Evaluation

of national guidelines and

implementation

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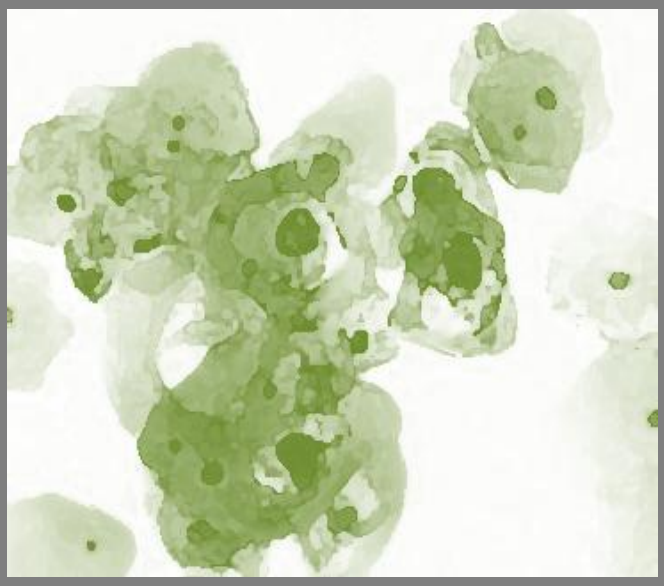
within the Dutch screening program

against cancer of the uterine cervix



Bert Siebers

Evaluation of national guidelines and implementation of new techniques within the Dutch screening program against cancer of the uterine cervix

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Evaluation of national guidelines and implementation of new techniques within the Dutch screening program against cancer of the uterine cervix

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In herinnering aan mijn ouders

Aan Rosan, Isabel, Carmen en Arthur

Contents

List of Abbreviations

HPV	Human Papillomavirus
CIN	Cervical Intraepithelial Neoplasia
SCJ	Squamo-columnar Junction
TZ	Transformation Zone
CIS	Carcinoma In Situ
PAP	Papanicolaou
TBS	The Bethesda System
SIL	Squamous Intraepithelial Lesion
HSIL	High-grade Squamous Intraepithelial Lesion
LSIL	Low-grade Squamous Intraepithelial Lesion
ASCUS	Atypical Squamous Cells of Undetermined Significance
AGUS	Atypical Glandular Cells of Undetermined Significance
ASC-H	Atypical Squamous Cells cannot exclude HSIL
AGC	Atypical Glandular Cells
LLETZ	Large Loop Excision of the Transformation Zone
VIA	Visual Inspection with Acetic Acid
LBC	Liquid-based cytology
HC2	Hybrid Capture 2
PCR	Polymerase Chain Reaction
NVVP	Nederlandse Vereniging Voor Pathologie
ECC-	Pap-smear without endocervical cells
CBO	Kwaliteitsinstituut voor de Gezondheidszorg
EM+	Endometrial cells present
HRT	Hormone Replacement Therapy
MI	Maturation Index
PALGA	Pathologisch Anatomisch Landelijk Geautomatiseerd Archief
RR	Relative Risk
BNC	Borderline Nuclear Changes
GP	General Practitioner
RCT	Randomized Controlled Trial
OR	Odds Ratio
TPR	Test Positive Rate
DRR	Detection Risk Ratio
PRR	Positive predictive value Risk Ratio
NETHCON	Netherlands ThinPrep versus Conventional cytology Trial

Chapter 1

General introduction and outline of this thesis

'The attack on disease must not be meddlesome; the desire to do something must be guided by sure argument that good will come of it'

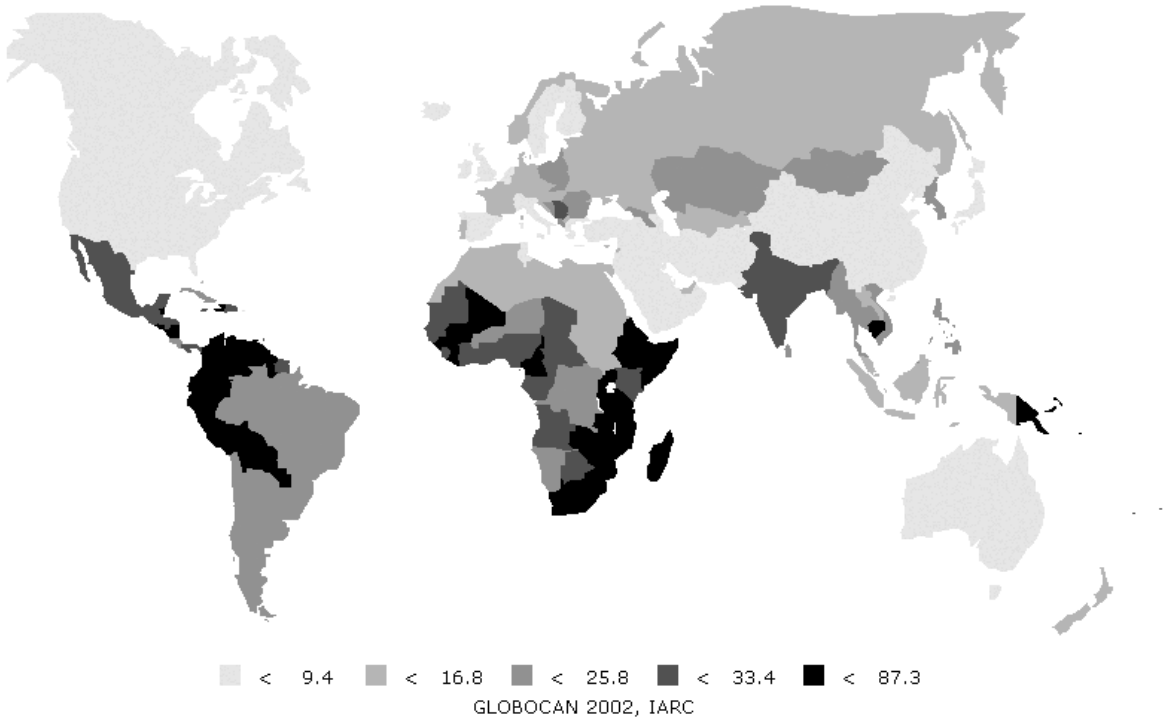
Gibson AG. The Physician's Art. Oxford: The Clarendon Press, 1933

General Introduction

1.1 Cervical carcinoma

Cervical carcinoma is an important health problem throughout the world. Each year an estimated 493,000 new cases are diagnosed of which more than 80 % occur in the developing countries. Consequently, cervical carcinoma is the second most common cancer among women globally [1,2] . Moreover, in many regions in developing countries it is the leading cancer, accounting for 15 % of the female cancers with a cumulative risk of 1.5 %. Eastern and South Africa, Melanesia, Latin America and the Caribbean show the highest incidence rates with incidence rates up to 43 per 100,000 (figure 1) [3]. The incidence in developed countries is much lower with age-standardized rates less than 14.5 per 100,000 women. However, this low incidence has been accomplished only after the introduction of screening programs in the 1960s and 1970s [4-11], resulting in substantial declines in cervical cancer incidence. Before this time, the incidence in Western countries was similar to developing countries today [2].

Figure 1: Incidence of cervical cancer worldwide, Globocan IARC 2002 [3]

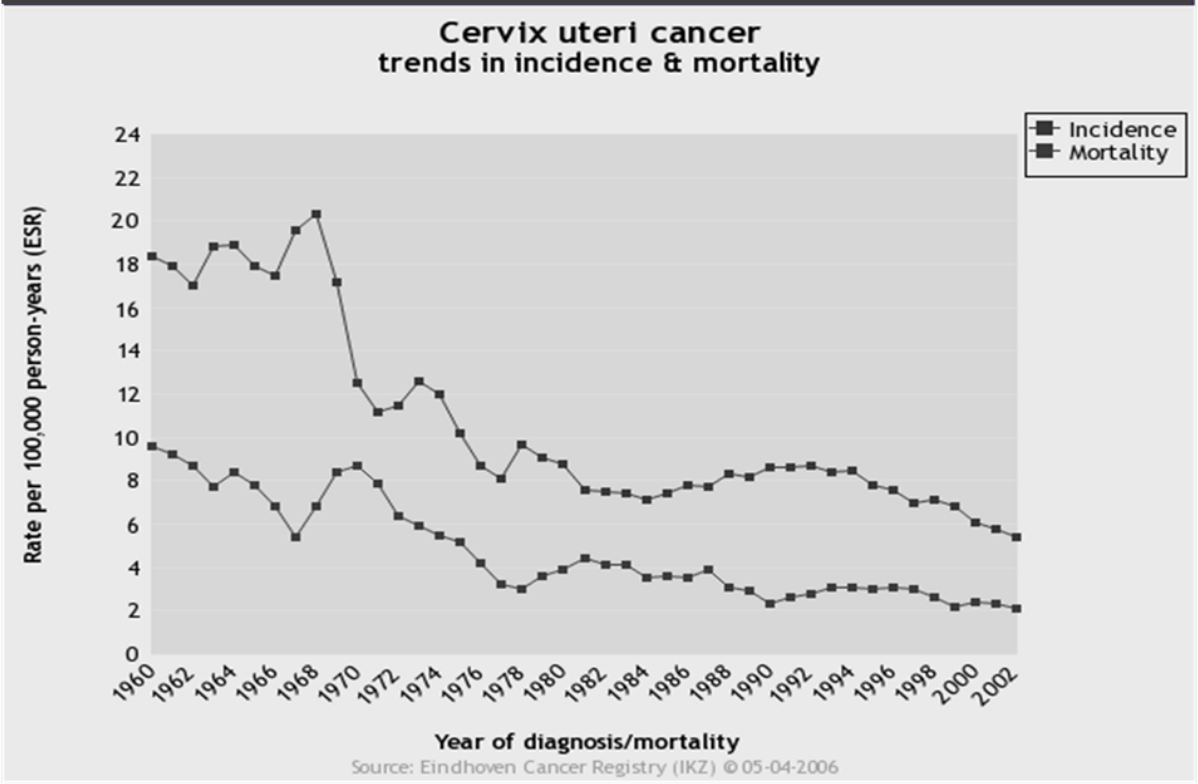


Major geographical variations in incidence are observed not only in developing areas of the world and between developed and developing countries, but also within Europe. In the 1990s, cervical cancer incidence varied by over factor 3, with the highest incidence rates in Poland and the former DDR (over 20 per 100,000) and the lowest rates in Italy, Switzerland, Spain and The Netherlands (less than 7 per 100,000) [12,13].

The mortality rate of cervical cancer is substantially lower as compared to the incidence. Worldwide, the mortality to incidence rate ratio varies between 0.47 – 0.55. Though survival rates differ between regions, even in developing countries the survival is rather good [2]. Nevertheless, an estimated 274,000 women die every year of cervical cancer, globally.

In The Netherlands, the incidence of and mortality from cervical cancer is among the lowest in Europe [11,13]. Cervical screening programs were gradually introduced in the early 1970s. However, it was not until 1988 that nationwide screening was performed. As shown in figure 2, the incidence and mortality started to decrease in the late 1960s and early 1970s [14]. This decrease continued in the following years and is attributed for a large part to treatment of screen-detected premalignant cervical lesions.

Figure 2: Trends in incidence and mortality in The Netherlands



The majority of cervical cancers are squamous cell carcinomas. However, the proportion of adenocarcinomas is generally higher in low incidence areas. In some western countries

adenocarcinoma even contribute up to 25% of cervical cancers. This is a consequence of cervical screening which is successful in the reduction of squamous cell carcinomas but has only marginal effect on adenocarcinomas. This has been explained by the localization of most cervical adenocarcinomas high in the endocervical canal, which is not easily sampled. In several countries an increased frequency of endocervical adenocarcinomas is found, especially in women of 35 years and younger [15-17].

1.2 Etiology of cervical cancer

Since the late 1970s, epidemiological studies recognized cervical cancer as being suggestive of a sexually transmitted process. A causal role was imputed to several infectious organisms, among which syphilis, gonorrhea and type 2 herpes simplex virus (HSV-2) [18]. However, during the 1990s strong evidence was found on the role of HPV in the carcinogenesis of cervical cancer, as already pointed out by Zur Hausen in the late 1970s [19]. HPV is a double-stranded 8 kb large DNA virus. Over 130 different genotypes of HPV have been identified of which 18 are associated with cervical carcinogenesis. These so called high-risk HPV (hrHPV) types include HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82. Infections with high-risk HPV is a necessary cause in the development of (pre-)cancerous cervical lesions. Cervical cancer is considered as a rare consequence of an infection with hrHPV [18]. HrHPV DNA can be found in virtually all cervical carcinomas, were HPV types 16, 18, 31 and 45 are the most common types [1,18,20-22]. Low-risk HPV (lrHVP) types such as HPV 6 and HPV 11 are associated with benign genital warts (condylomata accuminata).

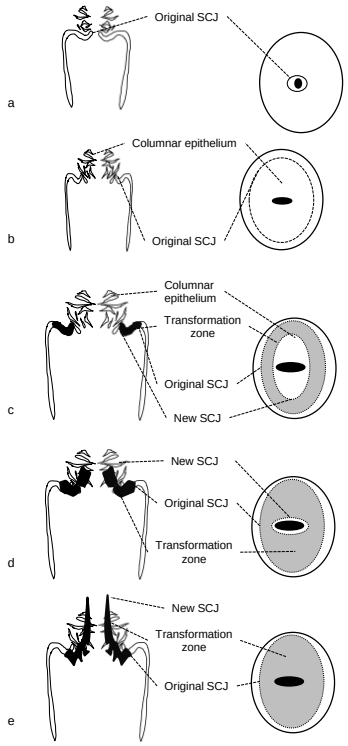
HPV infection is a very common sexually transmitted disease with a life time risk estimated to be up to 80% [18,23,24]. Estimates of the prevalence of HPV infections range from 2 % to 44% depending on the age range of the population studied and the sensitivity of the DNA assay used [25]. The cross-sectional prevalence of HPV is highest among young women and decreases spontaneously to a background level of 2 % to 8 % in most populations in women aged 35 years and above [1]. Risk factors for HPV infection are the lifetime number of sexual partners, promiscuity (also of the male partners), age at first intercourse, male circumcision status, smoking and oral contraceptive use [1,25].

Most HPV infections are transient and 80 % has been cleared after 12 to 18 months. However, some infections can become latent or persistent and these women are at increased risk for HPV associated disease. Persistence of HPV infection is among others associated with host immune factors [25,26].

1.3 Natural history of cervical cancer

Until recently it was thought that de natural history of a HPV-infection towards cervical carcinoma occurred via a continuum of long-lasting, consecutive stages of premalignant cervical intraepithelial lesions: CIN 1, CIN 2 and CIN 3. Currently an alternative concept is increasingly supported, in which clinically relevant high-grade lesions as CIN 2 and CIN 3 are induced rapidly within 2 to 3 years after infection with hrHPV. This first stage is then followed by a second stage of 10 to 12 years during which invasive cervical cancer develops [21]. Low-grade (CIN 1) lesions are generally more associated with transient, productive viral infections, whereas high-grade lesions (CIN 2 and CIN 3) occur when replicating immature, basal stem cells are infected with hrHPV subtypes which leads to continued replication of immature cells and accumulation of genetic abnormalities and instability [25]. Most low-grade lesions should therefore not be considered as a true precursor lesion of cervical cancer, but rather as a cytopathological effect of productive viral infection which is reflected in a high regression rate of these lesions [26-28]. High-grade lesions on the other hand will, when left untreated, ultimately progress to cancer in one-third to half of the cases [25,29]. The area of the squamocolumnar junction (SCJ), which is the border between ectocervical squamous and endocervical columnar epithelium, is especially susceptible for HVP-induced infection and transformation (Figure 3). Consequently most cervical lesions originate in this so called transformation zone. This is the area where the original columnar endocervical epithelium has been replaced by squamous epithelium via proliferation of reserve cells, a process termed squamous metaplasia. Primary HPV infections targeting proliferating basal cells of the squamous epithelium and multi-potent stem cells of the SCJ can result in viral persistence and CIN development whereas primary infection of differentiated, more superficial cells give rise to transient, productive viral infections, where the viral DNA will be lost with the shedding of infected cells and virions during the terminal differentiation process [24,,30-32]. In infected proliferating basal cells, HPV gene products such as E6 and E7 are responsible for deregulation of normal cell cycle mechanisms, ultimately leading to genomic instability and hyperproliferation. In benign and low-grade cervical

Figure 3: Squamocolumnar junction and transformation zone



lesions, the HPV genome is generally present in an episomal state. Progression to more severe CIN is associated with integration of the HPV genome in the genome of the host cell [33]. However it is still unclear whether integration represents the cause or just the consequence of genomic instability, leading to malignant transformation of CIN [21,24,30-34].

1.4 Terminology and classification of cervical lesions

The concept of precursor lesion of cervical carcinoma dates back to the nineteenth century, where Williams (1888) found areas of non-invasive epithelial changes adjacent to invasive cervical carcinoma [35]. Carcinoma in situ (CIS) was introduced as an entity by Broders in 1932 [36]. CIS was defined as undifferentiated carcinomatous cells, involving the full thickness of the cervical epithelium, however without disruption of the basal membrane.

One decade later, Papanicolaou and Traut [37] found that exfoliated cells in vaginal smears could be used for the diagnosis of CIS and cervical carcinoma in 1941 and introduced the cytological PAP classification system. The term dysplasia was introduced by Reagan in the 1950s as an intermediate between normal epithelium and CIS [38]. Dysplasia was categorized in three groups - mild, moderate and severe - which form a continuum with CIS and cervical carcinoma. Subsequently, Richart introduced the concept of cervical intraepithelial carcinoma (CIN) in the late 1960s [39]. CIN was subdivided into grades 1,2 and 3, where CIN 1 corresponds to mild dysplasia, CIN 2 to moderate dysplasia and CIN 3 to severe dysplasia and CIS.

Simultaneously, the National Cancer Institute organized a workshop in Bethesda, USA, on reporting of cervical cytology results. The cytological reporting system resulting from this workshop became known as the 1988 Bethesda system (TBS) [40]. The system was revised in 1991 and 2001 [41,42]. The introduction of the term squamous intraepithelial lesion (SIL) was the most important feature of TBS. It uses a two-tiered classification scheme consisting of LSIL (low-grade squamous intraepithelial lesion) and HSIL (high-grade squamous intraepithelial lesion). Condylomatous changes are categorized with low-grade CIN into LSIL. HSIL includes CIN 2 and CIN 3. Furthermore, the terms atypical squamous cells of undetermined significance (ASCUS) and atypical glandular cells of undetermined significance (AGUS) were introduced. These categories were replaced in TBS 2001 by ASC-US (atypical squamous cells of undetermined significance), ASC-H (atypical squamous cells cannot exclude HSIL) and AGC (atypical glandular cells).

Table 1: Histological and cytological terminology and classification systems (adapted from Bibbo [43])

Histology		Cytology		
Dysplasia terminology (WHO)	**CIN terminology (Richert)**	**Papanicolaou (PAP)**	**Bethesda 2001 (TBS 2001)**	**KOPACB (Netherlands)**
Normal	Normal	1	Negative for intraepithelial lesion or malignancy	P1/A1-2/C1-2
Atypia	Koilocytic atypia	2	ASC-US	P2-3/A3
			ASC-H	P2-3/A3
			AGC	A3/C3
Mild dysplasia	CIN 1	3a	LSIL	P4/A4/C4
Moderate dysplasia	CIN 2		HSIL	P5/A5/C5
Severe dysplasia	CIN 3	3b		P6/A6/C6
Carcinoma in situ		4		P7/C7
Invasive carcinoma	Invasive carcinoma	5	Carcinoma	P8-9/A7-8/C9

In the Netherlands the KOPACB classification system is in use nationally since 1996 [44,45]. This cytological system classifies the composition of the smear (K), inflammation (O), squamous abnormalities (P), other and endometrial abnormalities (A) and cylindrical abnormalities (C).

The adequacy is scored in the three tiered category B. KOPACB is more detailled than the PAP classification system but can be easily translated into PAP or Bethesda classification.

The British Society for Clinical Cytology (BSCC) adopted an alternative terminology in 1986. This BSCC terminology was revised in 2002. An overview of the various cytological and histological terminology is provided in table 1. Invasive cervical tumours are divided in three general categories: squamous, glandular and 'other' epithelial tumours.

1.5 Methods for screening and diagnosis of CIN and cervical cancer

There are several techniques for diagnosing CIN and cervical cancer. The method of choice is depending on the availability of resources and qualified and trained personnel but also on the test accuracy and expected follow-up compliance.

1.5.1 Visual inspection (VI, VIA, VILI, VIAM)

Visual inspection (VI) of the uterine cervix is used most often in low-resource settings. This technique of looking at the uterine cervix with the naked eye can be performed unaided by simple clinical examination of the cervix with a speculum and light source. The sensitivity of VI is too low for use as a primary screening test [49]. During the last decade a combination of visual inspection after application of diluted (3 % – 5 %) acetic acid to the cervix with a cotton swab is frequently used (VIA or Visual Inspection with Acetic acid). Test positivity is based on the appearance of acetowhite areas in or near the transformation zone. Sensitivity of VIA has been reported similar to cervical cytology, but with a lower specificity [50]. Visual inspection using Lugol's iodine (VILI) is a technique originally developed by Schiller in 1933. It was recognized that non-staining areas after application of Lugol's iodine were easier to interpret than acetowhite areas. In the only published report on performance of VILI [49] the sensitivity of VILI was higher than VIA with an equal specificity. The use of a low-level magnification (2-4x) for inspection of the cervix after application of acetic acid is called VIAM (visual inspection using acetic acid with low-level magnification). No differences in test performance has been found between VIAM and VIA [51].

1.5.2 Cytology

The concept of cytologic examination of exfoliated cervical cells was introduced by George Papanicoloau in the 1940s [52]. The Pap test is the most widely used cancer screening test in developed countries [53]. The test involves microscopic examination of fixed and stained

cervical cells which are scraped from the surface of the cervical transformation zone by special sampling devices. The cells are subsequently examined for abnormal morphologic cell changes.

A critical step in the preparation of a cytological specimen is proper sample collection, as one half to two thirds of false negative cervical cytology has been attributed to sampling errors [54]. Therefore, dedicated sampling devices such as the wooden or plastic spatula, with or without an extended tip and the Rovers® Cervex-Brush® have been designed. The Cytobrush is a sampling device, focusing on direct sampling of the endocervical canal. Recently, collection devices have been developed that sample the ecto- and endocervix simultaneously, such as the Rovers® Cervex-Brush® Combi. In order to prevent sampling errors, it is of utmost importance to obtain a sample of the complete transformation zone, since this is the region where the majority of CIN lesions occur.

Screening of a cervical smear is a complex procedure which is prone to screening and interpretation errors. These errors are highly correlated with the quality of the smear. The quality of a smear depends on adequate sampling but also on the presence of interpretation obscuring elements. Sampling adequacy is described in terms of cellularity as well as the presence of transformation zone indicators (endocervical or metaplastic cells) whereas obscuring elements concern excess of blood or inflammatory cells or bad fixation of the cells. Judgement of the quality of the slide is an important part of the diagnostic report.

1.5.2.1 Conventional cytology

In conventional cytology, the cervical cells are smeared onto a glass slide immediately after sampling. Cell fixation must be performed within a few seconds to prevent air-drying artifacts which hampers the cytological interpretation seriously and can lead to false diagnoses. Studies have shown that more than half of the sampled material remains on the sampling device, which is discarded and lost for analysis [55].
Nevertheless, though never proven in randomized clinical trials, there is convincing evidence from observational studies that screening with conventional cytology is effective in reducing the incidence of squamous cervical cancer [1,56]. However, the accuracy of conventional cervical cytology has been questioned the last decades. Accuracy of a test is defined by two aspects. One is the test specificity and the other the test sensitivity for detecting a given condition. Several meta-analyses showed that the cross-sectional test-validity (test-validity of a single test at one point in time to detect a defined end-point) of conventional cytology for CIN is rather limited [57-60]. The test sensitivity and specificity of conventional cytology is not

known precisely but estimates range from 50 % – 85 % and 60 % – 98 % respectively, depending on characteristics of the study group and study design [1,61].

1.5.2.2 Liquid-based cytology

Liquid-based cytology (LBC) is a new preparation technique that was introduced in the mid-1990s to improve the performance of the Pap test. The main difference with conventional cytology is the technique for transferring the exfoliated cervical cells from the sampling device to the microscope slide. The cells are not spread onto a glass slide by the sample taker as with conventional cytology but immersed into a vial by thoroughly rinsing the sampling device within a special liquid preservation fluid. The vial is transported to a specially equipped laboratory, where the microscopic slide is prepared. The result is that almost all sampled cells are transferred into the liquid. Next, a representative sample is transferred to a well defined circular area containing a limited number of cells which are evenly spread in a thin-layer. Though the various LBC systems differ in preparation technique, they all result in a thin, monolayer-like sample of well-preserved cells without obscuring blood, mucus and inflammatory cells. Advantages of the LBC technique are the availability of residual material for preparation of multiple smears or additional molecular testing, being a more proper target for automated screening and a possible increased detection of cervical lesions. Worldwide, various LBC systems are used. Most widely used systems are the ThinPrep system (Hologic Corporation, Marlborough, MA) and the AutoCytePrep system (currently known as SurePath; TriPath Imaging, Burlington, NC) [43].

The comparative performance of LBC and conventional cytology has been evaluated in numerous studies. Although there is reasonable agreement that LBC improves specimen adequacy and reduces screening time as compared to conventional cytology, controversy remains on the diagnostic performance of LBC, largely due to a lack of adequately designed studies [1,62-64].

1.5.3 Computer-assisted screening

Computer-assisted screening specifically aims on increasing the sensitivity of the Pap test by reducing false-negative results due to screening errors and better productivity by decreased screening time. This is thought to result in a reduction of the costs of screening programs and ultimately in a decreased incidence and mortality of cervical cancer. Automated screening devices help cytotechnologists to focus on abnormal cells on the slide, thus facilitating a correct diagnosis. On the other hand it should increase productivity by excluding normal

parts of the slides from manual screening by selecting only the most abnormal cells for interpretation by the cytotechnologist.

Computer-assisted screening is thought to perform at least as well as conventional screening and may be valuable in a sub-optimal screening environment by improving the sensitivity. On the other hand it may have no advantage in a high-quality setting other than a higher productivity [1,53,61]. First studies evaluating the ThinPrep Imager show an increased detection of computer-assisted screening as compared with conventional cytology [65].

1.5.4 Colposcopy

Colposcopy is a procedure, first described by Hinselmann in 1925, that allows observation of the uterine cervix under illumination and magnification (6x – 40x) after the application of normal saline for removing excess cervical mucous, 3% – 5% acetic acid and Lugol's iodine respectively. The cervical epithelium is examined for acetowhiteness, abnormal blood vessels and iodine uptake, identifying (pre-)malignant disease of the cervix.

Meta-analytical examination of 9 studies showed that the estimated sensitivity and specificity of colposcopy for detection of CIN 2+ was 96 % and 48 % respectively [62,66]. Colposcopy is not recommended for screening, because of its low specificity. However, colposcopy is an essential triage method for the management of women with abnormal cytology [61].

1.5.5 HPV testing

Recognition of the causal role of HPV infection in the development of cervical cancer has led to the development of several HPV testing systems. Most are based on hrHPV DNA testing. Hybrid Capture 2 (HC2; Digene Corp., Gaithersburg, Maryland, USA) is a commercially available test for the detection of 13 hrHVP types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) that is approved by the FDA for cervical cancer screening in combination with cytology for women over 30 years of age. Most other HPV tests are generally PCR tests such as the GP5+/6+ assay, PGMY09/11, SPF10 and Amplicor Human Papillomavirus Test (Roche Molecular Diagnostics). HPV-genotyping can be performed after PCR amplification with SPF10 or PGMY09-11 using for instance LiPA HPV typing systems. Furthermore there are the DNA micro-array detection system (Biomedlab Company, Seoul, South-Korea) and a viral mRNA detection systems such as NASBA and a commercially available test kit (PreTect HPV-Proover, NorChip AS, Kokkastua, Norway) [61]. The applicability of the different tests for use in clinical practice rather varies. This is because analytical sensitivity (minimum amount of DNA detectable) should be distinguished from clinical sensitivity (ability to detect clinical relevant

lesions). There is strong evidence that a high clinical sensitivity and specificity is not directly synonymous with presence or absence of hrHPV in a cervical sample. When the analytical sensitivity is too high this will result in decreased clinical specificity, since a low viral load is not associated with increased risk for CIN. When considering HPV tests for screening, a distinction between clinical relevant and irrelevant hrHPV infections should be made [67,68].

