Cervical cancer, quality issues in early detection and prognostic factors

Afra Zaal

Colofon

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Baarmoederhalskanker,
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(met een samenvatting in het Nederlands)

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Voor mijn ouders

Contents

Part II Prognostic factors in cervical cancer

1

General introduction

Etiology of cervical cancer

hrHPV infection

Cervical cancer is known to be caused by high-risk human papillomavirus (hrHPV) induced malignant transformation of cervical epithel-ium, and is preceded by cervical intraepithelial neoplasia (CIN)[1]. This precursor lesion will regresses spontaneously in the majority of women (Figure 1) when HPV is cleared, but 20% of women will have a persistent infection, of which 1-3% might ultimately develop into cervical cancer[2].

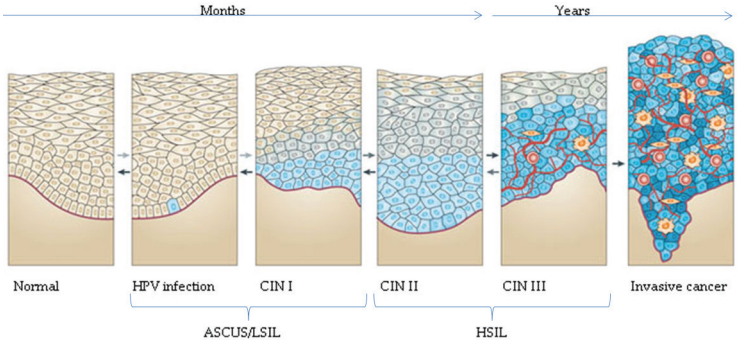


Figure 1. Schematic overview of progression and regression of cervical lesions in time (adapted from Nature Reviews Cancer).

The lifetime risk of cervical HPV infection is at least 80%[3]. The time between diagnosis of a high-grade (CIN2 or 3) cervical lesion to the diagnosis of cancer is largely unknown. The most accurate data we have is from an unethical observational study in which treatment was withheld from women with large CIN3 lesions. In this study, 31.3% of the CIN3 lesions progressed to cervical cancer within 30 years, as opposed to a cancer risk of 0.7% in women with adequate initial and follow-up treatment[4,5]. Current estimates of this lead time are based on cross sectional data from screening programs, showing a large variation in reported mean time spans (11.8 to 16 years)[6-8]. A recent mathematical model has incorporated the natural history of HPV infection and the time to development of CIN and has shown a median of 23.5 years between development of CIN2 or 3 and establishment of cervical cancer. In this model 1.6% of the lesions progressed to cancer within 10 years[9].

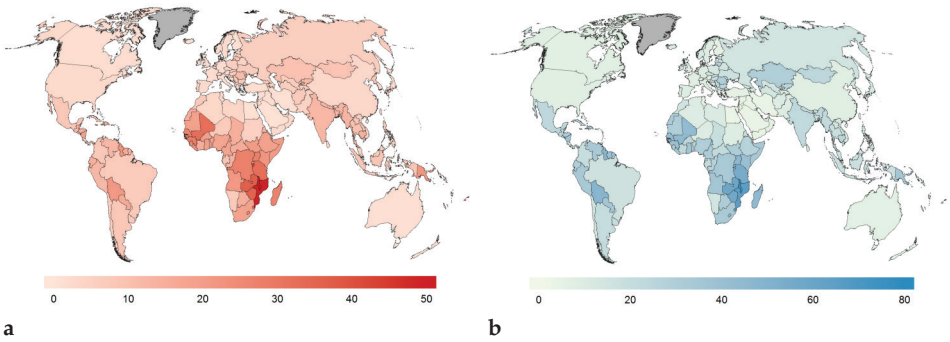
The human papillomavirus is an alpha papillomavirus, a double strain DNA virus consisting of early and late coding regions, the most important being the early 6-, and 7 coding regions. These coding regions are oncogenes and are responsible for the inactivation or loss of heterozygosity of the retinoblastoma (Rb) and p53 tumor

suppressor genes[10,11]. The late regions, which translate to capsid proteins, are used as a target in polymerase chain reaction (PCR) based tests to detect the HPV virus.
In 1970 the link between cervical cancer and a viral infection was first discovered, and in 2008 Professor dr. Harald zur Hausen received the Nobel prize for his research on the topic. Since then, a wide variety of HPV strains have been discovered, and a classification of high and low risk types has been established. Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 68, 73, and 82 are the currently known carcinogenic types[12].
The close relation between HPV infection and cervical pathology has created a window of opportunities for research on cervical cancer screening, prevention of disease and novel therapeutic agents.

Cofactors important for the development of cervical cancer
An important carcinogenic cofactor is tobacco use, which causes oxidative stress and thereby DNA damage[13]. Among other effects, smoking can cause inactivation of the fragile histidine 6 tumor suppressor gene[14], similarly to tobacco associated lung cancer. Also, it causes a decrease in epithelial cell-mediated immune response[15,16]. Furthermore, nicotine enhances cellular proliferation *in vitro*, suggesting that in an established tumor it might favor rapid tumor growth and lymphangiogenic spread[17]. Smoking cessation is effective but it takes up to 20 years for the effects on the cervical epithelium to be resolved[18].
A second important cofactor for the development of cervical cancer is infection with the human immunodeficiency virus (HIV), a risk that is correlated to the degree of immunosuppression. Eventually, HIV-positive women have a 9-fold increased risk of developing cervical cancer once they are diagnosed with AIDS[19,20], therefore the Center of Disease Control designated cervical cancer as an AIDS-defining condition in 1993[21].

Epidemiology of cervical cancer

Cervical cancer is the third most common type of cancer in women worldwide (after breast and colorectal cancer), with an estimated number of 528.000 newly diagnosed patients and 266.000 cancer deaths in 2012[22]. The vast majority (more than 85%) of cases occur in developing countries, reaching an age-standardised incidence rate (ASR) of 42.7 per 100.000 in Eastern Africa (Figure 2). Furthermore, there is an 18-fold difference in mortality rate across the world, reaching a devastating number of 27.6 deaths from cervical cancer per 100.000 in Eastern Africa. Unfortunately, the incidence of cervical cancer goes hand in hand with the worldwide AIDS epidemic.



a b

Figure 2. Estimated age-standardized incidence (a) and mortality rates (b) of cervical cancer per 100.000 women. Globocan cancer statistics 2012 (http://globocan.iarc.fr).

In the European Union it was the sixth most common type of cancer in women in 2012 (after breast, colorectal, lung, uterine and skin cancer), with an ASR of 9.6 per 100.000 and a mortality rate of 2.8 per 100.000[22]. In The Netherlands, in 2012, there were 735 new cases, and 215 patients died of the disease[23]. This number has decreased impressively after the introduction of cervical cancer screening in The Netherlands in the early 1970s, but has been stable in the last decade (Figure 3). Importantly the mortality from cervical cancer has not diminished either over the last decade.

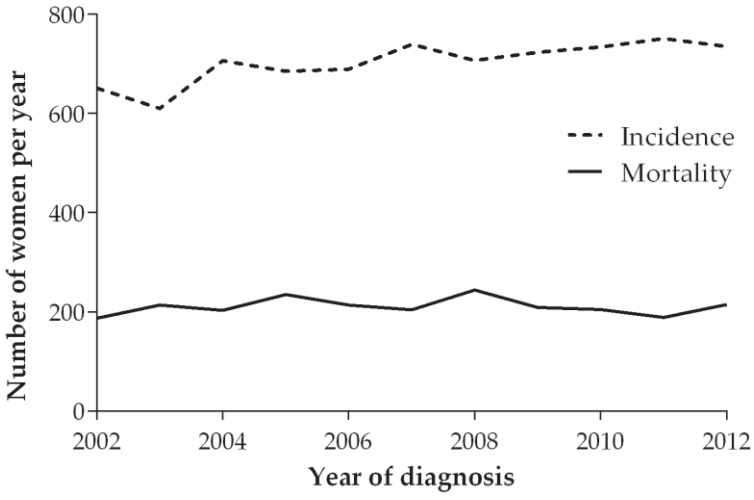


Figure 3. Number of new cervical cancer cases and deaths in The Netherlands per year (data from Netherlands Cancer Registry managed by Comprehensive Cancer Centre The Netherlands, 2014).

Cervical cancer prevention in The Netherlands

Primary prevention

A number of prophylactic and therapeutic vaccines have been developed against high risk HPV strains, and are currently implemented in clinical practice. In The Netherlands a program has been implemented in 2009 in which all 12-year old girls are vaccinated with a vaccine that protects against hrHPV types 16 and 18 (Cervarix©, GSK). The first effects of this vaccination on cervical cancer incidence are expected around 2024, since the process from first infection with the HPV virus to the diagnosis of cervical cancer takes at least 11.8-23.5 years[6-9].

Current cervical cancer screening

In The Netherlands a cervical cancer screening program is in place in which cervical cytology sampling is performed 5-yearly in women aged 30 to 60 years. The cytological smear (also known as the Pap smear) was named after Dr. Papanicolaou who demonstrated in 1941 that cervical cancer could be detected by this method[24]. The sensitivity of cervical cytology to detect high-grade premalignant cervical lesions is low (60 to 70%)[25-27], and much effort has been put into improving this sensitivity.

In 1996 the CISOE-A classification system was implemented in The Netherlands, which resulted in a substantial increase in quality and efficacy of screening[28].

Introduction of this system resulted in a decrease in the number of equivocal results and repeat recommendations, without a decrease in detection of high-grade lesions. The letters C (composition), I (inflammation), S (squamous), O (other and endometrium), E (endocervical cylindrical epithelium status) and A (adequacy) are used to indicate the composition of the smears. Each letter is scored with a number, corresponding to speficic information (Table 1), which is then translated to a pap score result (Table 2)[28].

This CISOE-A classification system can be easily translated into the British National Health Service (NHS) BSCC classification and the Bethesda system as used by the American Society for Colposcopy and Cervical Pathology (ASCCP) (Table 2)[28,29].

There is an algorithm available to triage women according to their cytology result. In short, women with a worse than borderline or mild dyskaryotic (>BMD) or twice a BMD cytology result are referred to a gynecologist. There is a second algorithm available incorporating the result of hrHPV testing. The main difference with the previous algorithm is that women with a BMD smear and a positive hrHPV test are directly referred to the gynecologist[30].

Table 1. Overview of the Dutch CISOE-A classification(Adapted from J Clin Pathol. Apr 2004; 57(4): 388–393, The Dutch CISOE-A framework for cytology reporting increases efficacy of screening upon standardization since 1996, S Bulk et al.)

Score	C	I	S	O	E
	Composition	*Inflammation*	*Squamous epithelium*	*Other, and endometrium*	*Endocervial columnar epithelium*
0	Inadequate	Not applicable	Not applicable	Not applicable	Not applicable
1	Endocervical epithelium	Viral infection	Normal	No other abnormalities	Normal
2	Squamous metaplastic cells	*Trichomonas vaginalis*	Abnormal squamous epithelial cells	Epithelial atrophy	No endocervical cells present
3	Endometrium	Bacterial infection	Atypical squamous metaplasia	Atypical repair reaction	Some atypical endocervical cells
4	Endocervical epithelium and squamous metaplastic cells	*Candida albicans*	Mild dyskaryosis	Mildly atypical endometrium	Mildly atypical endocervical epithelium
5	Endocervical epithelium and endometrium	*Haemophilus vaginalis*	Moderate dyskaryosis	Moderately atypical endometrium	Moderately atypical endocervical epithelium
6	Squamous metaplastic cells and endometrium	no inflammation	Severe dyskaryosis	Severely atypical endometrium	Severely atypical endocervical epithelium
7	Endocervical epithelium, squamous metaplastic cells, and endometrium	*Actinomyces israelii*	Carcinoma in situ	Adenocarcinoma endometrium	Adenocarcinoma in situ endocervical epithelium
8	Solely squamous epithelium	*Chlamydia trachomatis*	Microinvasive carcinoma	Metastasis malignant tumor	Not applicable
9	Not applicable	non-specific inflammation	Invasive squamous carcinoma	Not applicable	Adenocarcinoma endocervix

The smears are examined for five different aspects of the composition of the smear, and a score is assigned leading to the CISOE code. "A" indicates adequacy of the smear, which is graded as 1–3; 1, adequate; 2, adequate but suboptimal (reason specified by cytotechnologist); 3, inadequate.

Table 2. The Dutch CISOE-A classification compared with the Bethesda 2001 and Pap classifications. (Adapted from J Clin Pathol. Apr 2004; 57(4): 388–393, The Dutch CISOE-A framework for cytology reporting increases efficacy of screening upon standardization since 1996, S Bulk et al.)

CISOE-A			**PAP**		**Bethesda 2001**	
S	O	E			Epithelial	Glandular
0	0	0	0	Inadequate	Unsatisfactory for evaluation	
1	1	1–2	1	Normal	no intraepithelial lesion	
1	2	1–2	1	Normal	Atrophy, no intraepithelial lesion	
2–3	3	3	2	Borderline dyskaryosis	ASCUS/ASC-H	AGC
4	4	4	3a1	Mild dyskaryosis	ASC-H/LSIL	AGC favor neoplastic
5	5	5	3a2	Moderate dyskaryosis	HSIL	AGC favor neoplastic
6	6	6	3b	Severe dyskaryosis	HSIL	AGC favor neoplastic
7	–	7	4	Carcinoma in situ	HSIL	AIS
8–9	7–8	9	5	Carcinoma	Squamous cell carcinoma	Adenocarcinoma

S (squamous), O (other and endometrium), and E (endocervical cylindrical epithelium status).

hrHPV-based cervical cancer screening

In 2016, a novel cervical cancer screening program will be implemented in The Netherlands, using primary hrHPV screening and secondary cytology testing (to triage hrHPV positive women)[31]. This program will comprise five screening rounds at 30, 35, 40, 50 and 60 years of age (except for those who tested positive for hrHPV with normal cytology in the 5 years before, who will be retested at the age of 45, 55 and/or 65).

The decision of the Dutch Minister of Health to change the screening program was based on extensive research over the last decades. Large randomized trials have evaluated the performance of hrHPV testing[32-41] and show that hrHPV testing detects 30% more CIN2 and 3 lesions and cervical cancer (CIN2+). Furthermore, these trials report a lower (approximately 50%) detection rate of CIN3 lesions and cancer (CIN3+) in the following screening round among hrHPV negative women compared to women with normal cytology. The lower detection rate in the second round reflects earlier detection, rather than more detection of CIN lesions with this new approach. Lastly, in a recent trial with long-term follow-up it was shown that hrHPV-based screening provides better protection against cervical cancer than cytology[42].

It has been calculated[31] that implementation of this program will lead to an increase in referral of patients to the gynecologist for colposcopy from 3900 in the current, to 5200 patients per year in the novel program. Thus colposcopic examination by the

gynecologist is, and will become an even more important step in the efficacy of the cervical cancer screening program.

Colposcopy

In the early 1920's Professor dr. Hans Hinselmann (1884–1959) developed a technique to visualize the uterine cervix, and derived its name from the Ancient Greek words kolpos (κόλπος: tunic or vagina) and skopeo (σκοπέω: to regard or to look). Colposcopy is a visual technique used to examine the cervix, vagina and vulva with use of the application of acetic acid, which requires extensive training and experience. It has been shown, however that even highly experienced colposcopists do not perform better at colposcopy than averagely trained colleagues[43,44]. Colposcopy remains a subjective technique with a an average sensitivity to distinguish low- from high-grade lesions and cancer of around 55%[43,45-48]. This low sensitivity is associated with a high degree of inter- and intra-observer variability[49-51].

Over the years the colposcopic device has greatly been improved (Figure 4), and currently there are several available alternatives to conventional colposcopy, such as electrical impedance spectroscopy, digital imaging, optical coherence tomography, confocal microscopy and computer analysis coupled to recorded lesions[52,53]. These novel colposcopy devices and techniques have been developed to establish optimal performance of colposcopy with low inter- and intra-observer variability.

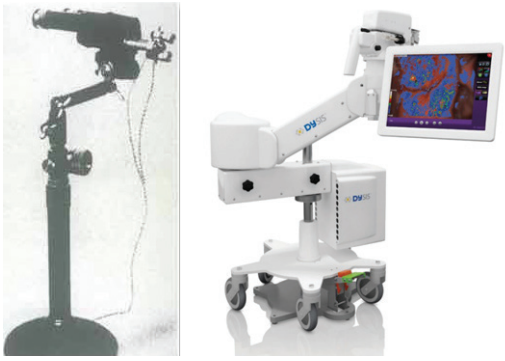


Figure 4. First colposcopy device as used by Prof. Hinselmann (left, printed from the cervical pathology textbook and atlas, Burghardt 1991) and novel digital spectral imaging device (right, DySIS Medical Ltd).

Clinical studies with the dynamic spectral imaging (DySIS) colposcope (DySIS™, Dynamic Spectral Imaging System; Forth Photonics, Livingston, UK) have shown promising results[54-57]. This spectral imaging system is developed to quantify optical features after application of acetic acid, in order to increase the objectivity of colposcopy and improve the detection of high-grade lesions.

The accuracy of histology (the 'gold standard' test for colposcopy) is hindered by the inconsistency in histological diagnoses among pathologists and the obvious sampling errors due to inaccurate colposcopic mapping of the lesion and tissue sampling[58-60]. Consequently, improvement of the sensitivity of colposcopy by minimising inter- and intra-observer variability would constitute a major improvement in the diagnosis of cervical cancer.

Cervical cancer staging

Cervical cancer staging dates back to 1928. Since then there have been various changes in the system, but the aim remains the same, which is having a uniform staging instrument that can be applied worldwide (even in low resource settings) to ensure optimal treatment, and offer possibilities for epidemiological evaluation and research (Table 3)[22,23,61].

