer excellent results in the treatment of CIN. Cryosurgery however, is easier to learn and to perform, the equipment is less costly and patient acceptance is high.
Strict and long-term cytologic follow-up is indicated after both destructive treatment methods.

In Chapter 11 the results and conclusions of the preceding chapters are synthesized and a "state of the art" of colposcopic assessment and management of CIN is formulated.

SAMENVATTING

In Hoofdstuk 1 worden algemene aspecten met betrekking tot de etiologie, diagnose en behandeling van cervicale neoplasie geïntroduceerd. De doelstellingen van de door beide auteurs, onafhankelijk van elkaar opgezette en in opvolgende perioden uitgevoerde onderzoeken worden samengevat.
Alle studies zijn gericht op de rol van de colposcopie in de diagnostiek of de behandeling van cervicale neoplasie.

In Hoofdstuk 2 wordt de morfologie van de epithelia van de portio cervicis uteri besproken. Een overzicht wordt gegeven van de oorzaken en gevolgen van het proces van plaveiselcel metaplasie en van de colposcopische en histopathologische stadia.

In Hoofdstuk 3 wordt de ontwikkeling van de huidige opvattingen over Cervicale Intraepitheliale Neoplasie (CIN) besproken. De historische bijdrage aan de vroege detectie van deze aandoening middels colposcopie, lugol-test en exfoliatie cytologie wordt toegelicht. Etiologische- en epidemiologische aspecten en theorieën omtrent de morfogenese en het natuurlijk verloop van CIN worden gepresenteerd.
Geconcludeerd wordt dat de bewijzen voor een virale etiologie van CIN overtuigend zijn, mede gezien de overeenstemming met de epidemiologische opvattingen dat een vroege sexarche en promiscuïteit verreweg de belangrijkste risicofactoren vormen. Preventieve maatregelen dienen zich daarom te richten op het venerische karakter van CIN. Verbetering van diagnostische mogelijkheden wordt bepleit, onafhankelijk van de methodiek, met als doel het klinisch beleid te optimaliseren.

In Hoofdstuk 4 wordt ingegaan op de geschiedenis van de colposcopie, op de procedure van een colposcopisch onderzoek en op de beoordeling van colposcopische bevindingen. Na een kritisch literatuuroverzicht worden de wijze van benadering van de colposcopische beoordeling en de ontwikkelde biopsiemethode geïntroduceerd, die werden gebruikt in de onderzoeken waarover in Hoofdstuk 5 en Hoofdstuk 6 wordt gerapporteerd.

In Hoofdstuk 5 worden de resultaten gepresenteerd van een onderzoek naar de diagnostische nauwkeurigheid van de beoordeling van colposcopisch gerichte proefexcisies. De patiënten-groep bestond uit 117 vrouwen, waarvan behalve een biopsie-diagnose ook een histopathologische beoordeling van een exconisatie- en /of uteruspreparaat beschikbaar was.
Na de cytologie tijdelijk te hebben gedefinieerd als een diagnostische methode, worden de verschillende methoden, afzondelijk en in combinatie, vergeleken

met de einddiagnose die per patiënt werd gedefinieerd als de hoogste beoordeling toegekend aan een histopathologisch preparaat.
Geconcludeerd wordt dat bij toereikende colposcopie het klinisch beleid, inclusief poliklinische, conservatief chirurgische behandelingen, kan worden gebaseerd op de histopathologische beoordeling van colposcopisch gerichte biopsieën. De algemeen geldende opvatting dat door combinatie van cytologie en colposcopie een verhoogde diagnostische nauwkeurigheid kan worden bereikt, wordt niet onderbouwd door de resultaten van het onderhavige onderzoek. Een dergelijke combinatie is wel zinvol ter verhoging van de detectiegraad van (pré)invasieve cervixaandoeningen.