Roughly three applicable strategies for HPV testing in cervical cancer prevention can be defined. Firstly, it can be used as primary screening test, alone or in combination with a Pap smear, for the detection of cervical cancer precursor lesions. In general the sensitivity of HPV screening is higher as compared to cytology but at the same time it is significantly less specific [63,69]. Combining HPV testing with cytology results in only slight improvement of sensitivity but at the expense of further loss in specificity. Several strategies are suggested to improve the specificity of HPV testing for primary screening. One of them is restriction of HPV screening to age-groups of 35 years or older [60]. Another is simply repeating the HPV test to identify persistent infections [61]. Beside that, HPV viral load [70-74], HPV genotyping [75] and cytology triage [73,76] have been proposed. Finally, the use of molecular markers such as mRNA testing for E6 or E7 oncogenes [77,78], immunostaining of overexpressed cell-cycle regulating proteins (e.g. p16) are promising methods for triage of HPV-positive women at increased risk for progressive CIN and cervical cancer [79]. The higher sensitivity of primary HPV screening as compared to cytological screening is a clear advantage. However, several potential disadvantages have been put forward. First there is the high unit cost and lower specificity of the HPV test as compared to cytology-based screening. This might result in unfavorable cost-efficacy since a relatively small decrease in specificity can have dramatic consequences on costs [61]. Besides that, there are some major negative psychosocial aspects of HPV testing. Public awareness on HPV and its role in cervical cancer is limited. Confusion and anxiety about the association with sexually transmitted infections as well as issues of fidelity and trust in relationships after communicating a positive HPV test results is common and may compromise screening participation [80]. On the other hand, HPV testing is suitable for self-sampling and has been propose as an alternative to cell collection by a clinician. Self-sampling is especially appealing for women who are reluctant to attend cervical screening for social or religious reasons and it is likely to improve compliance in these populations.

Another strategy for HPV detection in cervical cancer screening is the use of HPV testing for triage of women with equivocal (ASCUS) or LSIL cytological results. This strategy is allowed in follow-up of low-grade abnormalities in the Netherlands recently. Liquid-based cytology is especially suitable for this triage purpose since HPV testing can be easily performed on the residual material, avoiding a second visit for a HPV triage test. ASCUS triage using HC2 is significantly more sensitive for the detection of HSIL as compared to repeat cytology and

equally specific. HPV triage of LSIL lacks specificity and is not considered a useful management option in these cases. Up to now repeat cytology is considered still the best triage management option for LSIL [81,82]. Triage with other potential candidate molecular surrogate progression markers, such as cell cycle regulation proteins, viral integration markers and viral mRNA are under evaluation.

Finally, there is a potential role for HPV testing in the follow-up after treatment of CIN. This so called post-treatment testing procedure is based on the assumption that HPV DNA is commonly cleared after effective treatment of CIN. Persistence of HPV DNA is related with residual of recurrent CIN lesions and thus with treatment failure. Post-treatment HPV testing has been found a more sensitive and more rapid procedure for detection of residual or recurrent CIN during follow-up as compared to follow-up cytology with equal specificity [82].

1.5.6 Molecular markers

Several molecular markers are proposed for triage purposes and may potentially predict progressive behavior. However, research has been restricted to correlation studies so far and is not representative for applications in actual screening settings. Two widely used oncoproteins are Ki-67 and $p16^{INK4a}$. The proteins are up-regulated in dysplastic cervical epithelial cells through the hrHPV infection. Ki-67 (recognized by the monoclonal antibody Mib1) is expressed in dividing or proliferating cells. Normally, Mib1 positivity is only found in basal or suprabasal cell-layers. In dysplastic epithelium Mib1-positive cells are found throughout the whole thickness of the abnormal epithelium. $p16^{INK4a}$ overexpression is, just as overexpression of Mib1 considered to be a sensitive marker for CIN [43]. Potential applications are in the field of triage of women with minor or low-grade abnormalities, improving the accuracy and reproducibility of histological verification and prognostic prediction purposes.

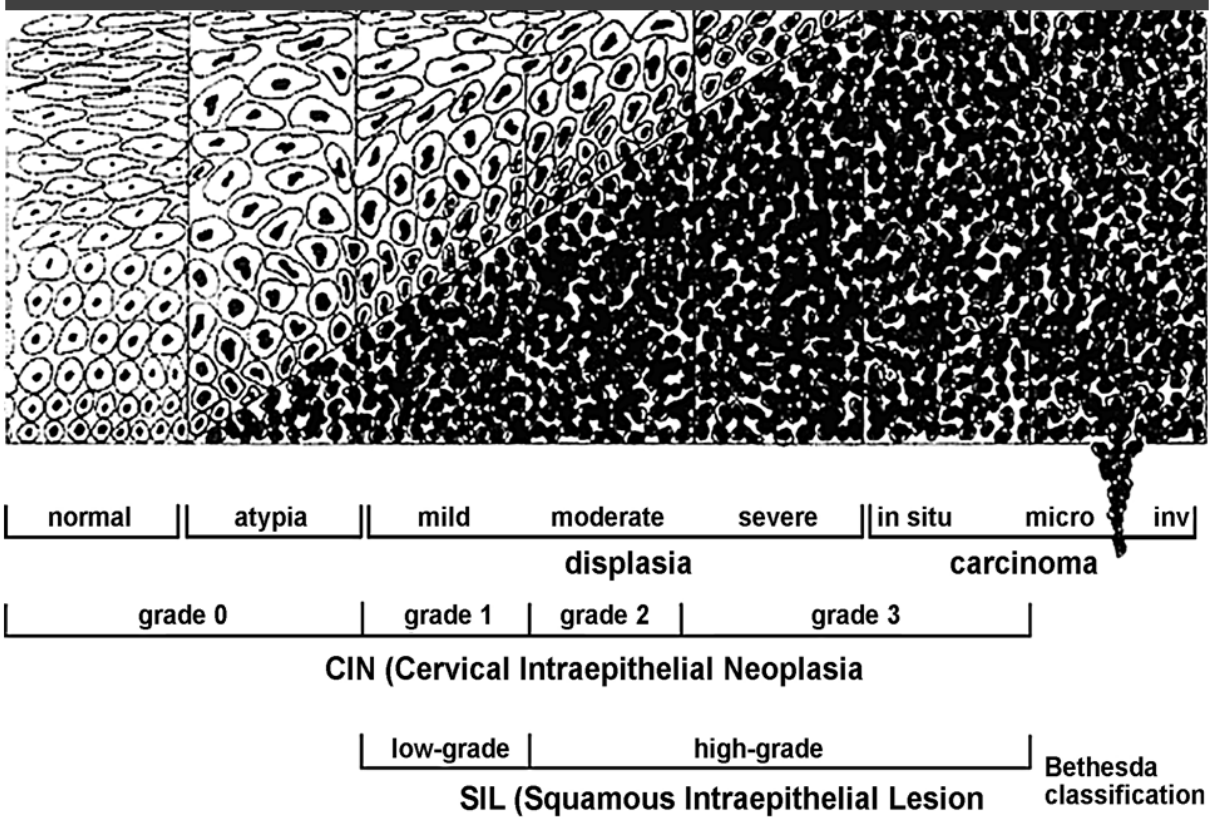
1.6 Histological diagnosis and treatment of cervical (pre) invasive disease

Women with abnormal findings on cytology or HPV testing are referred to the gynaecologist for colposcopic evaluation. The referral criteria vary from country to country. Colposcopy is a procedure where the uterine cervix is viewed with a stereoscopic microscope with a magnification between 6 and 40 times. After application of 3% acetic acid and Lugol's iodine (Schiller test) the transformation zone is visualized and inspected for abnormalities. When such abnormal areas are found, they are graded according to morphological features, such as acetowhiteness, margins, blood vessels and iodine uptake [1]. The role of

colposcopy is to identify the most abnormal area's for histological confirmation with a colposcopy directed biopsy or direct treatment.

In histology, intraepithelial squamous lesions are characterized by an abnormal cellular proliferation and maturation together with nuclear atypia. The morphologic epithelial alterations are classified in three categories: CIN 1 to CIN 3, which are mutually prone to a high intra- and interobserver variability [46]. In CIN 1 atypical basaloid cells and mitotic figures are found in the lower third part of the epithelium. HPV-induced cellular effects, such as koilocytotic changes are frequently found in these lesions. In CIN 2 lesions, atypical basaloid cells and mitotic figures occupy the lower two thirds of the epithelium. CIN 3 lesions show neoplastic changes or dysplastic cells and mitoses in two thirds to full thickness of the abnormal epithelium. The nuclear-to-cytoplasmatic ratio of the cells is increased substantially and the abnormal nuclei contain dense, hyperchromatic coarse chromatine. A schematic representation of the morphological changes in the consecutive CIN lesions is provided in figure 4. Invasive cervical epithelial tumours are subdivided in three categories: squamous, glandular and 'other' epithelial tumours.

Figure 4: Schematic representation of morphologic changes in CIN [24]



In general there are two techniques available for the treatment of CIN: destructive and excisional techniques. Destructive treatment can be only applied when pre-treatment biopsy has been performed and invasive cervical cancer has been ruled out. These techniques include CO_2-vaporation, cryotherapy, electro-cauterization and cold coagulation. Excisional techniques, in general followed by histological examination includes CO_2 laser excision, cold knife technique (including cone biopsy), Loop Electrosurgical Excision Procedure of the Transformation Zone (LEEP), Large Loop Excision of the Transformation Zone (LLETZ) and occasionally hysterectomy. LLETZ produces the least morbidity and the most adequate specimen for histological examination [47]. More than 90 % of the women are cured after this procedure [48].

The treatment of invasive carcinoma depends on the stage of the disease. For micro-invasive carcinoma (stage IA1) a cone biopsy may be considered as therapeutic procedure, whereas hysterectomy is the recommended treatment for higher stages of disease. In some cases of stage IA2 and IB1 disease, when preservation of fertility is desired, a large cone biopsy or trachelectomie with dissection of the lymph-nodes may be applied.

1.7 Population based screening programs

1.7.1 Principles of screening

A first definition of screening was formulated by the US Commission on Chronic Illness in 1957 where screening was described as 'the presumptive identification of unrecognized disease or defect by the application of tests, examinations or other procedures, which can be applied rapidly. Screening tests sort out apparently well persons who apparently have a disease from those who do not' [83]. Most recently the UK National Screening Committee (re)defined screening in the second NSC report (2000) as 'a public health service in which members of a defined population, who do not necessarily perceive that they are at risk of, or are already affected by, a disease or its complications, are asked a question or offered a test to identify those individuals who are more likely to be helped than harmed by further tests or treatment to reduce the risk of disease or its complications' [84].

The benefits of screening within the scope of disease prevention were recognized first in the 1940s, at the time when effective treatment for tuberculosis was introduced. The application of screening in secondary prevention of other diseases such as cancer emerged in the following years and led to the landmark publication of a World Health Organization Monograph of Wilson and Jungner in 1968 [86]. They prescribed a number of basic criteria to be

fulfilled before screening is introduced. These classic screening criteria are summarized in table 2. Cochrane and Holland [86] formulated some additional criteria concerning the test to be used within a screening setting. In order not to compromise the participation in the screening program, a screening test must be acceptable to the population screened, simple to perform and relatively cheap. Furthermore, it must be easy to interpret and have a good accuracy. Both the sensitivity of the test for finding a condition as well as the specificity for giving a negative result when the condition is absent should be as high as possible.

Table 2: Classic screening criteria Wilson and Jungner [86]

1. Important health problem
2. Accepted treatment for patients with disease leading to better prognosis
3. Facilities for diagnosis and treatment available
4. Recognizable latent or early symptomatic stage
5. Suitable diagnostic test available
6. Test is acceptable to population
7. Natural history of the condition, including development from latent to declared disease adequately understood
8. Agreement whom to treat
9. Cost of case-finding in relation to total costs of medical care
10. Case-finding is a continuous process

1.7.2 Cervical cancer screening

In general, the goal of cancer screening as a secondary prevention measure is to reduce the extent of treatment for and mortality of cancer [85]. If cancer screening focuses on the detection of (premalignant) precursor lesions, successful screening should be followed by a reduction in cancer incidence. When cancer screening is undertaken this should be performed only in an organized setting with quality assurance at all levels of the screening program, continuous monitoring of effects and knowledge of the benefits and disadvantages of the screening [85,88]. Benefits of cancer screening will be achieved only when compliance is high. Opportunistic screening activities may not achieve the benefits but rather cause unnecessary negative side-effects and should therefore be avoided. The effectiveness of

cancer screening depends on the sensitivity of the screening test, participation rate of the invited, frequency of screening, number of screening tests offered during a lifetime, compliance with follow-up and effectiveness of early treatment [88]. Negative side-effects mostly relate to sensitivity and specificity issues of the test and on side-effects of early treatment. Low sensitivity will give rise to false reassurance for those with a false-negative test result. Low specificity on the other hand will give rise to high numbers of false-positives cases, resulting in overtreatment, unnecessary anxiety and costs. Unfortunately, screening tests, even with all the safeguards applied, never will be perfectly accurate and will always be prone to a certain level of human and technical error. Even with the most extensive quality assurance mechanisms applied, errors will still occur. Accordingly, as also acknowledged in the NSC – Second Report 2000 [84], screening can harm as well as help.

Although the effectiveness of cervical cancer screening with cytology has never been proven by randomized controlled trials, it is generally accepted that it has reduced the incidence and mortality from cervical carcinoma in developed countries where cervical cancer screening programs have been implemented. Reductions of cervical cancer deaths of more than 70 % have been reported. Despite this success of the Pap test in decreasing the rate of cervical carcinoma in these countries, the public expectation of 100 % effectiveness has never been achieved in any screened population. Despite the introduction of various policies and new technologies to reduce the number of cervical cancer deaths even more, programs in western-Europe seem to have reached an irreducible minimum of cervical carcinomas. This is mainly caused by failures within the cervical screening programs. These include the failure of women to participate in screening at the recommended frequency or to participate at all, failures in smear taking, smear handling, screening and/or interpretation of abnormal cells, reporting failures, failures in follow-up compliance after cytological abnormalities and failures in treatment and follow-up after treatment [89]. All these issues have a more or less negative impact on the program sensitivity and thus on the effectiveness of cervical cancer screening. Several studies have shown that participation in screening has been suboptimal in more than half of the cases of cervical carcinoma [90-94] and that more than 50% of the cervical carcinomas occur in the 5% - 15% of women who have never been screened. Thus, compliance is an essential factor to the success of the cervical screening program and increasing the compliance is the best and most simple way of improving the effectiveness of cervical screening. Organized screening programs are considered to reach higher coverage rates as compared to opportunistic screening and are therefore more effective. As mentioned above, other reasons for failure of screening are inadequate sampling and/or erroneous smear interpretation. Though there have been increasingly adverse press reports focusing on these issues, these errors represent only 12 % - 23 % of the failures and are less important than lack of compliance. Nevertheless, it is clear that the

sensitivity of the Pap test is limited. This limited sensitivity is ascribed to either smear taking (sampling errors) or smear reading or laboratory errors (screening or interpretation error). Approximately two-third of the false negative results are due to inadequate sampling, whereas one-third is caused by screening or interpretation errors [93,94]. Even though the estimated sensitivity of the Pap-test is only very moderate and ranges from 30 % to 87 % [96], it is essential to appreciate that through the repetitive nature of the screening even a moderately sensitive test, such as the Pap test, can be able to reduce the incidence and mortality from cervical cancer to a low residual level [61,97]. Despite the limited role of sampling and screening errors in program failures, increasing attention is given towards new technologies, potentially reducing false-negative results in women who are screened.

While there is ample evidence supporting the advantages of cervical cancer screening, there are also negative consequences and potential harms of screening large numbers of healthy women in order to prevent significant disease in only a few. Firstly, the gynaecological examination and smear taking is experienced as uncomfortable by many women. Secondly, there is the psychological impact of a positive screening result. Approximately 5 % of all smears made in a cervical screening program are considered abnormal or need to be repeated for other reasons. However, in a considerable number of cases this concerns equivocal or low-grade abnormalities with very high regression rates, which often would have been left undetected without screening. Besides that, in many other cases it concerns real false-positive results. The psychological impact of a positive test, even when the result is only slightly abnormal, is considerable. It results in anxiety and fear among women which should not be underestimated. For many, the concept of 'pre-cancerous' lesion is equivalent with a real risk of having cancer which in its turn is associated with death. A majority of women referred for colposcopy after a positive screening result described feeling 'worried or alarmed' or even 'shocked, stunned and devastated' [98]. False-positive screening results lead to unnecessary interventions, with both human as well as financial costs. Overtreatment of false-positive cases and regressive premalignant lesions are considered major adverse effects of cervical cancer screening. Various complications after treatment have been reported. This included bleeding, infection, pain, cervical stenosis and cervical incompetence. Cervical incompetence has been associated with pre-term delivery [99]. The problem of false-positive results are related to the specificity of the screening test. In screening situations where the prevalence of disease is low, a small decrease in specificity will have dramatic consequences both on financial and human costs. Finally, in contrast to the impact of false-positive screening results there is the misunderstanding that a negative test implies no risk, rather than a low risk for cervical cancer giving in its turn, rise to false reassurance and possibly underestimation and -investigation of alarming clinical symptoms.

A prerequisite for an effective and efficiently working cervical screening program is that optimal performing screening tests are used but also that optimal follow-up protocols are defined and monitored.

1.7.3 Organization of cervical cancer screening

Cervical cancer screening programs can be either opportunistic or organized. In opportunistic screening programs there is no central data collection and participation depends on self-motivation. Screening intervals are accordingly variable. Screening programs in the USA, France, Germany and the Southern European countries are more or less opportunistic. Components of an effectively and well-organized screening program are the central coordination and data collection, a defined target population and defined screening interval. Women receive a personal invitation to participate and are re-invited in case of non-attendance (call/recall system) [95]. Furthermore clear and preferably nationally defined and monitored procedures for follow-up, after an evidence-based policy for follow-up has been established, are essential. Evaluation, audit and quality control and assurance addressing every stage of the screening process should be an integral part of organized high-quality screening program [85,100]. Organized screening also implies 'scientific analysis of outcome of the screening and quick reporting of these results to the population and screen providers' [101]. Organized cervical screening has been implemented among others in the UK, British Columbia, Canada, the Nordic countries and in the Netherlands [95]. The screening strategies vary across the different countries in terms of screening interval and the age-range of women which are invited. It is generally recognized that coverage – i.e., the participation rate of the invited population – is essential to the success of cervical screening. Coverage is generally higher in well-organized screening programs [85,95]. Moreover, adequate follow-up is an important feature of an efficient cervical screening program which is obviously better ensured in well-organized settings through fail-safe methods. Besides that, guidelines for quality assurance are applied more easily in an organized setting.

In the Netherlands, a pilot project for cervical screening started in the region of Utrecht in the early 70s of the last century. This pilot for systematic cervical screening was extended to 3 other regions (Utrecht, Nijmegen and Rotterdam) in 1976. Women aged 35 to 54 were invited centrally every 3 years. Based on the experiences in the pilot project, a nation-wide organized screening program was implemented in 1989. However, in the first half of the 90s it was recognized that the participation rate was very low (40 %-50 %). Also the follow-up compliance was unsatisfactory and follow-up policy varied due to a lack of clear-cut, nationally defined guidelines. As the existing program was considered ineffective, it was

reorganized in 1996. Changes focused on organization and the target population was extended to women aged 30 to 60 years with a 5-years screening interval. Criteria for minor cytologic abnormalities were also revised as well as repeat and referral policies. This resulted in a national guideline on cervical screening [102].

The development of new techniques within the field of cervical cancer screening, such as liquid-based cytology and automated screening at the beginning of this century , led to the question whether these techniques would be applicable within cervical screening and have potential in improving the Netherlands cervical screening program. Therefore a systematic, evidence-based review on these emerging techniques was performed and published in 2002 under the aegis of the 'Kwaliteitsinstituut voor de Gezondheidszorg CBO' and initiated by the Netherlands Pathology Society (NVVP) [103]. One of the conclusions of the advisory committee was that before the introduction of a liquid-based method (ThinPrep®) in the organized Dutch cervical cancer screening program 'further evaluation of the costs and benefits of the ThinPrep method should be undertaken to decide definitively whether to implement this method in the Netherlands population screening program' [104].

1.7.4 Quality assurance of screening

Good quality assurance is an important component of a high-quality screening program, concerning all stages of organized screening from invitation, compliance, cell collection, handling and staining of the cell sample, microscopic evaluation, and reporting up to follow-up management. A high-quality screening program is an effective and efficient working system with best possible patient care which depends on the quality of the chain of the whole process of consecutive activities in cervical screening [105]. When focusing on the performance of the cytological screening test and evaluation of follow-up management and follow-up guidelines, a high-quality program aims at a balance between a minimum number of false negative and positive test results on the one hand and at a balance between unnecessary and inadequate follow-up of suspicious cases on the other hand, considering economic resources as well. The main challenge is to achieve a maximal reduction in the incidence and mortality of cervical cancer at the cost of a minimal number of women being exposed to the potentially negative side effects of screening [106]. A paramount component of quality assurance of cervical cancer screening is monitoring and evaluation of existing guidelines. This implies also scientific analysis of the outcome of the screening and evaluation of new cancer screening tests with randomized trials before implementation in routine healthcare [101]. In the Netherlands, along with the reorganization of the cervical screening program in 1996, guidelines on cervical screening and quality assurance were introduced by

the Dutch Society of Pathology. These guidelines have been in place for several years now and need to be evaluated on their effectiveness.

1.8 Outline of the thesis

The aim of this thesis is to evaluate existing guidelines for follow-up management on the one hand and the accuracy of a new screening test, liquid-based cytology with the ThinPrep system (Hologic Corporation, Marlborough, MA) on the other hand, with the objective to define recommendations for improvement of the quality of cervical screening within the frame of the Netherlands cervical screening program.

The thesis addresses the following research questions:

1. Is the early guideline on the management of a negative Pap test without endocervical cells effective?
2. Is the reporting guideline for normal endometrial cells in asymptomatic postmenopausal women optimal?
3. How does the management guideline for repeated borderline abnormalities in the Dutch cervical screening program perform with respect to referral compliance and outcome?
4. How does liquid-based ThinPrep cytology perform as compared with conventional cytology in terms of cytological detection rates and what is the relative accuracy of this screening test in the Netherlands?

Chapter 2 deals with the question whether the early guideline on the management of a negative Pap test without endocervical cells (ECC-) has been effective. This guideline prescribed an early repeat of the Pap test after 6 months in case of a negative ECC- results. However, controversy exists about the clinical relevance of negative ECC- smears. In chapter 2 cross-sectional results were combined with short-term follow-up of negative ECC- smears to estimate the true prevalence of squamous lesions in women with ECC- smears and compare this with the true prevalence of squamous lesions in ECC+ smears. In Chapter 3 the existing reporting guideline for normal endometrial cells in postmenopausal women without clinical complaints is evaluated. The clinical relevance of these cells in smears of these women has been subject of debate for years and this finding may lead to a diagnostic dilemma. First, the prevalence rate of endometrial (pre)-malignancies in asymptomatic postmenopausal women with normal endometrial cells in their smear is examined and next this prevalence rate is compared with a control group existing of asymptomatic postmenopausal women

without normal endometrial cells in their smears. Chapter 4 deals with the compliance with the guideline for referral and outcome after repeated borderline, or equivocal test results. These borderline results are diagnosed frequently, but generally have only limited predictive value for high-grade lesions. Since this results in high economic costs and patient anxiety and possibly overtreatment, it is important to define the optimal management procedure. In the Netherlands a conservative follow-up management had been introduced in 1996 with repeat cytology at 6 and 18 months and referral for colposcopy in case of persistent borderline abnormalities. Compliance and outcome of this management procedure is studied in this chapter. Recent years new technology has impacted increasingly on cervical cancer screening. This was reflected in the CBO-guideline from 2002 where liquid-based cytology, HPV screening and automated screening had been evaluated with systematic, evidence-based review. Based on this CBO-guideline, a randomized controlled trial (Nethcon-trial) was set up to compare the diagnostic accuracy, adequacy and cost-effectiveness of the liquid-based ThinPrep cytology technique. In chapter 5 and chapter 6 the results from this trial are described and discussed. Finally, in chapter 7 the results of the various chapters are integrated in the moving field of cervical cancer screening and discussed in general.

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Chapter 2

Prevalence of squamous abnormalities in women with a recent smear without endocervical cells is lower as compared to women with smears with endocervical cells

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Abstract

Purpose of this study was to determine the prevalence rate ratio of squamous lesions in women with a recent smears without (ECC-) versus women having a smear with endocervical component (ECC+) and to estimate the true prevalence of these lesions in women with ECC- smears by addition of short-term follow-up results of negative ECC- smears. Results of initial smears in a 3 years period, as well as follow-up results of negative ECC- smears in the same period were retrieved. Women were categorized into two groups: having ECC- and ECC+ smears. The data were analysed for three outcome parameters, ASCUS or higher (ASCUS+), LSIL or higher (LSIL+) and HSIL or higher (HSIL+). Squamous abnormalities occurred far less frequent in women with initially ECC- than with ECC+ smears. Prevalence rate ratio (PRR) was 0.27 for ASCUS+, 0.39 for LSIL+ and 0.36 for HSIL+. Addition of follow-up results of negative ECC- smears, as a correction for false-negative ECC- smears, results in PRR's which are still significantly lower than 1, and most marked in sub-set HSIL+ (PRR=0.60). We conclude that the true prevalence of squamous lesions in women with a recent ECC- smears is significantly lower as compared to ECC+ smears. These findings lent support to the decision to abolish the repeat of ECC- smears in the Dutch population screening programme.

Introduction

In 1996 the Dutch Population Screening Program has been reorganized. Changes focused on reduction of opportunistic screening, redefinition of ASCUS and AGUS and changes in the organization of the screening program. The age-range was changed from 35-55 to 30-60 years, the screening interval was increased from 3 to 5 years, criteria for referral of the patient to the gynecologist or repeating of the smear were revised and the repeat advice for negative ECC- smears (adequate but limited by absence of endocervical component in the absence of abnormal cellular changes) was set at 6 months[1] . After a second negative ECC- smear the patient is referred to the 5 years scheme of the screening program. Recently in the U.S.A. the Bethesda 2001 workshop advised to classify an ECC- smear as satisfactory for evaluation. An early repeat is not advised in the Bethesda system in the U.S.A., where women are already advised to have a smear taken each year. In the Netherlands screening program, the Bethesda recommendations for the management of ECC- smears have been discussed and the Dutch early repeat advice for ECC- smears has been under reconsideration.

Generally a smear is thought to be most sensitive to detect abnormalities if the transformation zone (TZ) is fully sampled. The presence of endocervical cells is considered as a good marker for TZ-sampling. Also endocervical mucus and squamous metaplastic cells are described as TZ-markers [2-9]. In the literature there has been disagreement about the clinical relevance of negative ECC- smears. Results of most cross-sectional studies [2,3,8,10,11] suggest that negative ECC- smears are representing high percentages of false-negative (FN) smears due to sampling error. This is reflected in a significant lower prevalence of squamous abnormalities in ECC- smears as compared to ECC+ smears.

Retrospective studies show high proportions of negative ECC- smears preceding diagnosis CIN 3 and invasive carcinoma [12,-14]. A recent meta-analysis [15] concluded that squamous abnormalities are more likely to be found in ECC+ smears as compared to ECC- smears. Longitudinal studies, on the other hand, show no differences in detection-rate of squamous abnormalities after follow-up of ECC- and ECC+ smears [5,7,16,17]. These findings would not be expected if the group of ECC- smears consists of high numbers of false negative smears. It has been suggested that the reason for this discrepancy might be a lower prevalence of squamous abnormalities in women with ECC- smears [3,16,17]. The management of early repeat testing of negative ECC- smears in The Netherlands for the last 5 years can provide useful information for exploration of the true prevalence of squamous cervical lesions in ECC- smears. Therefore we performed a cross-sectional study design and added the results of short term follow-up of negative ECC- smears to the cross-sectional outcomes in order to correct

for false-negative ECC- smears. Because taking biopsies from the TZ, as the best golden standard, is obviously not feasible, we used cytologic follow-up results.
The first aim of this study was to determine the prevalence rate ratio (PRR) of squamous lesions in women with an initial ECC- smear versus ECC+ smear.

The second aim of the present study was to get a more accurate estimate of the true prevalence of cervical squamous lesions in women with ECC- smears as compared to ECC+ smears by correcting the PRR for false-negative results. We calculated the total number of abnormalities in women with ECC- smears by adding the abnormalities, found during short-term follow-up of ECC- smears to the number of abnormalities found in initial ECC- smears.

Study Population

Results of initial smears of 180,264 women, diagnosed in 3 regional pathology laboratories in East-Netherlands in a 3 year period (1997-1999), as well as the follow-up results of 15,021 of 15,796 negative ECC- smears in the same period were retrieved from the National Pathology Database (PALGA). Patients are identified by PALGA based on the first 4 characters of the name and date of birth. This results in a considerable number of administrative fusions. Therefore follow-up data were corrected for administrative fusions using additional personal data such as initial of the first name and place of birth. In some cases residence was used also. Using this methodology, cytologic diagnosis of the next following smear was determined. In case the next following examination was histology, this diagnosis was used as follow-up result. Smears showing epithelial atrophy were excluded from evaluation because of the known difficulties in recognizing endocervical and squamous metaplastic cells if combined with atrophic squamous cells [18]. Inadequate smears were also excluded. Smears of remaining 167,604 women were analyzed with respect to reported squamous abnormalities in two groups:

1. ECC-: squamous cells present, endocervical and squamous metaplastic cells absent
2. ECC+: endocervical cells and / or squamous metaplastic cells are present with sufficient number of squamous cells.