Table 3. FIGO staging of cervical cancer (adapted from Corrigendum to "Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium" International Journal of Gynecology and Obstetrics 108 (2010) 176, Sergio Pecorelli)

Stage I The carcinoma is strictly confined to the cervix (extension to the corpus would be disregarded)	
IA	Invasive carcinoma diagnosed only by microscopy, invasion ≤5.0 mm and extension ≤7.0 mm
IA1	Stromal invasion of ≤3.0 mm in depth and horizontal extension of ≤7.0 mm
IA2	Stromal invasion of >3.0 mm and not >5.0 mm with an extension of not >7.0 mm
IB	Clinically visible lesions limited to the cervix uteri or pre-clinical cancers greater than stage IA
IB1	Clinically visible lesion ≤4.0 cm in greatest dimension
IB2	Clinically visible lesion >4.0 cm in greatest dimension
Stage II Cervical carcinoma invades beyond the uterus, but not to the pelvic wall or to the lower third of the vagina	
IIA	Without parametrial invasion
IIA1	Clinically visible lesion ≤4.0 cm in greatest dimension
IIA2	Clinically visible lesion >4.0 cm in greatest dimension
IIB	With obvious parametrial invasion
Stage III The tumor extends to the pelvic wall and/or involves lower third of the vagina and/or causes hydronephrosis or non-functioning kidney	
IIIA	Tumor involves lower third of the vagina, with no extension to the pelvic wall
IIIB	Extension to the pelvic wall and/or hydronephrosis or non-functioning kidney
Stage IV The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum.	
IVA	Spread of the growth to adjacent organs
IVB	Spread to distant organs

Despite an extensive cervical screening program, and a decrease in incidence of cervical cancer, the incidence of advanced cervical cancer has not diminished over the years (Figure 5, data from The Netherlands Cancer Registry managed by Comprehensive Cancer Centre The Netherlands, 2014). Thus, despite an effective screening program, cervical cancer is still diagnosed in late-stages of disease in a substantial number of patients.

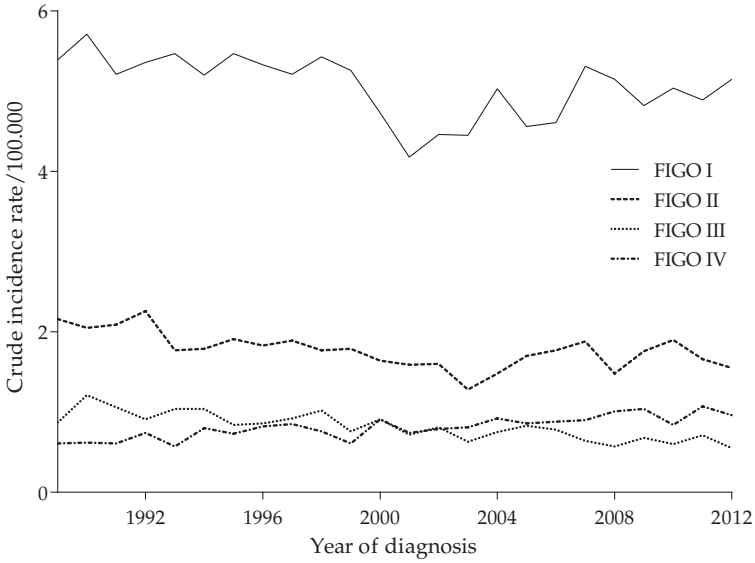


Figure 5. Crude incidence rate of cervical cancer per FIGO stage category and per year of diagnosis (data from Netherlands Cancer Registry managed by Comprehensive Cancer Centre The Netherlands, 2014).

Prognostic factors in cervical cancer

Lymph node metastasis

It is important to assess the lymph node status in cervical cancer, as it is a negative prognostic factor for survival[62] and it determines the choice of initial therapy, as well as the need for adjuvant treatment[63]. Currently, the gold standard for assessing the nodal status in cervical cancer is systematic pelvic lymph node dissection (LND)[64]. Such an extensive lymphadenectomy leads to lymphocyst formation in about 20% and to lymphedema in approximately 10% of patients with FIGO IB to IIA disease[65-67]. Cervical cancer is known to spread to the pelvic lymphatic system via the first draining lymph node, the sentinel lymph node (SN)[68]. If this node is tumour free, the other draining lymph nodes (non (n)SNs) are assumed not to contain tumour.

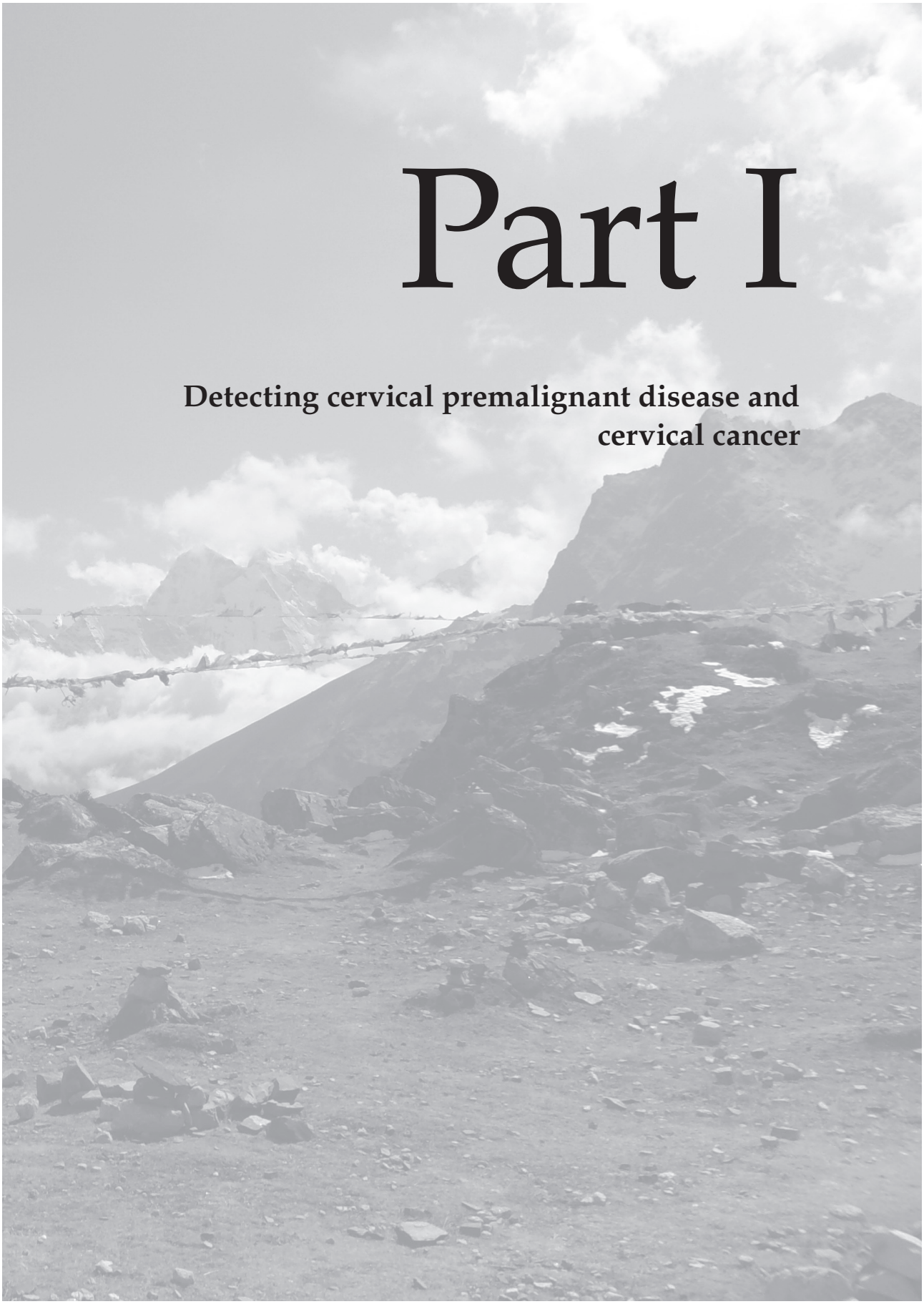
Though morbidity rates have decreased with implementation of laparoscopic lymphadenectomy[69], to further minimise these complications the sentinel node (SN) biopsy is currently being evaluated for adoption as the standard of care in early-stage cervical cancer[70,71]. This procedure entails detection and excision of the SN after submucosal injection into the cervix of a radioisotope tracer alone or combined with blue dye[72]. Essential in this procedure is optimal histopathological evaluation of the SN by serial sectioning and immuno-histochemistry (IHC)[73,74]. This technique has the added advantage of reliable detection of low-volume disease (LVD; micrometastasis (0.2-2 mm) or isolated tumour cells (<0.2 mm))[75].
Compared to pelvic LND, SN biopsy increases the detection rate of metastases up to 2.8 fold partly because of detection of aberrantly located nodes[76-78] and because of ultrastaging of the lymph nodes[79].

Serum markers
Cervical cancer disease recurrence is an important cause of cervical cancer mortality and morbidity[80]. The risk of recurrence ranges from 10-20% for stage IB1/IIA up to 72% for women with a stage IVA cervical tumor[81,82]. Early detection of recurrence is desired since cure may still be possible in selected cases with local and/or central recurrence[80,83,84].
Follow-up surveillance schemes commonly entail 5 years of ambulant visits with increasing time intermissions. To improve the efficacy of these follow-up programs, a range of serum biomarkers have been investigated. In these predictive studies, multiple outcome variables have been studied, including levels prior to clinical manifestation, detection of recurrence, disease specific and overall survival[85-96]. Furthermore, in the majority of these studies only a single marker is tested with an inherent heterogeneity between studies in design, populations, data collection and analysis. This hinders evaluation of combinations of promising biomarkers, and inter-study comparisons on their clinical efficacy.

Aims and outline of this thesis

Part One: First aim was to investigate the current and a novel colposcopy device in detecting premalignant cervical disease, and to describe the current situation in a subset of the Dutch population.
Firstly the quality of care indicators and treatment outcome of five Dutch colposcopy clinics are described (**Chapter 2**). Secondly, the clinical performance of a digital colposcopy device is described in a prospective trial (**Chapter 3**)**.** This study cohort is further analyzed in **Chapter 4,** showing a detailed analysis of a subset of patients with a specific HPV infection.
Chapter 5 depicts an in-depth analysis of the diagnostic process followed in a cohort of patients with cervical cancer.

Part Two: Second aim was to investigate prognostic factors once the diagnosis of cervical cancer has been established.
Chapter 6 describes the value of performing a lymph node dissection in addition to the sentinel node procedure. **Chapter 7** analyzes the prognostic utility of a panel of serum markers tested in a cohort of cervical cancer patients with extensive follow-up. Finally, in **Chapter 8** the results and conclusions of the conducted studies are discussed, and analyzed in the frame of the novel nationwide screening for cervical cancer.



Part I

Detecting cervical premalignant disease and cervical cancer

2

Quality assessment of colposcopy and subsequent treatment: poor recognition of cervical cancer

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Abstract

Objective: We aimed to assess quality of care with regard to recognition of cervical cancer, hospital access time and adherence to follow-up. Furthermore, a comparison between direct treatment ('see-and-treat') and colposcopically guided biopsy followed by treatment (two-step approach) was made.

Methods: This is a retrospective cohort study in five Dutch colposcopy clinics with 532 patients with a first colposcopy in 2007 because of abnormal cytology. Details on colposcopic impression, follow-up and all cytology and histopathology results were retrieved. Data were analyzed using SPSS.

Results: 233/532 (88.3%) of patients had a colposcopy within 10 weeks after index cytology. Sensitivity of colposcopy for the detection of CIN2+ lesions was 73.7%, and performing a biopsy increased sensitivity to 84.8%, with highest histology in the study period as gold standard. Treatment was performed in 338 (63.5%) of the patients, being 'see-and-treat' in 115 (21.6%) and two-step approach in 223 (41.9%) patients. These procedures did not differ in rate of overtreatment, incidence of invasive cervical cancer and recurrence. Only 108 (34.2%) patients had completed follow-up with a total of three smears at 6, 12 and 24 months after treatment. In total, 18 (3.6%) patients were diagnosed with invasive cervical cancer, of which only 5 (28%) were recognized at colposcopy.

Conclusions: Overtreatment rate, incidence of invasive cervical cancer and the number of recurrences or residual disease seem to be similar for 'see-and-treat' and two-step colposcopy with biopsy followed by treatment approach. Therefore, 'see-and-treat' is an optional approach for women with a suspected high-grade lesion and no wish to conceive. Follow up after treatment in this clinical cohort was very low, and asks for simplified post treatment monitoring. The finding that only 28% of the cervical cancer cases were recognized at colposcopy leads us to conclude that cancer recognition is poor and needs improvement.

Introduction

Cervical cancer has a well-defined precursor lesion, cervical intraepithelial neoplasia (CIN), which can be detected through cervical cytology sampling with or without testing for high-risk human papillomavirus (hrHPV). The national cervical cancer screening program in The Netherlands used cytology sampling 5-yearly in women aged 30 to 60 years. The efficacy of this screening program depends on a variety of factors, such as sensitivity of cytology or hrHPV detection, attendance rate of women, adherence to referral advice and efficacy of the gynecological examination and treatment.

The sensitivity of cervical cytology to detect high-grade premalignant cervical lesions is between 60 and 70%[25-27]. The attendance rate of the screening program in The Netherlands was 66% from 2004 through 2008. The five-year coverage of the target population (women aged 30 to 60) reached 78% in 2006[97] if opportunistic screening outside the screening program was included. The 2008 evaluation of the national cervical screening program showed that 0.7% of the screened women were directly referred to a gynecologist because of the smear result, worse than borderline or mild dyskaryosis (>BMD), and 2.5% were advised to repeat cytology because of a BMD result (of whom 31.9% were eventually referred because of persistent abnormal cytology). Of all women referred to the gynecologist, 89.5% actually turned up for colposcopy in 2008[98].

At the referral visit, colposcopic examination is performed including a colposcopically guided biopsy, endocervical sampling or curettage (ECC), or direct treatment of the lesion, depending on the colposcopic impression and local protocol. Based on these results, the colposcopist decides to perform (secondary) treatment or follow-up. Colposcopy is a subjective method with a sensitivity of approximately 55%[43,46,47], and much effort has been put into optimizing this performance rate by adequate instruction through national courses and the introduction of novel techniques, such as digital colposcopy[53,57,99].

Quality-of-care has gained much attention over the last years. Currently, Dutch colposcopy clinics report a set of indicators including 'time-to-colposcopy', percentage of cold knife conisations, percentage of treatments under non-local anesthesia and percentage of normal cervical cytology 6 to 12 months after treatment each year, in order to compare quality of care in the different centers[100].

In this retrospective cohort study with three years of follow-up, we aimed to assess the quality of care in colposcopy clinics with regard to the following indicators:
(1) time to colposcopy as patients find this an important indicator
(2) comparison between see-and-treat and 2-step colposcopy, with regard to over- and delayed treatment.
(3) adherence to follow-up protocols, as sensitivity of colposcopy is still not optimal.

This study was specifically done to identify areas for improvement of care.

Methods

Patients and follow-up algorithm

We retrospectively evaluated all consecutive patients in five colposcopy clinics (Erasmus MC, VUmc, UMCU, Antonius Hospital and RUNMC) who had had a first colposcopy because of abnormal cytology between January 1st up to December 31st 2007. Exclusion criteria were previous colposcopy or cervical treatment, colposcopy for vaginal intraepithelial lesions and endometrial pathology.

This cohort of women was followed longitudinally for three years up to December 31st 2010. Women had a follow-up of three to four years (depending on their date of accrual) according to local follow-up protocols. The baseline or index smear was defined as the smear leading to colposcopy. Of these women, the results of cytology, biopsy, ECC, treatment modality, treatment histology, follow-up smears at 6, 12 and 24 months and subsequent treatment were extracted from the hospital information systems and the nationwide network and registry of histopathology and cytopathology (PALGA, The Netherlands). A window of four months preceding, until four months after, the expected date of the 6, 12 and 24 month follow-up cytology was accepted.

At the RUNMC all women were treated by 'see-and-treat' policy, with the exception of patients with cervical cytology of mild dyskaryosis or less and impression of a low-grade lesion at colposcopy, or when the lesion impressed as a carcinoma. In that case, and in the majority of cases in the other hospitals, a biopsy with or without ECC was performed at the first colposcopy with subsequent recall and treatment if necessary.

Statistical analysis

All clinical data collected were analyzed using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). For all statistical tests a two-tailed p-value of ≤ 0.05 was considered significant. Standard summary statistics were used to describe the data. Sensitivity and specificity were analyzed based on two by two analysis, with final histology at the threshold of CIN2 as 'gold standard test'. Differences in recurrence rate and most abnormal histology was calculated by Chi-square testing. Non-parametric testing was performed for the continuous data on age, treatment and time-to-colposcopy, with Kruskal-Wallis for more than 3 variables, and post hoc Mann-Whitney U testing.

Results

In total, 532 women were included in this study (Table 1) with a median age at first colposcopy of 35.5 years [17.5-75.9]. Referral cytology was two consecutive BMD smears (268, 50.4%) or once >BMD (264, 49.6%). Patients had up to four colposcopies in the study period. In 34 (42.5%) of the 80 patients in whom the transformation zone (TZ) was not visible, an ECC was performed. Colposcopic impression was established as CIN grade 2 lesion or worse (CIN2+) in 198 (52.7%) patients. Notably, in 156 patients (29.3%) no colposcopic impression was documented. Patients who were treated (338, 63.5%) were subdivided into 'see-and-treat' (115, 21.6%) and 'two-step approach' (223, 41.9%). The former is defined as treatment without any preceding histology, whereas the latter is characterized by at least one histology sample prior to treatment. Within the latter group there were three cases in which only an endocervical curettage was performed, which showed insufficient material for diagnosis. Final histology, defined as the most abnormal histology result in the total study period, including diagnostic and therapeutic specimens, was CIN2 in 114 (23.0%), CIN3 in 209 (42.1%) and invasive cervical cancer in 18 (3.6%) of the patients.

Time-to-colposcopy and time-to-treatment was calculated and stratified according to cytology result and treatment category (Table 2). Median time-to-colposcopy was significantly shorter for patients with >BMD compared to those with BMD cytology (5.2 versus 7.0 weeks, p=0.000). In total, 78 patients (14.7%) had a colposcopy within 3 weeks and 438 patients (82.3%) within 10 weeks after the referral smear had been taken. Of the patients with >BMD cytology 56 (21.2%) had a colposcopy within 3 weeks and 233 (88.3%) within 10 weeks.