In Hoofdstuk 6 worden de resultaten gepresenteerd van een onderzoek naar de voorspellende waarde van diagnostische criteria in de colposcopie.
Een colposcopische impressie, gebaseerd op de scoringswijze, geïntroduceerd in Hoofdstuk 4, diende als colposcopische diagnose die werd vergeleken met de histopathologische diagnose gesteld op colposcopisch gericht genomen proefexcisies. De waarde van de afzonderlijke criteria die werden gebruikt in een proces van uitsluiting van diagnoses, wordt besproken. Door de nadruk te leggen op de kenmerken van het vaatpatroon zoals dit door de colposcoop kan worden waargenomen, en op de reactie van het epitheel op het deppen met azijnzuur, werd in een hoog percentage de histopathologische diagnose juist voorspeld.
De analyse van de scoringswijze wordt voorafgegaan door een verslaglegging van de resultaten van de herbeoordeling van de biopsieën. Slechts in 10,2% van 930 biopsieën werd de histopathologische beoordeling veranderd, hetgeen resulteerde in een verandering in einddiagnose in 35 van de 302 patiënten (11,4%). Achteraf gezien zou dit slechts in 3 gevallen een verandering van het therapeutisch beleid hebben betekend.

In Hoofdstuk 7 wordt de gang van zaken beschreven bij de routine evaluatie van patiënten met afwijkende cervix-cytologie, zoals die na 1 januari 1981 werd uitgevoerd in de Colposcopie polikliniek. Zowel voor de cytologie als de colposcopische impressie werd de nauwkeurigheid bepaald waarmee de histopathologische graad van CIN en het vaststellen van de aanwezigheid van een infectie met humaan papilloma virus (HPV) kan worden bepaald.
Geconcludeerd wordt dat de colposcopist de ernst van een CIN-lesie tenminste even betrouwbaar kon inschatten als de cytoloog en dat de colposcopist in staat was biopsieën te nemen uit de meest verdachte gebieden, hoewel hij tendeerde naar de beoordeling CIN II in gevallen die door de histopatholoog als CIN III werden bestempeld. Hoewel de resultaten van endocervix-curettages en blind genomen endocervix-biopsieën in geen enkel geval een verandering teweegbrachten in de histopathologische eindbeoordeling van de graad van CIN, waren zij zinvol bij het op de individuele patiënte en de individuele lesie toesnijden van de meest geëigende wijze van behandeling. In de onderhavige groep, bestaande uit 163 patiënten, werd door de cytoloog vrijwel nooit de verdenking geuit op een HPV-infectie; de colposcopist zag aanwijzingen hiervoor in 43% en de histopatholoog in 29% van de patiënten.
Voor de routine evaluatie van patiënten met een cytologische verdenking op

CIN, wordt een stroom-diagram voorgesteld, gebaseerd op een colposcopische benadering die op een zo conserverend mogelijke wijze een invasief cervix-carcinoom uitsluit of diagnostiseert dan wel de aanwezigheid of afwezigheid van CIN aantoont.

In Hoofdstuk 8 worden de redenen om CIN te behandelen en de algemene principes en methoden van behandeling beschreven en nader besproken. De nadruk wordt gelegd op relevante aspecten van cryocoagulatie en laser chirurgie met het oog op een in Hoofdstuk 10 gedocumenteerd prospectief gerandomiseerd onderzoek.

In Hoofdstuk 9 worden de opzet en de resultaten van een voedingsinterventie onderzoek naar de tendens tot progressie of regressie van CIN beschreven. In een prospectief, dubbel-blind onderzoek werd het effect op CIN nagegaan van orale toediening van foliumzuur gedurende 100 dagen. Een gunstig effect in de zin van het voorkómen van progressie of het stimuleren van regressie kon niet worden aangetoond bij patiënten waarbij cytologisch, colposcopisch en histopathologisch CIN was aangetoond. Evenmin bestond enige relatie tussen de genoemde tendensen bij het gebruik van foliumzuur en de aanwezigheid van een infectie met humaan papilloma virus of het gebruik van orale contraceptiva.