Data were analyzed for three outcome parameters, ASCUS+ (ASCUS, LSIL, HSIL and cancer), LSIL+ (LSIL, HSIL and cancer) and HSIL+ (HSIL and cancer). These cut-off points were considered relevant because of the clinical management of these lesions. The maximum term for follow-up of negative ECC- smears was set at 24 months.

Method

We compared the prevalence of ASCUS+, LSIL+ and HSIL+ in the two groups: women with smears with (ECC+) and without (ECC-) endocervical cells and determined the prevalence rate ratio (PRR = prevalence in ECC- / prevalence in ECC+) with 95% CI. Since we used a short follow-up period with a maximum of 24 months, we assumed that the abnormalities found after repeating negative ECC- smears were already present at the time of diagnosis of the negative ECC- smear and did not represent incidence disease. The number of abnormalities found in the repeat smears were added to the number of abnormalities initially found in ECC-. In this way we corrected for false negative ECC- smears. However, not all women with negative ECC- smear were repeated within 24 months. If we assume that the group of women who had a repeat smear and women without a repeat smear are comparable and thus the prevalence of squamous abnormalities is equally distributed among these two groups, we can calculate the expected total number of abnormalities in the ECC- group. The calculated total number of abnormalities in ECC- smears is found using the equation:

$A_1 + A_2 + (\%NRep / \%Rep) * A_2$

where A_1 is the number of abnormalities found in the initial ECC- smear, A_2 is the number of abnormalities found after repeating negative ECC-, %Nrep is the percentage not-repeated smears and %Rep is the percentage repeated smears. This calculated number abnormalities in ECC- results in an adjusted prevalence in which false-negative results of ECC- smears are taken into account. Adjusted prevalence rate ratios in ECC- versus ECC+ smears were computed, including 95% CI, using SPSS software.

Results

Proportions of squamous abnormalities found in women with ECC+ and ECC- smears as well as PRR's are shown in table 1 for the three outcome parameters: ASCUS+, LSIL+ and HSIL+. The overall number of ECC- smears in the studied period was 15985 (9.5 %). Squamous abnormalities are seen far less frequent in women with an initial ECC- smear (approximately one third) than in women with ECC+ smear. Prevalence rate ratios are significantly lower than 1 for all three outcome parameters. The found prevalence might be an underestimation of the true prevalence of squamous abnormalities in women with an ECC- smear, since false-negatives could be present in this group. In The Netherlands since 1996 all negative ECC- smears had a repeat advice of 6 months. Therefore short-term follow-up data for negative ECC- smears were available and correction for false-negative ECC- smears could be performed. Additional squamous abnormalities,

found after repeating negative ECC- smears, were added to the number of abnormalities found initially in ECC-, thus giving better insight in the true prevalence of abnormalities in women with ECC- smears. The number of squamous abnormalities, found after repeating negative ECC- smears is given in table 2. These squamous abnormalities were found in women who have had a repeat smear within a period of 24 months. The abnormality rate in repeat smears was independent of the time-interval between the initially negative ECC- smear and the repeat smear.

Table 1: Number, prevalence and prevalence rate ratio (95 % CI) for ASCUS+, LSIL+ and HSIL+ in initial ECC- smears versus ECC+ smears, subdivide in age categories

		ASCUS+		LSIL+		HSIL+		
		No *	Yes *	No *	Yes *	No *	Yes *	Total
All ages	ECC-	15,769 (98.6)	216 (1.4)	15,892 (99.42)	93 (0.58)	15,937 (99.7)	48 (0.30)	15,985
	ECC+	144,025 (95.0)	7,594 (5.0)	149,331 (95.5)	2,288 (1.5)	150,344 (99.16)	1,275 (0.84)	151,619
	PRR	0.27 (0.24 - 0.31)		0.39 (0.31 - 0.47)		0.36 (0.27 - 0.48)		
30-60	ECC-	13,221 (98.8)	164 (1.2)	13,320 (99.51)	65 (0.49)	13,357 (99.79)	28 (0.21)	13,385
	ECC+	122,312 (95.0)	6,465 (5.0)	126,964 (98.6)	1,813 (1.4)	127,765 (99.21)	1,012 (0.79)	128,777
	PRR	0.24 (0.21- 0.28)		0.35 (0.27 - 0.44)		0.27 (0.18 - 0.39)		
<30	ECC-	1,659 (98.0)	33 (2.0)	1,675 (99.0)	17 (1.0)	1,682 (99.41)	10 (0.59)	1,692
	ECC+	15,955 (94.6)	911 (5.4)	16,442 (97.5)	424 (2.5)	16,636 (98.6)	230 (1.4)	16,866
	PRR*	0.36 (0.26 - 0.51)		0.40 (0.25 – 0.65)		0.43 (0.23 – 0.82)		
30-39	ECC-	4,073 (98.6)	59 (1.4)	4,103 (99.3)	29 (0.70)	4,117 (99.64)	15 (0.36)	4,132
	ECC+	42,741 (94.7)	2,395 (5.3)	44,118 (97.7)	1,018 (2.3)	44,496 (98.6)	640 (1.4)	45,136
	PRR	0.27 (0.21 – 0.35)		0.31 (0.22 – 0.45)		0.26 (0.15 – 0.43)		
40-49	ECC-	5,946 (98.9)	67 (1.1)	5,987 (99.57)	26 (0.43)	6,002 (99.82)	11 (0.18)	6,013
	ECC+	54,458 (95.2)	2,744 (4.8)	56,591 (98.9)	611 (1.1)	56,895 (99.46)	307 (0.54)	57,202
	PRR	0.23 (0.18 – 0.30)		0.41 (0.27 – 0.60)		0.34 (0.19 – 0.62)		
50-59	ECC-	3,202 (98.8)	38 (1.2)	3,230 (99.69)	10 (0.31)	3,238 (99.94)	2 (0.06)	3,240
	ECC+	25,133 (95.0)	1,326 (5.0)	26,255 (99.3)	184 (0.70)	26,374 (99.75)	65 (0.25)	26,439
	PRR	0.23 (0.17 – 0.32)		0.44 (0.24 – 0.84)		0.25 (0.06 – 1.03)		
≥60	ECC-	889 (97.9)	19 (2.1)	897 (98.8)	11 (1.2)	898 (98.9)	10 (1.1)	908
	ECC+	5,758 (96.4)	218 (3.6)	5,925 (99.15)	51 (0.85)	5,943 (99.45)	33 (0.55)	5,976
	PRR	0.57 (0.36 – 0.91)		1.42 (0.74 – 2.71)		2.0 (0.99 – 4.0)		

* number (percentage)

No significant difference in abnormality rate was found in women with a repeat smear within 8 months and those having a repeat smear between 8 and 24 months. Squamous abnormalities can also be expected to be found in the group of women who did not have a repeat smear within this period. The overall percentage of repeated negative ECC- smears was 58.2 % (table 2), with oldest (50-59 and over 60 years) women having less frequently a repeat smear within 24 months. Based on groups characteristics, we assumed that women who had a repeat smear and women without a repeat smear after an initially negative ECC-

smear are comparable (mean age 42 years with and 43.4 years without repeat smear). Therefore we concluded that extrapolation of the follow-up results of negative ECC- to

Table 2: Number of abnormalities, additionally found after repeating initially negative ECC smears, subdivided in age categories and by repeat interval

Repeat smear after ECC- within 24 months*		ASCUS+		LSIL+		HSIL+	
		No	Yes	No	Yes	No	Yes
All ages	9179 (58.2 %)	8,968	211	9,124	55	9,160	19
30-60	7884 (59.6 %)	7700	184	7843	41	7868	16
<30	977 (58.9 %)	956	21	964	13	975	2
30-39	2510 (61.6 %)	2457	53	2491	19	2501	9
40-49	3801 (63.9 %)	3708	93	3786	15	3795	6
50-59	1573 (49.1 %)	1535	38	1566	7	1572	1
≥60	318 (35.8 %)	312	6	317	1	317	1
< 8 months	5186	5073	113 (2,18 %)	5160	26 (0,50 %)	5173	13 (0,25 %)
8–24 months	3993	3895	98 (2,45 %)	3964	29 (0,73 %)	3987	6 (0,15 %)

* number and percentage of initial negative ECC- smears

women without a repeat smear was justified. We made an estimate of the true prevalence of abnormalities in ECC- smears by using the above-mentioned calculation method. The adjusted prevalences and prevalence rate ratio's, including 95% CI for ASCUS+, LSIL+ and HSIL+ are shown in table 3. Adjusted prevalence of ASCUS+, LSIL+ and HSIL+ in women with ECC- smears was lower in comparison to women with ECC+ smears. Differences were most marked for the group of HSIL+. All PRR's were significantly lower than 1. PRR's for ASCUS+, LSIL+ and HSIL+ in ECC- were 0.72, 0.78 and 0.60 respectively. As age could act as a possible confounder, we stratified results on age. Age-category 30-60 years was analysed separately because this age-range is the target population of the screening program in The Netherlands as in many other countries. Table 1 shows statistically significantly lower percentages of squamous abnormalities in ECC- smears in all age-categories with exception of women over 60 years of age. Highest percentages of abnormalities were found in the youngest age-groups. This is especially true for categories LSIL+ and HSIL+ for both ECC+ as ECC- smears. Noteworthy is the high frequency of abnormalities in ECC- smears of women over 60 years, especially LSIL+ and HSIL+. In this group of women, 7 out of 10 cases (70 %) HSIL+ in the ECC- group were squamous cell carcinomas in contrast with the ECC+ smears, where HSIL+ consisted in 13 out of the 33 cases (39 %) of squamous cell carcinomas.

Table 3: Adjusted prevalence and prevalence rate ratio (PRR) for ASCUS+, LSIL+ and HSIL+ in ECC- versus ECC+ smears, subdivided in age categories

		% ASCUS+	% LSIL+	% HSIL+
All Ages	ECC-	3.6	1.2	0.51
	ECC+	5.0	1.5	0.84
	PRR*	0.72 (0.67 – 0.79)	0.78 (0.67 -0.90)	0.60 (0.48 – 0.75)
30-60	ECC-	3.5	1.0	0.41
	ECC+	5.0	1.4	0.79
	PRR*	0.70 (0.64 – 0.77)	0.71 (0.60 – 0.85)	0.52 (0.40 – 0.69)
<30	ECC-	4.0	2.3	0.77
	ECC+	5.4	2.5	1.4
	PRR*	0.76 (0.59 – 0.96)	0.92 (0.66 - 1.27)	0.56 (0.32 – 0.98)
30-39	ECC-	3.5	1.5	0.73
	ECC+	5.3	2.3	1.4
	PRR*	0.66 (0.56 – 0.78)	0.64 (0.50 – 0.83)	0.51 (0.36 – 0.74)
40-49	ECC-	3.5	0.81	0.33
	ECC+	4.8	1.1	0.54
	PRR*	0.74 (0.64 – 0.84)	0.76 (0.57 – 1.02)	0.62 (0.40 – 0.97)
50-59	ECC-	3.5	0.74	0.12
	ECC+	5.0	0.70	0.25
	PRR*	0.71 (0.59 – 0.85)	1.06 (0.70 – 1.63)	0.50 (0.18 – 1.38)
≥60	ECC-	4.0	1.5	1.4
	ECC+	3.6	0.85	0.55
	PRR*	1.09 (0.77 – 1.54)	1.81 (1.00 – 3.25)	2.59 (1.37- 4.91)

* PRR= prevalence rate ratio (95% CI)

The adjusted prevalence of ASCUS+, LSIL+ and HSIL+ in ECC- smears was lower compared to ECC+ smears for all age-categories, again with exception of women over 60 years and also in case of LSIL+ in women 50-59 (table 3). The adjusted PRR for diagnosis ASCUS+, LSIL+ or HSIL+ in ECC- was significantly smaller than 1 for the target population of the Dutch screening program (30-60), PRR= 0.70, 0.71 and 0.52 respectively. All age-strata, with exception of 60 years and older, showed lower estimates of the adjusted prevalence of abnormalities in ECC- as compared to ECC+ smears. However the number of cases in some of the age-strata were too small to show significant differences.

Table 4: Adjusted prevalence and prevalence rate ratio (PRR) for ASCUS+, LSIL+ and HSIL+ in ECC- versus ECC+ smears, considering recurrent ECC- as not repeated.

		% ASCUS+	% LSIL+	% HSIL+
All Ages	ECC-	4.4	1.3	0.58
	ECC+	5.0	1.5	0.84
	PRR*	0.88 (0.82-0.95)	0.88 (0.76-1.01)	0.68 (0.55-0.85)
30-60	ECC-	4.3	1.2	0.48
	ECC+	5.0	1.4	0.79
	PRR*	0.87 (0.80-0.94)	0.84 (0.71-0.99)	0.61 (0.47-0.78)
<30	ECC-	4.7	2.7	0.83
	ECC+	5.4	2.5	1.4
	PRR*	0.88 (0.70-1.09)	1.08 (0.80-1.46)	0.61 (0.36-1.04)
30-39	ECC-	4.2	1.7	0.82
	ECC+	5.3	2.3	1.4
	PRR*	0.78 (0.67-0.91)	0.74 (0.58-0.94)	0.58 (0.41-0.82)
40-49	ECC-	4.5	1.0	0.40
	ECC+	4.8	1.1	0.54
	PRR*	0.93 (0.83-1.05)	0.92 (0.70-1.20)	0.74 (0.49-1.13)
50-59	ECC-	4.5	0.93	0.15
	ECC+	5.0	0.70	0.25
	PRR*	0.91 (0.77-1.07)	1.33 (0.91-1.95)	0.63 (0.25-1.56)
≥60	ECC-	4.8	1.7	1.5
	ECC+	3.6	0.85	0.55
	PRR*	1.33 (0.97-1.82)	1.94 (1.09-3.43)	2.79 (1.50-5.20)

* PRR= prevalence rate ratio (95% CI)

Discussion

The prevalence of squamous abnormalities found in women with ECC+ smears amounts approximately three times the prevalence of these lesions in ECC- smears. This is in concordance with other published results. Bos *et al.* [3] reported a proportion of abnormalities in ECC+ smears amounting twice that of ECC- smears. Vooys *et al.* [8] found three times more HSIL+ in ECC+ smears as compared to ECC-. Martin-Hirsch *et al.* [12] reported a two- to three-fold detection rate in ECC+ smears in contrast with ECC- smears. These results might be the effect of a reduced sensivity of ECC- smears in detecting squamous abnormalities, as suggested in most references. However a truly lower prevalence of these lesions in women with ECC- can also explain the difference. Therefore we calculated an estimate of the prevalence of ASCUS+, LSIL+ and HSIL+ in women with ECC- smears after correction for false-

negative ECC- smears (adjusted prevalence). Results show that the adjusted prevalence of HSIL+ is significantly lower in women with ECC- as compared to ECC+ smears for age-categories all ages and 30-60 years, being the population screening program age range [PRR= 0.60 (0.48-0.75) and PRR= 0.52 (0.40-0.69) respectively]. Addition of less severe abnormalities (ASCUS+ and LSIL+) to the analysis resulted in less lower adjusted PRR's. There was one age-category showing opposite results. Women over 60 years showed more abnormalities having an ECC- smear, with the strongest effect seen in HSIL+. For this group of women a PRR= 2.59 (1.37-4.91) for detection of HSIL+ in ECC- was found. Closer examination of the category HSIL+ in ECC- in these older women showed that most of the lesions (seven of 10) were diagnosed as squamous cell carcinoma. It is likely that taking a smear from a carcinoma results in a smear without endocervical cells since the transformation zone is replaced by tumor. A reason might also be less accurate reporting of endocervical cells in these cases. All other age-categories showed lower adjusted prevalence's of squamous abnormalities in ECC- as compared to ECC+ smears. However the number of cases in some age-strata were too small to show significant differences.

The calculation method used in this study to determine the prevalence of abnormalities in ECC- smears can result in too low an estimate of the number of abnormalities in ECC- smears, since the repeat-smear after a negative ECC- can be a recurrent negative ECC- smear. One might state that a ECC- smear is inadequate for detection of cervical lesions and therefore women with recurrent negative ECC- smears at follow-up should be excluded from the group of women with follow-up after an initial ECC- smear. On the other hand, it is important to remember that ECC- smears are capable in detecting at least some squamous lesions, as shown in table 1. Exclusion of women with recurrent negative ECC- smears from the group with follow-up results will then again provide a too high estimate of the true prevalence of cervical abnormalities. In this respect we calculated also the adjusted prevalence in ECC- with exclusion of women having recurrent ECC- repeat-smears. An overview of thus calculated adjusted prevalence's in ECC- is given in table 4. Though probably too high, the adjusted prevalence rate ratio's for all ages and 30-60 are still significantly lower than one for ASCUS+, LSIL+ (30-60 years). For the most risk full category HSIL+ the prevalence rate ratios amounts PRR=0.68 (0.55-0.85) and PRR=0.61 (0.47-0.78) for all ages and 30-60 years respectively. We conclude that, after correction for false-negative ECC- smears, the true prevalence of cervical abnormalities in women with an ECC- smear is significantly lower as compared to women with an ECC+ smear and thus this group represents a low-risk subset.

This applies especially for severe lesions (HSIL+). The lower prevalence of abnormalities in women with ECC- smears as found in this study, suggests that women in the ECC- sub-category are less susceptible to external stimuli such as HPV infections. This might be caused

by a TZ, located higher in the endocervical canal or a TZ, which is transformed in a less sensitive epithelium through the squamous metaplastic pathway.

The results presented in this study support the management of ECC- smears as proposed by the Bethesda 2001 System. In case of limited resources there can be little justification for advising women at low risk to have a repeat smear. Resources can be better aimed at high risk categories. As a consequence early repeat testing of ECC- smears has recently been halted in the Dutch sceening program. Because of the anatomy of the TZ it is however of importance to continue to register the presence or absence of endocervical and squamous metaplastic cells for monitoring the quality of the performance of the smear-taker.

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Chapter 3

Normal appearing endometrial cells in cervical smears of asymptomatic postmenopausal women have predictive value for significant endometrial pathology

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Abstract

The objective of this study was to determine whether postmenopausal asymptomatic women with normal endometrial cells in their smear are at higher risk for endometrial pathology compared with women without these cells.

Histological follow-up outcome and otherwise cytological follow-up of 29,144 asymptomatic, postmenopausal women was determined. Presence of normal endometrial cells, age, use of hormones and reported elevated maturation index were assessed. Impact of each variable on outcome as well as the combined effect was evaluated.

Prevalence rate of (pre)malignant uterine disease was significantly higher when normal endometrial cells were found in the cervical smear (6.5%) as compared to smears without these cells (0.2%), resulting in a relative risk of 40.2 (95% CI 9.4 – 172.2). Neither age nor hormone use nor elevated maturation index showed significant impact the outcome.

Asymptomatic postmenopausal women with normal endometrial cells in their smear are at significant higher risk for (pre)cancerous endometrial lesion than women without these cells. These cases should be reported to the physician with an explicit comment that normal endometrial cells in a smear of a postmenopausal woman is an abnormal finding, possibly associated with significant endometrial pathology. It raises the question whether further gynecological examination would be more appropriate.

Introduction

In the Netherlands, endometrial adenocarcinoma is the most common malignancy of the female genital tract and the fourth most common cancer in females. With an incidence of 19 cases per 100,000 this disease accounts for 1400 new cases and 400 deaths each year. The incidence of endometrial carcinoma rises with increasing age. Therefore, most endometrial carcinomas are found in postmenopausal women. [1] Besides age, unopposed estrogenic stimulation due to obesity or exogenous hormone use and diabetes are associated with endometrial carcinoma. Atypical endometrial hyperplasia is considered as a precursor lesion of endometrial carcinoma. [2]

Even though the cervical smear is a poor screening tool for detection of endometrial carcinoma because of its low sensitivity (25% - 55%) [3], a cervical smear may provide important diagnostic information when atypical endometrial cells are detected. It has been shown that the presence of atypical endometrial cells has a significant correlation with significant endometrial disease (20 % - 55 %) [3-5]. However, the clinical significance of normal appearing endometrial cells in symptomatic as well as asymptomatic postmenopausal women has been subject of debate [6-8]. It has been claimed that detection of normal appearing endometrial cells in cervical smears of symptomatic postmenopausal patients has little, if any impact on subsequent patient management [6,8]. Finding these cells in smears of asymptomatic postmenopausal women, however, may lead to a diagnostic dilemma. Though the majority of patients with endometrial carcinoma or its precursor lesions are symptomatic at an early stage of the disease and present with abnormal uterine bleeding, asymptomatic patients with atypical hyperplasia or endometrial carcinoma have also been reported in frequencies varying from 2 % to 25 % [3,6,7,9-11].

The Bethesda 2001 System [17] recommends the reporting of normal appearing endometrial cells in all women from the age of 40 onward, with an optional educational comment that endometrial cells after menopause may be associated with benign endometrial changes, hormonal alterations and, less commonly, endometrial abnormalities. In these cases, clinical correlation is recommended.

In the Netherlands the guideline on cervical screening, as it is currently used, advocates the reporting of normal endometrial cells. In postmenopausal women a recommendation to the smear taker is added: in the presence of clinical symptoms that are associated with endometrial pathology, further gynecological investigation is advised. This was underscored by an additional alerting PAP classification PAP 3a (equivocal cytological findings or mild to

moderate abnormalities are found). However, recently this has been adjusted to PAP 1 (no abnormalies found).

This study focuses on the diagnostic dilemma arising when normal endometrial cells are found in cervical smears of asymptomatic postmenopausal women (EM+). The first aim was to examine the prevalence rate of endometrial (pre)malignancy in these women.

In most other studies, the prevalence rate of (pre)malignancy in the study population is provided but the findings are not related to a control group, that is to say postmenopausal women without endometrial cells in their smear (EM-). Therefore, the second aim of this study was to compare the prevalence rate of (pre)malignancy in asymptomatic postmenopausal women with normal endometrial cells in their smear (EM+) with the prevalence rate in women without these cells (EM-) in terms of prevalence rate ratio (PRR: prevalence rate in EM+ / prevalence rate in EM-). This reflects the relative risk (RR) for the presence of a (pre)malignant endometrial lesion when normal endometrial cells are found in the smear of an asymptomatic, postmenopausal woman.

Interacting variables such as increasing age, use of exogenous hormones (hormone replacement therapy (HRT)) and elevated maturation index (MI) of squamous cells in the smear have been described earlier [3,10-14]. Therefore, we examined the individual impact of these variables on the outcome as well as their combined effect on the PRR.

Materials and Methods

The results of 29,798 smears of women, participating in the Dutch cervical screening program and who were indicated as postmenopausal on their cytology requisition form (last menstruation more than one year ago) were retrieved from the files of the National Pathology Database (Pathologisch Anatomisch Landelijk Geautomatiseerd Archief: PALGA). All these smears were taken from the cervix and were considered as the initial smear.

All women in the Netherlands are invited to participate in the screening program every 5 year, starting on their 30th till they reach the age of 60. In case in the intervening 5-years period gynecological complaints arise, the woman is examined outside the screening program as a symptomatic patient. In this study screenees were, by definition, considered asymptomatic unless gynecological complaints or abnormal uterine bleeding were reported by the smear taker. On the requisition form this item is explicitly inquired. The Cervex-Brush® is the standard sampling device, used in the screening program. All cervical smears were

diagnosed in one of the three regional pathology laboratories in East-Netherlands in a 6-year period (1997-2002). The retrieved results included case history with cytological as well as histological follow-up. The cytological records of the initiating smear were investigated for reported normal appearing endometrial cells in the diagnosis and classified EM+ when these cells were indicated and EM- when not indicated. Cytologically detected abnormal endometrial cells in the initial smear as well as concurrently recorded gynecological complaints or abnormal uterine bleeding was assessed. In 641 cases, gynecologic complaints or abnormal uterine bleeding was indicated on the requisition form. These cases were considered symptomatic and therefore excluded. Thirteen cases with abnormal endometrial cells were excluded also. The remaining 29,144 cases were eligible for the study.

Follow-up was categorized in 4 groups: 1. no follow-up results present, 2. only negative follow-up smears (smears without endometrial cells) present, 3. histological follow-up, obtained within 18 months after the initial smear, showing normal or benign endometrial pathology (endometrial polyps, leiomyomata, simple or complex hyperplasia without atypia) and 4. histological follow-up, obtained within 18 months, showing (pre)cancerous endometrial lesions (atypical endometrial hyperplasia and endometrial adenocarcinoma or sarcoma). In cases with both histology as well as follow-up smears, only histology was counted. Histology was evaluated for a follow-up period of eighteen months in order to maintain a clear correlation between the initial smear and the consecutive histology.

Women with inconclusive or inadequate endometrial samples were evaluated by means of the results of their follow-up smears. Cases with normal histology or benign pathology as well as the cases with only negative follow-up smears were classified as outcome category A (outcome: normal/benign). Cases with histologically diagnosed endometrial (pre)malignancy were classified as outcome category B (outcome: (pre)malignant). Women without any kind of follow-up were excluded from analysis.

From the reports of the initial smears, use of exogeneous hormones (hormone replacement therapy (HRT)), diagnosed elevated maturation index of the squamous cells and the age at the time of initial smear taking was assessed. Further on, age was categorized in younger than 55 years and 55 years and older. Elevated maturation index of the squamous cells was dichotomous (high maturation index reported (HM+) / not reported (HM-)). Use of exogeneous hormones (HRT) was categoric (HRT-, HRT+ and HRT unknown).

Univariate analysis was first performed for calculation of prevalence rate of (pre)malignancy in EM+ and EM- as well as the prevalence rate ratio (PRR) and 95% confidence interval.

To stage the impact of age, HRT and HM on endometrial outcome, univariate analysis was performed for each of them. For assesment of combined impact of the variables, logistic regression analysis was applied. All statistical analyses were performed using SPSS software.

Results

During the 6-year study period, diagnoses of initial smears of 29,144 asymptomatic, postmenopausal women were evaluated for reported normal endometrial cells. Depending on the presence of histological or cytological follow-up, cases were categorized in four follow-up categories as shown in table 1.

Table 1: Follow-up and outcome categories of 29,144 asymptomatic, postmenopausal women

Outcome category	**-**	**Normal/Benign (A)**		**(Pre) malignant (B)**	**Total**
Follow-up categories	no follow-up*	negative follow-up smears*	normal/benign histology*	(pre) malignant histology*	
Asymptomatic / EM+	25 (44.6)	23 (41.1)	6 (10.7)	2 (3.6)	56 (100.0)
Asymptomatic / EM-	21,613 (74.3)	7,282 (25.0)	181 (0.6)	12 (0.04)	29,088 (100.0)
All asymptomatic cases	21,638 (74.2)	7,305 (25.1)	187 (0.6)	14 (0.05)	29,144 (100.0)

* number (percentage)

Two hundred one women (0.6 %) had histological sampling. A quarter (25.1 %) had at least one negative follow-up smear with a mean of 2.3 years between the initial smear and last negative smear, during which obviously no serious gynecological complaints developed, which provoked histological evaluation. Three out of four women (74.3 %) did not have any kind of follow-up. This is not surprising since the screening interval in the Netherlands is 5 years while the total follow-up period examined, was 3.2 years on average (range 0.7 – 6.7 years). Moreover, for a considerable part of the women the initial smear was the last smear taken within the cervical screening program since they had reached the age of 60 years.

Fifty-six women had normal endometrial cells reported in their initial smear. In these cases follow-up was performed more often. Eight of these women (14.3 %) were evaluated with histology, 23 women (41.1 %) had routine follow-up smears while 25 women (44.6 %) had no follow-up results in their case history.

Normal endometrial cells were detected in only 0.2 % (56 out of 29,144) of the initial smears and was therefore a rather uncommon finding.

In our study population, we were able to identify 14 cases with (pre)malignancy, diagnosed histologically within 18 months after the initial smear. At the time of the smear taking these women were reported to be asymptomatic. Detailed information on the outcome of the histological follow-up of both EM+ as well as EM- is provided in table 2. Two cases of (pre)malignancy were found in EM+. Both cases were endometrial adenocarcinomas. EM- showed 12 cases of endometrial (pre)malignancies: 8 adenocarcinomas, 3 atypical complex hyperplasias and 1 atypical simple hyperplasia.