Table 1. Baseline characteristics of the patients in this study

Characteristics		N (%)
Age	median years [range]	35.5 [17.5-75.9]
Center		
	AZ	78 (14.7)
	EMC	123 (23.1)
	UMCN	99 (18.6)
	UMCU	79 (14.8)
	VUmc	153 (28.8)
	Total	532 (100)
Intake cytology		
	BMD	268 (50.4)
	>BMD	264 (49.6)
Intake cytology		
	borderline dyskaryosis	121 (22.7)
	mild dyskaryosis	147 (27.6)
	moderate dyskaryosis	137 (25.8)
	severe dyskaryosis	91 (17.1)
	carcinoma in situ	31 (5.8)
	carcinoma	5 (0.9)
Colposcopies		
	1	399 (75.0)
	2	90 (16.9)
	3	31 (5.8)
	4	12 (2.3)
Transformation zone		
	not visible	80 (15.0)
	visible	353 (66.4)
	unknown	99 (18.6)
Impression at first colposcopy		
	<CIN2	178 (47.3)
	CIN2+	198 (52.7)
	unknown	156
Treatment category		
	no treatment	194 (36.5)
	see-and-treat	115 (21.6)
	two-step approach	223 (41.9)
Highest histology in total study period		
	normal	79 (15.9)
	CIN I	76 (15.3)
	CIN II	114 (23.0)
	CIN III	209 (42.1)
	Invasive cervcial cancer	18 (3.6)
	no/ insufficient histology	36

Table 2. Time between referral cytology, colposcopy or treatment, stratified according to referral cytology and treatment group. Borderline or mild dyskaryosis (BMD), Mann-Whitney U (MWU) test, follow-up (FU)

	Median time in weeks [range] from:	
	cytology - colposcopy	*colposcopy - treatment*
All	5.6[1.3-50.4]	4.8[0-188.1]
Referral Cytology		
BMD	7.0[0.9-488.4]	7.0[0-188.1]
>BMD	5.2[0-50.4]	3.9[0-175.1]
	p=0.000	**p=0.000**
		Kruskal Wallis test with post hoc MWU test. 2-tailed
Treatment		
no	7.0[0-488.4	NA
see-and-treat	4.8[1.3-36.5]	0[0-9.4]
two-step approach	6.5[1.7-50.4]	7.4[0.4-188.1]

The sensitivity and specificity of referral cytology, colposcopic impression, ECC, biopsy and first treatment for the detection of CIN2+ (Table 3) was determined. First, all factors were analyzed separately, and second factors were analyzed per cytology group, further stratified by colposcopic impression. Referral cytology, at the threshold of BMD was as sensitive for the detection of CIN2+ lesions as colposcopic impression at the threshold of CIN2 (67.7% [95 % CI 62.8-72.7] and 73.7% [68.0-79.4], respectively, p=0.489). Of note, as mentioned earlier there were 156 (29.3%) patients without documentation of colposcopic impression. These cases were excluded from this analysis. By performing colposcopically guided histological sampling (biopsy or ECC) the sensitivity significantly increased to 84.8% [95 % CI 80.1-89.4] (p=0.000) in the total study population. Among patients with BMD at referral cytology, colposcopic impression had a sensitivity of 60.3% [95 % CI 48.7-71.9]. Among patients with BMD at referral cytology, performing a biopsy or ECC significantly increased this sensitivity to 81.7% [95 % CI 73.9-89.6] (p=0.019). The sensitivity of colposcopic impression in patients with >BMD was 79.3% [95 % CI 73.1-85.5] and was significantly increased to 86.9% [95 % CI 81.2-92.5] (p=0.001) if histological sampling was performed.

The type of treatment is shown in Table 4. The first treatment was a LEEP in the majority of patients (315/532, 93.2%). As the number of treatments increased the type of treatment shifted to conisation or hysterectomy. Of all 532 women included in this study, 316 (59.2%) were treated by one to four LEEP procedures, 27 (5.1%) by a conisation, 2 (0.4%) by once or twice laser evaporation, 1 (0.2%) by crycoagulation and 11 (2.1%) by hysterectomy.

Table 3. Sensitivity and specificity of referral cytology, impression at first colposcopy and biopsy or endocervical curettage (ECC). Cut off was BMD or CIN2. True-positive (TP), true-negative (TN), false-negative (FN) and true-negative (TN)

	CIN2+ in final histology								
Factor (cut off)	*TP*	*FP*	*FN*	*TN*	*Total*	*Sensitivity (95% CI)*	*Specificity (95% CI)*	*PPV (95% CI)*	*NPV (95% CI)*
All									
Referral cytology (BMD)	231	30	110	125	496	67.7 (62.8-72.7)	80.6 (74.4-86.9)	88.5 (84.6-92.4)	53.2 (46.8-59.6)
Impression first colposcopy (CIN2)	171	26	61	86	344	73.7 (68.0-79.4)	76.8 (69.0-84.6)	86.8(82.1-91.5)	58.5 (50.5-66.5)
Biopsy/ECC 1 (CIN2)	195	0	35	136	366	84.8 (80.1-89.4)	100 (100-100)	100 (100-100)	79.5 (73.5-85.6)
Referral cytology BMD									
Impression first colposcopy (CIN2)	41	18	27	73	159	60.3 (48.7-71.9)	80.2 (72.0-88.4)	69.5 (57.7-81.2)	73.0 (64.3-81.7)
Biopsy/ECC 1 (CIN2)	76	0	17	113	206	81.7 (73.9-89.6)	100 (100-100)	100 (100-100)	86.9 (81.1-92.7)
and impression <CIN2									
Biopsy/ECC 1 (CIN2)	15	0	8	66	89	65.2 (45.8-84.7)	100 (100-100)	100 (100-100)	89.2 (82.1-96.3)
and impression CIN2+									
Biopsy/ECC 1 (CIN2)	26	0	4	17	47	86.7 (74.5-98.8)	100 (100-100)	100 (100-100)	81.0 (64.2-97.7)
Referral cytology >BMD									
Impression first colposcopy (CIN2)	130	8	34	13	185	79.3 (73.1-85.5)	61.9 (41.1-82.7)	94.2 (90.3-98.1)	27.7 (14.9-40.4)
Biopsy/ECC 1 (CIN2)	119	0	18	23	160	86.9 (81.2-92.5)	100 (100-100)	100 (100-100)	56.1 (40.9-71.3)
and impression <CIN2									
Biopsy/ECC 1 (CIN2)	21	0	7	10	38	75 (59.0-91.0)	100 (100-100)	100 (100-100)	58.8 (35.4-82.2)
and impression CIN2+									
Biopsy/ECC 1 (CIN2)	62	0	4	4	70	93.9 (88.2-99.7)	100 (100-100)	100 (100-100)	50 (15.4-84.6)

Table 4. Details of treatment. Large loop excision of the transformation zone (LEEP), laser evaporation (laser)

Type of treatment	N (%)			
	first (338)	*second (38)*	*third (7)*	*fourth (1)*
LEEP	315 (93.2)	23 (60.5)	3 (42.9)	1 (100)
conisation	18 (5.3)	8 (21.1)	1 (14.3)	
laser	2 (0.6)	1 (2.6)		
cryocoagulation	1 (0.3)			
hysterectomy	2 (0.6)	6 (15.8)	3 (42.9)	

Table 5. 'See-and-treat' versus two-step approach in terms of overtreatment and recurrence rates

	No treatement	See-and-treat	Two-step approach	Difference treatments
	N=194 (%)	*N=115 (%)*	*N=223 (%)*	*p value*
Highest histology at/following colposcopy 1 <CIN2	132/152 (86.8)	9/113 (8.0)	34/216 (15.7)	**0.047**
Highest histology total <CIN2	135/159 (84.9)	8/114 (7.0)	12/223 (5.4)	0.547
Highest histology total Cancer	8/160 (5)	4/114 (3.5)	6/223 (2.7)	0.675
Residual disease (31)/Recurrence (3)	NA	10/112 (8.9)	24/219 (11.0)	0.565

In table 5 the histological outcome and recurrence rates are specified per treatment group and were compared between the two treatment groups (see-and-treat and two-step approach group). First these data show that the number of patients with overtreatment (defined as normal or CIN1 as most abnormal histology in the total study period) is similar (7.0% versus 5.4%, p=0.547). Second, the percentage of patients with invasive cervical cancer was the same (3.5% versus 2.7%, p=0.675). Finally the rate of recurrence or residual disease was evenly distributed (8.9% versus 11.0%, p=0.565).

Table 6. Follow up cytology at 6, 12 and 24 months after treatment. Defined as normal, borderline or mild dyskaryosis (BMD) or worse than BMD. Residual or recurrent disease was defined as cervical intraepithelial neoplasia grade 1 or worse after treatment

Follow up	N (%)	
Number of cytology obtained at 6, 12 and 24 months		*of 316 pt with ≥ 20 mo FU*
0	26 (8.2)	
1	88 (27.8)	
2	94 (29.7)	
3	108 (34.2)	
6 Month cytology		
normal	202 (75.9)	
BMD	49 (18.4)	
>BMD	15 (5.6)	
NA	13	FU not needed; rec <6 mo, colpo, ca
missing	37	cytology should have been sampled
12 Month cytology		
normal	161 (85.2)	
BMD	24 (12.7)	
>BMD	4 (2.1)	
NA	27	
missing	100	
24 Month cytology		
normal	123 (88.5)	
BMD	13 (9.3)	
>BMD	3 (2.2)	
NA	35	
missing	142	
Number of normal cytology at 6, 12 and 24 months		
0	36 (12.4)	
1	95 (32.8)	
2	86 (29.7)	
3	73 (25.2)	
missing	26	no cytology at 6, 12 or 24mo
Three normal cytology in 30 months after treatment		*of 298 pt with ≥ 30 mo FU*
no	189 (63.4)	
yes	109 (36.6)	
Residual or recurrent disease after first treatment		
total	34 (10.3)	*of 331 treated pt (excluding 7 carcinomas)*
residual < 24 mo	31 (91.2)	
recurrence ≥ 24 mo	3 (8.8)	

Follow up data was retrieved for all patients (Table 6). The results at 6, 12 and 24 months were calculated for patients that were treated at least 20 months prior to the study closure date. The 8 patients that had cervical cancer diagnosed in the first treatment specimen were excluded, because the follow-up of cervical cancer was not part of this analysis. Only 108 (34.2%) patients had completed follow-up with a total of three smears taken at 6, 12 and 24 months after treatment. Patients were defined as not applicable (NA) if no cytology sampling was necessary at that timepoint (being patients with residual disease before the date of smear, with a colposcopy in between the smears or with cancer diagnosed before the date of smear). This number increased over time, from 13 at 6 months, 27 at 12 months to 35 at 24 months after treatment. Patients that defaulted visits (within a window of 8 months), were defined as missing. This number of patients with missing follow-up increased over time from 37 at 6 months, 100 at 12 months to 142 at 24 months after treatment. In total, 266/316 (84.2%) attended the 6 month follow-up, 189/316 (59.8%) the 12 month follow-up and 139/316 (44.0%) the 24 month follow-up. Of those patients that had a smear taken, normal cytology was detected in 202 (75.9%) patients at 6 months, in 161 (85.2%) at 12 months and in 123 (88.5%) at 24 months. Of patients treated at least 20 months before the end of study date 73 (25.2%) had three consecutive normal cytology smears 6, 12 and 24 months after treatment.

When the definition for normal cytology during follow-up was defined less strictly, namely as three consecutive normal cytology smears in the 30 months following treatment, there were 109 (36.6%) patients that met these criteria.

There were 18 (3.6%) women diagnosed with invasive cervical cancer in this cohort (supplementary table S1). In five (28%) of these patients the cervical tumor was clinically recognized at colposcopy. Thus, the colposcopist did not regognize cervical cancer in the majority (72%) of patients. Eight of all cervical cancer cases (44.4%) were detected by a biopsy, four of those had been clinically recognized. Four (22.2%) tumors were diagnosed in a see-and-treat specimen, of which one was recognized as a micro invasive tumor and was directly treated by conisation, the other were presumed high-grade CIN lesions. Six (33.3%) cases were diagnosed in the two-step approach group, none of those cancers were clinically recognized as such. The referral cytology of the patients with invasive cancer was carcinoma (3, 16.7%), carcinoma in situ (6, 33.3%), severe (7, 38.9%) or moderate dyskaryosis (2, 11.1%).

Discussion

Time-to-colposcopy

This study shows that > 80% of women underwent colposcopy within 10 weeks following the cervical smear. This was significantly shorter for > BMD smears than for BMD smears.

We calculated that the time between cytology sampling and date of colposcopy to be within 3 weeks for 14.7% and within 10 weeks for 82.3% of the patients. One of the reasons for the waiting times over three weeks might be that the former national colposcopy guideline advised to wait six weeks with colposcopy after having taken a cervical cytology specimen[30,101]. It is reassuring that the time-to-colposcopy is significantly shorter in patients with >BMD cytology, since those patients harbour a higher a priory risk for high-grade CIN or cancer than women with BMD cytology.

Comparison see-and-treat with two-step approach

First, we showed that the percentage of over-treatment was low at 7.0% for patients that received see-and-treat. This percentage is substantially lower than the over-treatment rate in 296/501 (59%) of immediately treated patients in a recent prospective trial comparing biopsy and selective recall with immediate LEEP (the TOMBOLA trial)[102]. There are two major differences with our study that could explain this 10-fold difference in rate of over-treatment. Firstly, our study population includes high-grade cervical abnormalities, whereas the TOMBOLA trial only included patients with BMD smears. Second, the threshold for treatment in the TOMBOLA trial was an abnormal transformation zone (colposcopic lesion with acetowhitening with or without capillary vessel patterns) whereas 'see-and-treat' was only performed in our study population if a high-grade CIN lesion was suspected, or if there was a high-grade cervical cytology smear. Also, our study is a retrospective cohort study, not a randomized study, most probably producing a selection bias in the sense that clinically more severe cases might be selected for immediate treatment. More comparable to our data is a recent cohort study in a 'see-and-treat' only clinic, which showed an overall overtreatment rate of 18.1%. In patients with an impression of a high-grade lesion the overtreatment rate was comparable to our data (8.1%)[103]. Overall, it is reassuring to see that in our study (an unselected clinical practice cohort in multiple colposcopy clinics) it seems that see-and-treat is used only in carefully selected patients and using a strict protocol, with a low risk of overtreatment.

Secondly, we investigated the follow-up, and as expected there was no difference in number of residual or recurrent disease in either treatment strategies. The time point of treatment was different, but the technical treatment procedures were similar, thus no differences in CIN2+ after treatment were expected. Finally we focused on invasive

cervical cancer (table S1). We show that there is no difference in cervical cancer incidence and percentage clinically recognized cancers in the 'see-and-treat' and the two-step approach group (treatment defined as all procedures up to the diagnosis of cancer, not the consecutive cancer treatment). Although it has been claimed that colposcopy will not differentiate between the degrees of CIN it would be capable of recognizing cancer. Disturbingly, the diagnosis of cancer was suspected in only five (of the 18 cancer cases) at the time of colposcopy. Despite the effectiveness of both see-and-treat and the two-step treatment as the latter approach at least potentially allows detection of cancer in the biopsy prior to treatment. Equally disturbing in this multicenter series is the fact that even with the two-step approach still 6 out of 14 cancer cases had undergone some sort of excisional treatment. Such local treatment first of all hampers or at least delays proper staging and the ability to determine the exact tumor size, especially in the case of fragmented excision. Further, it may impair or again delay tailored surgical treatment, especially minimally invasive and fertility-sparing treatment options.

Follow-up after treatment

This study shows that the follow-up rate after treatment was very low. The number of patients with complete follow-up was 108 (34.2%), and an even lower number of patients (73, 25.5%) had three consecutive normal cytology smears at 6, 12, and 24 months. This number is increased to 110 (35.9%) if optimal follow-up is less strictly defined as three normal cytology smears in the 30 months following treatment. There is a clear decrease in the number of patients who attended follow-up over time, from 88.4% at the 6 month visit, 69.1% at the 12 month visit to 43.1% at the 24 month visit. In a previous study among treated patients in the RUNMC only[104], a decrease in attendance rate was also detected, but there was only a decrease from 90% after one year to 80% after two years. Possibly, because our study is a retrospective strictly observational one without prospective data collection in all centers, the attendance rates we find are lower.

The median age of women in our study (35.2 [19.0-75.9]) was comparable to other colposcopy studies[46,53,99]. Furthermore, the percentage of detected lesions (68.8% CIN2+, 45.8% CIN3+ and 3.6% invasive cancers) was slightly lower than, but overall comparable to the 2008 evaluation of the national screening program (76.1% CIN2+, 59.6% CIN3+ and 4.6% invasive cancers)[98]. A possible explanation for the lower rates found in our study could be that our study population also includes patients with twice BMD, whereas the evaluation report[98] only includes >BMD cytology. Furthermore, we only included women with a first colposcopy whereas the analysis of the screening program includes women with previous colposcopy as well.

Although invasive cervical cancer is a rare disease, this study shows that in a regular colposcopic clinic, the incidence of cancer is 3.6%.

The sensitivity of colposcopy to detect high-grade cervical lesions (CIN2+) was higher in women with >BMD cytology compared to women with a BMD cytology result. This is in line with previous findings[43], showing that knowledge about the referral smear has an impact on the accurateness of colposcopy. In addition to this study, we show that performing a biopsy increases sensitivity, mostly in patients with a BMD result. These data would support a see-and-treat approach in case of >BMD cytology and impression of a high-grade lesion, because of the minimal effect of performing a biopsy on sensitivity. A major limitation of this retrospective study is the fact that in 156 patients, no colposcopic impression could be retrieved from the hospital information system. This clearly impacts the analysis, since it is very likely that only in those cases in which the colposcopist was certain of the diagnosis he/she recorded the colposcopic impression. As a consequence, probably the sensitivity of colposcopic impression is higher in our study (73.7%) compared to the literature (60-70%)[25-27]. Furthermore, this was a strictly observational study, and we did not perform a structural revision of histology or cytology samples, which might have induced under- or overestimation of results.