In Hoofdstuk 10 worden de opzet en de resultaten beschreven van een prospectief gerandomiseerd onderzoek waarin een vergelijking werd gemaakt tussen poliklinische cryocoagulatie en laser evaporatie bij patiënten met CIN, wat betreft genezingseffect, complicaties en bijwerkingen.
De patiënten waren geselecteerd na een volledige colposcopische evaluatie. In het onderhavige onderzoek werden met cryocoagulatie betere resultaten bereikt en waren de complicaties en bijwerkingen geringer in aantal dan met laser evaporatie. Deze weliswaar belangrijke verschillen waren statistisch niet significant. De behandelingsresultaten van laser chirurgie werden beter na adequate locale verdoving.
In de dagelijkse praktijk kunnen met beide poliklinische methoden voor de behandeling van CIN uitstekende resultaten worden verkregen. Cryocoagulatie is echter gemakkelijker aan te leren en uit te voeren, de apparatuur is minder duur en de acceptatie door de patiënten is goed.
Na het toepassen van beide behandelingsmethoden is stricte en langdurige cytologische follow-up geïndiceerd.

In Hoofdstuk 11 worden de resultaten en conclusies van de voorgaande hoofdstukken samengevoegd en wordt de huidige stand van zaken betreffende de colposcopische beoordeling van CIN en het te voeren beleid geformuleerd.

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ADDENDUM

Relationship between a diagnostic mode and the final histopathologic diagnosis (see Chapter 5). Results are given for the total group of patients (N = 117), for satisfactory colposcopy (N = 62) and for unsatisfactory colposcopy (N = 55). For all tables: INV = microinvasive carcinoma and macroinvasive carcinoma taken together.

Table 5.3-A. Cytology and final diagnosis (N = 117).

Cytology	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	2	1	3	0	1	7
CIN I	1	0	1	0	0	2
CIN II	1	3	10	4	1	19
CIN III	2	1	21	47	9	80
INV	0	0	1	2	6	9
Total (n)	6	5	36	53	17	117

Table 5.3-B. Cytology and final diagnosis in satisfactory colposcopy (N = 62).

Cytology	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	2	1	2	0	1	6
CIN I	1	0	0	0	0	1
CIN II	1	3	6	2	1	13
CIN III	0	0	13	27	1	41
INV	0	0	0	1	0	1
Total (n)	4	4	21	30	3	62

Table 5.3-C. Cytology and final diagnosis in unsatisfactory colposcopy (N = 55).

Cytology	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	0	0	1	0	0	1
CIN I	0	0	1	0	0	1
CIN II	0	0	4	2	0	6
CIN III	2	1	8	20	8	39
INV	0	0	1	1	6	8
Total (n)	2	1	15	23	14	55

Table 5.3-D. Biopsy and final diagnosis (N = 117).

Biopsy	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	6	0	2	2	0	10
CIN I	0	5	1	0	0	6
CIN II	0	0	33	7	0	40
CIN III	0	0	0	44	4	48
INV	0	0	0	0	13	13
Total (n)	6	5	36	53	17	117

Table 5.3-E. Biopsy and final diagnosis in satisfactory colposcopy (N = 62).

Biopsy	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	4	0	0	0	0	4
CIN I	0	4	0	0	0	4
CIN II	0	0	21	1	0	22
CIN III	0	0	0	29	1	30
INV	0	0	0	0	2	2
Total (n)	4	4	21	30	3	62

Table 5.3-F. Biopsy and final diagnosis in unsatisfactory colposcopy (N = 55).

Biopsy	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	2	0	2	2	0	6
CIN I	0	1	1	0	0	2
CIN II	0	0	12	6	0	18
CIN III	0	0	0	15	3	18
INV	0	0	0	0	11	11
Total (n)	2	1	15	23	14	55

Table 5.3-G. Colposcopic impression and final diagnosis (N = 117).

Colposcopic impression	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	6	0	0	0	0	6
CIN I	0	5	2	0	0	7
CIN II	0	0	28	4	0	32
CIN III	0	0	5	49	4	58
INV	0	0	1	0	13	14
Total (n)	6	5	36	53	17	117

Table 5.3-H. Colposcopic impression and final diagnosis in satisfactory colposcopy (N = 62).