Table 2: Histological follow-up results of 201 cases of asymptomatic, postmenopausal women

Histologic diagnosis	EM+ n (%)	EM- n (%)	Total
Normal histology	6 (75.0)	100 (51.8)	106 (52.7)
Endometrial polyps	0 (0.0)	54 (28.0)	54 (26.9)
Simple hyperplasia without atypia	0 (0.0)	25(13.0)	25 (12.4)
Complex hyperplasia without atypia	0 (0.0)	2 (1.0)	2 (1.0)
Simple hyperplasia with atypia	0 (0.0)	1 (0.5)	1 (0.5)
Atypical complex hyperplasia	0 (0.0)	3 (1.6)	3 (1.5)
Endometrial adenocarcinoma	2 (25.0)	8 (4.1)	10 (5.0)
Total	8 (100.0)	193 (100.0)	201 (100.0)

The prevalence rates for uterine (pre)malignancy in EM+ and EM- as well as the prevalence rate ratio (PRR) for EM+ versus EM- are shown in table 3. The prevalence rate of (pre)malignancy in EM+ was 6.5 %. In contrast, the prevalence of (pre)malignancy in EM- was 0.2 % resulting in a RR (or PRR) for EM+ versus EM- of 40.2 (95% CI = 9.4 – 172.2).

To determine which other factors have impact on the outcome (pre)malignancy, univariate analysis was performed first (table 3). Variables included EM+ or EM-, age < 55 or ≥ 55 years, HRT and diagnosed elevated maturation index (MI). The only relevant variable was found to be the finding of normal endometrial cells in the initial smear ($p<0.001$). Age ≥ 55 years was nearly significant ($p=0.07$). HRT and maturation index were not significant in the univariate analysis, although we faced sparse data.

Table 3: Univariate analysis of prevalence rates of (pre)malignancy in EM+ and EM- in smears of 7,506 women with cytologic or histologic follow-up

	Cytologic and histologic follow-up		
	Outcome A Normal/Benign*	Outcome B (pre)malignant*	total
EM+	29 (93.5)	2 (6.5)	31 (100.0)
EM-	7,463 (99.8)	12 (0.2)	7,475(100.0)
Prevalence rate ratio		40.2 (9.4 – 172.2) #	
P value		< 0.001	
Age < 55 years	3,393 (99.9)	3 (0.1)	3,396 (100.0)
Age ≥ 55 years	4,099 (99.7)	11 (0.3)	4,110 (100.0)
P value		0.07	
HRT-	6,140 (99.9)	9 (0.1)	6,149 (100.0)
HRT+	635 (99.7)	2 (0.3)	637 (100.0)
HRT unknown	717 (99.6)	3 (0.4)	720 (100.0)
P value		0.21	
Maturation index: HM-	7,231 (99.8)	14 (0.2)	7,245 (100.0)
HM+	261 (100.0)	0 (0.0)	261 (100.0)
P value		0.48	

* number (percentage)

PRR (95% CI)

Finally a multivariate forward stepwise logistic regression analysis was performed to investigate combined impact of the other variables. Presence of normal endometrial cells appeared to be the sole prognostic parameter predictive of endometrial (pre)malignancy.

Discussion

Although the cervical smear is not the first test of choice for the detection of endometrial abnormalities, cytologically detected endometrial cells may provide useful information. This is especially true for abnormal endometrial cells. The significance of normal appearing endometrial cells in smears of postmenopausal women is still debated. Some studies found a clear association between the presence of normal epithelial endometrial cells in smears of both symptomatic and asymptomatic postmenopausal women and uterine (pre)malignancy [3,5,7,10,11,13,15]. Other studies however, failed to demonstrate this association or found that the presence of these cells had no consequences with regard to patient management since most of these patients presented with clinical symptoms [4,6,8]. However, asymptomatic cases of endometrial carcinoma or hyperplasia have been reported previously [9,10,13,15]. In our study

population of asymptomatic, postmenopausal women, we identified 14 cases of uterine (pre)malignancy within 18 months after the initial smear, underscoring that not all patients with uterine cancer or a precursor lesion are necessarily symptomatic in an early stage of the disease.

We chose to include cases with negative follow-up smears (smears without normal or abnormal endometrial cells) in outcome category A (normal / benign), together with normal or benign histology. This is in contrast with many other studies, where only histological follow-up is considered conclusive. The rationale for this is that we think that women, who had been evaluated with follow-up smears were monitored medically and did not develop alarming clinical symptoms in that specific period that would have provoked invasive endometrial sampling. Therefore we think it is valid to assume that these cases have a normal clinical outcome. A second reason is the possibility of introducing a selection bias when using histological follow-up results only, as stated by Chang *et al.* [16] and Mount *et al.* [12]. Cases that were found to be at higher risk during follow-up are evaluated by endometrial sampling. In contrast, cases that are less likely to have endometrial disease during follow-up are excluded from analysis, resulting in a biased outcome. Though we tried to minimize selection bias, we cannot exclude this completely, since EM+ was examined more extensively with follow-up as compared to EM-.

The finding of normal endometrial cells in our postmenopausal asymptomatic study population is a rather uncommon finding. We only found 56 cases EM+ in a population of 29,144 women (0.2 %). This is in line with other studies, where normal endometrial cells were found in low proportions also (Sarode *et al.*[14]: 0.24 %, Cherkis *et al.*[7]: 0.23 %, Mount *et al.*[12]: 1.1 %). The higher proportion EM+ smears in the study of Mount *et al.* was probably related to a high frequency of women who took HRT.

In this study, (pre)malignant uterine disease was found in 6.5 % of the women who were diagnosed with normal endometrial cells in their smear (EM+). In order to facilitate comparison of the results of the present study with the outcomes of other studies, a review of proportions of (pre)malignancies found in other studies is provided in table 4. In the studies shown, the reported endometrial (pre)malignant disease in asymptomatic postmenopausal women with EM+ smears ranges 0.0 % - 5.4 %. The prevalence rate of (pre)malignant endometrial disease found in our study population corresponds fairly well with the results of some earlier studies, with exception of the studies performed by Gomez-Fernandez *et al*[6] and Ashfaq *et al* [8]. Both were unable to demonstrate any cases of (pre)malignancy in asymptomatic women with EM+ smears. The authors concluded that reporting of normal endometrial cells had no clinical relevance and could in fact account for a diagnostic

dilemma. This conclusion cannot be supported by the outcome of the present study where 6.5 % of asymptomatic postmenopausal women with normal endometrial cells in their smears were diagnosed with endometrial adenocarcinoma within 18 months.

Table 4: Results of other studies correlating normal endometrial cells (EM+) in smears of postmenopausal, asymptomatic women with endometrial pathology

	Follow-up clinical, cytological or histological evaluation	
Reference	(pre)malignancy n (%)	Total n
Chang et al., 2001 [16]	2 (1.0)	217
Ashfaq et al., 2001 [8]	0 (0.0)	24
Gomez-Fernandez et al., 1999 [6]	0 (0.0)	24
Siebers (present study)	2 (6.5)	31
	Follow-up endometrial histology	
Reference	(pre)malignancy n (%)	Total n
Montz, 2001 [13]	5 (5.4)	93
Kerpsack et al., 1998 [9]	1 (3.6)	28
Ng et al.,1974 [15]	6 (4.3)	140

As it stands, the prevalence rate of (pre)malignant disease in EM+ is not particularly informative. To put the significance of normal endometrial cells in smears of postmenopausal, asymptomatic women into perspective, the prevalence rate in EM+ should be related to prevalence rate in EM-, resulting in PRR or RR of EM+ for finding severe endometrial pathology. To our knowledge, the only other study using EM- as control group is the study described by Chang *et al*. [16]. In this study, we also provide the RR of EM+ for (pre)malignant outcome. The RR is the risk of (pre)malignant uterine disease in the presence of normal endometrial cells in the cervical smear (EM+) compared with those without these cells. As shown in table 3 the RR was found to be 40.2 (95% CI 9.3 – 172.2). Chang *et al.* [16] found a relative risk of 5.36 (95% CI 1.3 – 22.1). However, their study population comprised not only asymptomatic women, but also symptomatic women and moreover their study population had another age distribution as 40 % of their cases was 60 years and older.

Univariate analysis shows that neither HRT, nor cytologically diagnosed elevated maturation index had a significant impact on the detection of uterine (pre)malignancy. Age showed a borderline significance. This is surprising since age is known to be an important risk factor for severe endometrial pathology. Our study population, however, comprises a relative young

population, due to the nature of the selected cases. All women were screenees participating in the Dutch screening program. These women had their last invitation when they reached the age of 60 years. The effect of age might be more strongly marked when evaluating women of 60 years and older. However, we could not reliably define an asymptomatic study population older than 60 years.

We found that the finding of normal endometrial cells in smears of asymptomatic, postmenopausal screenees results in a high relative risk (40.2) for the detection of atypical hyperplasia or endometrial carcinoma. Focusing on only endometrial adenocarcinoma as primary outcome the relative risk was found to be even higher. There is a 60 times greater probability of detecting endometrial carcinoma for EM+ than for EM- (95% CI 13.3 – 273). This is the result of the fact that both cases of (pre)malignancy in EM+ were endometrial carcinomas whereas only 8 out of the 14 cases of (pre)malignancies found in EM- were diagnosed as adenocarcinoma. The risk for detection of any kind of endometrial abnormality whatsoever (endometrial polyps, leiomyomata, hyperplasia with or without atypia or endometrial malignancy) was in our study population 5.2 higher (95% CI 1.3 – 20.1) for EM+ than for EM-.

In the guidelines of the Dutch Screening program, the finding of normal endometrial cells in smears of postmenopausal women does not prompt the physician to further evaluation. Nevertheless, a specific comment is given, recommending further gynecologic investigation when a patient has or develops complaints that are suspicious for endometrial pathology, such as abnormal bleeding. The results of this study however show that 6.5 % of asymptomatic postmenopausal women with EM+ are diagnosed within 18 months as having endometrial (pre)malignancy and that the risk for detection of such lesions is up to 40 higher for EM + as compared to EM-. This underscores the importance of reporting the presence of normal endometrial cells in cervical smears of asymptomatic women in their postmenopause. Moreover, the question may arise whether direct examination of these women by a gynecologist would be preferable. This study shows that the prevalence of normal appearing endometrial cells in an asymptomatic, postmenopausal screening population is very low and therefore the number of women, referred for further evaluation would be limited. In addition, the results also indicate that the relative risk for endometrial (pre)malignancy is significant. Unfortunately, the relative small number of events may limit the validity of the results of the present study. However, it is our opinion that the results confirm the importance of making a clear statement to the physician that the finding of normal endometrial cells in a smear of a postmenopausal woman, even when she is asymptomatic, is an abnormal finding that may be associated with significant endometrial pathology. A definite conclusion whether it would be appropriate to refer these asymptomatic postmenopausal women for gynecological

evaluation based on the finding of normally appearing endometrial cells would have to be determined by a more extensive study.

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Chapter 4

Referral compliance, outcome and predictors of CIN after repeated borderline cervical smears in the Netherlands

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Abstract

Borderline cytological abnormalities are diagnosed in high frequencies but have limited predictive value for high-grade cervical lesions, resulting in high costs, patient's anxiety and over treatment. A conservative management strategy for the Dutch diagnostic equivalent of borderline nuclear changes (BNC) was introduced in The Netherlands in 1996, with repeat cytology at 6 and 18 months and referral for colposcopy if BNC is persistent. The objective was to analyse compliance with the current guidelines for referral, as well as the outcome after repeated BNC. Concurrently we investigated whether other variables are predictive for high-grade lesions.

We retrieved 1,898 eligible cases of repeated BNC with 4 years follow-up from the national pathology database (PALGA) and performed a nationwide survey.

The management strategy for women with repeated BNC in The Netherlands has been accepted and supported. Seventy-seven percent (77%) of the patients had visited a gynaecologist within one year and only 4.3% were lost to follow-up. We found that 25.2 % of the patients had a low-grade lesion or worse (CIN 1+) and 10.2 % had a high-grade lesion or worse (CIN 2+), among which were four malignancies. The only variable associated with CIN or worse was age. Women under 40 years were found to be at higher risk. This finding may be used for prioritizing women for colposcopy on basis of their age. More stringent use of the diagnosis of BNC, higher thresholds for colposcopically directed biopsy and introduction of HPV triage, combined with more specific new techniques or combination of techniques such as molecular markers for P16, MIB-1 and L1 may reduce the unnecessary high referral rate and over treatment of healthy women.

Introduction

Cytological screening of the uterine cervix aims at the detection and treatment of premalignant and early invasive lesions in order to reduce mortality from cervical cancer. The quality of a screening program depends among others on an acceptable ratio between positive and negative – or unintended – effects. A well-known negative effect is over treatment caused by false positive test results.

Equivocal cytological abnormalities such as borderline nuclear changes (BNC) from the UK [1] and atypical squamous cells of undetermined significance (ASC-US) and atypical glandular cells (AGC) from Bethesda 2001[2] account for considerable proportions of false positive results [2-13]. While being least predictive for high-grade lesions, these equivocal abnormalities are usually found in high proportions [3,4,6,7]. ASC-US is defined as "cytologic changes that are suggestive of a squamous intraepithelial lesion, but lack criteria for a definitive interpretation" [2] where as BNC is used in cases where "the pathologist has genuine doubt as to whether or not the cells are dyskaryotic" [1].

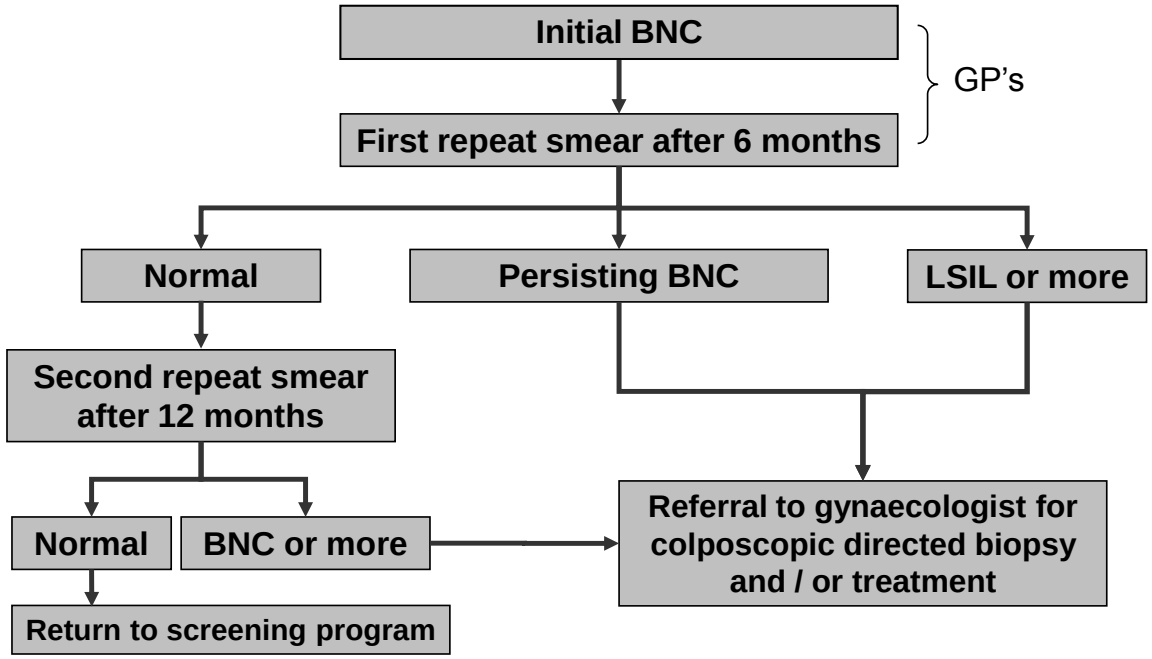
Strategies for management of equivocal abnormalities vary from country to country, but are all aiming at the reduction of the number of patients receiving unnecessary treatment. Immediate colposcopy, with or without resection, conservative management with cytological follow-up and HPV triage are the most applied strategies [3,10,13-16].

The Dutch equivalent of BNC and ASC-US / AGC is PAP 2, an additional class provided in the Dutch CISOE-A classification system [17] and used within the national screening program. Historically the proportion of PAP 2 exceeded 10 %, since inflammatory cellular changes were also included in this category [18,19]. These women were seen every 12 months for a repeat smear until a negative smear was obtained, or until they were referred to a gynaecologist when the abnormality progressed to a high-grade lesion. The result was that the Dutch cervical screening program was considered highly inefficient and cost ineffective by the Dutch Health Care authorities. As a consequence, the screening program was reorganized in 1996. A major change concerned the redefinition of equivocal cytological abnormalities (PAP 2) with more precise criteria [1,20], corresponding with Bethesda's ASCUS and the British BNC. The main goal was to decrease the proportion PAP 2 and concurrently increase the positive predictive value of this cytological diagnosis [18]. At the same time the management of these abnormalities was changed. For triage of these women, a conservative management with repeat cytological testing was chosen: women with an initial BNC test result are advised to have a repeat test after 6 and 18 months, taken by the general practitioner (GP) (figure 1). When this repeat test shows persisting or progressing abnormality,

the patient will be referred to a gynaecologist for colposcopic evaluation. When colposcopy is abnormal, a directed biopsy is performed and when colposcopy proves normal the patient is referred back to the GP.

At the time of introduction of the new management guidelines, Dutch gynaecologists feared overloading of their colposcopy clinics with women with persistent equivocal abnormal cytology, not harbouring any significant cervical lesions. General practitioners (GPs) were also sceptical about the benefit of referring women with repeated BNC results for colposcopy and some were even reluctant to do so. This major change in the cervical screening program has now been implemented for several years but until now the compliance with the current guidelines for referral and the outcome after repeated BNC have not been evaluated [18], whilst good follow-up compliance is one of the critical elements for adequate performance of cervical screening [21,22].

Figure 1: Management of borderline nuclear changes (BNC) in the Netherlands



We undertook this study to

1. determine the compliance of GPs to refer women with repeated BNC results for colposcopy
2. evaluate the significance of repeated BNC by determining the detection rate of CIN 1 or worse (CIN 1+: CIN 1, CIN 2, CIN 3 and carcinoma) and CIN 2 or worse (CIN 2+: CIN 2, CIN 3 and carcinoma)
3. concurrently investigate the value of other predictors for CIN 1+ or CIN 2+ in patients with repeated BNC Pap tests including age [8,11,23,24], cytological type of borderline abnormality [4,25-28], concurrent infection or inflammation (among which HPV-associated cellular changes) [27,29,30], reported clinical symptoms and prior smear history of the patient [28].

Study Population

The patient population was retrieved from the national pathology database (PALGA), a nationwide network for registry of histology and cytopathology in The Netherlands. Since 1990 every pathology department in The Netherlands supplies this database with all their cytology and histology results. Women advised to visit a gynaecologist, based on a repeated cytological diagnosis PAP 2 (BNC) in 2000 (n=2,885) were selected from PALGA. This included case history with an average of 4 years of follow-up results (range 3.75 years – 4.75 years). Women who had the cytological diagnosis CIN 1 or worse in the previous 5 years and who were not evaluated histologically, and thus not treated for the lesion, were excluded. Cases with persistent borderline test results that had been taken by a gynaecologist were excluded also. This was done because we were interested in incident cases with persistent BNC that had been obtained by a GP, in order to also assess the GP referral compliance in The Netherlands. This resulted in 1,898 cases eligible for analysis.

Methods

For evaluation of the adherence to the current guidelines for referral, the cases were categorized, based on histological or cytological follow-up results:

1. adequately referred patients: patients who were examined by a gynaecologist in the first year, following the second BNC result by means of histological sampling or cervical smear taking;
2. referral unknown: neither histology nor cytology that had been taken by a gynaecologist within the first follow-up year was found;
3. no follow-up: patients without any follow-up results in the 4 years period.

Follow-up histology of the uterine cervix was used for assessment of the outcome after repeated BNC. However, when no histology was obtained, the result of the last follow-up cytology was used as the outcome. Patients with more than one histology result had their outcome based on the most severe (highest grade) histological diagnosis. Cases with histologically proven CIN 1 or worse and CIN 2 or worse as well as cases without histology but with a final cytological diagnosis consistent with CIN 1 or worse or CIN 2 or worse were classified CIN 1+ and CIN 2+ respectively.

Finally, for identification of other variables impacting on the outcome, the following parameters were assessed and classified: age (in decades) at the time of the second BNC result, type of atypical cells (atypical squamous cells, atypical metaplastic cells or atypical glandular cells), concurrent inflammatory disease or infection, diagnosed in the second BNC smear (bacterial, viral (HPV associated changes), other micro-organisms (monilia or trichomonas), non-specific inflammation), clinical symptoms as these had been reported by the GP as well as the number of smears taken prior to the second BNC test result (1, 2-5 or >5). These parameters were used for univariate and logistic regression analysis with SPSS in order to identify relevant and independent risk factors.

Results

The study population comprised 1,898 women who had their smears taken by a general practitioner and who were advised to visit a gynaecologist based on repeated BNC test results. Characteristics of the study population are displayed in table 1. Mean age was 44.1 +/- 9.1 years, with median age of 45 years at the time of the second BNC diagnosis. In 68.4 % of the cases the second BNC diagnosis was based on the finding of atypical squamous cells. In 27.7 % this was based on atypical metaplastic cells. Only 3.9 % had repeated atypical glandular cells (AGC). Concurrent specific infection (HPV, bacterial or other infection (monilia or trichomonas)) was found in 19.2 % (364 out of 1,898) of the patients, among which 183 cases with cellular changes suggestive for HPV-infection (9.6 %). However, in most samples (54.6 %) no signs of inflammation or infection were found. Clinical complaints had been reported in 13.0 % of the cases at the time that the second BNC smear was taken. The mean number of cytological examinations, taken prior to the second BNC smear, was 5.8. Most women had 2-5 previously performed Pap tests in their history.

Table 1: Descriptive statistics of the 1,898 cases in the study population at time of the second BNC smear

2nd BNC	Years (sd)	%	(n)
Age			
Median	45.0		
Mean (sd)	44.1 (9.1)		
10-19		0.2	(4)
20-29		3.7	(71)
30-39		24.5	(465)
40-49		40.9	(776)
50-59		25.3	(481)
60-69		5.0	(95)
70-79		0.3	(6)
Cytological type of diagnosed atypical cells			
Atypical squamous cells		68.4	(1,299)
Atypical metaplastic cells		27.7	(525)
Atypical glandular cells		3.9	(74)
Inflammatory disease			
Signs of HPV-infection		9.6	(183)
Bacterial infection		6.9	(131)
Other infections		2.6	(50)
Non-specific inflammation		26.2	(497)
No inflammation or infection		54.6	(1,037)
Clinical symptoms			
Reported		13.0	(247)
Not reported		87.0	(1,651)
Number of preceding smears			
Median	5.0		
Mean (sd)	5.8 (4.5)		
< 2		10.7	(203)
2-5		49.0	(930)
> 5		40.3	(765)

As shown in table 2 one thousand four hundred and seventy four (1,474) women visited a gynaecologist within a year, resulting in an adequate referral rate of 77.7 % (1,474 out of 1,898 women). Follow-up of 343 patients showed neither histology nor cytology had been taken by a gynaecologist within the first year. It is unknown whether these patients had been visiting a gynaecologist in the first year and had colposcopy without sample taking or whether they were followed by their GP (referral unknown). Only a minority of 81 patients (4.3 %) were lost to follow-up.

Table 2: Compliance with the referral advice and 4-year follow-up outcome of CIN 1 or worse (CIN 1+) and CIN 2 or worse (CIN 2+) of 1,898 cases with repeated BNC smear results

	Cases in analysis	Cytological outcome			Histological outcome			4-year follow-up outcome		
	% (n)	Negative % (n)	CIN 1+ % (n)	CIN 2+ % (n)	Negative % (n)	CIN 1+ % (n)	CIN 2+ % (n)	Negative % (n)	CIN 1+ % (n)	CIN 2+ % (n)
Adequately referred	77.7 (1.474)	25.2 (372)	0.3 (5)	0.1 (2)	45.3 (668)	29.1 (429)	11.6 (171)	70.6 (1.040)	29.4 (434)	11.7 (173)
Referral unknown	18.1 (343)	84.0 (288)	0.3 (1)	0.3 (1)	9.3 (32)	6.4 (22)	3.5 (12)	93.3 (320)	6.7 (23)	3.8 (13)
Cases with follow-up	95.7 (1.817)	36.3 (660)	0.3 (6)	0.2 (3)	38.5 (700)	24.8 (451)	10.1 (183)	74.8 (1.360)	25.2 (457)	10.2 (186)
Lost to follow-up	4.3 (81)	-	-	-	-	-	-	-	-	-

Thus 1,817 cases with either histological or cytological follow-up were eligible for analysis of outcome. The outcome after 4 years of follow-up is shown in table 2. Outcome was based on histology in 1,151 cases (63.3 %). In the remaining 666 cases (36.7 %) the outcome was based on cytology. Outcome CIN 1+ and CIN 2+ comprised almost exclusively of histologically confirmed lesions (98.7 % and 98.4 % respectively). Only 6 cases of CIN 1 or worse – among which 3 cases of CIN 2 or worse – were diagnosed by cytology without histological confirmation.

Overall, CIN 1+ was detected in 25.2 % of the cases (457 out of 1,817). CIN 2+ was found in 10.2 % (186 out of 1,817) of the cases. Notably, among these cases were 4 women (0.2 %) with carcinoma (one invasive squamous cell carcinoma, two micro invasive squamous cell carcinomas and one endocervical adenocarcinoma).

In total 1,151 women underwent histological sampling under colposcopic guidance, of which 61 % (700) showed histologically no signs of an intraepithelial lesion. These cases represent false positives, which had received unnecessary histological sampling.

For identification of other risk factors for outcome CIN 1+ or CIN 2+ after repeated BNC, a univariate analysis was performed. The results are shown in table 3. Variables that impacted both outcome CIN 1+ as well as CIN 2+ were: age ($p < 0.001$), the number of Pap tests in the prior history ($p < 0.001$) and concurrently diagnosed infections or inflammatory disease ($p = 0.001$ and $p = 0.005$ for CIN 1+ and CIN 2+ respectively). Cellular changes suggestive for HPV infection were associated with outcome CIN 1+ and to a lesser extend with CIN 2+. Type of atypical cells was not predictive for CIN 1+ ($p = 0.09$) or CIN 2+ ($p = 0.84$). The same was true for clinical symptoms as reported by the general practitioner (CIN 1+ $p = 0.14$; CIN 2+ $p = 0.35$).

Logistic regression analysis with age, cytological cell type, concurrently detected infection or inflammatory disease, clinical symptoms and number of Pap tests in the medical history as independent variables, indicated that the number of smears in the history as well as concurrent inflammation or infection were strongly age-related and became insignificant when controlled for age. Therefore, age was shown to be the sole relevant variable related to outcome CIN 1+ as well as CIN 2+. Younger patients under 40 years of age, were shown to be at higher risk of harbouring a clinical relevant cervical lesion.

Table 3: Univariate analysis of other risk factors impacting outcome of 1,817 cases

	CIN 1+					CIN 2+				
	no % (n)		yes % (n)		p-value	no % (n)		yes % (n)		p-value
Age category										
10-19	50.0	(2)	50.0	(2)		75.0	(3)	25.0	(1)	
20-29	47.8	(32)	52.2	(35)		76.1	(51)	23.9	(16)	
30-39	61.5	(273)	38.5	(171)		80.9	(359)	19.1	(85)	
40-49	77.9	(580)	22.1	(165)	< 0.001	91.7	(683)	8.3	(62)	< 0.001
50-59	85.3	(394)	14.7	(68)		96.3	(445)	3.7	(17)	
60-69	82.2	(74)	17.8	(16)		94.4	(85)	5.6	(5)	
70-79	100.0	(5)	0.0	(0)		100.0	(5)	0.0	(0)	
Cytological cell type										
Atyp. squamous cells	73.4	(912)	26.6	(331)		89.6	(1114)	10.4	(129)	
Atyp. metaplastic cells	77.6	(389)	22.4	(112)	0.09	89.8	(450)	10.2	(51)	0.84
Atyp.l cylindrical cells	80.8	(59)	19.2	(14)		91.8	(67)	8.2	(6)	
Inflammatory disease										
HPV	63.5	(106)	36.5	(61)		83.2	(139)	16.8	(28)	
Bacterial	72.7	(88)	27.3	(33)		86.8	(105)	13.2	(16)	
Other	71.4	(35)	28.6	(14)	0.001	81.6	(40)	18.4	(9)	0.005
Non-specific	79.9	(385)	20.1	(97)		91.3	(440)	8.7	(42)	
None	74.7	(746)	25.3	(252)		90.9	(907)	9.1	(91)	
Clinical symptoms										
No	75.4	(1194)	24.6	(389)		90.0	(1425)	10.0	(158)	
Yes	70.9	(166)	29.1	(68)	0.14	88.0	(206)	12.0	(28)	0.35
Nr of preceding smears										
1	62.1	(113)	37.9	(69)		80.8	(147)	19.2	(34)	
2-5	74.1	(655)	25.9	(229)	< 0.001	89.9	(795)	10.1	(89)	< 0.001
> 5	78.8	(592)	21.2	(159)		91.7	(689)	8.3	(62)	

Discussion

The optimal management of BNC is the subject of ongoing discussion. Basically there are two conflicting conditions that should be met: on the one hand there is the need to identify as much as possible CIN, on the other hand there is the urge to minimize the number of unnecessarily treated false positive cases. Various strategies for triaging BNC have been proposed. In The Netherlands a conservative management strategy has been chosen in 1996 with repeat cytological testing after 6 months and 18 months and persistent BNC referred for colposcopy. A critical element in this management strategy is an appropriate and high follow-up compliance [21,22]. The current study aimed at assessing follow-up compliance and outcome after repeated BNC results in The Netherlands.