Sensitivity and specificity of cytology screening itself cannot be determined from these data, since we only investigated women with abnormal cytology that were referred for colposcopy, and actually attended the colposcopy. We have no data on false-negative cytology, or results of women that did not attend colposcopy, estimated in previous studies to be around 10.5%[98]. Conisation and LEEP treatment have shown similar efficacy in the treatment of CIN lesions[105], but more healthy tissue is removed by conisation[106,107], resulting in more complications like cervical stenosis[108], and cervical incompetence causing preterm delivery[109]. Therefore, it is advised to perform conisation only in those cases with suspicion of a (micro-)invasive cervical lesion or in case of glandular disease, since in these cases LEEP can induce a thermal artefact precluding accurate interpretation of margins. It is reassuring that only in 27 (5.1%) of all women referred for colposcopy a conisation was performed, with only one case of overtreatment (histology <CIN2, treatment because of a prolapsed cervix, rather than for presumed cervical pathology). This number has reduced significantly over the years, being up to 29.4 % in 2005[110].

In conclusion, this retrospective evaluation in five hospitals in The Netherlands shows that there are several issues still to be addressed in the colposocpic management of women with abnormal cervical cytology: 1. Time from index cytology to colposcopy is generally more than 3 weeks, but shorter in patients with a more severe cytological

outcome. 2. Documentation of colposcopic impression, an essential part of the examination, and was low. This might have resulted in a sensitivity bias and hampers adequate evaluation of the quality of care. 3. Since colposcopic examination is not be able to exclude cancer, immediate excisional treatment should be avoided in women who might need individualized radical surgical treatment. Colposcopic training should focus more on the recognition of signs of malignancy. 4. Only a minority of patients in this 'real life' cohort has completed full follow-up as recommended in our national guidelines. In the wake of low sensitivity of colposcopy, both to detect CIN and cancer, more attention needs to be paid to this safeguard, e.g. by a well established call and re-call system.

Table S1. Details of 18 cancer cases, colposcopic impression, diagnostic procedures and histological subtype

n	Impression first colposcopy	Details of interventions prior to diagnosis	Diagnosis Invasive Cancer
see-and-treat			
4	invasive cervical cancer		conisation microinvasive SCC
	unknown	direct LEEP CIN3	re LEEP microinvasive SCC
	unknown		direct LEEP microinvasive ACC
	unknown		direct LEEP microinvasive SCC
secondary treatment			
14	invasive cervical cancer		biopsy macroinvasive SCC
	invasive cervical cancer		biopsy macroinvasive SCC
	invasive cervical cancer		biopsy macroinvasive SCC
	invasive cervical cancer		biopsy macroinvasive SCC
	CIN3		biopsy macroinvasive SCC
	CIN3	biopsy and ECC CIN2	LEEP macroinvasive SCC
	CIN3	biopsy CIN3, LEEP CIN3; colposcopy 2 impression unknown, biopsy CIN3, colposcopy 3 impression normal, biopsy CIN3	hysterectomy multifocal microinvasive SCC
	CIN3	biopsy CIN3	conisation macroinvasive SCC and ACC
	CIN2		biopsy microinvasive SCC
	normal		biopsy macroinvasive SCC
	unknown		biopsy macroinvasive SCC
	unknown	biopsy CIN3	conisation multifocal microinvasive SCC
	unknown	biopsy CIN3	LEEP microinvasive SCC
	unknown	biopsy CIN3, LEEP CIN3; colposcopy 2 impression CIN3, biopsy CIN3	conisation microinvasive SCC

3

Dynamic Spectral Imaging Colposcopy: Higher Sensitivity for Detection of Premalignant Cervical Lesions

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Abstract

Objective: To validate the dynamic spectral imaging (DySIS) colposcope's color-coded map in discriminating high- from low-grade cervical lesions and non-neoplastic tissue.

Design: Prospective, comparative, multicentre clinical trial.

Setting: The colposcopy clinics of three Dutch hospitals.

Population: Women of 18 years or over with an intact cervix, referred for colposcopy.

Methods: During a 3-minute image acquisition phase, the DySIS colposcope was used as a regular video colposcope: the colposcopist located and graded potential lesions based on conventional colposcopic criteria. Subsequently, a color-coded map was calculated and displayed, representing localisation and severity of the cervical lesion. Biopsies were collected from all atypical sites, as identified by digital mapping and/or conventional colposcopy. Furthermore, one additional biopsy was taken.

Main outcome measures: Histologically confirmed high-grade cervical disease (CIN2+).

Results: In total 275 women were included in the study: 183 women were analyzed in the 'according-to-protocol' (ATP) cohort and 239 women in the 'intention-to-treat' (ITT) cohort. In the ATP cohort, the sensitivity of DySIS colposcopy to identify women with high-grade (CIN2+) lesions was 79% (95% CI 70- 88) and the sensitivity of conventional colposcopy was 55% (95% CI 44-65) ($P = 0.0006$, asymptotic McNemar test). When the DySIS color-coded map was combined with conventional colposcopy, the sensitivity was 88% (95% CI 82-95).

Conclusions: DySIS colposcopy has a significant higher sensitivity to detect cervical lesions than conventional colposcopy. If the color-coded map is combined with conventional colposcopic examination, the sensitivity increases further.

Introduction

Colposcopy is a visual technique used to identify cervical lesions after the application of acetic acid. It requires extensive training and experience; however, recent studies have suggested that higher level of experience in colposcopy do not increase sensitivity of conventional colposcopy[43,44]. The variation in the reported performance for colposcopy is high, with the average sensitivity of colposcopy to distinguish low- from high-grade lesions and cancer being around 55%[43,45-48]. Furthermore, the low to average sensitivity and specificity of colposcopic examination is also associated with a high degree of inter- and intra-observer variability[49-51].

Histology is the gold standard to grade cervical lesions detected by colposcopy, therefore punch biopsies or direct loop excision ('see-and-treat') are required for definite diagnosis. This sampling of the cervix is often stressful and painful for the woman and does not provide an immediate test result. Besides, the accuracy of the 'gold standard' itself is hindered by the variability in histological diagnosis among pathologists and the obvious sampling errors through the inaccuracy of colposcopy and tissue sampling[58-60]. Therefore, to be able to establish a 'base-line' sensitivity for the colposcopic assessment by minimising inter- and intra-observer variability would constitute a major improvement in colposcopic practice and the cervical cancer diagnostic chain in general.

Previous studies with the dynamic spectral imaging (DySIS) colposcope (DySIS™, Dynamic Spectral Imaging System; Forth Photonics, Livingston, UK) have shown promising results[54-57]. Quantifying rather than qualifying optical features after application of acetic acid may increase the objectivity of colposcopy and, therefore, improve detection of high-grade lesions. Hence, we designed our study to validate the capacity of the DySIS colposcope alone or in combination with conventional colposcopy to discriminate high- from low-grade cervical lesions and non-neoplastic tissue and to improve the colposcopic performance through digital documentation and analysis of sequences of colposcopic images. In addition, we compared these images with the visual interpretation of the colposcopist and histology results.

Methods

Enrollment

This study was designed as a prospective multicenter comparative clinical trial, with the participation of the colposcopy clinics of three Dutch hospitals; the VU University Medical Center in Amsterdam, the Reinier de Graaf Hospital in Voorburg and

the Sint Antonius Hospital in Nieuwegein. All relevant ethic boards approved the protocol, and the study was registered in the Dutch trial registry (ISRCTN66112760). Consecutively, women of 18 years or over who were referred to these clinics for colposcopy were invited to participate in the study. Inclusion criteria were abnormal cervical cytology (i.e. at least borderline nuclear abnormalities) or follow-up of a cervical intraepithelial neoplasia (CIN) grade 1 or 2 lesion. Signed informed consent was obtained from all women before any study procedures. Exclusion criteria were previous surgery on the cervix, pelvic radiotherapy, current pregnancy and pregnancy in the last 3 months.

The DySIS colposcope

The DySIS colposcope (Figure 1) is a digital imaging instrument used to visualise the cervix during a colposcopic examination, allowing different color-filtering and magnification options. The basic technical characteristics of the DySIS colposcope (DySIS™ v2.1, Forth Photonics Ltd, Livingston, UK) used in this study are: digital video camera resolution 1024×768 pixels; white bright LED illumination; field of view (approximately) 25×35 mm; 10x to 27x magnification; polarised glare-free images; green and blue digital filter. Additionally, there is the option to measure and map the dynamics (i.e. rise-time, intensity and persistence) of the acetowhitening effect, for every point of the cervix. Modelling of the measured dynamic curves by the DySIS colposcope provides a per point analysis of the acetowhitening effect.

Once acetic acid has been applied, the DySIS colposcope starts automatically with the measurement of the acetowhitening effect. Although the DySIS acquisition period lasts approximately 3 minutes, the acetowhitening effect can last several minutes more[54,111,112], which is the reason why no second DySIS assessment can be performed immediately after the first. At the end of the examination, the DySIS information is concisely presented in the form of a color-coded map, which can be overlaid onto the color image of the tissue (Figure 2), to assist the identification of the location and severity of cervical lesions. The DySIS colposcope and the measurement principles and procedures have been described in detail previously[55,57,112].

Study procedures

Before participating in this study, colposcopists and supervising colposcopists had to attend an instruction meeting. Colposcopists had to perform at least 20 colposcopies with the DySIS colposcope, supervising colposcopists at least five.

Before colposcopy, a cervical sample for high-risk human papillomavirus (hrHPV) and hrHPV viral load testing was collected and stored in Universal Collection Medium® (UCM) (Qiagen Corp., Gaithersburg, MD, USA). The sampling was

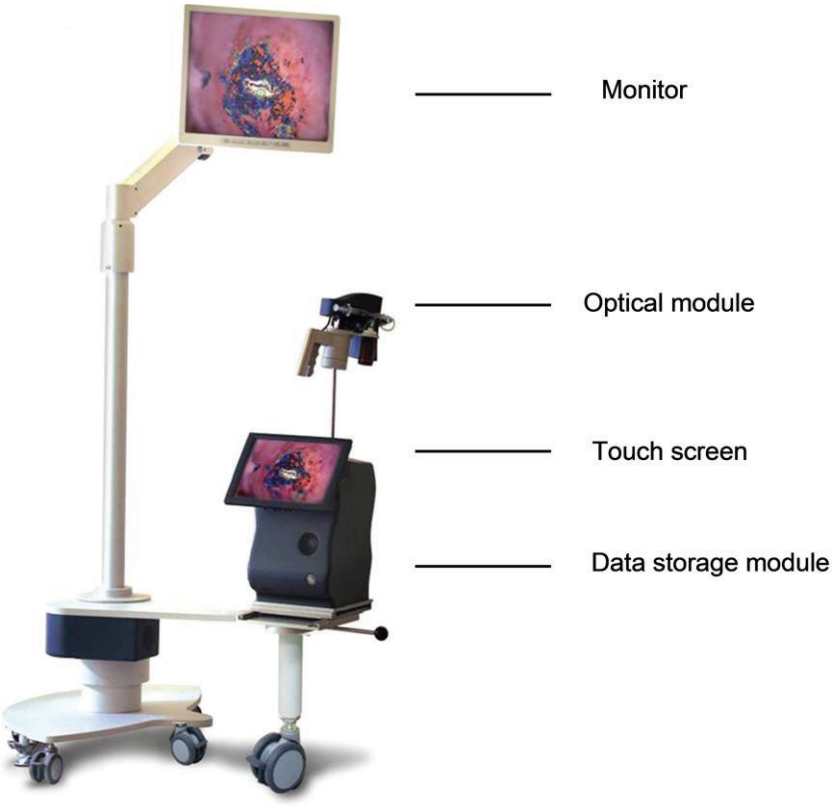

Figure 1. Dynamic spectral imaging colposcope (DySIS™, Dynamic Spectral Imaging System; Forth Photonics, Livingston, UK).

carried out with special attention to avoid excessive bleeding that would impair the colposcopic examination. Subsequently, colposcopy was performed or supervised by expert colposcopists, according to national colposcopy guidelines[101], using the DySIS colposcope as a regular video colposcope during the 3-minute data acquisition phase. After completion of the data acquisition, the colposcopic impression was digitally recorded by the colposcopist, with annotation of the most atypical location and predicted severity of the lesion. Up to this point, the colposcopist was blinded to the DySIS analysis of the images, and if desired, the colposcopist was able to continue the colposcopic inspection by (re)applying acetic acid and/or iodine.

The collected images were digitally analyzed by the DySIS colposcope, and the resulting quantitative color-coded map of the acetowhitening effect was subsequently revealed and overlaid on the image of the cervix, but not before the entry of the colposcopist's final predictions. Based on comparison with pre-determined threshold

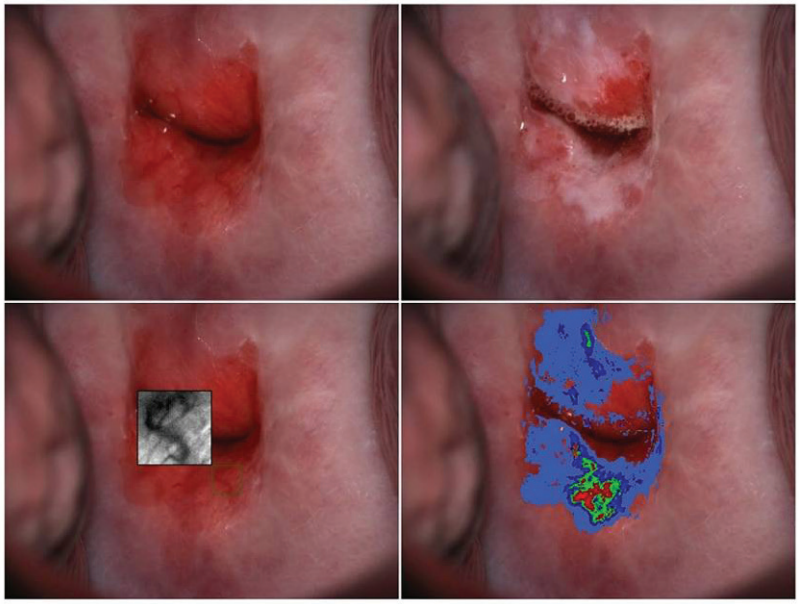

Figure 2. Examination with the dynamic spectral imaging colposcope. Top left, cervix without acetic acid; top right, cervix after the application of acetic acid; bottom left, green filter to enhance vessel viewing; bottom right, color-coded map with red indicating the most severe acetowhite area.

values,[57] the DySIS color-coded map provided a prediction for the presence and grade of neoplasia, and indication of the most atypical site for biopsy sampling accordingly. The color-coded map was compared with the colposcopist's own impression and punch biopsies were taken from all identified suspicious sites. These included those indicated by the colposcopist and the DySIS colposcope as well as one additional control biopsy of apparently normal cervical tissue on the opposite side of the lesion(s). If both colposcopist and colposcope evaluated the cervix as normal, one biopsy was taken from the transformation zone at the 12 o'clock position, to ensure that no lesions were missed and to reduce ascertainment bias. In 27 of the women no punch biopsies were collected, but a loop electrosurgical excision procedure (LEEP) was performed immediately ('see-and-treat' procedure). The colposcopic examination data were saved for further evaluation and the biopsy sampling procedure was recorded on video and later reviewed to obtain objective evidence on whether the tissue sample was collected from the annotated area. Taking punch biopsies from a rather small lesion can easily lead to a biopsy sampling error, something that may also happen during conventional colposcopic examinations. In both situations, this may lead to under detection of high-grade cervical disease. In this study we corrected for this by

taking an additional biopsy from every woman and ensuring that at least one biopsy sample was collected from women who were not suspected for high-grade disease.
Finally, all women were given two questionnaires: one to evaluate demographics and risk factors and another one to evaluate patient satisfaction[113,114]. Subsequent treatment and follow-up of the women was performed at the discretion of the attending physician according to national guidelines[101].

Clinical specimen handling

High-risk HPV and viral load were tested in the cervical sample using the GP5+/6+ polymerase chain reaction (PCR) enzyme immunoassay test and real-time PCR respectively according-to-protocols routinely running in the VU University Medical Center laboratory and as previously described[115,116].
All histology was independently reviewed by a pathologist specialising in gynecological pathology (FK). In case of disagreement between original assessment and review (defined as no neoplasia/ low-grade lesion [CIN0-1] versus high-grade lesion [CIN2-3, adenocarcinoma in situ or carcinoma]), a third expert reviewer (CM) graded the lesion (19.0% of all tissue samples), blinded to all previous results, and final diagnosis was determined by the majority decision.

Statistical analysis

Before the start of the study, a power analysis was performed based on the presumption that conventional colposcopy has a sensitivity of 70% and DySIS colposcopy a sensitivity of 80%. Therefore, 200 women would be needed for this study to detect this difference (80% power, 5% alpha).
All clinical data collected were analyzed using 2x2 tables, chi-square tests, asymptotic McNemar tests[117] and 95% CI (SPSS software package version 15.0; SPSS, Chicago, IL, USA). For all statistical tests a two-tailed *P*-value ≤0.05 was considered significant. For data analysis two cohorts were formed: the according-to-protocol (ATP) cohort and the intention-to-treat (ITT) cohort. The data in the ITT cohort, even though protocol criteria (e.g. not all DySIS indications for high-grade lesions were sampled or the device was used even though there was a hardware problem) were not adhered to in all women, was analyzed to approximate the performance of DySIS colposcopy under clinical conditions (*clinical performance*). The ATP cohort is a subset of the ITT cohort where the protocol was strictly adhered to for all women and reflects the device performance (*proof of principle*).

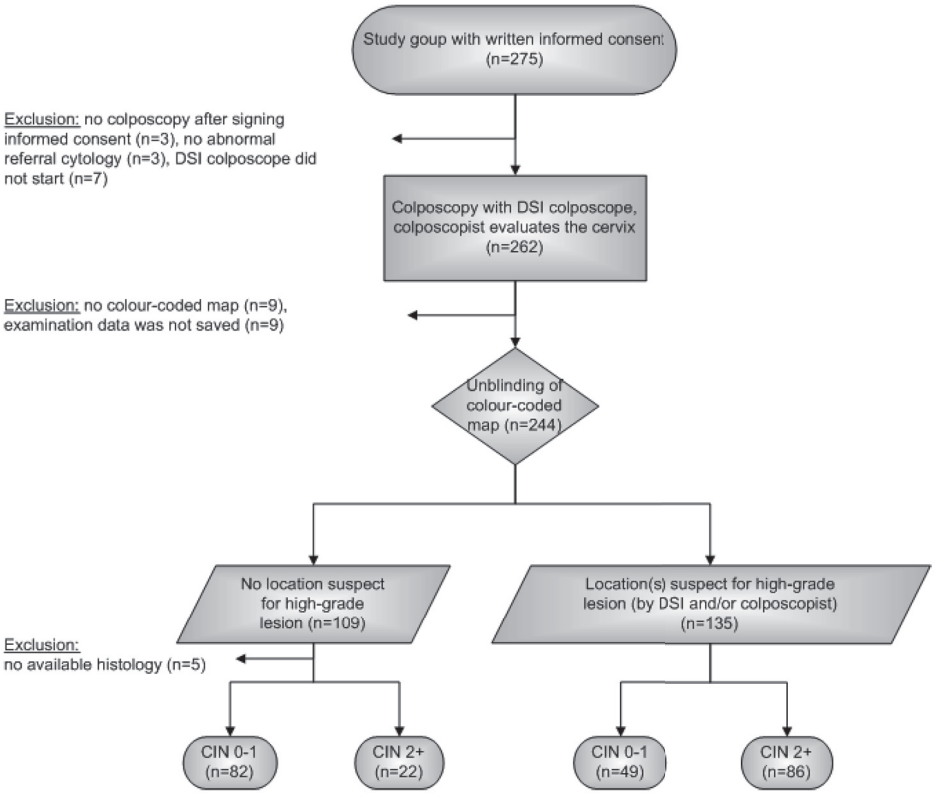

Figure 3. Study profile (intention-to-treat cohort).