Colposcopic impression	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	4	0	0	0	0	4
CIN I	0	4	2	0	0	6
CIN II	0	0	16	1	0	17
CIN III	0	0	3	29	0	32
INV	0	0	0	0	3	3
Total (n)	4	4	21	30	3	62

Table 5.3-I. Colposcopic impression and final diagnosis in unsatisfactory colposcopy (N = 55).

Colposcopic impression	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	2	0	0	0	0	2
CIN I	0	1	0	0	0	1
CIN II	0	0	12	3	0	15
CIN III	0	0	2	20	4	26
INV	0	0	1	0	10	11
Total (n)	2	1	15	23	14	55

Table 5.3-J. Cytology + biopsy and final diagnosis (N = 117).

Cytology + biopsy	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	2	0	0	0	0	2
CIN I	1	1	1	0	0	3
CIN II	1	3	13	1	0	18
CIN III	2	1	21	50	3	77
INV	0	0	1	2	14	17
Total (n)	6	5	36	53	17	117

Table 5.3-K. Cytology + biopsy and final diagnosis in satisfactory colposcopy (N = 62).

Cytology + biopsy	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	2	0	0	0	0	2
CIN I	1	1	0	0	0	2
CIN II	1	3	8	0	0	12
CIN III	0	0	13	29	1	43
INV	0	0	0	1	2	3
Total (n)	4	4	21	30	3	62

Table 5.3-L. Cytology + biopsy and final diagnosis in unsatisfactory colposcopy (N = 55).

Cytology + biopsy	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	0	0	0	0	0	0
CIN I	0	0	1	0	0	1
CIN II	0	0	5	1	0	6
CIN III	2	1	8	21	2	34
INV	0	0	1	1	12	14
Total (n)	2	1	15	23	14	55

Table 5.3-M. Colposcopic evaluation and final diagnosis (N = 117).

Colposcopic evaluation	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	6	0	0	0	0	6
CIN I	0	5	0	0	0	5
CIN II	0	0	30	2	0	32
CIN III	0	0	5	51	1	57
INV	0	0	1	0	16	17
Total (n)	6	5	36	53	17	117

Table 5.3-N. Colposcopic evaluation and final diagnosis in satisfactory colposcopy (N = 62).

Colposcopic evaluation	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	4	0	0	0	0	4
CIN I	0	4	0	0	0	4
CIN II	0	0	18	0	0	18
CIN III	0	0	3	30	0	33
INV	0	0	0	0	3	3
Total (n)	4	4	21	30	3	62

Table 5.3-O. Colposcopic evaluation and final diagnosis in unsatisfactory colposcopy (N = 55).

Colposcopic evaluation	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	2	0	0	0	0	2
CIN I	0	1	0	0	0	1
CIN II	0	0	12	2	0	14
CIN III	0	0	2	21	1	24
INV	0	0	1	0	13	14
Total (n)	2	1	15	23	14	55

Table 5.3-P. Cytology + colposcopic evaluation and final diagnosis (N = 117).

Cytology + colposcopic evaluation	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	2	0	0	0	0	2
CIN I	1	1	0	0	0	2
CIN II	1	3	13	0	0	17
CIN III	2	1	21	51	1	66
INV	0	0	2	2	16	20
Total (n)	6	5	36	53	17	117

Table 5.3-Q. Cytology + colposcopic evaluation and final diagnosis in satisfactory colposcopy (N = 62).

Cytology + colposcopic evaluation	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	2	0	0	0	0	2
CIN I	1	1	0	0	0	2
CIN II	1	3	8	0	0	12
CIN III	0	0	13	29	0	42
INV	0	0	0	1	3	4
Total (n)	4	4	21	30	3	62

Table 5.3-R. Cytology + colposcopic evaluation and final diagnosis in unsatisfactory colposcopy (N = 55).

Cytology + colposcopic evaluation	Final diagnosis					
	negative	CIN I	CIN II	CIN III	INV	Total (n)
negative	0	0	0	0	0	0
CIN I	0	0	0	0	0	0
CIN II	0	0	5	0	0	5
CIN III	2	1	8	22	1	34
INV	0	0	2	1	13	16
Total (n)	2	1	15	23	14	55

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