The data, used in the study were retrieved from the national pathology database, which contains pathology results from all 70 laboratories for pathology in The Netherlands, thus representing the nationwide practice. We showed that in The Netherlands the follow-up compliance was very high: only 4.3 percent of the patients with persistent BNC were lost to

follow-up during a 4-year period. The majority of the patients had no delay in diagnosis (77.7 %) and visited a gynaecologist within the first follow-up year. This might be an underestimation, since referral for colposcopy does not necessarily yield a cytological or histological result. A patient may have been referred back to the GP without sample taking in case of normal colposcopy, as illustrated in figure 1. The referral compliance rate of at least 77.7 % as found in the present study is in contrast with the findings of Bos *et al.*[18]. Their results indicated that less than 50 percent of the women with repeated BNC were referred for colposcopy. Their study, however, assessed the referral compliance in 1996, the year in which the new management guidelines had been implemented. It is likely that GP's at that time still had to be convinced of the usefulness of referring women for colposcopy after repeated BNC. The results of the present study indicate that the current management protocol has been accepted to a large extent by GPs. Not only acceptance by GPs, but clear guidelines on the management of cervical abnormalities as set on a national level, as well as the use of follow-up guidance systems might have influenced the referral compliance positively. The effectiveness of the management protocol for BNC, as used in The Netherlands depends to a large extend on a high follow-up and referral compliance. The results show that this condition has been met.

The diagnostic borderline categories (BNC as well as ASC-US) is not easy to interpret as it may reflect a heterogeneous population of abnormalities with a mixture of differently behaving biological processes [22]. For example, atypia secondary to stimuli other than HPV infection, mimicking a cervical lesion and cell populations showing abnormalities that have been caused by transient HPV infections may be diagnosed as BNC and account for false positive results during follow-up. On the other hand, BNC may also represent sub-optimally sampled cervical lesions that may progress or regress, all resulting in different outcomes depending on the time of follow-up. Comparison with results of other studies is therefore hampered, also because different classification systems are in use. Besides differences in diagnostic accuracy, frequency of cytologically detected minimal abnormalities as well as variations in prevalence of underlying CIN in study populations and different lengths of follow-up make comparison and interpretation difficult.

The results of the present study show that 25.2 % of the cases with repeated BNC results had concomitant CIN 1+ or developed this during the 4-year follow-up period. In 10.2 % of the cases high-grade lesions (CIN 2+) were found. Among these lesions there were four cases of cervical carcinoma (0.2 %). The detection rate of CIN 1+ or CIN 2+ after repeated BNC results is provided in only a limited number of other studies, mainly concerning BNC from the UK. These studies reported detection rates of CIN 1+ ranging from 34 % up to 80 %. CIN 2+ was reported in rates varying from 14.9 % to 35 % [4,5,10,11,27]. Another study from The Netherlands [16]

found an equal proportion of CIN 2+ after two consecutive BNC results (10.0 %) as in the present study.

Most other studies provide information on CIN or worse after a single or initial BNC smear. In these studies CIN 1+ was found in 10 % - 46 % and CIN 2+ in 1.7 % - 14 % [7,8,9,12,13,20,23,24,26,29,30]. One study, focusing on atypical squamous metaplastic cells [25] even found CIN 1+ in 62 % and CIN 2+ in 44 %. Carcinoma following a borderline result was reported in only a limited number of studies and in low frequencies, ranging from 0.1 % up to 3.8 % [9,12,20,27,29,31].

The detection rate of CIN 1+ as found in the present study is lower as compared to other studies that evaluated detection rates after repeated BNC results. Indeed, the detection rates we found are more in line with the results from studies that explored outcome after an initial BNC result. This was an unexpected finding since we investigated the outcome of a sub-selection of women with persistent BNC, who are thought to be at increased risk for underlying CIN. Despite the clear and more precise criteria for BNC as these had been set on a national level within the reorganized national screening program, the diagnostic accuracy of BNC in The Netherlands is apparently lower than in other countries, resulting in a relatively low predictive value for CIN or worse. Continuing education and monitoring of the predictive value of BNC is an important tool to improve the accuracy of this category. As has been stated above, diagnostic imperfection is not the sole cause for low accuracy of BNC. Prevalence of CIN in the study population, regression and progression of lesions, length of follow-up and stringency with which BNC is applied in daily practice, all play a role. In order to minimize the influence of variables such as regression rates and length of follow-up, it is more appropriate to look at CIN 2+ as outcome, since the regression rate of minimal abnormalities and CIN 1 is thought to be very high (47 % - 68 %) [7]. However, in The Netherlands the detection rate of CIN 2+ after repeated BNC is also relatively low as compared to other studies. The question is whether the practice of referring patients with repeated BNC is inefficient in The Netherlands. Although more stringent use of BNC could improve the predictive value, it is important to keep in mind that, despite the fact that detection of a high-grade lesion in only 10 percent of the patients with repeated BNC is not very high and thus reflects a relatively low individual risk, the diagnostic category BNC accounts for a considerable number of high-grade lesions due to the high frequency of this cytological diagnosis. Kinney *et al*. [32] showed that 39% of the biopsy-confirmed high-grade disease was detected in follow-up to ASC-US. Besides the considerable number of cases with CIN 2 and CIN 3, we also identified four cases with cervical carcinoma, stressing the importance of close follow-up of this diagnostic category.

However, the relatively low predictive value of repeated BNC for CIN or carcinoma has a major disadvantage. Many women without any cervical lesions are referred for colposcopy, leading to considerable anxiety in women and unnecessary costs for society. Beside this, a large proportion of these women underwent invasive procedures like histological biopsy and LEEP/LLETZ. In our study 1,151 out of 1,817 women with follow-up (63 %) had histological sampling of which 700 (61 %) had no abnormality detected. This tremendous over treatment is clearly due to the low specificity of colposcopy, since histological samples are taken under colposcopic guidance. Using higher thresholds for colposcopy along with continuing education may decrease this over treatment of otherwise healthy women.

In the current study, CIN 1+ and CIN 2+ on follow-up of BNC was age dependent and we were not able to identify other independent predictors for CIN 1+ or CIN 2+. Cellular changes suggestive for HPV infection and other infections of the uterine cervix, as well as the number of Pap smears in the medical history were shown to be age related. It is well known that prevalence of HPV is higher in young, sexually active women. We found that women under 40 years are at significantly higher risk for CIN 1+ or CIN 2+ after the diagnosis BNC as compared to older women. Age has shown to have prognostic value in other studies also [3,8,10,11,22,23,24,30,31]. Peri- and postmenopausal alterations such as changing hormonal levels may result in equivocal atrophic changes in older women and lead to overcalling of otherwise normal cellular changes. Selection on age, especially of women older than 40 years of age, might prove to be useful in defining a subcategory of patients who are at low risk for CIN 2+ and can remain under careful cytological surveillance.

In contrast to other studies, which have reported atypical squamous metaplastic cells and atypical glandular cell as high-risk subcategories [3,4,25-28], we found no significant differences in outcome between atypical squamous cells, atypical metaplastic cells or atypical glandular cells in our study population. Sub typing of the PAP 2 category seems to provide no additional value in The Netherlands diagnostic practice.

As the predictive value of BNC is low, it might be useful to triage these women before referral for colposcopy with HPV detection [14]. HPV triage is undergoing evaluation in The Netherlands and may prove to be a cost-effective improvement with substantially lower burden for women, as measured by the number of referrals for colposcopy [33-35]. However, specificity of HPV testing has proven to be low [36,37]. Research of other, new techniques or markers for high-grade disease such as $p16^{INK4A}$, MIB-1 or HPV L1 capsid protein may also help to improve specificity.

In conclusion we state that the management protocol for referring women with repeated BNC in The Netherlands has been accepted and supported. The observed over treatment of healthy women must be reduced. This may be realized by prioritizing women for colposcopy on the basis of their age, but also by a more stringent use of BNC by the pathologist, use of higher thresholds for colposcopically directed biopsy by the gynaecologist and the introduction of HPV triage, possibly in combination with other molecular biomarkers such as $p16^{INK4A}$, MIB-1 or HPV L1 capsid protein. Ongoing research for the value of these techniques is needed.

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Chapter 5

Cytologic detection of cervical abnormalities using liquid-based compared with conventional cytology: a randomized controlled trial

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Abstract

OBJECTIVE To compare test positivity rates of liquid-based and conventional cytology.

METHODS This study was a cluster randomized controlled trial with family practice as the unit of randomization, performed within the Dutch national cervical screening program. Women aged 30–60 yrs (n=89,784) recruited from 246 family practices were included. One-hundred twenty-two practices (49,222 individuals) were randomly assigned to the experimental arm and 124 practices (40,562 participants) to the conventional arm. Inclusion was performed in a 3-year period between April 2003 and July 2006. Cytological test positivity rates of liquid-based compared with conventional cytology was compared in terms of crude and adjusted odds ratios, applying a per-protocol analysis.

RESULTS Crude ratios of the odds of test positivity rates of liquid-based compared with conventional cytology for atypical squamous cells of undetermined significance or more severe, low-grade squamous intraepithelial lesion or more severe, and high-grade squamous intraepithelial lesion or more severe were 0.95 (95% confidence interval [CI] 0.82-1.10), 1.00 (95 % CI 0.83-1.20), and 0.97 (95% CI 0.77-1.22), respectively. Liquid-based cytology resulted in fewer unsatisfactory tests (odds ratio 0.30, 95% CI 0.23-0.38). The results did not change when the odds ratios were adjusted for age, study site, study period and urbanization level. Of 128 women screened with liquid-based cytology one unsatisfactory preparation is avoided.

CONCLUSIONS This study found no statistically significant difference in cytological test positivity rates between liquid-based and conventional cytology. However, liquid-based cytology resulted in significantly fewer unsatisfactory tests.

Introduction

Although successful in reducing the incidence of and mortality from cervical carcinoma, the diagnostic accuracy of screening with conventional Pap tests is hampered by the occurrence of both false-negative and false-positive results. Besides sampling issues during test taking, erroneous results are in a great part due to problems with sample preparation and cytologic interpretation. Liquid-based cytology has been developed to address these issues[1-3].

Numerous studies have been done comparing the performance of liquid-based cytology with conventional cervical cytology; however these studies resulted in substantial controversy about whether liquid-based cytology performs better than conventional cytology. Although most studies reported an increased detection of squamous intraepithelial lesions (SIL) and decreased inadequacy rates, several systematic reviews yielded contradictory results depending on the choice of the outcome measure and selection criteria for inclusion of individual studies [4-11].

We initiated a large-scale population-based cluster randomized controlled trial (RCT), including almost 90,000 cases. The objective was to prospectively test the cytologic test positivity rates of atypical squamous cells of undetermined significance or more severe (ASCUS+), low-grade squamous intraepithelial lesions or more severe (LSIL+) and high-grade squamous intraepithelial lesions or more severe (HSIL+) of the ThinPrep system (using the ThinPrep 3000 Processor, Cytyc Corporation, Boxborough, MA) in comparison with conventional cervical cytology. For practical reasons, we used family practices as unit of randomization in the cluster design. This report presents the baseline outcomes in terms of odds ratio (OR) for the cytological test positivity rates of ASCUS+, LSIL+ and HSIL+, taking cluster design into account and applying a per-protocol analysis.

Materials and methods

The randomized controlled trial was performed within the framework of the national cervical screening program in two regions in the Netherlands, in collaboration with local gynecologists, pathologists, and family physicians. The screening program invites women aged 30-60 years every five years to have a Pap test done by a family physician. Two clinical laboratory sites (PAMM Laboratories, Eindhoven and Radboud University Nijmegen Medical Centre, Nijmegen) participated in the trial. All family practices feeding the study sites were eligible for random assignment to the experimental arm (preparation of the test using the

liquid-based system) or control arm (preparation of the test using conventional cervical test preparation). Women who were visiting their family practice for participation in the national cervical screening program were all included in the study and received a conventional Pap test or a liquid-based sample according to the random allocation of their respective family practice. Ethical approval for this study was obtained by the Dutch Ministry of Health, Welfare and Sport.

The sample size for this study was calculated based on the baseline assumption of 0.6% of HSIL+ in the participants and liquid based cytology detection of a 33% increase in cervical intraepithelial neoplasia 2 at $\alpha = 5\%$ and $\beta = 20\%$. With these parameters, we initially computed the sample size of 28,269 by ignoring the clustering of women within practices. To account for the clustering effect, we assumed from the previous routine data from the two sites, an intraclass correlation coefficient of 0.05 with average cluster size of 250 and standard deviation of 200. This led us to the coefficient of variation of 0.8 and a design effect of 1.59 [12]. By multiplying the design effect by sample size without clustering effect, we obtained a sample size of 44,947 women to be screened in each arm.

The inclusion of 89,960 women screened started in April 2003 and was completed in July 2006. One hundred seventy-six participants were excluded from analysis because their GP was not randomly assigned. Identification data, clinical data, and the screening results of the remaining 89,784 participants were stored in the local pathology databases.

Allocation was based on clusters rather than on individuals, with family practice as the unit of randomization. This was done to prevent contamination by patient preference (selection bias) and for other practical reasons. All practices connected to the two study sites were ranked by postal code, and subsequently, the codes 0 (conventional) or 1 (liquid-based) were allocated using a binomial random number generator [13]. The family practices in the catchment areas of the two study sites were stratified by level of urbanization (high urbanization meaning an urban area with more than 100,000 inhabitants) by sorting on postal code. They were assigned to one of the study arms by assigning them at random to conventional or liquid-based screening by the study database manager. All practices participated in the randomization procedure and agreed with the outcome of randomization after being informed by mail. Family practices allocated to the experimental arm were provided with material for test taking with the liquid-based system. Practices allocated to the control arm were provided with the conventional test-taking material. Adherence to the assignment was checked periodically during the study.
For obvious reasons blinding for the method could not be realized for sample taking and test reading.

Family physicians or their assistant took the cervical samples. At the start of the trial, all family practices were informed about the study and consented to participation. Next, the practices that converted to liquid-based cytology received additional training, either by a regional course or by in-practice training by the manufacturer.

All cervical samples were obtained using the Rovers Cervex-Brush (Rovers Medical Devices BV, Oss, the Netherlands). Conventional tests were prepared in the usual way, whereas liquid-based cytology users were instructed to rinse their cell samples in PreservCyt (Cytyc Corporation) transport medium according to the manufacturer's instructions by rotating the brush in the solution 10 times while pushing against the PreservCyt vial wall [1]. At the laboratory, liquid-based samples were prepared using the ThinPrep 3000 Processor.

At the start of the trial, one of the participating laboratories had experience with screening liquid-based slides for 1 year; the other laboratory did not have previous experience with liquid-based cytology. Before implementation of the liquid-based method in the laboratories, cytotechnologists and cytopathologists attended a 3-day training course, provided by the manufacturer. The course finished with a test, which was mandatory before starting to screen liquid-based cytology slides. During the learning stage a minimum of 200 liquid-based slides, taken from the routine workload, were screened within a multiple screening protocol by two cytotechnologists until cytologic consensus was reached. After these 200 liquid-based slides, cytotechnologists had a final test, and when they passed they were allowed to screen liquid-based cytology independently. Technical operators received instruction for operating and maintenance of the ThinPrep 3000 Processor from Cytyc Corporation.

Both liquid-based and conventional slides were randomly examined by the trained cytotechnology staff and routinely reported using the Dutch CISOE-A classification, which can be translated to the Bethesda 1991 subcategories (ASCUS/AGUS, LSIL and HSIL) [14,15]. Abnormal slides with diagnosis HSIL+ were reviewed by a senior cytotechnologist and a trained pathologist as were slides with diagnosis ASCUS/AGUS/LSIL combined with advice for referral to a gynecologist. Cases of ASCUS/AGUS/LSIL with repeat advice followed a multiple screening protocol, with review by a senior cytotechnologist.
Cytological diagnoses were categorized in four diagnostic categories:

1. normal (including benign cellular change)
2. ASCUS/AGUS
3. 3. LSIL (low-grade intraepithelial squamous lesions with addition of low-grade glandular lesions)
4. 4. HSIL/carcinoma (high-grade intraepithelial squamous lesions or squamous cell carcinoma with addition of adenocarcinoma in situ and cervical adenocarcinoma)).

All participants from the randomized practices were included in an intention-to-treat analysis. Only those participants who had the proper test (ie, the study arm their family practice had been assigned to by randomization) were included in the per-protocol analysis. Proportions were compared by using X2 tests whereas continuous variables were compared by Student t-test. The test positivity rates of the experimental (liquid-based cytology) arm relative to the control arm were assessed for the cytologic outcome of ASCUS, LSIL, ASCUS+ (ASCUS, LSIL, HSIL and carcinoma), LSIL+ (LSIL, HSIL and carcinoma) and HSIL+ (HSIL and carcinoma) taking intracluster coefficients into account for assessment of the confidence intervals. Additionally, unsatisfactory rates were analyzed.

Crude and adjusted (controlling for age, urbanization level, study period [defined as first and second half of the study, using the median preparation date as separator] and clinical laboratory site) odds ratios (ORs) for cytological outcomes were computed using univariable and multivariable logistic regression analysis, also taking the cluster design into account. The number needed to screen was computed as the reciprocal of the risk difference ($1/(rate_{liquid-based} - rate_{conventional})$). Analyses were performed with SPSS 14.0.2 (SPSS Inc., Chicago, IL) and Stata 9.2 (StataCorp LP, College Station, TX) software.

Results

As shown in Figure 1 and Table 1 there were 89,784 participants, recruited from 246 practices included in the intention-to-treat analysis and 85,076 participants from 246 practices in per-protocol analysis. The number of practices was evenly distributed over the two study arms (122 in the experimental arm and 124 in the control arm). Nevertheless, the overall distribution of individuals between the two study arms was unbalanced, with more samples examined in the experimental (liquid-based cytology) arm (n=49,222) than in the control arm (n=40,562). This was mainly caused by an uneven distribution of liquid-based and conventional slides at site 1 (PAMM laboratory) (57.7% liquid-based compared with 42.3% conventional), due to allocation, by chance, of six large (n>1,000) practices to liquid-based compared with only one to the control arm. The largest clinical laboratory (site 1) examined almost twice the number of slides (57,045) as compared with site 2 (32,739). In site 1, proportion of liquid-based cytology preparation was similar in high-urbanization areas as compared with low-urbanization areas (site 1: 57.9% liquid-based in high-urbanization compared with 57.5% in low-urbanization area; P=0.37). In site 2, more liquid-based preparations were processed from

Fig. 1. Flow diagram of enrolment and allocation in the trial and test positivity rates.

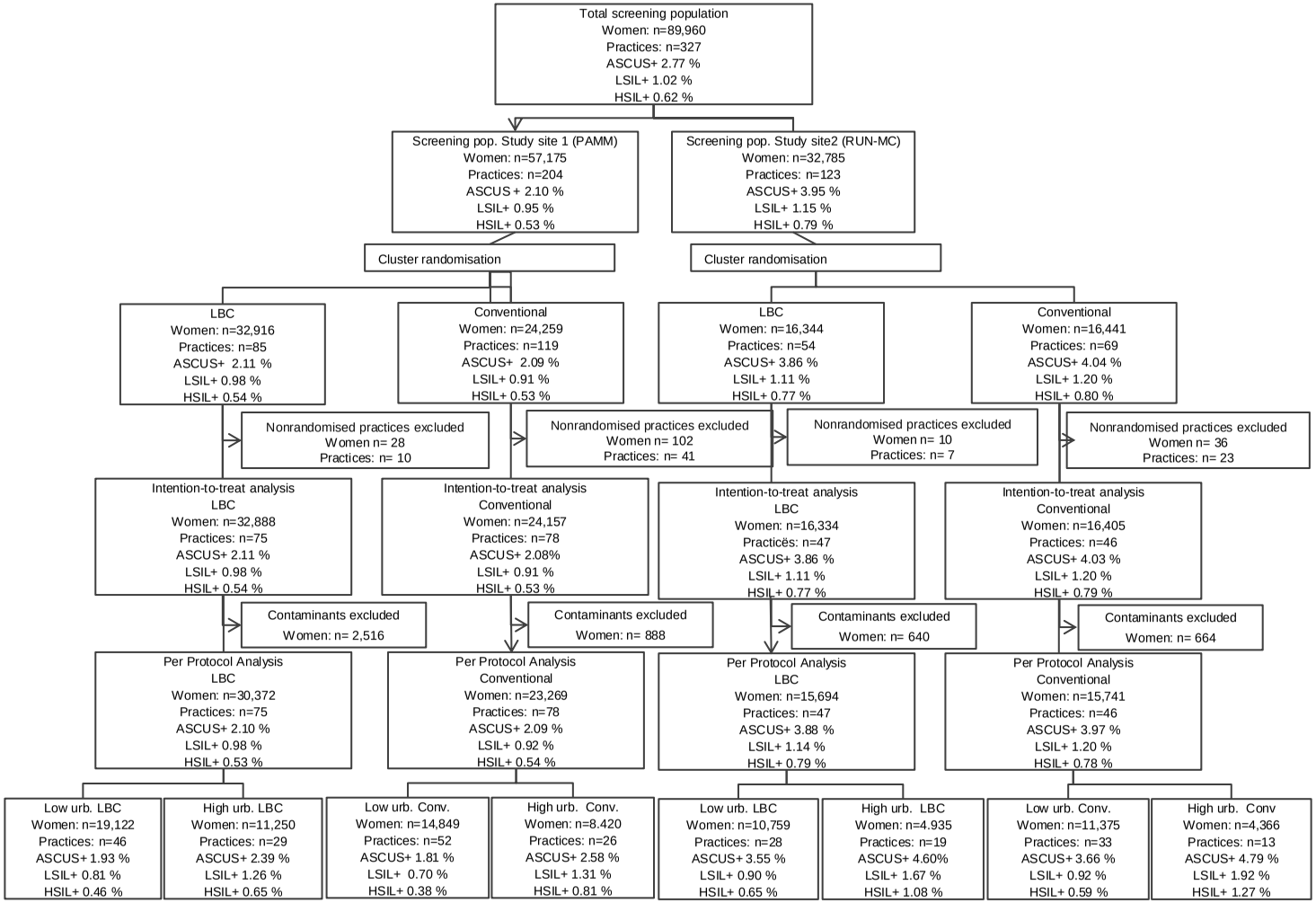

Table 1: Population characteristics (Intention-to-treat analysis)

	Urbanization	Liquid-based n (%)	Conventional n (%)	P difference	Total
Study site					
Site 1 (PAMM)	High	12,206 (57.9)	8,877 (42.1)	.37*	
	Low	20,682 (57.5)	15,280 (42.5)		
Subtotal site 1		32,888 (57.7)	24,157 (42.3)		57,045
Site 2 (RUN-MC)	High	5,036 (52.3)	4,602 (47.7)	< .001*	
	Low	11,298 (48.9)	11,803 (51.1)		
Subtotal site 2		16,334 (49.9)	16,405 (50.1)		32,739
Age					
Less than 30		325 (56.9)	246 (43.1)		
30-34		10,364 (55.7)	8,233 (44.3)		
35-39		7,233 (56.0)	5,673 (44.0)		
40-44		8,959 (55.5)	7,181 (44.5)		
45-49		5,935 (54.7)	4,910 (45.3)	< .001*	
50-54		6,183 (53.2)	5,450 (46.8)		
55-59		8,698 (53.4)	7,602 (46.6)		
More than 59		1,525 (54.6)	1,267 (45.4)		
Less than 45		26,881 (55.8)	21,333 (44.2)		
45 or more		22,341 (53.7)	19,229 (46.3)	< .001*	
Mean (y)		43.8 y (±9.2)	44.1 y (±9.2)	< .001†	
25th percentile		35	35		
50th percentile		44	44		
75th percentile		50	50		
Number of cases in intention-to-treat analysis		49,222 (54.8)	40,562 (45.2)		89,784
Number of cases in per-protocol analysis		46,066 (54.1)	39,010 (45,9)		85,076
Practice characteristics					
No of practices		122	124		246
Age‡ (y)		43.9 (39.8 to 47.8)	44,2 y (38.9 to 50.3)	.099†	
	High	48 (55.2)	39 (44.8)	.195*	
	Low	74 (46.5)	85 (53.5)		

*Chi-square test; †Student t-test; ‡means are averages over practices; range in practices

practices in high-urbanization areas (52.3% liquid-based in high-urbanization areas and 48.9% in low-urbanization areas, P<.001). Women aged younger than 45 years were relatively more often examined with the experimental method (55.8% liquid-based cytology) as compared to women aged 45 years or older (53.7% liquid-based cytology).

The crude ORs, taking the cluster effect into account, for the various cytologic diagnostic categories are shown in Table 2. Only women with a satisfactory index test were included for calculation of proportions of test positivity. The ratios of the odds for test positivity of liquid-based compared with conventional cytology were never significantly different from unity. In contrast the crude OR of the unsatisfactory rate was 0.30 (95% CI 0.23-0.38), indicating that in the experimental arm, significantly fewer tests were classified as unsatisfactory as compared

with the control arm. We also performed an intention-to-treat analysis on the dataset but this did not change the results.

As shown in the flow diagram (Fig. 1) test positivity rates of the various cytologic categories varied significantly with the study site (p<.001) as well as with level of urbanization (p<.001). Test positivity rates were higher for all three cytologic cutoffs in study site 2. The same was seen for high-urbanisation level, both in study site 1 as well as study site 2. The odds ratios for cytologic abnormalities never differed significantly from unity. These findings did not vary significantly by laboratory, urbanization or study period (data not shown).

Table 2: Per-Protocol analysis: Crude rates of cytological test positivity and unsatisfactory samples of liquid-based compared with conventional method by category of cytological abnormality and unsatisfactory tests and Odds Ratios of liquid-based compared with conventional cytology, taking the cluster design into account

Cytological category	Liquid-based		Conventional		OR (95% CI)
	n	%	n	%	
ASCUS/AGUS	769	1.67	700	1.81	0.92 (0.77 - 1.10)
LSIL	191	0.42	154	0.40	1.04 (0.82 - 1.33)
ASCUS+	1,243	2.71	1,099	2.85	0.95 (0.82 - 1.10)
LSIL+	474	1.03	399	1.03	1.00 (0.83 - 1.20)
HSIL+	283	0.62	245	0.64	0.97 (0.77 - 1.22)
Subtotal	45,913		38,576		
Unsatisfactory	153	0.33	434	1.11	0.30 (0.23 - 0.38)
Total	46,066		39,010		

OR, odds ratio; CI, confidence interval; ASCUS, atypical squamous cells and atypical glandular cells of undetermined significance; LSIL= low-grade squamous epithelial lesion; HSIL= high-grade squamous epithelial lesion; ASCUS+= ASCUS/AGUS or more severe; LSIL+= LSIL or more severe; HSIL+= HSIL or more severe

To adjust for potentially confounding variables (age, site, urbanization level, and experience with liquid-based cytology) we used logistic regression. Table 3 provides the crude ORs as well as adjusted ORs (adjusted for differences in age, study site, study period and urbanisation level). Again, none of the diagnostic categories showed a significant difference between the two study arms. The unsatisfactory rate in the liquid-based cytology arm, however, remained significantly lower as compared with the unsatisfactory rate in the control arm (OR 0.29, 95% confidence interval [CI] 0.23-0.38)). The number needed to screen to observe an additional cervical abnormality was not statistically significantly different from zero. Per 128 women screened with liquid-based cytology, one unsatisfactory preparation is avoided (number needed to screen -128, 95% CI -111 to -151).

Table 3: Per-protocol analysis: Crude odd ratios and adjusted odd ratios for observing cytological abnormalities (defined at three cytological cutoffs) or unsatisfactory tests in liquid based compared with conventional cytology, taking the intracluster coefficient into account

Cytologic detection	Crude OR (95% CI)	Adjusted OR* (95% CI)
ASCUS+	0.95 (0.82 to 1.10)	0.97 (0.88 to 1.07)
LSIL+	1.00 (0.83 to 1.20)	0.98 (0.84 to 1.15)
HSIL+	0.97 (0.77 to 1.22)	0.96 (0.79 to 1.18)
Unsatisfactory	0.30 (0.23 to 0.38)	0.29† (0.22 to 0.37)

OR, odds ratio; CI, confidence interval;
*adjusted for age, study site, urbanization level and study period
† Statistically significant

Discussion

In this large-scale population–based, cluster randomized controlled trial including almost 90,000 cases, we found no difference in performance between the liquid-based method (experimental arm) and conventional cytology (control arm) in terms of cytological test positivity rates for the various cutoff points. The cluster randomization of practices resulted in unequal numbers of subjects in the two arms. The overrepresentation of number of cases in the experimental arm in clinical laboratory site 1 was caused by some large centers of family practices that had been assigned to the experimental arm. These centers were operating in a high-urbanization area that resulted in an overrepresentation of liquid-based tests in this stratum. Potential confounding, due to unequal distribution of factors and the clustering, was controlled for by logistic regression with and without correction for design effect.