Results

Between 1 July 2008 and 1 September 2009, 275 consecutive women were recruited from the three hospitals. Of these 275 women, 36 (13.1%) were excluded. The main reasons for exclusion were unsaved examination data (n = 9, 25.0%) and no color-coded map available (*n* = 9, 25.0%). This resulted in an ITT cohort of 239 women. A detailed flow-chart describing the study profile is presented in Figure 3. Management of 56 (20.4%) women did not strictly adhere to the protocol and could therefore only be analyzed in the ITT cohort, resulting in an ATP cohort (subset of the ITT cohort) of 183 women.

Table 1. Reasons for complete exclusion or exclusion from according-to-protocol cohort (these women were still included in the intention-to-treat cohort)

Reason	n (%)
Complete exclusion	
Examination data was not saved	9 (25.0)
No colour-coded map	9 (25.0)
DSI colposcope did not start	7 (19.4)
No available histology	5 (13.9)
No abnormal referral cytology	3 (8.3)
No (DSI) colposcopy after signing informed consent	3 (8.3)
Total	36 (100)
Exclusion from according-to-protocol cohort	
Transformation zone not (completely) visible with DSI	19 (33.9)
No biopsy from DSI colposcope high-grade location	14 (25.0)
Image quality unsatisfactory	7 (12.5)
Hardware failure	6 (10.7)
Too much blood	3 (5.4)
DSI colposcope starts too late*	2 (3.6)
Miscellaneous	5 (8.9)
Total	56 (100)

* That is, the measurement of the acetowhitening effect did not start automatically after the application of acetic acid

Main reasons for exclusion from the ATP cohort were inability to visualise 75% or more of the transformation zone during the DySIS image collection (n = 19, 33.9%) and no available histology from a high-grade location indicated by the color-coded map (n = 14, 25.0%) (Table 1). The baseline characteristics of the study population, partly derived from the demographics and risk factors questionnaire, can be seen from Table 2. No significant differences were observed between the ATP and ITT cohorts.

In the ATP cohort a total of 332 punch biopsies, 18 endocervical curettages and 84 treatment specimens (mainly loop electrosurgical excision procedure) were obtained. In total, 153 control biopsies were taken from apparently normal tissue of which 39 (25.5%) were classified as high-grade disease. Altogether, 86 women (47.0%) had a high-grade lesion, including three women with cervical adenocarcinoma. The adenocarcinomas were correctly identified by both DySIS and the colposcopist as high-grade disease.

Table 2. Baseline characteristics

	ATP cohort (n = 183) (%)	ITT cohort (n = 239) (%)
Centre[1]	96 (52.5)	127 (53.1)
A	54 (29.5)	74 (31.0)
B	33 (18.0)	38 (15.9)
C		
Age (years)		
mean [range]	36.6 [18.7 - 62.6]	36.7 [18.7 - 62.6]
median	35.4 yrs	35.3 yrs
Indication colposcopy		
Abnormal cytology	166 (90.7)	219 (91.6)
Follow-up CIN1-2	17 (9.3)	20 (8.4)
Result last smear[2]		
Normal	4 (2.2)	5 (2.1)
BMD cytology	118 (64.5)	153 (64.0)
>BMD cytology	61 (33.3)	81 (33.9)
hrHPV test		
negative	54 (29.5)	73 (30.5)
positive	123 (67.2)	158 (66.1)
Test not performed	6 (3.3)	8 (3.3)
Current smoker	69 (37.7)	87 (36.4)
Mean age first sexual contact, years [range]	16.9 [9 - 30]	17.0 [9 - 30]
Mean number sexual partners in last year [range]	1.3 [0-5]	1.2 [0-5]
Condom use		
Always	10 (5.5)	13 (5.4)
Sometimes	35 (19.1)	46 (19.2)
Never	122 (66.7)	154 (64.4)
Missing / not applicable	16 (8.7)	26 (10.9)
Mean number of pregnancies [range]	1.3 [0-5]	1.3 [0-9]

[1] Centre A: VU University Medical Centre, Amsterdam; Centre B: Reinier de Graaf Hospital, Voorburg; Centre C: Sint Antonius Hospital, Nieuwegein
[2] Follow-up CIN1-2, BMD, borderline or mild dyskaryosis, >BMD cytology, worse than borderline or mild dyskaryosis

The performance of DySIS colposcopy, conventional colposcopy or a combination of the two in identifying high-grade CIN lesions was assessed on a per-patient basis (Table 3). Sensitivity, specificity, positive and negative predictive values with 95%CI were calculated and are represented in Table 4. In the ATP cohort the DySIS colposcope identified correctly 68 of the 86 women with histologically confirmed high-grade disease, whereas conventional colposcopy was able to identify 47 women with high-grade disease, resulting in sensitivities of 79% (95% CI 70-88) and 55% (95% CI 44-65), respectively. This difference is statistically significant (P = 0.0006, asymptotic McNemar test). When used in combination, DySIS and conventional colposcopy detected 76 of the 86 examples of high-grade disease, resulting in an overall sensitivity of 88% (95% CI 82-95). The specificity of DySIS colposcopy was 77% (95% CI 69-86) and of conventional colposcopy 85% (95% CI 77-92) (P = 0.144, asymptotic McNemar test). DySIS combined with conventional colposcopy had a specificity of 69% (95% CI 60-78).

In the ITT cohort, of the 108 women with histologically confirmed high-grade disease the DySIS colposcope identified 70 correctly, whereas conventional colposcopy detected 56. This resulted in sensitivities of 65% (95% CI 56-74) for DySIS colposcopy and 52% (95% CI 42-61) for conventional colposcopy, also a statistically significant difference (P = 0.039, asymptotic McNemar test). When the two techniques were combined, 86 incidences of high-grade disease were correctly identified, resulting in a sensitivity of 80% (95% CI 72-87). The specificity of DySIS colposcopy in this cohort was significantly lower than that of conventional colposcopy: 70% (95% CI 62-78) versus 82% (95% CI 75-88) (P = 0.011, asymptotic McNemar test). The combination of DySIS with conventional colposcopy led to a specificity of 63% (95% CI 54-71) in this cohort.

The high-grade disease missed by DySIS or conventional colposcopy was compared with the detected high-grade disease (Table 5). In the ATP cohort, 11/18 (61.1%) of the women with high-grade disease missed by DySIS colposcopy were hrHPV positive, compared with 59/68 (86.8%) of the women whose disease was correctly identified (P = 0.016, chi-square test). Furthermore, only 1 of the 18 women (5.6%) whose high-grade disease was missed by DySIS was hrHPV type 16 positive, whereas 31/68 (45.6%) women with the correctly identified disease were hrHPV 16 positive (P = 0.003, chi-square test), suggesting a trend that the lesions missed by DySIS colposcopy are less clinically relevant. For conventional colposcopy, there were no differences between the missed and detected disease in overall hrHPV or hrHPV-16-positive status.

Table 3. Cross-tabulation of the results

	ATP cohort			ITT cohort		
	CIN0-1	*CIN2+*	*Total*	*CIN0-1*	*CIN2+*	*Total*
DSI colposcope						
Prediction N/LG	75	18	93	92	38	130
Prediction HG	22	68	90	39	70	109
Total	97	86	183	131	108	239
Colposcopist						
Prediction N/LG	82	39	121	107	52	159
Prediction HG	15	47	62	24	56	80
Total	97	86	183	131	108	239
DSI and conventional colposcopy combined						
Prediction N/LG	67	10	77	82	22	104
Prediction HG	30	76	106	49	86	135
Total	97	86	183	131	108	239

HG; high-grade, N/LG; normal / low-grade
Rows: Prediction according to DSI or conventional colposcopy or a combination of the two.
Columns: Histology result (after revision: 'golden' standard).

Table 4. Sensitivity, specificity, positive and negative predictive values

	n	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
According to protocol cohort					
DSI colposcope	183	79 (70-88)	77 (69-86)	76 (67-84)	81 (73-89)
Colposcopist	183	55 (44-65)	85 (77-92)	76 (65-86)	68 (59-76)
DSI and colposcopist combined	183	88 (82-95)	69 (60-78)	72 (63-80)	87 (80-95)
Intention to treat cohort					
DSI colposcope	239	65 (56-74)	70 (62-78)	64 (55-73)	71 (63-79)
Colposcopist	239	52 (42-61)	82 (75-88)	70 (60-80)	67 (60-75)
DSI and colposcopist combined	239	80 (72-87)	63 (54-71)	64 (56-72)	79 (71-87)

PPV; positive predictive value, NPV; negative predictive value

Finally, the patient satisfaction questionnaire was completed by 178 (97.3%) women in the ATP cohort. The main result was that DySIS colposcopy was no extra burden for the majority of the participating women, compared with conventional colposcopy. No adverse events were reported during the study period.

Table 5. Characteristics of missed versus detected high-grade disease

	Missed by DSI n (%)	Detected by DSI n (%)	Missed by colposcopist n (%)	Detected by colposcopist n (%)
According-to-protocol cohort				
Total	18 (100)	68 (100)	39 (100)	47 (100)
Mean age, yr [range]	38.8 [22.7-55.7]	36.3 [18.7-57.9]	38.4 [20.9-57.9]	35.5 [18.7-52.2]
Centre[1]				
A	7 (38.9)	35 (51.5)	21 (53.8)	21 (44.7)
B	8 (44.4)	20 (29.4)	9 (23.1)	19 (40.4)
C	3 (16.7)	13 (19.1)	9 (23.1)	7 (14.9)
Indication colposcopy				
Abnormal smear	17 (94.4)	66 (97.1)	38 (97.4)	45 (95.7)
Follow-up CIN1-2	1 (5.6)	2 (2.9)	1 (2.6)	2 (4.3)
Result last smear[2]				
Normal	0 (0)	0 (0)	0 (0)	0 (0)
BMD cytology	8 (44.4)	34 (50.0)	23 (59.0)	19 (40.4)
>BMD cytology	10 (55.6)	34 (50.0)	16 (41.0)	28 (59.6)
hrHPV positive	11 (61.1)	59 (86.8)	30 (76.9)	40 (85.1)
hrHPV 16 positive	1 (5.6)	31 (45.6)	15 (38.5)	17 (36.2)
Current smokers	7 (38.9)	21 (30.9)	16 (41.0)	12 (25.5)
Mean age sexual debut [range]	16.8 [13-22]	16.8 [9-30]	16.2 [14-21]	17.3 [9-30]
Mean number of pregnancies [range]	0.88 [0-3]	1.4 [0-5]	1.2 [0-3]	1.4 [0-5]
Intention to treat cohort				
Total	38 (100)	70 (100)	52 (100)	56 (100)
Mean age [range]	37.4 [22.1-55.7]	36.3 [18.7-57.9]	38.1[20.9-57.9]	35.4 [18.7-52.2]
Centre				
A	17 (44.7)	37 (52.9)	28 (53.8)	26 (46.4)
B	15 (39.5)	20 (28.6)	13 (25.0)	22 (39.3)
C	6 (15.8)	13 (18.6)	11 (21.2)	8 (14.3)
Indication colposcopy				
Abnormal smear	35 (92.1)	67 (95.7)	48 (92.3)	54 (96.4)
Follow-up CIN1-2	3 (7.9)	3 (4.3)	4 (7.7)	2 (3.6)
Result last smear[2]				
Normal	1 (2.6)	0 (0)	1 (1.9)	0 (0)
BMD cytology	16 (42.1)	36 (51.4)	29 (55.8)	23 (41.1)
>BMD cytology	21 (55.3)	34 (48.6)	22 (42.3)	33 (59.9)
hrHPV positive	27 (71.1)	61 (87.1)	40 (76.9)	48 (85.7)
hrHPV 16 positive	9 (23.7)	33 (47.1)	20 (38.5)	22 (39.3)
Current smokers	13 (34.2)	22 (31.4)	19 (36.5)	16 (28.6)
Mean age sexual debut [range]	17.0 [13-22]	16.8 [9-30]	16.4 [14-21]	17.3 [9-30]
Mean number of pregnancies [range]	1.1 [0-5]	1.4 [0-5]	1.3 [0-5]	1.3 [0-5]

[1] Centre A: VU University Medical Centre, Amsterdam; Centre B: Reinier de Graaf Hospital, Voorburg; Centre C: Sint Antonius Hospital, Nieuwegein
[2] Follow-up CIN1-2, BMD, borderline or mild dyskaryosis, >BMD cytology, worse than borderline or mild dyskaryosis

Discussion

In the ATP cohort, DySIS colposcopy has a statistically significant better sensitivity to detect high-grade lesions than conventional colposcopy (79% versus 55%, $P = 0.0006$), without any statistically significant differences in the specificity, confirming the *proof of principle* of DySIS colposcopy. If the DySIS colposcope is used in combination with conventional colposcopic examination, the sensitivity increases to almost 90%.

A substantial number of women could only be analyzed in the ITT cohort, which reflects the *clinical performance*. In this cohort the sensitivity of DySIS colposcopy was also significantly higher than of conventional colposcopy (65% versus 52%, $P = 0.039$). Combining DySIS with conventional colposcopy resulted in a sensitivity of 80% (95% CI 72-87) in this cohort. So, even when the DySIS colposcope cannot be used completely or adequately, but is used in combination with conventional colposcopy, the clinical sensitivity can be increased significantly (from 52% to at least 80%) in comparison with conventional colposcopy alone, highlighting the clinical value of the DySIS colposcope. Naturally, this increase in sensitivity means a loss of specificity (from 82% to 63%, $P = 0.011$). Also, the prevalence of high-grade disease was quite high in our population (47%). In a population with a lower prevalence of cervical disease, it is likely that the sensitivity will be lower, arguing for a good selection of colposcopy clinic referrals.

Even though for the initial power analysis we assumed a sensitivity of 70% by conventional colposcopy to detect high-grade cervical lesions, our study yielded a sensitivity of only 55%, which is in accordance with other studies on colposcopic efficacy and was probably the result of the measures taken to reduce ascertainment bias[43,45-48]. The addition of DySIS to conventional colposcopy therefore results in an almost 50% increase in sensitivity; from 55% to 79% by DySIS colposcopy alone if all preconditions for an optimal examination are met, and to 80% in combination with conventional colposcopy regardless of the circumstances or individual user adequacy. Hence, the higher sensitivity of DySIS colposcopy alone or in combination with conventional colposcopy improves the detection of high-grade cervical lesions significantly and guides cervical sampling. This is emphasised further by the fact that the high-grade lesions that were missed by DySIS were more often hrHPV-16-negative (and therefore less clinically relevant) than the high-grade lesions that DySIS colposcopy identified successfully. The reason for this is that it seems that hrHPV 16 infections result in more intense acetowhite lesions, which are more easily detected by DySIS colposcopy[118]. With the implementation of HPV vaccination programs in many developed countries, the prevalence of hrHPV 16 is expected to decrease. However, it is unclear if there are other hrHPV types with similar acetowhitening

features, because the prevalence of non-type 16 hrHPV is quite low. We think there will probably still be a role for DySIS colposcopy in vaccinated populations.

Limitations

A possible restraint of the study was that the DySIS colposcope was used for the conventional colposcopic examination as well. Those colposcopists who were not accustomed to using video colposcopes needed some time to become familiar with this method of colposcopy (i.e. observing the cervix on a screen rather than directly through eye-pieces). However, we think that the training before the study was sufficient to have this unfamiliarity resolved. Furthermore, the performance of the colposcopists in this study is similar to that typically reported for colposcopy[43,45-48], indicating that the performance of conventional colposcopy using the DySIS colposcope is not hindered by the device itself.

The main drawback of the DySIS colposcope is the inherent difficulty in exploiting its full potential in certain situations; for example, during the examination it is recommended that the speculum is attached to the device, which stabilises the field of view and corrects immediately for small movements of the cervix. Although a necessity for a correct DySIS examination, this sometimes hindered the complete view of the transformation zone, especially in women with a retroverted uterus; one of the main reasons for exclusion from ATP analysis. However, in clinical practice, after the data acquisition, the speculum can be released from the device and the colposcopist can review those areas which could not be analyzed during the DySIS examination, as would be done with any other colposcope. Therefore it is not a problem during a routine colposcopic examination outside the study protocol.

Furthermore, no second DySIS examination can be performed directly after the first because of the acetowhitening effect which can last up to 45 minutes and that can interfere with a DySIS measurement[54,111,112]. This is not always practical in the day-to-day clinic routine. Sometimes it would have been convenient to repeat the examination, for instance when during the first examination only a part of the transformation zone could be visualised. Furthermore, there are a number of circumstantial conditions that need to be met before a correct examination with the DySIS colposcope can be performed. As DySIS measures the backscattered light of the cervix, no other light (like the overhead lamps that are often used while inserting the speculum) should shine directly onto the cervix during the examination. Besides, no mucus or blood should be present on the cervix, because this interferes with the measurement and limits the visualisation of the lesion (as it does also in conventional practice). This may pose a particular challenge when a cervical scrape is taken before the colposcopy. Sometimes it can also be difficult to interpret whether the color-

coded map indicates a high-grade lesion. The color-coded map that is displayed over the image of the cervix uses red, yellow and white pixels to indicate predicted high-grade lesions, and the red pixels of small lesions can be difficult to visualise on the reddish background of the cervix.
Another limitation of the study is that no follow-up data were available, so the true prevalence of cervical disease might have been underestimated. We have tried to correct for this ascertainment bias by always sampling one additional biopsy.

Conclusion

In both the ATP and ITT analyses DySIS colposcopy has a higher sensitivity in the detection of high-grade cervical lesions than conventional colposcopy. The main drawbacks are its limited usefulness in some situations: e.g. when only part of the cervix can be visualised at one time or when there is an excess of blood or mucus on the cervix. Therefore it has been shown to be most effective in combination with a trained colposcopist, attaining a sensitivity of up to 88% in the ATP cohort and 80% in the ITT cohort. In this setting, the limitations of the DySIS colposcope can be overcome: when DySIS colposcopy fails or conditions are suboptimal, the colposcopist uses the DySIS colposcope as a regular video colposcope. This signifies that in suboptimal conditions the DySIS colposcope can be a valuable asset to the colposcopic examination.

Disclosure of interest

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that (1) none of the authors have received support from Forth Photonics Ltd, Livingston, UK for the submitted work; (2) CB is a stockholder and EP an employee of Forth Photonics Ltd. UK at the time of this study; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) none of the authors have non-financial interests that may be relevant to the submitted work. JL and MK received support from Forth Photonics Ltd, Livingston, UK to travel to study meetings in Athens. Furthermore, JL received a travel grant from Forth Photonics Ltd, Livingston, UK to visit a conference on colposcopy.

Contribution to authorship

RV was the project leader and designed the study with JL, MK, BH, CB, EP, PS and CM. JL, AZ and MK drafted the manuscript. JL, AZ, MK, BH, GG and JS were responsible for the colposcopies and collection of the data. PS supervised the hrHPV testing. FK and CM were responsible for revising the histology samples. All authors critically reviewed the manuscript.

Details of ethics approval

The ethic boards of the three participating clinics approved the protocol (number 2007/098). Signed informed consent was obtained from all women before any study procedures. The study was registered in the Dutch trial registry (ISRCTN66112760).

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4

Agreement between colposcopic impression and histological diagnosis among HPV16 positive women: a clinical trial using dynamic spectral imaging colposcopy

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Abstract

Objective: To investigate the agreement between conventional colposcopic impression, dynamic spectral imaging (DySIS) colposcopy and histology, for human papillomavirus type 16-positive (HPV16+) and non-16 high-risk (hr) HPV+ women.

Design: Prospective, comparative, multicenter clinical trial.

Setting: Three colposcopy clinics in The Netherlands. Population: Women (n=177) aged 18 years or over with an intact cervix, referred for colposcopy.

Methods: The colposcopist graded the lesion by using the DySIS colposcope as a regular video colposcope. Subsequently the DySIS impression was displayed and biopsies were taken from all abnormal areas as well as from a random (normal) site. A cervical smear was taken for HPV typing.

Main Outcome Measures: Histologically confirmed high-grade cervical intraepithelial neoplasia or cancer (CIN2+), positive for HPV16 or for any other hrHPV type.

Results: The DySIS colposcope identified more CIN2+ cervical lesions among HPV16+ women than in non-16 hrHPV+ women (P = 0.032 regardless of final histology and P = 0.009 among women with CIN2+). Consequently, the sensitivity of the DySIS colposcope for detecting CIN2+ lesions was higher in HPV16+ women than in non-16 hrHPV+ women (97% versus 74%, P = 0.009). No such differences were seen for the colposcopist impression. In addition, mainly smaller cervical lesions are missed by the colposcopist.

Conclusions: The sensitivity of DySIS colposcopy for CIN2+ is higher in HPV16+ than in non-16 hrHPV+ women. Furthermore, regardless of HPV16 status, the sensitivity of DySIS for CIN2+ is higher than that of the colposcopist, probably because colposcopists tend to miss smaller cervical lesions.

Introduction

Uterine cervical cancer is the third most frequent invasive cancer in women worldwide (updated after publication[22])and is caused by malignant transformation of cervical epithelium induced by high-risk human papillomavirus (hrHPV) through premalignant stages of cervical intraepithelial neoplasia (CIN)[1].

The incidence and mortality rates of cervical cancer in developed countries have been reduced at least partly as a result of population-based screening programs and subsequent treatment of premalignant cervical lesions[119]. The efficacy of these programs depends strongly on the recognition of high-grade cervical abnormalities by colposcopy after referral. Unfortunately the sensitivity of colposcopy to detect high-grade cervical lesions is low, around 55%[43,46,58,120]. It has previously been shown by us and others that the sensitivity of colposcopy can be significantly improved when the dynamic spectral imaging (DySIS) colposcope is used[57,99].

Recently in a sub-study of the ALTS trial (atypical squamous cells of undetermined significance [ASCUS]-low-grade squamous intraepithelial lesion[LSIL] Triage Study)[121] Jeronimo et al.[118] observed that HPV16 positive (HPV16+) lesions produce more defined visual abnormalities at colposcopy than lesions caused by other hrHPV types, regardless of final histological diagnosis. As a consequence, it has been hypothesised that HPV16+ lesions are detected earlier than other lesions[122].

To further explore the relationship between hrHPV infection, colposcopic appearance and histology, we compared lesions related to various hrHPV-types using the DySIS colposcope as most sensitive instrument in a clinical study population[99] and evaluated the ability of DySIS to correctly classify high-grade cervical lesions in HPV16+ women.

Methods

Patients

This study was designed as a sub-study of the DySIS colposcope validation trial[99], a prospective multicenter comparative clinical trial in three Dutch outpatient clinics (VU University Medical Center, Amsterdam; Reinier de Graaf Hospital, Voorburg and Sint Antonius Hospital, Nieuwegein). In this trial women aged 18 years and over who were referred to the outpatient clinic for regular colposcopy were consecutively included. The indication for colposcopy was an abnormal cytological test result (twice borderline or mild dyskaryosis [BMD] or once worse than [>] BMD) or follow-up of an untreated CIN1 or 2 lesion. BMD corresponds to ASCUS/ASC-H (cannot exclude high-grade squamous intraepithelial lesion)/LSIL and >BMD equates to high-grade squamous intraepithelial lesion[28]. For this sub-study, only women with an adequate

HPV test result, who were treated 'according-to-protocol', were evaluated (n = 177, 96.7% of the 'according-to-protocol' cohort). Further details on patient selection, trial design and training of the colposcopists have been reported previously[99].
The institutional review boards of the three participating clinics approved the protocol and the study was registered in the Dutch trial registry (ISRCTN66112760). Signed informed consent was obtained from all women before any study procedures.

Study procedures
After taking a cervical scrape for HPV testing, colposcopy was performed using the DySIS digital colposcope (DySIS® v2.1, DySIS Medical Ltd (formerly Forth Photonics Ltd), Livingston, UK) using a standard 3% acetic acid solution. The DySIS digital colposcope enables the calculation of a color-coded map which is based on quantifying the dynamics (temporal behavior and intensity) of acetowhitening recorded for a three-minute period for every location on the cervix. During the procedures in the trial, the DySIS color-coded map was displayed only after the colposcopist's impression (most atypical site and grading) had been digitally recorded. Impression was defined and recoded in the final data analysis as normal to low-grade (N/LG) or high-grade (HG). For DySIS results analysis, the colors red yellow and white were considered as indicative for the presence of high-grade disease.
A desktop version of the DySIS software was used to perform area measurements on the collected data; the standardised set-up of the colposcope (field of view and working distance) allows the direct quantification and comparison of the size of the examined area. Expert users (JL, MK, AZ) used a graphic tool to indicate the area of the cervix on each colposcopic image and high-grade lesions were subsequently quantified in terms of pixels count based on the DySIS color-coded map. The total examination area was 825 mm^2 and equalled 786 432 pixels. In this area, 1 mm^2 equals 953.25 pixels, so one pixel area is 0.001049 mm^2. When translating from image pixels to mm one has to keep in mind that the DySIS system calculates the pixels from a two-dimensional image, constructed from the three-dimensional surface of the cervix. Therefore the actual size in mm can slightly differ from the size calculated by DySIS, but can still be considered as a good approximation for the purpose of comparison[123].

Histopathological evaluation
Histology was considered the 'gold standard' in this study. Biopsies were taken from all sites that were suspect for high-grade disease (indicated by either the colposcopist or DySIS) and in addition a 'random' biopsy was taken from all women. This random biopsy was taken from apparently normal cervical tissue on the opposite side of

the lesion(s). If both colposcopist and the DySIS colposcope evaluated the cervix as normal, one biopsy was taken from the transformation zone at the 12 o'clock position, to reduce the possibility of missing a lesion. Women diagnosed with high-grade disease were treated according to current national guidelines[101].

Independent histopathology review of all biopsy and treatment specimens was performed by a pathologist specialised in gynecological pathology (FK). In case of disagreement between original assessment and review (defined as N/LG [CIN0/1] versus HG or cancer [CIN2+]) a third expert reviewer (CM) graded the lesion blinded to all previous results. The final diagnosis, defined as the most abnormal diagnosis in all specimens (biopsy, endocervical curettage, large loop excision of the transformation zone, cold-knife conisation or hysterectomy, whichever applicable) was based on a majority decision, and was classified as CIN0/1, CIN2+ or CIN3+.

HPV detection and typing

Cervical scrapes were collected in Universal Collection Medium (Qiagen Corporation, Gaithersburg, MD, USA) and DNA was extracted from the cervical smear specimens using proteinase K digestion according to standard procedures[124]. Detection and genotyping of HPV was performed using the clinically validated GP5+/6+-polymerase chain reaction with an enzyme immunoassay readout followed by reverse line blot analysis[115,116] of enzyme immunoassay-positive cases to identify hrHPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and low-risk (lr)HPV genotypes (6, 11, 26, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 70, 71, 72, 73, 81, 82, 83, 84 and 89).

Statistical analysis

All clinical data collected were analyzed using SPSS (software package version 15.0, Chicago, IL, USA). For all statistical tests a two-tailed *P*-value ≤ 0.05 was considered significant. Difference in colposcopic impression and histological outcome in women positive for HPV16 only or co-infected with any other HPV type (HPV16+) compared with women negative for HPV16 but positive for at least one other hrHPV type (non-16 hrHPV+) was calculated using two-sided Fisher's Exact Testing. Mann–Whitney U (MWU) testing was performed to assess whether the number of 'HG' pixels in the DySIS color-coded map (a reflection of the lesion size) was related to the HPV16 status and the ability to correctly classify a lesion as HG.

Results

Baseline characteristics

A total of 183 women were included in the 'according-to-protocol' cohort of the DySIS validation study[99]. Of these, six cases were excluded from the current analysis because no HPV sample was taken or because of testing failure, resulting in a cohort of 177 women. The HPV test was positive in 133 (75.1%) of the 177 women, and subsequent typing resulted in lrHPV+ (10), non-16 hrHPV+ (80) or hrHPV16+ (42). In one hrHPV+ case the type could not be determined (HPV X).

The population baseline characteristics can be seen from Table 1, stratified according to HPV testing and typing results. The majority (*n* = 160; 90.4%) of women were referred for colposcopy because of an abnormal cytological test result whether or not in combination with a positive hrHPV test. In all other women (*n* = 17; 9.6%) the indication for colposcopy was follow-up of cervical lesions. Of the 177 women included, four (2.3%) had a normal, 113 (63.8%) a BMD and 60 (33.9%) had a >BMD cervical cytology referral result. Final histology was N/LG in 92 (52.0%) and HG in 85 (48.0%) women.

HPV typing

The prevalence of the individual HPV types amongst the 132 HPV positive women was as follows; HPV16 (*n* = 42; 31.8%), HPV31 (*n* = 21; 15.9%), HPV51 (*n* = 16; 12.1%), HPV52 (*n* = 13; 9.8%), HPV18 (*n* = 12; 9.1%), HPV42 (*n* = 12; 9.1%), HPV56 (*n* = 11; 8.3%), HPV66 (*n* = 10; 7.6%), HPV33 (*n* = 9; 6.8%), HPV45 (*n* = 9; 6.8%), HPV59 (*n* = 8; 6.1%), HPV6 (*n* = 8; 6.1%), HPV39 (*n* = 7; 5.3%), HPV73 (*n* = 7; 5.3%), HPV58 (*n* = 6; 4.5%), HPV54 (*n* = 6; 4.5%), HPV67 (*n* = 3; 2.3%), HPV35 (*n* = 2; 1.5%), HPV53 (*n* = 2; 1.5%). HPV type 26, 82, 11, 30, 40, 70 and 81 were each detected only once, (0.8%). Both single and multiple infections were present and the above prevalence was calculated per individual HPV type. Multiple hrHPV infections were detected in 24 (30.0%) of the non-16 hrHPV+ women and in nine (21.4%) of the HPV16+ women (Table 1).

Table 1. Baseline characteristics and study findings for all 177 women in this study, stratified according to result of HPV typing (the total of the stratified groups is 176, because the HPV typing failed in one woman)

Characteristics	Total	HPV-	lrHPV+	non-16 hrHPV+	HPV16+
	n=177	*n=44 (25.0%)*	*n=10 (5.7%)*	*n=80 (45.5%)*	*n=42 (23.9%)*
Median age [range]	33.6 [18.7-62.6]	40.0 [21.4-61.6]	39.9 [23.4-45.8]	33.2 [18.6-62.5]	33.6 [19.8-50.6]
Mean pregnancies (SD)	1.2 (1.3)	1.5 (1.5)	0.8 (0.8)	1.1 (1.2)	1.4 (1.4)
Mean sex partners (SD)					
in total	2.9 (1.1)	2.5 (1.2)	3.3 (1.0)	2.8 (1.1)	2.9 (1.1)
in last year	1.3 (0.9)	1.1 (0.8)	1.4 (1.3)	1.3 (0.9)	1.4 (1.0)
Smoking n (%)	66 (37.3)	15 (34.1)	4 (40.0)	31 (38.8)	16 (38.1)
Condom use n (%)					
always	9 (5.1)	0 (0)	1 (10.0)	4 (5.0)	4 (9.5)
sometimes	33 (18.6)	6 (13.6)	3 (30.0)	15 (18.8)	9 (21.4)
never	119 (67.2)	31 (70.5)	4 (40.0)	54 (67.5)	29 (69.0)
missing/NA	16 (9.1)	7 (15.9)	2 (20.0)	7 (8.8)	0
Menopausal status n (%)					
pre/peri	152 (85.9)	37 (84.1)	7 (70.0)	68 (85.0)	39 (92.9)
post	13 (7.3)	5 (11.4)	1 (10.0)	5 (6.3)	2 (4.8)
unknown	12 (6.8)	2 (4.5)	2 (20.0)	7 (8.8)	1 (2.4)
Indication for colposcopy n (%)					
Abnormal cytology	129 (72.9)	32 (72.7)	7 (70.0)	59 (73.8)	30 (71.4)
Abnormal cytology & HPV+	31 (17.5)	2 (4.5)	2 (20.0)	20 (25.0)	7 (16.7)
Follow up CIN1	15 (8.5)	8 (18.2)	1 (10.0)	1 (1.3)	5 (11.9)
Follow up CIN2	2 (1.1)	2 (4.5)	0	0	0
Last cytology n (%)					
Normal	4 (2.3)	2 (4.5)	0	1 (1.3)	1 (2.4)
BMD	113 (63.8)	28 (63.6)	7 (70.0)	57 (71.3)	21 (50.0)
> BMD	60 (33.9)	14 (31.8)	3 (30.0)	22 (27.5)	20 (47.6)
Median biopsies* [range]	2.0 [0-4]	2.0 [0-3]	2.0 [0-4]	2.0 [0-4]	2.0 [0-3]
ECC n (%)	18 (10.2)	7 (15.9)	1 (10.0)	10 (12.5)	0
SCJ n (%)					
on ectocervix	153 (86.4)	35 (79.5)	9 (90.0)	67 (83.3)	42 (100.0)
in endocervix	12 (6.8)	4 (9.1)	0	8 (10.0)	0
visualized after spreading	11 (6.2)	4 (9.1)	1 (10.0)	5 (6.3)	0
not completely visualized	1 (0.6)	1 (2.3)	0	0	0

Table 1. Continued

Characteristics	Total	HPV-	lrHPV+	non-16 hrHPV+	HPV16+
	n=177	*n=44 (25.0%)*	*n=10 (5.7%)*	*n=80 (45.5%)*	*n=42 (23.9%)*
Histology n (%)					
no CIN	43 (24.3)	20 (45.5)	3 (30.0)	16 (20.0)	4 (9.5)
CIN1	49 (27.7)	14 (31.8)	2 (20.0)	26 (32.5)	6 (14.3)
CIN2	16 (9.0)	1 (2.3)	1 (10.0)	9 (11.3)	5 (11.9)
CIN3	66 (37.3)	9 (20.5)	4 (40.0)	28 (35.0)	25 (59.5)
AdCa	3 (1.7)	0	0	1 (1.3)	2 (4.8)
Histology group n (%)					
Normal/LG	92 (52.0)	34 (77.3)	5 (50.0)	42 (52.5)	10 (23.8)
HG	85 (48.0)	10 (22.7)	5 (50.0)	38 (47.5)	32 (76.2)
Mean HPV types (SD)	NA	NA	1.4 (0.5)	1.8 (1.1)	1.6 (1.2)
Mean lrHPV types (SD)	NA	NA	1.4 (0.5)	0.3 (0.7)	0.3 (0.6)
Mean hrHPV types (SD)	NA	NA	NA	1.6 (0.9)	1.3 (0.8)
Multiple hrHPV types n (%)	NA	NA	NA	24 (30.0)	9 (21.4)

AdCa, adenocarcinoma of the cervix; NA, not applicable; peri, perimenopausal; post, postmenopausal; pre, premenopausal; SCJ, squamocolumnar junction. *In case of direct treatment there was no biopsy taken.