Neither the crude nor the adjusted ORs were found to differ significantly from unity in the per-protocol analysis, suggesting that the test positivity rates of liquid-based cytology are similar to conventional cytology. On the other hand, we found a strong reduction in unsatisfactory rates in the experimental liquid-based arm as compared to conventional cytology (OR 0.29, 95% confidence interval 0.23 to 0.38). Applying an intention-to-treat analysis on the data set did not change results, indicating that the per-protocol analysis did not alter the outcome.

There were striking differences in test positivity rates between the two participating clinical laboratory sites as well as between women living in low- and high-urbanization areas. The difference in test positivity rates between the study sites may reflect differences in cytological interpretation of the laboratory, but may also be the result of differences in the prevalence of cervical abnormalities. The relation we found between urbanization level and the prevalence

of abnormalities of the squamous and glandular epithelium corroborates the results obtained by other investigators[16]: the higher the urbanization level the higher the prevalence of cervical epithelial lesions. To evaluate a potential learning effect for liquid-based cytology, we analyzed the results from the first half of the trial as well as the second half, but we did not find a significant effect on the ORs.

Most previously performed studies used a split sample design. Although looking perfectly controlled, this study design has raised concerns with respect to a possible disadvantage for liquid-based cytology when the collected cellular material is split, with a conventional test made first, and the residual material immersed in the fixative solution[5]. Studies using a two-cohort design (in which conventional tests and liquid-based samples are taken from women belonging to separate but similar populations) frequently found higher test positivity rates for liquid-based cytology[17-23]. In contrast, we found no difference in test positivity rates between liquid-based and conventional tests, irrespective of the diagnostic cutoff value. Whereas we used a randomized study design, the other studies compared cytological detection rates with historical cohorts. Most of these studies reported a substantial and statistically significant increase in cytologically detected abnormalities for liquid-based cytology, with the most impressive increase found in screening centers with low rates of abnormalities[20,24]. The present study was also performed in a low-risk screening population, but we did not find higher detection rates with liquid-based cytology. The higher detection rates reported with the liquid-based technique in other studies may be caused by the introduction of the liquid-based technique, creating a higher awareness and enthusiasm for the new technique (intention bias). Also improved quality control, coinciding with the introduction of the new technique, may have resulted in an increased detection of cytological abnormalities[8]. Finally, when using historical data as a control group, differences in the study populations may have biased the results. On the other hand, it may also be the case that the quality of conventional screening in the Netherlands is so high that introduction of the new technique has little additional value.

Only two other randomized controlled trials have been published[25,26]. The study from Obwegeser[25] was unpowered (n=1,999) and found no difference in test positivity rates between liquid-based and conventional cytology. Ronco et al[26] found a significantly higher test positivity rate for liquid-based cytology as compared with conventional cytology (relative frequency 1.57, 95% CI 1.13 - 2.18). However, this higher test positivity rate in liquid-based cytology was at the expense of a reduced positive predictive value.

Several other studies found higher rates of LSIL and lower rates of ASCUS/AGUS[11,16-18,20]. This observation was not found in the present study since both ASCUS and LSIL detection rates did not differ significantly between the liquid-based and conventional study arm.

We did find significantly lower unsatisfactory rates when using liquid-based cytology as preparation technique, which will be advantageous in settings with high proportions of unsatisfactory tests. However, in the Netherlands the unsatisfactory rate for conventional tests is already very low, which reduces the added value of liquid-based cytology in terms of absolute reduction of the number of unsatisfactory tests. Use of the liquid-based method results in this study in a reduction of unsatisfactory tests of 8 per 1,000 tests.

A clear additional benefit of the liquid-based method is the availability of residual material for human papillomavirus reflex testing in case of ASC-US or LSIL[3,27]. However, presently, negative triage of ASC-US and LSIL in the Netherlands is not allowed on program tests but only for the follow-up tests of borderline and low-grade program tests.

The present study does not yet allow the conclusion that the diagnostic accuracy of liquid-based and conventional cytology is equal with respect to histologically defined outcomes. It may be theoretically possible that liquid-based cytology would be more sensitive for cervical intraepithelial neoplasia and that the conventional Pap test is less specific or vice versa. Therefore, for definite conclusions, comparison with a blindly verified reference standard is needed to assess the relative sensitivity and positive predictive value for histologically confirmed cervical intraepithelial neoplasia and cancer. These results will be available after completion of the follow-up period and be the subject of a future report.

Our conclusions are that both methods perform equally well in terms of test positivity rates within the setting of the Dutch cervical screening program. The liquid-based method does result in fewer unsatisfactory tests, but in the framework of the Netherlands cervical screening program, this adds little extra because unsatisfactory rates for conventional screening are already very low. However, the liquid-based technique does offer other additional advantages such as availability of material for reflex human papillomavirus testing and other molecular tests.

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Chapter 6

NETHCON randomized controlled trial: performance of liquid-based compared with conventional cytology to detect cervical cancer precursors

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Abstract

CONTEXT Liquid-based cytology (LBC) has been developed as an alternative for conventional cervical cytology. Despite numerous studies and systematic reviews controversy remains about the diagnostic accuracy of LBC.

OBJECTIVE To assess the performance of LBC compared with conventional cytology in terms of detection of histological confirmed cervical intraepithelial neoplasia (CIN).

DESIGN AND SETTING Cluster randomized controlled trial with family practice (FP) as unit of randomization, performed within the Dutch cervical screening program between April 2004 and July 2006, with 18 months of follow-up per patient and ending in January 2008.

PARTICIPANTS Screenees, aged 30-60 years (n=89,784), recruited from 246 FPs and randomized to LBC (122 practices, 49,222 individuals) or conventional cytology (124 practices, 40,562 individuals).

INTERVENTION Screening using LBC or conventional PAP test. Review of all follow-up of screen-positive women blinded to the type of cytology and the initial result.

MAIN OUTCOME MEASURES Intention-to-treat and per-protocol analysis of the detection rates (DR) of, and positive predictive values (PPV) for histological verified CIN in both cytology systems. Outcomes are presented as crude and adjusted rate ratios (adjustment for age, urbanization, study site and period).

RESULTS The adjusted DR ratios for CIN grade 1 or more severe (CIN1+) was 1.01 (95% CI 0.85 – 1.19), for CIN2+ 1.00 (0.84 – 1.20), for CIN3+ 1.05 (0.86 – 1.29) and for carcinoma 1.69 (0.96 – 2.99). The adjusted PPV ratios, considered at several cytological cutoffs and for various outcomes of CIN never differed significantly from unity.

CONCLUSION This study indicates that LBC does not perform better than conventional PAP test in terms of relative sensitivity and PPV for detection of cervical cancer precursors.

Introduction

The conventional PAP test (CP) is considered suboptimal due to false-negative and false-positive test results. This is caused by poor quality of sampling and preparation, (obscuration by blood or inflammation, bad cell fixation and inhomogeneous distribution of cells) as well as errors in detection- and interpretation. Liquid-based cytology (LBC) was developed as an alternative for the CP. With LBC, the cervical cells are collected with a traditional sampling device and rinsed into a vial with preservation solution rather than being smeared on a slide[1-3]. Because only a representative portion of the sample is used in LBC, the residual material in the vial may be used for ancillary testing such as reflex HPV testing and other molecular tests[3].

The accuracy of LBC has been compared with conventional cytology in numerous studies, however with disparate results. Recent systematic reviews concluded that there is still insufficient evidence that LBC is superior or inferior in the detection of high-grade lesions, due to the lack of well designed comparative studies[4-7].

The objective of this prospective trial was to compare the screening performance of LBC and CP in terms of test positivity rates, histological detection rates and positive predictive values. The evaluated LCB method was the ThinPrep® system (Hologic Corporation, Marlborough, MA). The cytological results of the trial, in terms of differences in test positivity and specimen adequacy, were recently published[8]. The present report focuses on the histological detection rates and positive predictive values.

Methods

Study Design and Study Population

Participants in this randomized controlled trial were women aged 30 to 60 years, participating in the Dutch cervical screening program. Women are invited for a Pap test every 5 years. The sample is taken by the family physician. The NETHCON trial was performed by two clinical laboratories (PAMM Laboratories, Eindhoven (study site 1) and Radboud University Nijmegen Medical Centre, Nijmegen (study site 2)) in collaboration with local gynecologists, pathologists and family physicians. Ethical approval was obtained by The Dutch Ministry of Health, Welfare and Sport. Informed refusal was offered with an information folder.

FPs, feeding the clinical study sites, were randomly assigned to the experimental arm (LBC (ThinPrep® Pap test)) or the control arm (CP). All screened women from the randomized FPs were included in the study.

Screen positive women were followed prospectively for 18 months after the initial screening test. When available, histologic follow-up was used as a reference standard. Abnormal cases, in particular women with minor abnormalities, without histologic follow-up were followed with follow-up smears. The main outcomes were the ratios of the detection rates of histologically confirmed CIN or cervical carcinoma in LBC versus CP. The absolute test sensitivity cannot be assessed in a RCT, unless the reference standard is applied to all screened subjects. However, the ratio of the detection rates (DR) equals relative sensitivity. As the prevalence of disease is equal in both arms due to randomization, the ratios of the PPVs reflect differences in specificity. Thus, a second outcome were the ratios of PPVs of cytologically or histologically confirmed outcome of CIN1/LSIL or more severe (CIN1+/LSIL+) or CIN2+/HSIL+, where verified cytology outcomes are added to those with histological outcomes.

The calculation of the sample size was documented previously[8] and was based on 0.6% detection of CIN2+ using CP and an expected 33% increase using LBC with $\alpha = 5\%$ and $\beta = 20\%$, an intraclass correlation coefficient of 0.05, an average cluster size of 250 and a standard deviation of 200. This resulted in a coefficient of variation of 0.8 and design effect of 1.59 [9]. By multiplication of the design effect by sample size without cluster effect, a sample size of 44,947 women in each arm was obtained.

Recruitment started in April 2003 and was completed in July 2006 after enrollment of 89,960 women. 176 Cases were excluded from analysis because the FP had not been randomized. Local pathology databases were used for data storage. Initial cytological cytology results were linked with the cytologic and histologic follow-up outcome assessed within an 18-month period. Follow-up data were retrieved from the local and national pathology databases, which contain the results of all histology and cytology specimen examined in The Netherlands[10].

Cluster randomization

A cluster randomization was chosen for practical reasons and to prevent contamination by preference of patient or physician (selection bias). The FPs connected to the two study sites served as the units of randomization. Stratification by urbanization level (areas with low and high level defined as containing less or more than 100,000 inhabitants) was done by ranking according to postal code. Subsequently, the FPs were allocated to CP or LBC using a

binomial random number generator[11]. All practices were included in the randomization procedure and informed by mail on the results of the randomization. They all agreed with the outcome. Adherence of the FPs to their assignment was checked periodically.

Blinding

The gynecologists, pathologists, cytotechnologists and other study personnel who were involved in the follow-up and review of histology and cytology were blinded for the cytology system used for cytological screening to prevent selective assessment bias.

Test taking

All FPs were informed about the study before the start of the trial and consented with participation. Practices who were assigned to the LBC arm received written instructions about sample collection with LBC and an additional training, either by a regional course or by in-home instruction by the manufacturer. Sample taking was done by the family physician or by their assistant.

The Rovers® Cervex-Brush® (Rovers Medical Devices B.V., Oss, The Netherlands) was used for sample taking in both study arms. The conventional Pap tests were prepared in the traditional way by spreading the sampled cells quickly onto a glass slide and performing cell fixation within a few seconds. LBC samples were prepared according to the manufacturer's instruction by transferring the sampled cells from the Cervex-Brush into PreservCyt® transport solution by firmly rotating and pushing the brush against the vial wall 10 times. LBC samples were processed at the laboratory with the ThinPrep® 3000 Processor[1,12].

Cytology

The introduction phase for LBC started with a 3-day training course for cytotechnologists and pathologists which was provided by the manufacturer and was finished with a test. During the learning stage, a minimum of 200 LBC slides were taken from the routine workload and screened. All slides were rescreened by another cytotechnologist. Primary screening of LBC was not allowed before the learning stage was successfully finished with a final test. A training course for the technical operators of the ThinPrep 3000 was also provided by Hologic Corporation. One study site had already screened LBC slides for one year before the start of the study. The other study site had no prior experience with screening LBC. Smears were screened and classified by cytotechnologists according to the CISOE-A classification system. This Dutch classification system can be easily translated into the Bethesda 1991 subcategories

ASCUS/AGUS, LSIL and HSIL[12,13]. Borderline and low-grade abnormalities were reviewed by a supervising cytotechnologist and high-grade abnormalities were reviewed by both a supervising cytotechnologist and cytopathologist. Cytological test results were categorized as:

1. Within Normal Limits
2. ASCUS/AGUS (atypical squamous or glandular cells of undetermined significance)
3. LSIL (low-grade intraepithelial squamous lesions or low-grade glandular lesions)
4. HSIL (high-grade intraepithelial squamous lesions, squamous cell carcinoma, adenocarcinoma in situ (AIS) or cervical adenocarcinoma)

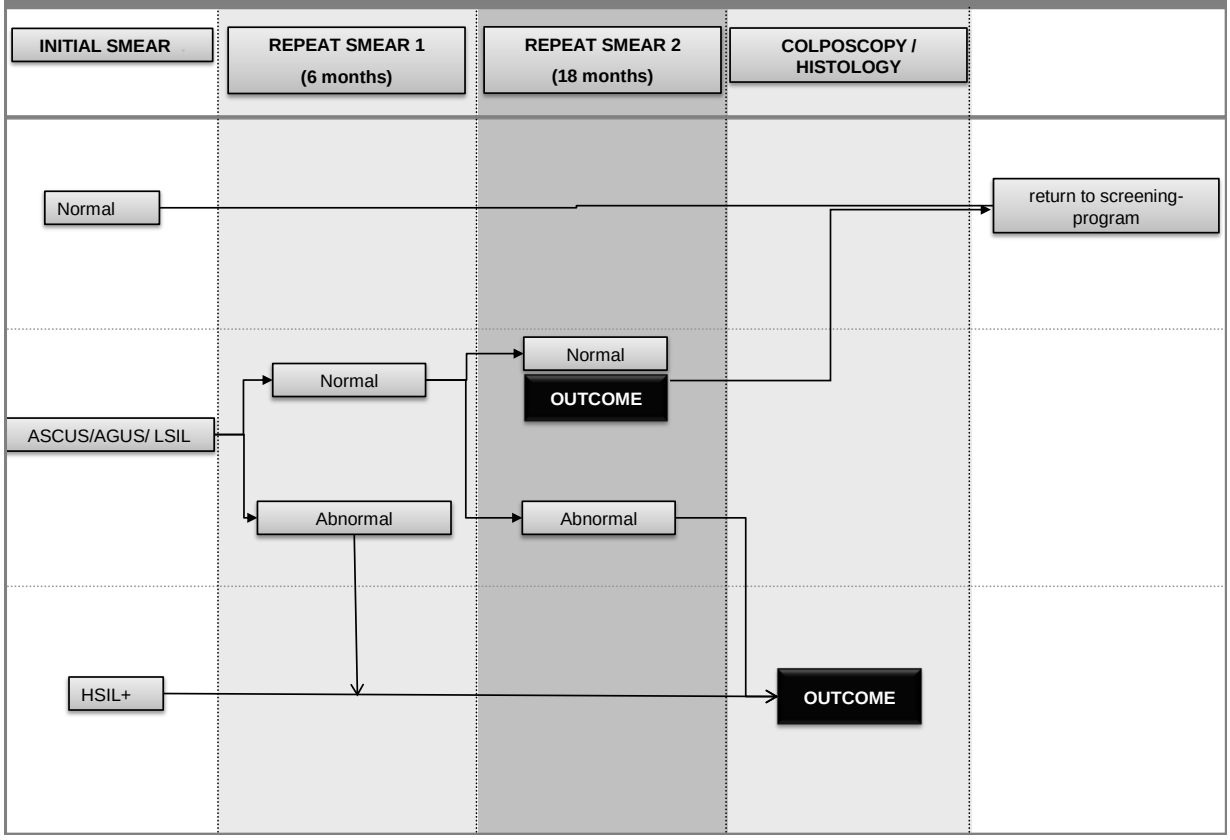
Referral, follow-up and outcome

Screen positive cases had follow-up tests in accordance with the guidelines of the Dutch Society of Pathologists (NVVP)[12] and the Dutch Society of Obstetrics and Gynecology (NVOG). Follow-up tests included cytological testing, colposcopy or histology. The follow-up protocol as practiced in the Netherlands is shown in figure 3. Women with equivocal or low-grade cytological abnormalities (ASCUS/AGUS or LSIL) on the initial test were offered repeat cytology. When both the first test (at 6 months) and the second test (at 18 months) are normal, the patient was referred back to the screening program. When the initial abnormality is found to persist or progress in the first or second repeat test, the patient is advised to visit a gynecologist for colposcopy. Histology is taken from colposcopically abnormal areas. High-grade cytological abnormalities (HSIL or more) on initial or repeat test are immediately referred to a gynecologist for colposcopy and further histological evaluation.
FPs received a reminder when follow-up tests are not performed within a previously defined time-frame, according to routine national procedures.
Assessment of the primary final outcome was based on blinded review of all histological follow-up. The secondary final outcome was also based on reviewed histological follow-up, but in cases where there was no histological follow-up, this outcome was based on blinded review of follow-up cytology.

Review of the reference standard (histology or cytology)

Cervical histology was blindly reviewed in all test positive cases where histology was performed within the time frame of 18 months. The most severe diagnosis was registered when more histological specimen were available in the follow-up period. When no histology was available within 18 months, the most severe cytology was reviewed and used for assessment of the secondary outcome. Review of histology was done by a panel of four experienced pathologists who were blinded for the cytological system, the original

Fig. 3. Referral and follow-up protocol in accordance with the guidelines of the Dutch Society of Pathologists (NVVP) and the Dutch Society of Obstetrics and Gynecology (NVOG)



cytological and histological findings and all follow-up data. Review of cytology was done by experienced cytotechnologists, using the same protocol. Discrepancy or concordance between the original diagnosis and review diagnosis was assessed using four classes:

1. WNL (Within Normal Limits)
2. ATYPIA/CIN1 (atypia or CIN1 and their glandular equivalents for histology and ASCUS/AGUS/LSIL for cytology)
3. CIN2/CIN3 (CIN2 or CIN3 and their glandular equivalents for histology and HSIL/AIS for cytology)
4. Malignant (comprising squamous- and endocervical adenocarcinoma).

In case of different outcomes within the same class, the reviewed histological diagnosis was used as outcome. In case the review diagnosis fell in another class compared to the original diagnosis, a second experienced pathologist (or cytotechnologist in case of cytology) did a blinded review. When this second review diagnosis fell in the same class as the original or first review diagnosis, this second review diagnosis was used as outcome. However, if the second review did not concur with the two previous assessments, the case was discussed using a double headed microscope by the two reviewers and a consensus diagnosis was reached. This consensus diagnosis was used as the final outcome. The final outcome was categorized

as CIN1+/LSIL+ (CIN1, CIN2, CIN3, carcinoma or the cytological equivalents) or CIN2+/HSIL+ (CIN2, CIN3, carcinoma or the cytological equivalents).

Statistical analysis

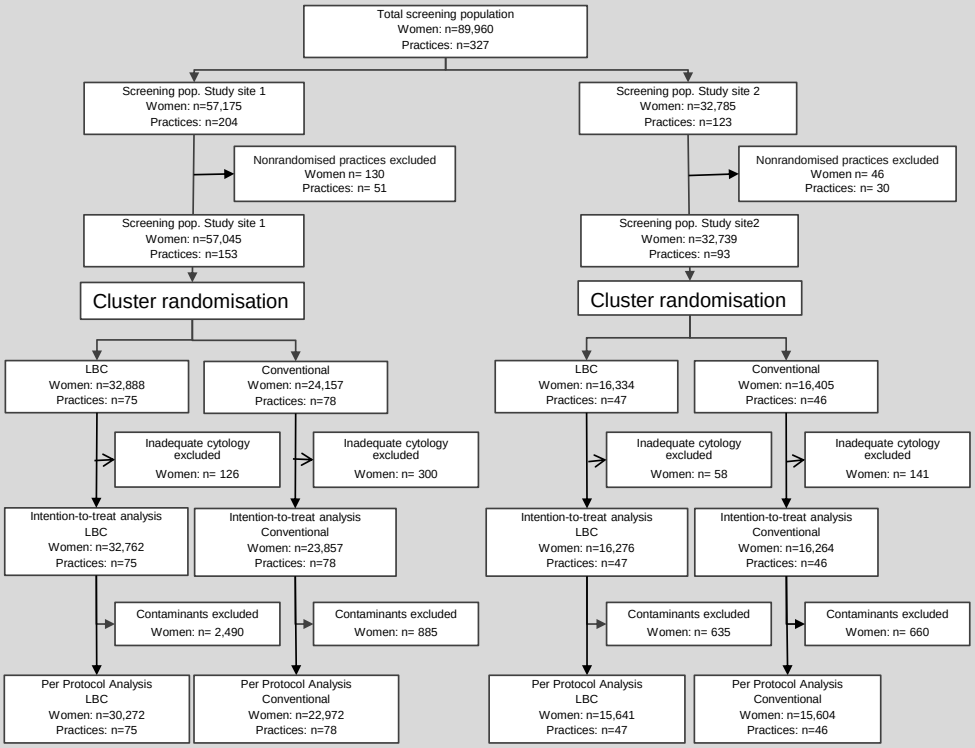
Only participants from randomized practices were included in the intention-to-treat analysis. The per-protocol analysis included only participants who received the test determined by randomization. X^2-tests were used for comparison of proportions. Crude rate ratios were computed as ratios of the DRs or PPVs. Odds ratios for finding a verified outcome in LBC versus CP, adjusted for confounding factors were computed by logistic regression. The following confounding factors were included in multivariate analyses: age, urbanization level, study site and period. Period was defined as first and second half of the study, using the median preparation date as separator. Odds ratios were converted into rate ratios using established methods[14-16].
The ratios of the DR of verified cervical abnormalities in the LBC relative to the CP arm was assessed for the primary histological outcome of CIN1+, CIN2+, CIN3+ and carcinoma. The cluster design was taken into account for calculation of 95% confidence intervals. Statistical testing was two-sided and significance was defined at $p<0.05$. Binomial exact 95% confidence intervals (CI) were computed around proportions. Analyses were performed with Stata 10.0 (StataCorp LP, College Station, TX) software.

Results

A total of 89,960 women, recruited from 327 FPs were enrolled in the trial. The flow of participants through the trial is shown in figure 1. FPs that were not connected to one of the study sites but nevertheless requested cytological assessment on an occasional base had not been randomized and were excluded from analysis (176 cases from 81 practices), leaving 89,784 participants from 246 FPs for evaluation (49,222 individuals from 122 practices with LBC; 40,562 individuals from 124 practices with CP). In the intention-to-treat analysis, another 625 inadequate smears were excluded. This left 89,197 individuals for evaluation (49,038 individuals in LBC arm; 40,121 individuals in CP arm). Moreover, in the per-protocol analysis 4,670 participants who received another test than the one to which their FP was assigned (contaminants) were excluded resulting in 84,489 participants from 246 practices (45,913 with LBC and 38,576 with CP). Cases that had been lost to follow-up were withdrawn from both analyses.

Despite a balanced distribution of FPs over the study arms (122 in the LBC arm and 124 in the conventional arm), differences were found in the number of participants over the two study

Fig. 1 Participants flow of enrollment and allocation in the trial including verified histological follow-up results



arms (table 1). By chance, 6 large practices (with 1000 women or more) belonging to study site 1 (Eindhoven) and operating in urban areas were allocated to the LBC arm whereas only one was allocated to the CP arm. Study site 1 is a high-volume laboratory and evaluated considerably more slides compared to study site 2. LBC was performed more often in high- than low-urbanization areas (56.1% LBC in high- versus 54.2% LBC in low-urbanization areas). A higher proportion of younger participants was examined with LBC as compared with older participants. The differences were statistically significant ($p<0.001$).

In the intention-to-treat analysis (table 2) 2,474 women with cytological abnormalities were identified (1.321 in LBC, 1.153 in CP arm). The follow-up results of women with abnormal cytology are given in the flowchart in figure 2. Most cases (56.4%) were followed cytologically (LBC 55.7%, CP 57.2%). Histology was performed in 36.3% of the cases (LBC 36.3% and CP 36.3%). Six cases had only colposcopy during follow-up and 171 cases (6.9%) were lost to follow-up (LBC 7.3% and CP 6.4%). None of the differences in follow-up procedures between LBC and CP were statistically significant. Outcome verification of cytological borderline and low-grade abnormalities was mainly based on revised cytological follow-up (71.3%) whereas high-grade cytological lesions were generally verified with histology (91.2%). Only 3.8% of the

Table 1: Population characteristics

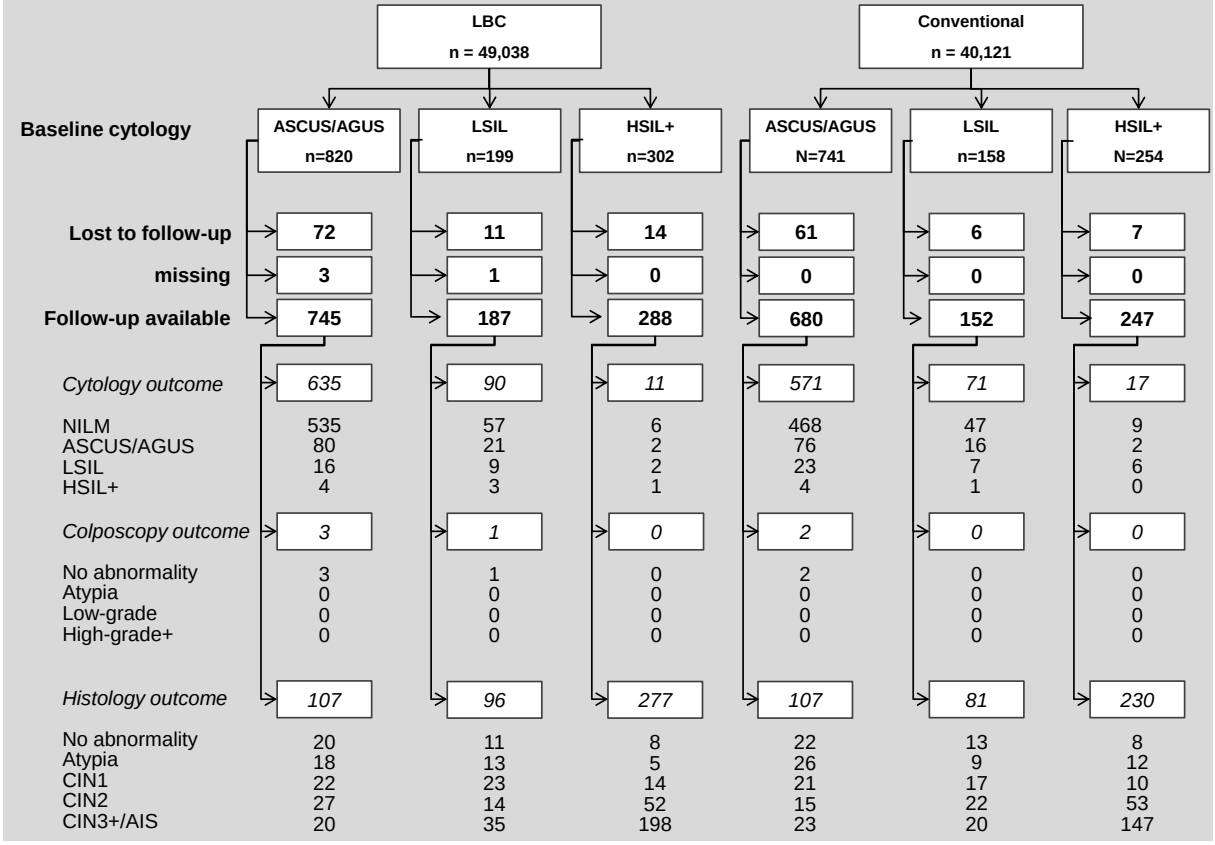
Variable		LBC n (%)	CP n (%)	p-value difference	Total
Study site	Site 1 (Eindhoven)	32,888 (57.6)	24,157 (42.4)	< 0.001*	57,045
	Site 2 (Nijmegen)	16,334 (49.9)	16,405 (50.1)		32,739
Urbanization	High	17,242 (56.1)	13,479 (43.9)	< 0.001*	30,721
	Low	31,980 (54.2)	27,083 (45.9)		59,063
Age	<30 y	325 (56.9)	246 (43.1)		571
	30-34 y	10,364 (55.7)	8,233 (44.3)		18,597
	35-39 y	7,233 (56.0)	5,673 (44.0)		12,906
	40-44 y	8,959 (55.5)	7,181 (44.5)		16,140
	45-49 y	5,935 (54.7)	4,910 (45.3)	< 0.001*	10,845
	50-54 y	6,183 (53.1)	5,450 (46.9)		11,633
	55-59 y	8,698 (53.4)	7,602 (46.6)		16,300
	>59 y	1,525 (54.6)	1,267 (45.4)		2,792
Study period	Period 1	24,250 (54.1)	20,563 (45.9)		44,813
	Period 2	24,972 (55.5)	19,999 (44.5)		44,971
Number of cases in intention-to-treat analysis		49,222 (54.8)	40,562 (45.2)		89,784
Number of cases in per-protocol analysis		46,066 (54.1)	39,010 (45,9)		85,076
Cluster characteristics					
No of practices		122	124		246
Urbanization	High	48 (55.2)	39 (44.8)	0.195*	87
	Low	74 (46.5)	85 (53.5)		159

*Chi-square test

Table 2: Intention-to-treat analysis: Follow-up and verification of test-positive cases

	LBC		CP			Total
ASCUS/AGUS and LSIL	**n**	**% of test positives**	**n**	**% of test positives**	**p-value difference**	
Cytology	725	71.1	642	71.4		71.3
Colposcopy	4	0.4	2	0.2		0.3
Histology	203	19.9	188	20.9	0.343	20.4
Cases with follow-up	932	91.5	832	92.5		92.0
Lost to follow-up	83	8.1	67	7.5		7.8
missing	4	0.4	0	0.0		0.2
Subtotal	**1,019**	**100.0**	**899**	**100.0**	**1,918**	**100.0**
HSIL+						
Cytology	11	3.6	17	6.7		5.0
Colposcopy	0	0.0	0	0.0		0.0
Histology	277	91.7	230	90.6	0.145	91.2
Cases with follow-up	288	95.4	247	97.2		96.2
Lost to follow-up	14	4.6	7	2.8		3.8
Subtotal	**302**	**100.0**	**254**	**100.0**	**556**	**100.0**
Overall						
Cytology	736	55.7	659	57.2		56.4
Colposcopy	4	0.3	2	0.2		0.2
Histology	480	36.3	418	36.3	0.297	36.3
Cases with follow-up	1,220	92.4	1,079	93.6		92.9
Lost to follow-up	97	7.3	74	6.4		6.9
missing	4	0.3	0	0.0		0.2
Total	**1,321**	**100,0**	**1,153**	**100,0**	**2,474**	**100,0**

Fig. 2 Flowchart of the follow-up outcome of abnormal cytological test results (intention-to-treat)



initial HSIL+ lesions were lost to follow-up as compared to 7.8% of borderline and low-grade cytology. All follow-up histology and cytology was reviewed and the result of the review was used as study outcome. Cases that were lost to follow-up were excluded from analysis.

Table 3 presents the intention-to-treat and per-protocol results of the detection rates (DRs) of histological verified CIN or carcinoma in LBC and CP, as well as the crude and adjusted DR ratios. Irrespective of the grade of the initial cytological abnormality that triggered further follow-up, DR ratios were close to one and none significantly differed from unity.

Table 4 provides the correlation between the baseline cytological result and the verified outcome (histology, colposcopy or cytology) for LBC and CP in the intention-to-treat analysis. With LBC, ASCUS/AGUS resulted in 2.7% (CI: 1.6% - 4.1%) in the detection of CIN3+/severe dysplasia or cancer, in 4.2% (CI: 2.8% - 5.9%) in detection of CIN2/moderate dysplasia, in 5.1% (CI: 3.6% - 6.9%) in CIN1/LSIL, and in 88.1 % (CI: 85.5% - 90.3%) in absence of CIN/SIL. For CP these figures were 3.5% (CI: 2.3% - 5.2%) for CIN3+/severe dysplasia or cancer, 2.6% (CI: 1.6% - 4.2%) for CIN2/moderate dysplasia, 6.5% (CI: 4.7% - 8.6%) for CIN1/LSIL and 87.4% (CI: 84.6% - 89.8%) in absence of CIN/SIL. An LBC result of LSIL resulted in 18.7% (CI: 13.4% - 25.8%) in an outcome of CIN3+/severe dysplasia or cancer, 9.1% (CI: 5.4% - 14.2%) in CIN2/moderate

Table 3. Detection rates (DR) of verified histological confirmed CIN or cervical cancer in LBC and in CP and the crude and adjusted DR ratios (DR_{LBC}/DR_{CP})

Intention-to-treat analysis						
Verified histological outcome	**DR in LBC (N=48,941)**		**DR in CP (N=40,047)**		**Crude DR ratio**	**Adjusted* DR ratio**
	N	**%**	**n**	**%**	**(95% CI)**	**(95% CI)**
CIN 1+	405	0.83 (0.75-0.91)	328	0.82 (0.74-0.91)	1.01 (0.88 – 1.17)	1.01 (0.85 – 1.19)
CIN 2+	346	0.71 (0.63-0.78)	280	0.70 (0.62-0.78)	1.01 (0.86 – 1.18)	1.00 (0.84 – 1.20)
CIN 3+	253	0.52 (0.45-0.58)	190	0.47 (0.41-0.54)	1.09 (0.90 – 1.31)	1.05 (0.86 – 1.29)
Carcinoma	30	0.06 (0.04-0.08)	14	0.03 (0.02-0.05)	1.75 (0.91 – 3.31)	1.69 (0.96 – 2.99)
Per-protocol analysis						
Verified Histology	**DR in LBC (N=45,818)**		**DR in CP (N=38,504)**		**Crude DR ratio**	**Adjusted DR ratio**
	n	**%**	**n**	**%**	**(95% CI)**	**(95% CI)**
CIN 1+	382	0.83 (0.75-0.92)	312	0.81 (0.72-0.90)	1.03 (0.89 – 1.19)	1.02 (0.85 – 1.21)
CIN 2+	327	0.71 (0.64-0.80)	270	0.70 (0.62-0.79)	1.02 (0.87 – 1.20)	1.00 (0.83 – 1.20)
CIN 3+	235	0.51(0.45-0.58)	181	0.47 (0.40-0.54)	1.09 (0.90 – 1.33)	1.04 (0.85 – 1.28)
Carcinoma	27	0.06 (0.04-0.09)	13	0.03 (0.02-0.06)	1.75 (0.90 – 3.38)	1.66 (0.90 – 3.03)

CIN1+, cervical intraepithelial neoplasia grade 1 or more severe; CIN2+, cervical intraepithelial neoplasia grade 2 or more severe; CIN3+, cervical intraepithelial neoplasia grade 3 or more severe; DR, detection rate; CI, confidence interval)
*(adjusted for age, study site, urbanization level and study period and taking the cluster design into account)

dysplasia, in 17.1% (CI: 12.0% - 23.3%) in CIN1/LSIL and in 55.1% (CI: 47.7% - 62.3%) no CIN or a verified outcome less than LSIL was found. For CP these figures were 13.8% (CI: 8.8% - 20.0%), 14.3% (CI: 9.3% - 21.1%), 15.8% (CI: 10.4% - 22.6%) and 55.9% (CI: 47.6% - 64.0%) respectively. HSIL+ in LBC resulted in 87.1% (CI: 83.3% - 91.0%) (n=251) in verified high-grade cervical lesions as compared to 81.0% (CI: 76.0% - 85.9%) (n=200) with CP. The PPVs of LBC and CP and their ratios for different levels of test positivity and outcome thresholds are presented in table 5 for both the ITT and PP approaches. The PPVs of LBC and CP were comparable since both the crude and adjusted positive predictive value ratios never differed significantly from unity, irrespective of the cytological or verified outcome cut-off value.

Comment

The performance of LBC and CP were prospectively compared in terms of detection rates of and positive predictive values for cervical cancer precursors. This was done in a large-scale,

Table 4. Baseline cytology versus verified follow-up outcome (blindly verified histology, colposcopy or blindly reviewed final cytology). Absolute numbers and proportion of tests positives with verified outcome (=n/N, with 95 % confidence intervals).

Intention-to-treat		**Verified follow-up outcome**				
Baseline Cytology		**WNL/ATYPIA or ASCUS**	**CIN1/LSIL**	**CIN2/moderate dysplasia**	**CIN3+/severe dysplasia+**	**P-value** [#]
ASCUS/ AGUS	LBC (N=725)	n=656 **88.1%** (85.5-90.3)	n=38 **5.1%** (3.6-6.9)	n=31 **4.2 %** (2.8-5.9)	n=20 **2.7 %** (1.6-4.1)	0.224
	CP (N=680)	n=594 **87.4%** (84.6-89.8)	n=44 **6.5%** (4.7-8.6)	n=18 **2.6 %** (1.6-4.2)	n=24 **3.5 %** (2.3-5.2)	
LSIL	LBC (N=187)	n=103 **55.1%** (47.7-62.3)	n=32 **17.1%** (12.0-23.3)	n=17 **9.1%** (5.4-14.2)	n=35 **18.7%** (13.4-25.8)	0.330
	CP (N=152)	n=85 **55.9%** (47.6-64.0)	n=24 **15.8 %** (10.4-22.6)	n=22 **14.3%** (9.3-21.1)	n=21 **13.8%** (8.8-20.0)	
HSIL+	LBC (N=288)	n=21 **7.3%** (4.6-10.9)	n=16 **5.6%** (3.2-8.9)	n=52 **18.1%** (13.8-23.0)	n=199 **69.1%** (63.4-74.4)	0.084
	CP (N=247)	n=31 **12.6%** (8.7-17.3)	n=16 **6.5%** (3.7-10.3)	n=53 **21.5%** (16.5-27.1)	n=147 **59.5%** (53.1-65.7)	
Per-protocol						
ASCUS/ AGUS	LBC (N=696)	n=613 **88.1%** (84.4-90.4)	n=35 **5.0%** (3.5-6.9)	n=28 **4.0%** (2.7-5.8)	n=20 **2.9%** (1.8-4.4)	0.529
	CP (N=640)	n=563 **88.0%** (82.2-90.4)	n=38 **5.9%** (4.2-8.1)	n=18 **2.8%** (1.7-4.4)	n=21 **3.3%** (2.0-5.0)	
LSIL	LBC (N=179)	n=98 **54.8%** (47.2-62.2)	n=31 **17.3%** (12.1-23.7)	n=16 **8.9%** (5.2-14.1)	n=34 **19.0%** (13.5-23.5)	0.416
	CP (N=149)	n=85 **57.1%** (48.7-65.1)	n=23 **15.4%** (10.0-22.3)	n=21 **14.1%** (8.9-20.7)	n=20 **13.4%** (8.4-20.0)	
HSIL+	LBC (N=269)	n=21 **7.8%** (5.0-11.9)	n=15 **5.6%** (3.2-9.2)	n=51 **19.0%** (14.8-24.7)	n=182 **67.7%** (63.2-74.7)	0.233
	CP (N=238)	n=30 **12.6%** (8.7-17.5)	n=14 **5.9%** (3.2-9.7)	n=52 **21.9%** (16.8-27.6)	n=142 **59.7%** (53.1-66.0)	

(WNL, Within normal limits; ATYPIA/ASCUS, atypical epithelium or atypical squamous cells of undetermined significance; CIN1/LSIL, Cervical intraepithelial neoplasia grade1 or low-grade squamous epithelial lesion; CIN2/moderate dysplasia, Cervical intraepithelial neoplasia grade 2 or cytological moderate dysplasia; CIN3+/severe dysplasia+, Cervical intraepithelial neoplasia grade 3 or more severe or cytological severe dysplasia or more severe)

[#] Chi^2 test

population-based, cluster randomized controlled trial including almost 90,000 participants. Strength of the present study is that it is to our knowledge the largest high quality study to date, performed in a population-based setting with blind verification of follow-up outcomes of all test positive cases contrasting with many previous studies which often suffer from methodological flaws [4,5]. Despite careful cluster randomization, the distribution of individuals over the two study arms was unbalanced. This was the result of allocation of a few large FP centers to the experimental arm. These centers were mainly serving densely populated areas. However, we controlled for possible confounding by applying multi-variate logistic regression with correction for cluster effects.

As shown in a previous publication, no differences were found in the cytologic test positivity rates between LBC and CP [8]. Nevertheless, these cytologic findings contribute insufficient evidence to claim equal diagnostic accuracy. In both the intention-to-treat and per-protocol

Table 5. Positive predictive values (in italics, computed from the number of true positives=n over the number of verified test positives=N) at three cutoffs for verified follow-up outcome in LBC and CP, crude and adjusted* PPV ratios (PPV_{LBC} / PPV_{CP})

	LBC **n** ***PPV (95% CI)***	**CP** **n** ***PPV (95% CI)***	**Crude PPV ratio (95% CI)**	**Adjusted PPV ratio# (95% CI)**
Intention-to-treat analysis				
Baseline cytology ASCUS+	N=1,220	N=1,079		
CIN1+/LSIL+	n=440 *36.1% (33.4 – 38.8)*	n=369 *34.2% (31.4 – 37.0)*	1.05 (0.94 – 1.18)	0.97 (0.81 – 1.18)
CIN2+/HSIL+	n=354 *29.1% (26.5 – 31.6)*	n=285 *26.4% (23.8 – 29.0)*	1.10 (0.96 – 1.25)	0.99 (0.80 – 1.22)
Baseline cytology LSIL+	N=475	N=399		
CIN1+/LSIL+	n=351 73.9% (69.9 – 77.9)	n=283 70.0% (66.5 – 75.4)	1.04 (0.95 – 1.13)	1.03 (0.74 – 1.42)
CIN2+/HSIL+	n=303 63.8% (59.5 – 68.1)	n=243 60.9% (56.1 – 65.7)	1.05 (0.94 – 1.16)	1.03 (0.66 – 1.78)
Baseline cytology HSIL+	N=288	N=247		
CIN2+/HSIL+	n=251 87,1% (83.3 – 91.0)	n=200 81.0% (76.0 – 85.9)	1.08 (0.99 – 1.16)	1.08 (0.67 – 1.75)
Per-protocol analysis				
	LBC **n** ***PPV (95% CI)***	**CP** **n** ***PPV (95% CI)***	**Crude PPV ratio (95% CI)**	**Adjusted PPV ratio# (95% CI)**
Baseline cytology ASCUS+	N=1,144	N=1,027		
CIN1+/LSIL+	n=412 *36.0% (33.2-38.9)*	n=349 *34.0% (31.1-37.0)*	1.06 (0.94 – 1.19)	0.99 (0.81 – 1.21)
CIN2+/HSIL+	n=331 *28.9% (26.3-31.7)*	n=274 *26.7% (24.0-29.5)*	1.08 (0.94 – 1.24)	0.99 (0.79 – 1.23)
Baseline cytology LSIL+	N=448	N=387		
CIN1+/LSIL+	n=329 73.4% (69.1-77.5)	n=272 70.3% (65.5-74.8)	1.04 (0.96 – 1.14)	1.06 (0.76 – 1.49)
CIN2+/HSIL+	n=283 63.2% (58.5-67.6)	n=235 60.7% (55.7-65.6)	1.04 (0.93 – 1.16)	1.04 (0.77 – 1.40)
Baseline cytology HSIL+	N=269	N=238		
CIN2+/HSIL+	n=233 86.6% (82.0-90.4)	n=194 81.5% (76.0-86.2)	1.06 (0.98 – 1.15)	1.09 (0.66 – 1.79)

CIN1/LSIL or more severe; CIN2+/HSIL, CIN2/HSIL or more severe; ASCUS+, atypical squamous/glandular cells of undetermined significance or more severe; LSIL+, low-grade squamous epithelial lesion or more severe; HSIL+, high-grade squamous epithelial lesion or more severe; PPV, positive predictive value; CI, confidence interval)
#adjusted for age, study site, urbanization level and study period, taking clustering into account

analyses was demonstrated that LBC was not superior to CP regarding detection rates of histological confirmed outcomes. The same was found for the positive predictive values. Altogether, these findings provide strong evidence that the performance of LBC is not superior to that of CP when applied within a well-organized and quality-controlled cervical screening program.

These findings are partly in contrast with the results of other studies comparing performance of LBC and CP [17-39]. Most studies used a non-randomized study design and compared only cytological test positivity rates. Only a limited number focused on biopsy-confirmed cervical lesions or (blind) gold standard verification.

The results of various systematic reviews varied depending on the quality criteria for inclusion of individual studies [4-7]. Our results are in line with those of a recently published meta-analysis, including only clinical studies with complete gold standard verification or randomized screening trials with nearly complete verification of cytological abnormalities [5].

A randomized study design was applied in only three other studies [40-42]. One study was underpowered (n=1.999) but also found no difference in performance of LBC and CP [40]. Another, large-scale study (n=45.174) from Ronco et al. [41] found no statistically significant difference for detection of CIN2+ between LBC and CP but reported a reduced positive predictive value for LBC. This was, in contrast to the current trial, the result of an increased frequency of minor cytological abnormalities with LBC without an increase in high-grade CIN on histology. In accordance to the present study they reported a significant decrease in unsatisfactory rates too. Finally, in a smaller study (n=13.484) from Strander et al. [42] LBC detected significantly more high-grade lesions but this was at the expense of a 30% increase in abnormal cytology samples.

In contrast, the present study found no difference in sensitivity in terms of histological detection rates of cervical lesions or in positive predictive value between LBC and CP, indicating that the accuracy of both methods is comparable [44]. This may be caused by high quality standards of conventional screening in the Netherlands.

Because of randomization, it can plausibly be assumed that the prevalence of CIN was equal in both arms. Therefore, the lack of difference in DR and PPV in this RCT demonstrates that LBC is neither more sensitive nor more specific in detecting cervical cancer precursors than the conventional PAP smear. A reduced unsatisfactory rate was found when using LBC [8] even though the added value was limited since the unsatisfactory rates were already low. On the other hand, the cost of an individual LBC test is higher as compared to a CP test, but in unequivocal cases it has the possibility of concomitant testing on the residual material for the presence of hrHPV or other molecular cell cycle related biomarkers.

In conclusion, LBC is neither more sensitive nor more specific in detecting cervical intraepithelial neoplasia or cancer.

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Chapter 7

General discussion

General discussion

The primary goal of cervical cancer screening is to decrease the incidence of cervical cancer by early detection of premalignant cervical lesions. In the Netherlands a reorganized cervical screening program has been active since 1996. The reorganization focused on organizational changes, changes in the screening interval and age-range as well as on the redefinition of follow-up policies. These were laid down in national guidelines. An important component of quality assurance of organized cervical screening is monitoring and evaluation of existing guidelines. In this thesis some of the national management and reporting guidelines as defined during the 1996 reorganization of the cervical screening program were evaluated. Furthermore, the performance of another preparation technique for cervical smears - liquid-based cytology (LBC) – for use within the cervical screening program was investigated. For this purpose we studied the accuracy and adequacy of the ThinPrep system, one of the liquid-based preparation techniques.

In chapter 2 we investigated whether the first management guideline from 1996 with an advice to repeat a negative ECC- Pap test (missing endocervical component in the absence of cellular abnormalities (Pap1)) after 6 months has been effective. In Chapter 3 the reporting guideline for the presence of normal endometrial cells in asymptomatic postmenopausal women was evaluated. In chapter 4, the compliance and outcome after repeated borderline smears were studied and discussed. Lastly, in chapter 5 and 6 the cytological detection rates and relative accuracy of LBC as compared with conventional cytology were investigated. In this final chapter the main findings of the various studies will be discussed within the scope of the quickly changing field of cervical cancer screening. The next years, the current program will be prone to serious changes due to new developments, of which the most important are HPV testing, automated screening and HPV-vaccination which all will have their impact. The conclusions discussed here should therefore be considered in the context of these future developments.

In general, in this thesis it was demonstrated that evaluation of the early guidelines was useful in proposing recommendations on measures for a more effective follow-up management in the cervical screening program. Furthermore, the study on the performance of LBC in the cervical screening program showed that the relative accuracy of the ThinPrep technique is equal to that of conventional cytology. However, this technique offers other advantages that could make implementation of LBC in the cervical screening program profitable.

In chapter 2 the prevalence of squamous cervical lesions in women with a recent negative ECC- smear was compared with the prevalence of these lesions in women with a ECC+

smear. In order to estimate the true prevalence, short-term follow-up results of negative ECC- smears were additionally used. This was possible because the first follow-up guideline from 1996 advised women with a negative ECC- test result to have a follow-up smear made after 6 months. In case the follow-up smear was a recurrent negative ECC- smear, the woman was referred back to the 5-years scheme of the screening program. The effectiveness of this follow-up protocol was tested because the clinical relevance of negative ECC- smears was disputed in literature. This was especially relevant because negative ECC- results are diagnosed rather frequently in the Netherlands (in about 10 percent of the cases) and thus results in a considerable burden for the screening population and laboratories on the one hand and on public resources on the other hand. The conclusion of this study was that the estimated true prevalence of cervical lesions in women with a recent negative ECC- smear is significantly lower as compared to women with a recent negative ECC+ Pap test and that there is no justification for these women at low risk to have a repeat smear. This conclusion was in concordance with the change in the management for negative ECC- smears as proposed by the Bethesda 2001 System in which a negative ECC- smear is considered satisfactory. Based on the new insights provided by this and another important evaluation study from the Netherlands [1] as well as other international studies [2,3], early repeat testing for ECC- smears in the Netherlands has been abolished in 2002. This guideline was slightly adjusted in 2007. When the uterine cervix has not been visualized during smear taking or was found to be abnormal, a negative ECC- is considered inadequate and diagnosed as Pap 0 and repeated in short term (after 6 weeks). However, administrative failures in the reporting of the cervical status during smear taking in combination with a negative ECC- diagnosis, also leads to an inadequate test result (Pap 0). The impact of this policy-change should be monitored and evaluated closely as well as the outcome of inadequate test results that are based on the absence of transformation zone components combined with an abnormal or unseen / unreported cervical status. The increasing use of LBC may also have impact on the effectiveness of the adjusted guideline for ECC- smears, since some studies reported an increase in ECC- results after implementation of LBC. Most of these studies used a split-sample design in which a conventional smear is made first and the residual material is used for LBC subsequently [4-8] resulting in a possible disadvantage for LBC. In direct-to-vial studies this increase in ECC- was not seen [4,9]. However, our study on the accuracy and adequacy of the ThinPrep technique (NETHCON trial) found an increase in ECC- results when using LBC (results not yet published). Careful monitoring and evaluation of the effectiveness of this issue is strongly recommended.

The clinical relevance of normal endometrial cells in cervical smears of asymptomatic postmenopausal women – being equivalent with the older age-cohorts in the cervical screening program – is also disputed worldwide. Though a cervical smear is not a screening

tool for the detection of endometrial lesions, these lesions can be diagnosed occasionally through the finding of abnormal endometrial cells in the Pap test. This can be considered as an extra benefit of cervical cancer screening. The finding of normal endometrial cells in smears of women in their postmenopause however, especially when there are no clinical complaints such as abnormal bleeding, may lead to a diagnostic dilemma. In the Netherlands, reporting of normal endometrial cells is advocated. An additional remark for the clinician is given when normal endometrial cells are found in smears of postmenopausal women: 'in the presence of clinical symptoms that are associated with endometrial pathology, further gynecological investigation is advised'. In the 1996 guidelines this was underscored by the alerting Pap classification Pap3a. This policy was changed during 2004. Reporting of the alerting Pap3a classification was abandoned and replaced by a Pap1 classification. Even though the additional remark was maintained, an important signal to the clinician has been lost with the abolishment of the alarming Pap class, because many clinicians only look at the Pap-class of the test result. The Bethesda 2001 System also advocates reporting of normal endometrial cells in women older than 40 years of age with an optional educational comment that 'endometrial cells after menopause may be associated with benign endometrial changes, hormonal alterations, and, less commonly, endometrial abnormalities'. Clinical correlation is recommended. In chapter 3 it is shown that only very few asymptomatic postmenopausal women are diagnosed with normal appearing endometrial cells in their smear (0.2 %). In 6.5 % of these cases an endometrial (pre)-malignancy was found. As compared with women with smears without endometrial cells the Relative Risk was 40 times higher. These results would justify a direct referral to a gynaecologist since the number of women concerned is only very limited while the relative risk is significant. However, the number of cases in the study is limited which could diminish the validity of the results. Therefore it is recommended to perform a more extensive study, using the worldwide unique PALGA database, to come to a more definite conclusion whether it would be appropriate to refer these cases for gynaecological evaluation directly. In the mean time it would make sense to reconsider the reporting of the Pap3a diagnosis for these cases in order to provide the clinician with an extra alert. In view of new developments in the field of cervical screening it would be a loss if the incidental detection of endometrial lesions would be complicated by the introduction of new technology such as LBC or automated screening. The impact of these new technologies with regard to this issue should be evaluated carefully.

With the reorganization of the screening program in 1996, the cytological criteria for borderline abnormality were redefined. In the period before, the proportion of borderline cytology exceeded 10 % as inflammatory changes were classified Pap 2 as well. In 1996, the threshold for borderline abnormality was elevated in such way that inflammatory changes were classified as normal. As a result the frequency of borderline cytology decreased

sharply[10]. Along with the redefinition of the borderline abnormalities, the follow-up management was changed also. Where in former days women with borderline cytology were seen every 12 months until the abnormality progressed or normalized, women with an initial borderline abnormality have cytological triage after 6 months since 1996. Persistent borderline abnormalities are referred for colposcopy. In case the initial abnormality normalized after 6 months, a second repeat smear after the next 12 months is made. The expectation was that the cytological triage after 6 months would improve the predictive value and reduce unnecessary smear-taking and costs as compared to the old situation. On the other hand there was scepticism concerning the demand for colposcopy and effectiveness of this new follow-up guideline. The effectiveness depends on a high referral compliance and acceptable predictive value for significant cervical lesions. In chapter 4 we studied these items and found that the referral compliance was high, despite earlier scepticism. A minimum of 77.7 % of women with repeated borderline cytology was adequately referred for colposcopy, indicating that the management protocol had been accepted for the greater part by the general practitioners. The predictive value of repeated borderline cytology was found to be 25.2 % for CIN 1 or more severe and 10.2 % for CIN 2 or more. These figures are lower than generally found in studies performed outside the Netherlands and suggest that the accuracy of borderline cytology is lower as compared to other countries. Careful monitoring of the predictive value of borderline abnormalities and continuous education is of utmost importance for improving the predictive value without compromising the sensitivity of screening. In our study we found four cases of cervical carcinoma after repeated borderline cytology, emphasizing the significance of this cytological diagnosis. However, this was at the cost of a considerable level of over-diagnostics and over-treatment, harming many healthy women and leading to unnecessary costs. This could be improved by prioritizing women for colposcopy based on their age, clear-cut use of criteria for borderline cytology, higher thresholds for colposcopic directed biopsy, but most important, HPV triage eventually combined with other molecular testing. Recently negative HPV-triage has been implemented in the follow-up procedure for borderline and mildly dysplastic cytology. The follow-up smear after an initial borderline smear is allowed to be additionally HPV-triaged. LBC is especially attractive for this purpose, since next to cytological assessment the HPV test can be performed on the same cell sample. Women with HPV-negative smears and normalized cytology are referred back to the next round of the screening program. Women with HPV-negative smears and persistent borderline cytology are followed cytologically after 12 months instead of being referred to the gynaecologist. HPV-triage on the initial borderline smear is not allowed. This together illustrates the quickly changing landscape in which cervical cancer screening in the Netherland is performed. The effectiveness of the implementation of HPV-triage has to be monitored and compared to the earlier guideline. Optimal registration of the results of HPV-triaging is a prerequisite in which

PALGA should play a vital role. Attention should also be focused on continuing research on the additional value of other molecular biomarkers for optimizing the specificity of HPV-triage.