HPV16+ versus non-16 hrHPV+ cervical lesions

Regardless of histological outcome, DySIS indicated a higher percentage of lesions as being high-grade in HPV16+ than in non-16 hrHPV+ women (73.8% versus 52.5%, P = 0.032; Table 2). Among HPV16+ women all ten women with CIN0/1 lesions were correctly classified by DySIS as N/LG, whereas among non-16 hrHPV+ women 14 (33.3%) of the 42 CIN0/1 lesions were incorrectly classified as HG (P = 0.046). Moreover, the sensitivity of DySIS to detect CIN2+ lesions was higher among HPV16+ women than among non-16 hrHPV+ women (97%, 95% confidence interval (CI) 84-100) versus 74% (57-87), P = 0.009). For women with CIN3+ lesions sensitivity figures were similar, although not statistically significant (96%, 95% CI 81-99) versus 79%, 61-90, P = 0.103). In contrast, no significant differences in sensitivity for CIN2+ were observed for the individual colposcopist impression between HPV16+ and non-16 hrHPV+ women, either when all histology was combined, or when stratified per histological outcome (P = 0.274 to P = 1.0; Table 2).

Table 2. Colposcopic impression of DySIS and the colposcopist, defined as normal or low-grade (N/LG) versus high-grade (HG) cervical intraepithelial neoplasia

A Prediction DSI	**Negative**	**lrHPV+**	**Non-16 hrHPV+**	**HPV16+**	**Total**	**Fisher's Exact Test, two sided (HPV16 vs non-16hrHPV+)**
	n (%)	*n (%)*	*n (%)*	*n (%)*		
All histology						
N/LG	33 (75)	5 (50)	38 (47.5)	11 (26.2)	87	
HG	11 (25)	5 (50)	42 (52.5)	31 (73.8)	89	p=0.032
Total	44	10	80	42	176	
CIN0/1						
N/LG	28 (82.4)	4 (80)	28 (66.7)	10 (100)	70	
HG	6 (17.6)	1 (20)	14 (33.3)	0 (0)	21	p=0.046
Total	34	5	42	10	91	
CIN2+						
N/LG	5 (50)	1 (20)	10 (26.3)	1 (3.1)	17	
HG	5 (50)	4 (80)	28 (73.7)	31 (96.9)	68	p=0.009
Total	10	5	38	32	85	
CIN3+						
N/LG	4 (44.4)	1 (25.0)	6 (20.7)	1 (3.7)	12	
HG	5 (55.6)	3 (75.0)	23 (79.3)	26 (96.3)	57	p=0.103
Total	9	4	29	27	69	
B Prediction Colposcopist	**Negative**	**lrHPV+**	**Non-16 hrHPV+**	**HPV16+**	**Total**	**Fisher's Exact Test, two sided (HPV16 vs non-16hrHPV+)**
	n (%)	*n (%)*	*n (%)*	*n (%)*		
All histology						
N/LG	32 (72.7)	9 (90)	50 (62.5)	24 (57.1)	115	
HG	12 (27.3)	1 (10)	30 (37.5)	18 (42.9)	61	p=0.566
Total	44	10	80	42	176	
CIN0/1						
N/LG	28 (82.4)	5 (100)	35 (83.3)	9 (90)	77	
HG	6 (17.6)	0 (0)	7 (16.7)	1 (10)	14	p=1.0
Total	34	5	42	10	91	
CIN2+						
N/LG	4 (40)	4 (80)	15 (39.5)	15 (46.9)	38	
HG	6 (60)	1 (20)	23 (60.5)	17 (53.1)	47	p=0.630
Total	10	5	38	32	85	
CIN3+						
N/LG	3 (33.3)	3 (75.0)	9 (31.0)	13 (48.1)	28	
HG	6 (66.7)	1 (25.0)	20 (69)	14 (51.9)	41	p=0.274
Total	9	4	29	27	69	

Results are shown for all 177 women regardless of histological outcome. Furthermore, results are displayed stratified for final histology of CIN0/1, CIN2+ and CIN3+. Colposcopic impression was compared in non-16 hrHPV+ versus HPV16+ lesions using Fisher's exact test.

Table 3. Sensitivity and specificity (with 95% CI) of DySIS and the colposcopist for the detection of CIN2+ lesions

	Treshold of CIN2+ histology	
	Sensitivity (95% CI)	*Specificity (95% CI)*
DSI		
total population	80% (70 - 88)	77% (67 - 85)
non-16 hrHPV+	74% (57 - 87)	67% (50 - 80)
HPV16+	97% (84 - 100)	100% (69 - 100)
Colposcopist		
total population	55% (44 - 66)	85% (76 - 91)
non-16 hrHPV+	61% (43 - 76)	83% (69 - 93)
HPV16+	53% (35 - 71)	90% (55 - 100)

Results are displayed for the total population, and for the non-16 hrHPV+ and HPV16+ patients respectively.

The sensitivity and specificity with 95% CI for lesion detection at the threshold of CIN2+ was calculated for the total study population and specified according to HPV16 status (Table 3). Although not statistically significant, there was a trend towards more DySIS-guided biopsies from HPV16+ than from non-16hrHPV+ women (n = 26; 61.9% versus n = 38; 47.5%, P = 0.181). Similarly, a colposcopist-guided biopsy was obtained more frequently among HPV16+ than among non-16 hrHPV+ women (37, 88.1% versus 58, 72.5%, P = 0.066).

The HG lesion size is given in Table 4, stratified per HPV16 status. High-grade lesion size was defined as the number of pixels indicated as HG by the DySIS color-coded map. Therefore the size of those lesions that were indicated as N/LG by the DySIS color-coded map but as HG by the colposcopist are missing (Table 4). These data show that there were no differences in mean number of HG pixels according to HPV16 status (mean rank 32.86 versus 27.42, P = 0.225 Mann-Whitney U test). However, CIN2+ lesions defined as HG by the colposcopist were significantly larger than those who were defined as N/LG by the colposcopist (mean rank 35.74 versus 21.02, p=0.001 Mann-Whitney U test).

Table 4. Number of HG pixels of missed (N/LG) and detected (HG) HPV16+ and non-16 hrHPV+ CIN2+ lesions

	Impression			
	DSI N/LG	**DSI HG**	**Colpo N/LG**	**Colpo HG**
Non-16 hrHPV$^+$ CIN2$^+$ (*n* = 38)				
Median HG pixel number [range]	*n* = 10	*n* = 28 2632 [40–65 796]	*n* = 15 384 [40–8870]*	*n* = 23 4350 [20–65 796]**
HPV16+ CIN2+ (*n* = 32)				
Median HG pixel number [range]	*n* = 1	*n* = 31 680 [4–24 948]	*n* = 15 562 [4–10 225]***	*n* = 17 1636 [8–24 948]

The number of HG pixels of those lesions that were indicated as N/LG by the DySIS map but as HG by the colposcopist are missing, therefore the total number of women with a known amount of HG pixels is indicated with asteriks: **n* = 10, ***n* = 20, ****n* = 14.

Discussion

Our data are in partial agreement to previous findings that visual appearance among HPV16+ women is more defined, although this was previously thought to be irrespective of the severity of the lesion[118]. In this study we demonstrate that this effect is dependent of final histology. Furthermore, we add to these data by using a clinically significant outcome measure of N/LG versus HG lesions, instead of an impression of normal versus low-grade or worse (LG+)[118].

The fact that colposcopic impression was recorded prior to biopsy taking, and that random biopsies were collected from all women in addition to colposcopically guided biopsies, add credit to our results. Histology in our study is a more reliable 'gold standard' than in a previous study in which only colposcopically guided biopsies were taken[118]. Furthermore, our cohort of women were referred for colposcopy because of abnormal cytology or follow-up of CIN lesions and this reflects the actual population referred for colposcopy in regular clinical practice. We find a relatively high percentage of HPV negative women with a high-grade lesion (n=10, 22.8%) compared with other studies. This might have been caused by a false-negative HPV test because two of these ten women, with a CIN3 lesion, did indeed have a positive hrHPV test on the biopsy sample. An additional explanation could be that the scrapes used for HPV testing were taken directly before colposcopy, and were therefore taken with more caution, resulting in a false-negative test result. However, to keep the analysis uniform throughout the study, we did not correct the status of these patients to hrHPV positive.

Remarkably, we were only able to reproduce the findings by Jeronimo et al.[118] using DySIS digital colposcopy. With visual colposcopy we did not observe a difference in either sensitivity, or specificity for HG lesions caused by different HPV types. Within the limitations of this study we cannot fully explain why DySIS classifies lesions more accurately among HPV16+ women. We explored possible explanations such as the number of biopsies that had been taken, but as a random biopsy was collected in all women this could not have influenced the outcome. Furthermore, a possible confounder, cytology status, has been shown not to differ amongst the missed and detected lesions among the HPV16+ and non-16hrHPV+ women. Also the amount of multiple hrHPV infections was similar in both groups, and 'HG' pixel count (lesion size) was not related to HPV16 status. Hence a plausible remaining explanation is that HPV16+ lesions show more intense/durable acetowhitening, an effect that is directly used by the DySIS software algorithms to map the lesion[112].

HPV16 is one of the most oncogenic hrHPV types[125-127] so it is reassuring for the colposcopic practice that HPV16 caused lesions can be effectively detected by DySIS digital colposcopy. In this era of vaccination of young women against HPV16, the prevalence of HPV16 infections is expected to decrease; however, it is not likely that this will affect the population over the next 15-20 years, when the vaccinated women of today come of age. It has been argued[118] that this reduction may eventually lead to the deterioration of colposcopic performance, because this depends largely on the experience of the practitioners. New technologies such as DySIS digital colposcopy will maintain colposcopic performance to a high standard. Furthermore, it may be clinically relevant that DySIS seems to detect smaller cervical lesions. It can be speculated that by detection and early treatment of these smaller lesions the number of complications of treatment (premature birth and premature rupture of membranes during pregnancy) can be diminished. However, it remains to be elucidated how relevant the detection of small HG lesions is since small HG lesions are more likely to regress spontaneously than large HG lesions.

Conclusion

We studied the agreement between colposcopic impression and final histology for HPV16+ and non-16 hrHPV+ women in a prospective cohort study in which women referred for colposcopy were evaluated by both conventional colposcopy and digital colposcopy using the DySIS digital colposcope[99]. It has been shown that the DySIS digital colposcope improves the sensitivity of colposcopy[57,99]. Our main finding in this study is that lesions are better detected among HPV16+ women than among non-16 hrHPV+ women at colposcopy with DySIS and that this effect is associated with CIN

grade. Furthermore, we show that the HPV16 status does not affect the performance of the colposcopist. It is mainly the lesion size that determines the accuracy of the colposcopic impression, and, as generally anticipated, the colposcopic performance is worse when HG lesions are smaller.

Disclosure of interest

Besides CB and EP none of the authors received financial support from DySIS Medical Ltd (formerly Forth Photonics Ltd), Livingston, UK for the submitted work. CB was a stockholder and EP is an employee of DySIS Medical Ltd (formerly Forth Photonics Ltd) UK at the time of this study; their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and none of the authors have non-financial interests that may be relevant to the submitted work. JL and MK received support from DySIS Medical Ltd (formerly Forth Photonics Ltd), Livingston, UK for investigator meetings. Furthermore, JL and AZ received travel support from DySIS Medical Ltd (formerly Forth Photonics Ltd), to visit a conference on colposcopy.

Contribution to authorship

AZ and JL drafted the manuscript with assistance of JB for statistical analysis. JL, AZ, MK, WH, GG and JS were responsible for the colposcopies and collection of the data. PS supervised the hrHPV testing. FK and CM were responsible for revising the histology samples. RV was the project leader and designed the clinical study with JL, MK, WH, CB, EP, PS and CM. All authors critically reviewed the manuscript.

Details of ethics approval

The ethic boards of the three participating clinics approved the protocol (number 2007/098). Signed informed consent was obtained from all women prior to any study procedures. The study was registered in the Dutch trial registry (ISRCTN66112760).

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digital colposcope and the patient insurance coverage. Their representatives had a role in the study design and they critically appraised the manuscript, but they had no role in data collection or final data analysis. All authors had full access to all data in the study. The corresponding (AZ) and last author (RHMV) had the final responsibility for the decision to submit for publication.

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5

Prologue

With this case series we would like to illustrate the difficulties and challenges in diagnosing cervical cancer. We have included cases in which one or more suboptimal factors can be identified at the various levels of the diagnostic process, from the attendance of the patient to cervical screening up to the accuracy of clinical and histopathological examination.

Patient related window of opportunities

A 39 year old woman did not attend screening for one round and was diagnosed with invasive cervical cancer thereafter. In 2001 she had a pap 1 smear. She defaulted the second screen. In 2009 cervical cytology showed a pap 4. Direct LEEP was performed because of a suspected high-grade cervical lesion. The examination showed a grade 2, FIGO IB1 squamous cell carcinoma without LVSI. The tumor diameter was 9mm and the surrouding tissue contained a high-grade squamous intraepithelial lesion (HSIL). She was treated with a robot assisted radical hysterectomy. She did not have any lymph node metastasis and is disease free up to the present.

Clinician related window of opportunities

A 29 year old woman, not yet called for the cervical screening program had a intrauterine device inserted during which a large cervical tumor was overlooked. In 2008 she had a intrauterine device containing levonorgestrel inserted by a gynecologist. After two days she visited her general practitioner to have the device removed because of severe bleeding. The bleeding persisted, and she was seen by a second gynecologist. At the physical examination a large cervical tumor (around 6 cm in width) was detected and biopsies confirmed the diagnosis of a grade 2 FIGO IIIB squamous cell carcinoma without LVSI. There were no enlarged lymph nodes on radiological examination. She was treated with chemoradiation, and she is free of recurrence at the moment.

A 79 year old women was being analyzed since May 2009 by a urologist because of haematuria, in hindsight a symptom of an unrecognized cervical tumor. Since May 2009 she was being analyzed by a urologist for haematuria. In November of the same year she visited her general practitioner, because of unrelated complaints and after further inquiring she mentioned persistent vaginal blood loss. Examination by the general practitioner showed excessive bleeding and she was referred to the gynecologist the same day. The gynecological examination revealed a tumorous cervix, and directed biopsies confirmed the diagnosis of a grade 3 squamous cell tumor, FIGO IIB without LVSI. The tumor was 4cm in width and spread to the parametrium. She was treated with radiotherapy only because of kindey failure and to date she is free of recurrence.

(Cyto)Pathologist related window of opportunities

46 year old asymptomatic woman, participating in the five yearly cervical screening program had a delayed diagnosis because of false-negative cytology. In 2006 she had a pap smear showing borderline dyskaryosis, in 2007 she had normal cytology but was hrHPV positive. The repeat cytology in 2008 showed moderate dyskaryosis. Colposcopy was performed in January 2009, the impression was a high-grade lesion and a direct LEEP was performed. The pathological examination showed a grade 2 squamous cell carcinoma of at least 9 mm in width (due to fragmentation difficulties to analyze), without LVSI. She was diagnosed with a stage IIB tumor of 5 cm in width with no enlarged lymph nodes on radiological examination and was treated with chemoradiation. She is free of recurrence to date.

A 48 year old woman, participating in the cervical screening program and treated for a cervical lesion, which was wrongfully interpreted as a high-grade squamous intraepithelial lesion (HSIL). She had normal cytology in 1995, 1996 and 1999. In 2006 she had a cytology smear showing carcinoma in situ, colposcopically guided biopsy showed a HSIL, and a subsequent LEEP was performed showing the same HSIL. She had normal cytology in 2007 and 2008, during regular follow-up. Because of complaints of abnormal bleeding and vaginal discharge a colposcopy with directed biopsy was performed later in 2008, showing grade 3 squamous cell cancer with LVSI. The exterior of the cervix appeared normal, also by examination by the gynecological oncologist, however, a subepithelial tumor of 5cm in width was identified, extending to the parametria. The LEEP specimen from 2006 was revised and found, in hindsight, suspicious of an invasive carcinoma. She was diagnosed with a FIGO stage IVB cervical cancer with enlarged lymph nodes on radiological examination. Despite chemoradiation she had disease progression and died of disease in the same year.

Combination of factors

A 28 year old symptomatic woman was diagnosed in 2011 with invasive cervical cancer, recognized after several gynecological examinations and inappropriate hormonal treatment. She sought counselling for preimplantation genetic diagnosis (PGD). She had no complaints, had a presumed normal cervical cytology and apart from a presumed ectropic cervix there were no abnormalities seen during the routine gynecological examination. She was switched from local (ethinylestradiol/ etonogestrel ring) to oral contraceptive (ethinylestradiol/ levonorgestrel), after which she reported severe vaginal bleeding. Hormonal imbalance was thought to be the cause of this excessive blood loss. The oral contraceptive pill was stopped and she was treated with tranexamic acid. Because of persisting complaints she was

re-examined, cervical cytology showed suspicion of carcinoma and at colposcopy a 5 cm tumor was recognized, showing squamous cell carcinoma on biopsy. She was staged FIGO IB2 with positive lymph nodes on radiological examination and was treated with chemoradiation and is disease free to date.

A 32-year old woman was diagnosed with invasive cervical cancer two years after a normal cytology smear. She presented at her general practitioner in 2009 with post coital and intermenstrual bleeding since 6 months. In 2007 she had had normal cytology in the cervical screening program. The general practitioner saw an ectropion of the cervix at clinical examination which was treated with trichloroacetic acid without prior cytological examinaton. As this treatment was not effective, she was referred to the gynecologist. Gynecological examination revealed a cervical tumor of 3.5cm in width. A cytological smear showed suspicion of carcinoma and biopsies confirmed the diagnosis of a grade III squamous cell carcinoma of the cervix. She had a FIGO stage Ib1 tumor, with LVSI, but no remarklable lymphadenopathy at radiological examination. She was treated by a robot assisted radical hysterectomy, was node negative, and is free of disease to date.

A 35 year old woman, not participating in the cervical screening program had a delayed diagnosis because of unrecognized complaints. She visited the gynecologist in 2005 because of irregular vaginal bleeding and was treated with a intrauterine device containing levonorgestrel. In 2008 she had a pap smear showing severe dyskaryosis. Colposcopically directed biopsy showed a HSIL and a subsequent conisation showed a gr 2 squamous cell carcinoma of 3 cm in width. She was clinically staged with a FIGO stage IB1 cervical cancer, she had a robot assisted radical hysterectomy and adjuvant radiotherapy because of microscopic parametrial invasion. She has no had a disease recurrence to the present.