Liquid-based cytology, of which ThinPrep is one of the representatives, is widely practiced in the Netherlands. Nevertheless, use of LBC within the cervical screening program was not allowed until recently. The reason was the lack of sufficient evidence for the superiority of LBC as compared to conventional cytology. The conclusion of the evidence-based review 'CBO guideline cervical cytology' – on the applicability of automated screening, LBC and HPV-detection in the cervical screening program – which has been published in 2002, was that 'further evaluation of the costs and benefits of the ThinPrep method should be undertaken to decide definitively whether to implement this method in the Netherlands population screening program'. Several publications indicated that the absence of high-quality studies hampered drawing definitive conclusions on the accuracy and adequacy of LBC [11-13]. This was the motive to start a cluster randomized controlled trial in the Netherlands (Nethcon). This trial focuses on the accuracy, adequacy and cost-effectiveness of the ThinPrep technique in comparison to conventional cytology. In chapter 5 the cytological test positivity and inadequacy rates were studied. In chapter 6 the diagnostic accuracy of the ThinPrep method was investigated. The large-scale trial, that included almost 90,000 participants detected no difference in test positivity rates between LBC and conventional cytology. On the other hand a significant reduction in unsatisfactory rate for LBC was found. The results as such provided insufficient evidence to conclude that the accuracy of LBC and conventional cytology is equal. Correlation with a reference standard of the test positive cases is required. This was further studied in chapter 6. This was expressed in terms of ratios of verified histological detection rates and positive predictive values. No difference was found in histological detection rates nor in positive predictive values between the two study arms. Therefore, the diagnostic accuracy of ThinPrep as compared to conventional cytology is considered to be equal. As mentioned, LBC was found to result in reduced unsatisfactory rates. However, in the scope of the Dutch cervical screening program, this advantage of LBC is limited since the unsatisfactory rate of conventional cytology is already low. The main advantage of LBC in the setting of the Dutch cervical screening program is the availability of residual material for HPV testing and other molecular tests. This is of increasing concern since the role of HPV testing is expected to grow in the next years. LBC assists adjunctive testing and can achieve greater laboratory efficiency by increasing throughput as a result of a reduced screening time. HPV testing is presently implemented in the follow-up management of initially borderline and low-grade program smears as a negative triage tool. It is not unlikely that the results of presently running studies, which are focusing on combined HPV/cytology testing [14] and on reflex HPV triage of borderline and low-grade program smears will have consequences for the role of HPV-testing within the screening program in the near future. As

been mentioned before, other advantages of LBC comprise the reduced screening time and screening comfort for cytotechnologists and the positive experiences of general practitioners with LBC. Other new developments in cervical cancer screening profit from the increasing use of LBC too. LBC is especially suited for automated screening, a technique that will be used increasingly in the near future. A possible higher productivity and higher sensitivity for the detection of CIN by the combination of LBC and automated screening may prove to be favorable. However, future research should clarify whether implementation of this new technology combined with LBC in the screening program is accurate, adequate and cost-effective. In summary, accuracy of LBC is equal as compared with conventional cytology and implementation of LBC in the cervical screening program should therefore be based on cost-technical and other qualitative considerations and viewed in the perspective of other new developments in cervical screening.

Future perspective

Cervical cancer screening is a secondary prevention measure that has proven to be capable in effectively reducing the incidence and mortality of cervical cancer. However, a further reduction is hindered by issues of adherence, failures in the screening program and a limited sensitivity of the screening test. As a matter of fact, the most effective and challenging way to minimize the number of cervical cancer cases through screening still seems to be the improvement of adherence and coverage[15]. Besides that, skilled quality management is considered more important by some authors than novel screening approaches, especially in developing countries [16]. Nevertheless, new technology will unquestionably going to play a role in cervical cancer prevention in the near future.

Since the recognition of persistent infection with hrHPV as the necessary cause of cervical cancer and its precursor lesions, a strategy of primary prevention through prophylactic HPV vaccination has become available. Two prophylactic vaccines have been developed. Firstly the bivalent Cervarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) – protecting against infection from oncogenic HPV types 16 and 18 – and secondly the quadrivalent HPV vaccine Gardasil (Sanofi Pasteur MSD)), protecting against oncogenic HPV types 16 and 18 and low risk HPV types 6 and 11, which are associated with anogenital warts. HPV16 and 18 are considered to be responsible for approximately 70% of the cervical cancers. Thus a maximum reduction of 70% may be achieved. This can be accomplished only when vaccination coverage is high and women are vaccinated before their sexarche. The results of randomized, double-blind, placebo controlled phase III clinical trials show that the vaccines are highly immunogenic. The antibody titers persist for at least 7 years after vaccination. However, the long-term prophylaxis is not yet known. The necessity of booster injections can

only be assessed during long-term follow-up. Clinical trials show that the efficacy for protection against persistent HPV16/18 infections in HPV16/18 negative women amounts 90% or more [17]. Additionally, cross-protection against other hrHPV types, such as HPV31/45 has been reported. Protection against CIN was high but not 100%. However, it will take longer follow-up time before current clinical trials will be conclusive about efficacy of vaccination in reduction of cervical carcinoma. Possible threats for the effectiveness of vaccination is type replacement (replacement with other hrHPV types than the types that are targeted by the vaccine within the vaccinated population) and development of escape mutants (vaccine resistant mutant subtypes). Finally, long-term adverse effects are unknown and should be monitored carefully also. Despite these uncertainties The Dutch Health Council approved introduction of the HPV vaccine in the National Vaccination Program for 12-year old girls, starting in 2009 [18]. The preventive effect of HPV vaccination will not be seen within the next 10 to 15 years and will not eliminate screening requirements. Moreover, screening will remain important to protect vaccinated women against non-HPV16/18 hrHPV induced cervical carcinomas. However, deteriorated compliance for cervical screening may become a threat as women may assume to be fully protected by HPV vaccination. Self-sampling followed by HPV-testing might be an attractive option to maintain acceptable participation rates. Primary prevention through HPV vaccination will have a significant impact on the rate and distribution of abnormal cytology and thus on the secondary prevention strategy. Guidelines for cervical screening will need revision in long term and the optimal screening policy within the context of effective HPV vaccination will have to be assessed. The challenge will be to achieve a cost-effective synergy between HPV vaccination and cervical cancer screening.

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Summary

Cervical carcinoma is still one of the leading causes of death from cancer in women worldwide. In the Netherlands, the incidence and mortality from cervical carcinoma is among the lowest in Europe as a result of cervical cancer screening with the so-called Pap test. Cervical cancer screening has been organized in a national program in the Netherlands since 1988. The program consists of various stages including invitation, smear-taking, cell processing and microscopic evaluation by the laboratory, reporting, follow-up management and possibly treatment. Screen-detected premalignant cervical lesions can be treated effectively, thus preventing the development of cervical cancer. Persistent infections with high-risk human papilloma virus (hrHPV) types are a necessary cause for development of (pre)-cancerous cervical lesions. HPV infection is very common with a life time risk of 80%. However, most infections are transient and cervical cancer is a rare consequence of a hrHPV infection.

The goal of cancer screening is reduction of incidence and mortality of cancer. Cancer screening is most effectively performed within an organized setting with quality assurance at all levels and continuous monitoring of effects. However, a 100 % effectiveness has never been achieved in any screened population, despite the introduction of various policies and new technologies. Failures in any stage of the screening program results in a reduced effectiveness. This gives rise to several negative side-effects such as false reassurance in case of false-negative and to overtreatment, unnecessary anxiety and costs in case of false-positive tests. Therefore optimally performing screening tests and best possible follow-up protocols must be used and monitored. Nationally defined guidelines help to achieve a maximal reduction in the incidence of cervical cancer at the cost of a minimal number of women exposed to the negative side-effects of screening. Monitoring and evaluation of these guidelines are an essential component of quality assurance of cervical screening. Implementation of new tests or procedures should be evidence-based and preferably evaluated with randomized trials. The aim of this thesis is to evaluate existing guidelines for follow-up management on the one hand and the accuracy of liquid-based cytology (LBC) with the ThinPrep system, a new cytological test for cervical screening on the other hand with the objective to define recommendations for improvement of the quality of the Dutch screening program.

In **chapter 2** the effectiveness of the first guideline on the management of a negative (Pap1) test without endocervical cells (ECC-) from 1996 has been evaluated. The clinical relevance of ECC- results have been questioned in literature. Since the first follow-up guidelines adviced woman with a negative ECC- smear to have a repeat smear after 6 months, we could estimate the true prevalence of cervical lesions in women without endocervical cells in their smears as compared to women with endocervical cells by adding the results of short-term

follow-up of negative ECC- smears to the cross-sectional prevalence. The results show that the estimated true prevalence of squamous lesions in women with recent ECC- smears is significantly lower as compared with women with ECC+ smears and that there is no justification for these women at low risk to have a repeat test after 6 months. In accordance to the Bethesda 2001 System and based on new insights, the early repeat testing for ECC- smears in the Netherlands has been abolished in 2002 and the guideline on the management of ECC- smears was adjusted slightly again in 2007. Careful monitoring and evaluation of the effectiveness of this guideline is strongly recommended.

Chapter 3 describes a study on the reporting guideline for normal endometrial cells in asymptomatic postmenopausal women. The relevance is also disputed worldwide. The finding of normal endometrial cells in postmenopausal women without any clinical complaints may lead to a diagnostic dilemma. In the Netherlands an additional remark for the clinician is provided. The policy of an alerting Pap3a classification was changed during 2004 when this was replaced by a Pap1 classification. This was the reason why we started a study to determine whether postmenopausal asymptomatic women with normal endometrial cells in their smear are at higher risk for significant endometrial pathology as compared with women without these cells in their smears. We found a 40.2 times significant higher relative risk and therefore these cases should be reported to the physician with an explicit comment that this is an abnormal finding, possibly associated with significant endometrial pathology. However, the relative small number of cases may limit the validity of the results and it is recommended to perform a more extensive study to come to a more definite conclusion whether it is appropriate to refer these cases for gynaecological examination. In the mean time the reporting of these cases as Pap3a should be reconsidered.

The management guideline for repeated borderline abnormalities with respect to referral compliance and outcome is investigated in **chapter 4**. With the reorganization of the cervical screening program in the Netherlands in 1996, cytological criteria for borderline abnormality (Pap2) were redefined as well as the follow-up management. A conservative management with cytological triage after 6 months was chosen. Women with persistent or progressing borderline lesions are referred to a gynaecologist for colposcopic evaluation. Compliance with the referral guidelines and the outcome after repeated borderline abnormalities had not been evaluated. In this study we found that the referral compliance was high (78 %). The predictive value of persistent borderline abnormality was found to be 25.2 % for CIN 1 lesions or more severe and 10.2 % for CIN 2 or more severe abnormality. This is lower than usually found in other studies performed outside the Netherlands suggesting that the cytological accuracy of borderline cytology in the Netherlands is moderate. Careful monitoring of the predictive value of borderline abnormalities and continuing education of cytotechnologists

is crucial for improving the predictive value without compromising the sensitivity of screening. We suggest prioritizing women for colposcopy based on age, but more important is a clear use of criteria for borderline cytology, higher thresholds for colposcopic directed biopsy and HPV triage perhaps combined with other molecular testing on remaining cell material. With respect to HPV testing, use of liquid-based cytology (LBC) is especially attractive. Residual LBC material can be used for additional testing such as HPV or molecular testing. Negative HPV-triage is implemented recently in the follow-up management of borderline abnormality and mild dysplasia in the Netherlands. Monitoring of the effectiveness of this HPV-triage, a good registration of the HPV test results should be given attention in the near future. The additional value of molecular biomarkers is subject of continuing research worldwide and therefore not yet implemented in the Dutch guidelines.

In **chapter 5 and chapter 6**, the performance of LBC ThinPrep cytology as compared with conventional cytology as well as the relative accuracy of this screening test as applied within the Dutch screening program is evaluated. Until recently use of LBC within the screening program was not allowed because of insufficient evidence for the superiority of LBC compared with conventional cytology. The Nethcon cluster randomized controlled trial started in the Netherlands in 2003 with the objective to evaluate the accuracy, adequacy and cost-effectiveness of the ThinPrep technique in comparison to conventional cytology when applied in the Dutch cervical screening program. The trial included almost 90,000 participants from 246 randomized family practices. In **chapter 5** the cytologic test positivity rates of LBC was compared with conventional cytology in terms of crude and adjusted odds ratios in a per protocol analysis. Independent on the cytological cut-off for abnormality, no difference in cytological detection rates were found between LBC and conventional cytology. However, LBC did result in a statistically significant reduction of unsatisfactory smears. The actual relative accuracy of LBC can be assessed only after correlation of the test-positive cases with a reference standard. Generally, the reference standard for a cytological diagnosis is histology. Preferably this reference standard (histology) is blindly reviewed. This is studied in **chapter 6**. The diagnostic accuracy of LBC compared with conventional cytology is assessed and expressed in terms of ratios of verified histological detection rates and positive predictive values in a per-protocol analysis. The results show that there is no statistically significant difference in diagnostic accuracy between LBC and conventional cytology. Nevertheless, LBC has some important advantages: reduced unsatisfactory rates, availability of residual material for HPV testing and other molecular tests, reduced screening time and screening comfort for cytotechnologists and preference of the smear-takers for LBC. Besides that, LBC is especially suited for automated screening.

In the **chapter 7**, the results are discussed in general and placed in sight of emerging future developments within the Dutch cervical screening program. The recent availability of two prophylactic HPV vaccines will have a significant impact on future screening both worldwide as in the Netherland. Despite several limitations and uncertainties, HPV vaccination is promising in reducing the incidence of cervical cancer in future. Vaccination of 12 years old girls with the bivalent HPV vaccine (Cervarix) has started in the Netherlands in 2009. Even in vaccinated populations, screening still will have an important role, yet this will be in a revised design.

In summary, this thesis has shown that the field of cervical screening is changing very rapidly. Good monitoring and evaluation of every change is important as well as evaluation of new technology before introduction in the national screening program.

Samenvatting

Baarmoederhalskanker of cervixcarcinoom is wereldwijd nog steeds een van de belangrijkste oorzaken van sterfte aan kanker bij vrouwen. Nederland behoort bij de Europese landen waar de incidentie en sterfte aan baarmoederhalskanker het laagste is als gevolg van een screeningprogramma voor baarmoederhalskanker. Hierbij wordt gebruik gemaakt van de zogenaamde Paptest. Dit bevolkingsonderzoek (BVO) op baarmoederhalskanker wordt in Nederland sinds 1988 landelijk uitgevoerd en is nationaal georganiseerd. Het BVO is een ketenproces bestaande uit het uitnodigen van de vrouw, het maken van een uitstrijkje door de huisarts of de praktijkassistente, de beoordeling van het uitstrijkje door het laboratorium, rapportage van de uitslag via de huisarts aan de vrouw en eventueel vervolgonderzoek en soms behandeling door de gynaecoloog. Het doel van screening is het vroegtijdig opsporen van (pre)maligne afwijkingen van de cervix. Deze kunnen effectief behandeld worden waardoor de ontwikkeling van een cervixcarcinoom kan worden voorkomen. (Pre)maligne epitheliale afwijkingen van de cervix ontstaan als gevolg van een persisterende infectie met een hoogrisico humaan papilloma virus (hrHPV). Infectie met het HPV-virus komt erg vaak voor. Geschat wordt dat 80 % van de vrouwen ooit in haar leven besmet raakt met een HPV-virus. De meeste HPV infecties zijn voorbijgaand, maar een enkele keer persisteert de infectie en dit kan uiteindelijk resulteren in de ontwikkeling van (pre)maligne afwijkingen van de cervix. Dit wordt echter beschouwd als een zeldzame complicatie van besmetting met een hrHPV.

Het doel van screening op kanker is het reduceren van de incidentie en mortaliteit van deze ziekte. Deze is het meest effectief wanneer screening in een georganiseerde setting wordt uitgevoerd, met kwaliteitsbewaking op elk niveau van de keten en met continue monitoring van de effecten. Ondanks de toepassing van verschillende screening-strategieën en de introductie van nieuwe technologieën is het nog nooit gelukt om in een gescreende populatie 100 % effectiviteit te bereiken. Dit is het gevolg van fouten die kunnen plaatsvinden op elk niveau van het screeningprogramma. Dit resulteert in ongewenste negatieve bijeffecten, zoals een onterechte geruststelling bij een fout-negatieve en onnodige ongerustheid en overbehandeling in geval van een fout-positieve uitslag. Daarom is het van belang een zo betrouwbaar mogelijke screeningstest te gebruiken evenals de meest optimale follow-up protocollen. Nationale richtlijnen zijn essentieel in het vinden van een balans tussen maximale reductie van kanker ten koste van zo weinig mogelijk negatieve bijeffecten. Het monitoren en evalueren van deze richtlijnen zijn een essentieel onderdeel van de kwaliteitsbewaking van cervixscreening. Daarnaast moet de implementatie van nieuwe testen of procedures ‘evidence-based’ zijn en bij voorkeur onderzocht door middel van gerandomiseerde klinische trials. Het doelstelling van deze thesis is het evalueren van enkele van de bestaande follow-up richtlijnen en onderzoeken van de accuratesse van liquid-based cytology (LBC) ofwel dunnelaag cytologie met de ThinPrep methode. Dit moet

resulteren in aanbevelingen voor verdere verbetering van de kwaliteit van het Nederlandse bevolkingsonderzoek naar baarmoederhalskanker.

In **hoofdstuk 2** wordt de effectiviteit onderzocht van eerste richtlijn uit 1996 met betrekking tot de uitvoering van vervolgonderzoek na een negatieve (Pap1) uitstrijk waarin geen endocervicale cellen werden gevonden, een zogenaamde ECC- uitstrijk. De klinische relevantie van een ECC- uitslag zonder verdere afwijkingen is ondanks veel onderzoek nog steeds onduidelijk. De eerste richtlijn voor ECC- uitstrijken, met herhaling van het onderzoek na 6 maanden, biedt een goede mogelijkheid om de daadwerkelijke prevalentie van afwijkingen in vrouwen met een ECC- uitstrijk te schatten en te vergelijken met ECC+ uitstrijken. Dit is mogelijk door de resultaten van de korte termijn follow-up van ECC- uitstrijken toe te voegen aan de cross-sectionele prevalentie. De resultaten laten zien dat de geschatte prevalentie van plaveiselcellige afwijkingen bij vrouwen met een recente ECC- uitstrijk significant lager is in vergelijking met vrouwen met een recente ECC+ uitstrijk. Er bestaat derhalve geen goede rechtvaardiging om deze groep van laag-risico vrouwen een vervroegd herhalingsadvies te geven. Het vervroegde herhalingsadvies bij een ECC- uitstrijk is in 2002 afgeschaft maar in 2007 weer in geringe mate aangepast. Zorgvuldige monitoring en evaluatie van de effectiviteit van deze nieuwste richtlijn voor ECC- wordt aanbevolen.

In **hoofdstuk 3** worden de resultaten gepresenteerd van een studie over de richtlijn voor rapportage van normale endometriumcellen in uitstrijken van postmenopauzale vrouwen zonder klinische klachten. De relevantie van deze cellen is wereldwijd onderwerp van discussie. Deze cellen horen normaal niet voor te komen in uitstrijken van postmenopauzale vrouwen maar aangezien zij niet afwijkend zijn kan dit leiden tot een diagnostisch dilemma. In Nederland is het beleid om in deze gevallen een opmerking aan de uitslag toe te voegen dat deze bevinding niet normaal is. Tot 2004 werd in deze gevallen een Pap3a gegeven, daarna werd dit vervangen door een Pap1. Deze aanpassing van de richtlijn was de reden voor het onderzoek naar de waarde van normale endometrium cellen bij postmenopauzale vrouwen zonder klinische klachten. Dit onderzoek liet zien dat vrouwen in de postmenopauze bij wie normale endometriumcellen in de uitstrijk werden gevonden een 40.2 keer hoger relatief risico hadden op de aanwezigheid van (pre)maligne endometrium laesies. De conclusie is dat deze bevinding aan de clinicus moet worden gerapporteerd met de toevoeging van een expliciete opmerking dat dit een abnormale bevindingen is welke mogelijk geassocieerd is met significante endometrium pathologie. Een beperking van deze studie is het relatief kleine aantal cases hetgeen de validiteit verminderd. Een direct verwijsadvies van deze patiënten wordt om deze reden op dit moment nog niet aanbevolen anders dan nadat deze bevindingen in een grotere studie worden bevestigd. In de tussentijd

is het verstandig de afschaffing van de Pap3a uitslag te heroverwegen, teneinde de clinicus extra te attenderen.

De follow-up richtlijn met betrekking tot persisterende borderline afwijkingen (Pap2) wordt in **hoofdstuk 4** onderzocht. Tijdens de reorganisatie van het bevolkingsonderzoek in 1996 werd in Nederland, tegelijk met de cytologische criteria voor een Pap2, het follow-up beleid geherdefinieerd. Er werd in Nederland gekozen voor een conservatief follow-up beleid met cytologische triage na 6 maanden. Wanneer de afwijking persisteert of progressief is, wordt de vrouw naar de gynaecoloog verwezen voor verder onderzoek (colposcopie). De naleving van dit verwijsbeleid noch de uitkomsten hiervan waren sinds de invoering onderzocht. Uit deze studie blijkt dat de naleving van het verwijsadvies met 78 % hoog is. De positief voorspellende waarde van een persisterende Pap2 uitslag was 25,2 % voor CIN 1 of ernstiger en 10,2 % voor CIN 2 of meer. Dit is lager dan over het algemeen bij andere buitenlandse studies wordt gevonden. Dit suggereert dat de accuratesse van een Pap2 diagnose in Nederland matig is. Om de voorspellende waarde van de Pap2 diagnose te verbeteren zonder de sensitiviteit van de screening aan te tasten is deelname aan nascholingsprogramma's van cytologisch analisten van groot belang, evenals een nauwkeurige bewaking van de voorspellende waarde. Verder is priorering van vrouwen op basis van leeftijd een mogelijkheid. Echter, een meer stringent gebruik van de criteria voor Pap2, het gebruik van een hogere drempelwaarde voor colposcopisch geleide biopten door de gynaecoloog of toepassing van HPV triage, eventueel gecombineerd met moleculair onderzoek van het restmateriaal van de uitstrijk ligt meer voor de hand. Gebruik van dunnelaag cytologie heeft een groot voordeel boven de conventionele uitstrijk met betrekking tot de uitvoering van aanvullende testen zoals HPV- en moleculaire testen. Na het maken van een cytologisch preparaat kan het overgebleven materiaal voor aanvullende testen worden gebruikt. Negatieve triage met behulp van een HPV test is sinds kort in Nederland toegestaan in het follow-up traject van geringe afwijkingen. De effectiviteit van deze nieuwe triage methode moet goed gecontroleerd worden. Verder is een goede registratie van de resultaten van de uitslag van de HPV test van groot belang. Onderzoek naar de toegevoegde waarde van het gebruik van moleculaire biomarkers bij de triage van cytologisch geringe afwijkingen is momenteel wereldwijd nog onderwerp van studie en in Nederland daarom nog niet vastgelegd in de richtlijn.

Hoofdstuk 5 en 6 handelen over de prestatie van LBC ThinPrep cytologie in vergelijking met conventionele cytologie. Tevens wordt de relatieve accuratesse van de ThinPrep methode onderzocht indien deze wordt toegepast binnen het Nederlandse bevolkingsonderzoek naar baarmoederhalskanker. Dit was tot voor kort niet toegestaan omdat er onvoldoende bewijs was voor de geclaimde superioriteit van LBC. Daarom werd in Nederland in 2003 de Nethcon

gerandomiseerde gecontroleerde trial gestart met als doel de accuratesse, geschiktheid en kosten-effectiviteit van de ThinPrep methode te vergelijken met conventionele cytologie. Voor de trial werden 90.000 deelneemsters geïncludeerd afkomstig uit 246 gerandomiseerde huisartspraktijken. In **hoofdstuk 5** wordt de cytologische test positiviteit van ThinPrep door middel van een per protocol analyse vergeleken met conventionele cytologie, uitgedrukt in ruwe en gecorrigeerde odds ratio's. Er wordt voor geen enkel cytologisch afkappunt verschil in cytologische test positiviteit gevonden tussen de twee technieken. De ThinPrep LBC techniek geeft wel significant minder uitstrijken van onvoldoende kwaliteit. De werkelijke relatieve accuratesse van LBC ten opzichte van conventionele cytologie kan alleen worden bepaald als de test-positieven worden vergeleken met een referentie standaard. Als referentie standaard voor cytologie wordt over het algemeen histologie gebruikt. Deze referentie standaard (histologie) is dan bij voorkeur blind gereviseerd. De relatieve accuratesse van LBC wordt onderzocht in **hoofdstuk 6**. Deze wordt uitgedrukt in ratio's van de geverifieerde histologische detectie percentages en de positief voorspellende waarden in een per protocol analyse. De resultaten tonen aan dat er geen statistisch significant verschil is in diagnostische accuratesse tussen ThinPrep en conventionele cytologie. Desalniettemin blijkt het gebruik van de ThinPrep methode diverse andere voordelen te hebben. Het percentage onvoldoende kwaliteiten is lager dan bij conventionele cytologie, er is rest materiaal beschikbaar voor de uitvoering van een (reflex) HPV test of andere moleculair biologische test, de tijd van screening is korter, het screenen van een dunnelaag preparaat is voor de cytologisch analist veel comfortabeler en het gebruik van LBC heeft de sterke voorkeur van de praktijkassistenten en de huisartsen. Daarnaast is dunnelaag cytologie bij uitstek geschikt voor computer ondersteund screenen.

In **hoofdstuk 7** worden de resultaten van de verschillende hoofdstukken in bredere zin bediscussieerd in het licht van toekomstige ontwikkelingen binnen het bevolkingonderzoek baarmoederhalskanker in Nederland. De recente beschikbaarheid van twee profylactische HPV vaccins zal wereldwijd en in Nederland een grote invloed hebben op de toekomstige screening naar baarmoederhalskanker. Ondanks verschillende beperkingen en allerhande onzekerheden is HPV vaccinatie veelbelovend met betrekking tot een verdere reductie van de indicentie van baarmoederhalskanker in de nabije toekomst. In het voorjaar van 2009 is in Nederland gestart met de vaccinatie van meisjes van 12 jaar met één van de beschikbare HPV vaccins welke is gericht tegen 2 hrHPV typen. Desalniettemin zal ook in een gevaccineerde populatie screening op baarmoederhalskanker nodig blijven, zij het in een aangepaste vorm.

Samengevat laat deze thesis zien dat het gebied van cervix screening onderhevig is aan snelle veranderingen waarbij een goede monitoring en evaluatie van elke aanpassing van

groot belang is, evenals onderzoek naar nieuwe technieken, voordat deze worden geïntroduceerd in het landelijke screening programma.

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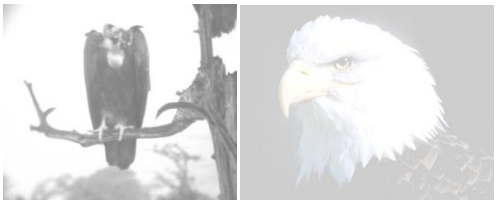
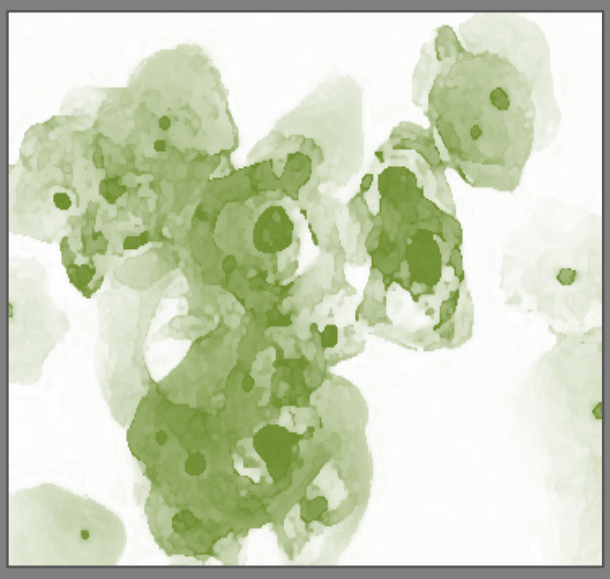
Curriculum Vitae

Bert Siebers werd geboren op 23 januari 1963 als zoon van Henk Siebers en Jenny Hasselo te Almelo alwaar hij zijn jeugd doorbracht. In 1982 behaalde hij zijn VWO-diploma aan de Almelose Rijksscholengemeenschap Erasmus. De opleiding HLO werd in 1982 gestart in Hengelo en Enschede maar in 1983 verliet hij zijn Twentse vaderland en vervolgde zijn opleiding in Nijmegen. In 1986 werd het diploma HLO cyto-histologie behaald, waarna hij zijn dienstplicht vervulde bij de Terreinmeetkundige Dienst van de Veldartillerie van de Koninklijke Landmacht. In 1987 startte hij zijn carrière als cytologisch analist op het laboratorium voor cytopathologie van de afdeling Pathologie van het UMC St Radboud. In 1989 begon hij aan de Katholieke Universiteit Nijmegen met de doctoraalstudie Gezondheidswetenschappen, afstudeerrichting Toxicologie, begonnen waarvoor hij in 1992 afstudeerde. Van 1992 tot 1996 was hij werkzaam bij het Screening Center Nijmegen en van 1996 tot heden keerde hij terug in de schoot van de afdeling Pathologie van het UMC St Radboud. Zijn functie ontwikkelde zich van cytologisch analist via 'Coördinator BVO' naar 'Beleidsmedewerker BVO/Coördinator informatisering en automatisering medisch inhoudelijke en administratieve zaken' en uiteindelijk tot 'Junior onderzoeker/Stafmedewerker Pathologie' waarbij werd gewerkt aan diverse onderzoeksprojecten. In deze tijd werd de fundering voor dit proefschrift gelegd.

Hij is sinds 1993 getrouwd met Rosan Kelly en samen hebben zij 3 kinderen gekregen: Isabel (1996), Carmen (1997) en Arthur (1999).





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