A 44-year old woman was finally diagnosed with cervical cancer after a long history of 18 cervical smears and 6 histological samples of the cervix. In 1998, at the age of 35 she had her first cytology smear showing severe dyskaryosis, repeat smear showed a carcinoma in situ and colposcopy with directed biopsies showed a HSIL lesion which was treated by cryocoagulation. She had three borderline or mild dyskaryotic smears in the follow-up, and had a second colposcopy in 1999. The quality of the biopsies was insufficient and a conisation was advised, which the patient refused. In the follow-up the cytology remained mild to moderate dyskaryotic. She was persistent in her refusal of surgical treatment, and colposcopically guided biopsies in 2000 showed HSIL. Up to 2003 she had 5 smears taken, one normal and four showing borderline or mild

dyskaryosis. A LEEP was performed in 2003 showing HSIL, after which she had five normal follow-up smears up to 2007. In the mean time she had two benign cervical polyps removed in 2004 and 2006. In 2008 she presented with complaints of irregular vaginal bleeding and abnormal discharge. She was again referred to a gynecologist and was diagnosed with a 6cm large cervical tumor at the first examination, which may have been a fast growing tumor, or had been overlooked by previous examiners. Cytology showed signs of carcinoma and biopsies showed grade II squamous cell cancer. Revision of the cytology smear from 2007 showed severe dyskaryosis. She was staged with FIGO IIIb disease. Despite chemoradiotherapy she had disease recurrence in 2008, and succumbed in 2009.

5

The diagnostic process of cervical cancer: areas of good practice and windows of opportunity

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Abstract

Objective: Despite an extensive screening program in The Netherlands, some cases of cervical cancer are still diagnosed in late-stages of disease. The aim of the present study was to investigate which elements in the diagnostic process of cervical cancer may be improved.

Methods: This is a retrospective study of 120 patients with cervical cancer diagnosed between January 1st 2008 and June 1st 2010 at the University Medical Center Utrecht. Patient charts, referral information and pathology results were analyzed.

Results: 39.1% of cancer cases were screen or interval detected; the other 60.9% of patients had not been screened, either due to non-attendance or because they fell outside the age range for screening. The final diagnosis of cervical cancer was established by biopsy in 77 (64.2%) and by excision of the cervical transformation zone in 35 (29.2%) of the patients. Fifteen (43%) of the excisions could have been avoided if no 'see-and-treat' had been performed, whereas the other 20 patients (57%) had a premalignant histology result prior to the excision procedure.

Conclusions: Cervical cancer screening aims at early detection of precursor lesions to decrease the incidence of cancer. This in-depth analysis suggests that improvement of quality of care is to be expected from correctly recognizing cervical cancer by physicians and adjustments of the screening program to reach younger women and non-responders. Furthermore, great care should be taken with the use of diagnostic excisional procedures as they might impede fertility sparing surgery.

Introduction

Cervical cancer is currently regarded as a preventable or at least early recognizable disease, but it still accounts for 12% of all female cancers, and for 7.5% of female cancer deaths. In 2012, there were an estimated 528.000 new cases and 266.000 deaths worldwide (Globocan). Almost nine out of ten (87%) of the deaths occur in less developed countries. Due to screening programs in the western world, cervical cancer has become a rare disease with a diminishing incidence. In The Netherlands, the overall 5-year survival of patients diagnosed between 2001 and 2005 was 67%. This 5-year survival drops from around 90% for smaller tumor lesions (Stages IA-IB) to less than 70% for more advanced-staged tumors (patients diagnosed between 2003 and 2009) (Source: Netherlands Cancer Registry managed by Comprehensive Cancer Center The Netherlands).

High-risk human papillomavirus (hrHPV) infection has been recognized as an imperative cause of cancer. Invasiveness is preceded by cytologically detectable premalignancy (cervical intraepithelial neoplasia, CIN)[1]. In The Netherlands, as in other countries, a cervical screening program is in place that invites women aged 30-60 years to be screened 5-yearly. The main purpose of the screening program is to detect CIN lesions, to offer treatment, and thereby to prevent the development of invasive cervical cancer. Currently this program entails primary cytology screening, which will be adapted in the near future to primary hrHPV screening with reflex cytology testing. Women with a cytological diagnosis worse than borderline or mild dyskaryosis (>BMD) or a persistent BMD are referred to a gynecologist[30]. The current screening program suffers from a relatively low attendance rate (66%)[97], a low sensitivity of cytology (60-70%)[25-27] and a low sensitivity of colposcopy (55%)[43,46,47]. Despite this extensive screening program, cervical cancer is, even in The Netherlands, still diagnosed in an inoperable stage in approximately 40% of the cases. In 2012 the incidence rate of FIGO stage IIA and higher was 3.53/100.000 on a total cervical cancer incidence of 8.68/100.000 (Source: Netherlands Cancer Registry managed by Comprehensive Cancer Center The Netherlands). In 2012, the Dutch cervical cancer incidence was 735, and 215 patients died of the disease[23]. Earlier and more accurate recognition of abnormalities might further improve prognosis for patients with cervical cancer. In this study, we aimed to identify areas of good practice of cervical cancer detection and areas that could be improved. To this end we performed an analysis of the screening history and diagnostic pathway in an aselect cohort of patients with cervical cancer. We analyzed the diagnostic pathway from complaints or referral because of abnormal cytology until the choice of treatment.

Methods

Patients

In this retrospective cohort study we analyzed all 120 women with newly diagnosed cervical cancer between January 1st 2008 and June 1st 2010 in our region. Through The Netherlands Cancer Registry managed by Comprehensive Cancer Center The Netherlands), the hospital information system, as well as the pathology and gynecology department registration, we identified 129 patients with invasive cervical cancer referred to the academic gynecological oncological department at the University Medical Center Utrecht (UMCU), The Netherlands where all of the cervical cancer patients in the region are treated. Patients were referred for review of histopathology, diagnostic evaluation and/or treatment. Nine patients were excluded from the study because of insufficient information or because it was not a first diagnosis of cervical cancer. The patient records and referral information from the gynecologist, and, if available, from the general practitioner were reviewed. We analyzed the files for complaints that could be related to cervical cancer (vaginal blood loss, abnormal discharge, abdominal pain, micturition problems and weight loss), and identified when the cancer lesion had been first suspected or recognized. Tumor stage was established according to the International Federation of Gynecology and Obstetrics (FIGO) guidelines[61].

Pathology

The cytological and histological results of all patients were retrieved from the nationwide network and registry of histopathology and cytopathology (PALGA, The Netherlands), including classification, histological type, differentiation grade, parametrial invasion, lymphovascular invasion (LVI) and lymph node invasion. Cytological results were reported according to the Dutch CISOE-A classification, which easily translates into Pap and/or Bethesda classification[28]. The cytology specimens were not revised on a regular basis, but all histological tumor specimen were revised at the Department of Pathology of the UMCU.

Patient grouping

Patients were divided into five groups based on their participation in the cervical screening program: 'pre-screening', 'screen-detected', 'interval diagnosis,' 'non-participants' and 'post-screening'. The 'pre-screening' group consisted of women <30 years who were not yet invited for population screening. Women who were diagnosed as a result of the cervical screening program were assigned to the 'screen-detected' group. Even if they missed one screening examination, if the tumor was detected in the next screening round they were considered screen-detected. The

'interval diagnosis' group consisted of women between 30-60 years who had normal cytology within 5.5 years before the diagnosis of cervical cancer.
Women between 30-60 years were assigned to the 'non-participant' group when the tumor was not 'screen-detected' and the last cytology was >5.5 years before the diagnosis of cervical cancer. Finally, women >60 years were assigned to the 'post-screening' group.
Furthermore, we merged the FIGO stages to microinvasive (FIGO stage IA), localized (IB-IIA2) and advanced-stage (>IIA2). The date of diagnosis was defined as the date of the first histology sample that showed invasive cervical cancer.

Statistical analysis
Data collected were analyzed using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). For all statistical tests a two-tailed p-value ≤ 0.05 was considered significant. Baseline characteristics were calculated using percentages and median values with range. Differences between groups were analyzed using chi-square testing for the categorical variables, and Mann-Whitney U testing for the non-parametric variables. Kaplan-Meier survival probability estimates with log rank testing were used to describe time to compare disease specific survival.

Results

Patient data
The patient and tumor characteristics are presented in Table 1. The median age of the 120 patients at diagnosis was 47 years. Reasons for the first visit to the gynecologist were complaints in 88 (73%) or abnormal cytology in 32 (27 %) of the women. In total 98 (83.1%) women reported complaints at the time of diagnosis (this includes 10 screen-detected cancers). Next to the 28 (23.3%) screen-detected cancers, there were 19 (15.8%) women with an interval diagnosis and 32 (26.7%) cancers detected among non-participants. Of the 28 screen-detected cases, 11 (39.3%) were detected in the first screening round. Per definition all patients with interval diagnosis had normal cytology within 5.5 years prior diagnosis. Of the 32 non-participants, 17 (53.1%) never had any cytology sampling, 12 (37.5%) had normal cytology more than 5.5 years prior to diagnosis, 2 (6.2%) had BMD cytology with repeat advised and 1 (3.1%) was advised to attend for colposcopy more than 6 months prior to diagnosis.
Most (75.8%) of the tumors were of squamous origin, grade II (66.7%) and FIGO stage IB (48.3%). LVI was known for 109 of the cases of whom 45 (41.3%) were positive (in 11 patients LVI could not be determined in the diagnostic specimens, and there

Table 1. Patient characteristics of the 120 patients in this study. Screening history does not include the cytology smear that has led to diagnosis, only those samples prior to the diagnostic smear. The non-screening category consists of pre and post screening, interval diagnosis and non-participants

Patient characteristics	Total N=120
Age at diagnosis median [range]	47.2 [24.2-87.8]
Complaints N (%)	98/118 (83.1)
Vaginal blood loss	84/98 (85.7)
Vaginal discharge	16/98 (16.3)
Abdominal pain	11/98 (11.2)
Screening category N (%)	
pre-screening	6 (5.0)
screen-detected	28 (23.3)
interval-diagnosis	19 (15.8)
non-participant	32 (26.7)
post-screening	35 (29.2)
Screening history N (%)	
no cytology in NL	47 (39.2)
cytology >5.5 y prior to diagnosis	36 (30.0)
negative cytology <5.5y prior to diagnosis	30 (25.0)
repeat advised	6 (5.0)
colposcopy advised >6m prior to diagnosis	1 (0.8)
Tumor characteristics	
Histologic type N (%)	
SCC	91 (75.8)
ACC	18 (15.0)
ASCC	7 (5.8)
Small cell	4 (3.3)
Differentiation grade N (%)	
I	12/111 (10.8)
II	74/111 (66.7)
III	25/111 (22.5)
FIGO stage N (%)	
IA	10 (8.3)
IB-IIa2	66 (55)
>IIa2	44 (36.7)
LVI N (%)	45/109 (41.3)
LN metastasis in PA N (%)	15/55 (27.3)
Lymphadenopathy radiology N (%)	43/113 (38.1)
Parametrial invasion N (%)	4/41 (9.8)

was no surgical treatment). 55 patients underwent lymphadenectomy of whom 15 (27.3%) women had lymph node metastasis. Of the 65 patients that did not undergo lymphadenectomy, 61 were radiologically evaluated, of whom 32 patients (52%) had suspicious nodes.

Treatment was performed according to national guidelines, and is detailed in supplementary Table S1. Two women refrained from treatment, of which one participated in a trial on therapeutic HPV vaccination. Follow up was recorded until December 2013. 30 (25.2%) of the women died of disease, three women died of other causes (pancreatic, lung and endometrial cancer respectively) and in one patient the cause of death was unknown.

Duration of diagnostic pathway

Duration of the diagnostic pathway for the individual patients is shown in Table 2. The median time from first complaints to diagnosis was known for 80 patients and was 3.8 months. The median time between first abnormal cytology result and diagnosis was 5.5 months. Figure 1 shows that 58% of the patients (48/83) were diagnosed with cervical cancer within two years after their first abnormal smear, 60% within five years (50/83) and the remaining patients thereafter. Per definition, these data could not be calculated for women without abnormal cytology prior diagnosis. There was no difference in time between first abnormal cytology to diagnosis between screen and interval detected cancers and cancer diagnosis among non-participants.

It took a median of 0.9 month from the first abnormal premalignant histopathological result to diagnosis. In 96 cases cytology had been sampled prior to the diagnosis of cancer, in the remaining 24 cases immediate histological sampling was performed because of suspicion of invasive cancer.

Table 2. Median time to diagnosis from cytology and histology results. The diagnostic histological specimen is not included in these analysis. Only the premalignant histology results prior to diagnosis

Time in months median[range] to diagnosis	
Complaints	
First complaints N=80	3.8 [0-60.1]
Cytology	
First abnormal cytology N=83	5.5 [0.1-333.5]
Last normal cytology N=51	60.7 [0.1-238.2]
Histology	
First abnormal histology N=35	0.9 [0.1-193.4]
Last normal histology N=6	21.8 [0.4-236.3]

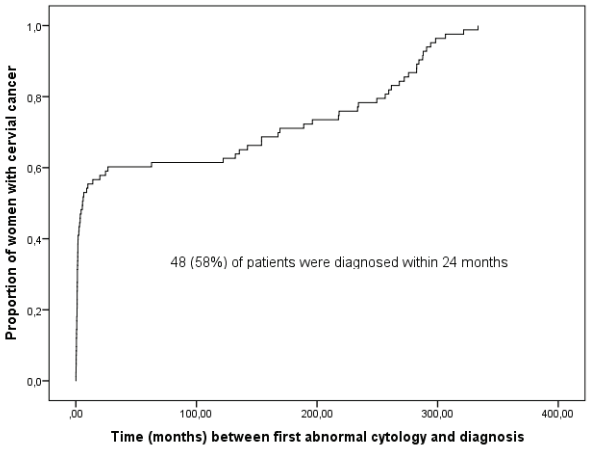


Figure 1. Time from first abnormal cytology smear to the diagnosis of cervical cancer, stratified for mode of detection of the tumor (screen or interval detected versus non-participants).

Cytology

The results of the cytology samples preceding the diagnosis are summarized in Table 3. A median of 1.5 smears were taken before diagnosis. The most abnormal cytology result prior to diagnosis was BMD (11.4%), moderate (16.7%) or severe dysplasia (25%), carcinoma in situ (17.7%) or invasive carcinoma (14.6%). Interestingly, 13.5% of the patients had normal cytology before diagnosis.

Diagnostic procedures

The information on the different diagnostic procedures at the referral visit are shown in Table 4. 55 (47%) of the patients underwent a colposcopy, the others patients had a gynecological speculum examination with biopsy sampling at the outpatient clinic which was sufficient for the diagnosis. Furthermore, the colposcopic impression could be retrieved for 51% of these 55 patients. The majority of women (47.9%) were diagnosed with invasive cervical cancer at the first visit to the gynecologist or oncological gynecologist. The remainder needed one (30.8%) or two (12.8%) visits to perform colposcopy or examination under anaesthesia. One patient had up to 10 visits to the gynecologist, with complaints (in hindsight) related to cervical cancer.

Table 3. Information on cytology smears prior diagnosis

Cytology including diagnostc smear	Total N=120
Number of pap smears median [range]	1.5 [0-21]
Number of pap smears N (%)	
0	24 (20.0)
1	36 (30.0)
2	17 (14.2)
3	19 (15.8)
4	5 (4.2)
5	5 (4.2)
>5	14 (11.7)
Worst cytology result N/96 (%)	
unsatisfactory	1 (1.0)
normal	13 (13.5)
BMD	11 (11.4)
moderate dysplasia	16 (16.7)
severe dysplasia	24 (25.0)
carcinoma in situ	17 (17.7)
invasive carcinoma	14 (14.6)
Last cytology result N/96 (%)	
unsatisfactory	4 (4.2)
normal	19 (19.8)
BMD	5 (5.2)
moderate dysplasia	15 (15.6)
severe dysplasia	24(25.0)
carcinoma in situ	16 (16.7)
invasive carcinoma	13 (13.5)
Ever had normal cytology N (%)	51/96 (53.1)

Histology

All histopathological specimens were analyzed in a similar fashion (Table 5). In 81(67.5%) women the pathologic examination of the first histological sample showed invasive cervical cancer. The remaining 39 (32.5%) women had a premalignant lesion (CIN III in 26(66.7%)) or adenocarcinoma in situ detected prior to the diagnosis of invasive cervical cancer. We retrieved the details of the first histological specimen that led to the diagnosis of invasive cervical cancer. This was a punch biopsy (64.2%), LEEP (20.0%), conisation (9.2%), endocervical curettage (4.2%) or hysterectomy specimen (2.5%). The colposcopic impression was related to FIGO stage and type of histological specimen and can be seen from supplementary Tables S2 and S3. A LEEP or conisation procedure had been performed in both patients with early-stage disease as well as in patients with advanced tumor stage. The colposcopic impression (not described for all patients) was premalignant for those patients that underwent a LEEP or conisation procedure.

Table 4. Details on colposcopy and gynecologist visits, not all parameters are applicable or known for all 120 patients. If the denominator is not 120 it is specified in the table

Details on colposcopy and gynecologist visits	
Colposcopy prior to diagnosis N (%)	55/117 (47)
Number of colposcopies prior to diagnosis N/117 (%)	
0	62 (53)
1	48 (41)
2	4 (3,4)
3	3 (2,6)
Impression at first colposcopy N/55 (%)	
unknown	27 (49,1)
normal	2 (3.6)
high-grade lesion	12 (21.8)
invasive cancer	14 (25.5)
Number of visits prior to diagnosis median [range]	1 [0-10]
Number of visits prior to diagnosis N/117 (%)	
0	56 (47.9)
1	36 (30.8)
2	15 (12.8)
3	2 (1.7)
4	4 (3.4)
5	1 (0.9)
6	2 (1.7)
10	1 (0.9)

Differences between screen and non-screen detected cancer

Univariate analysis was performed in order to relate patient and tumor characteristics to the mode of detection. For the purpose of this analysis we merged patients that were screen-detected or had an interval diagnosis and compared them with the non-participants. The women outside the population based screening program (aged below 30 or above 60 years of age) were excluded from this analysis. Complaints ($p < 0.001$), lymphadenopathy at radiological examination ($p = 0.011$), and number of pap smears ($p < 0.001$) differed significantly between the participant and non-participant group.

Table 5. Information on histology results prior to diagnosis (top half), and results including the diagnostic specimen

Cervical histology prior to cancer diagnosis		Cervical histology including diagnostic sample	
Number of samples median [range]	0 [0-6]	Type first histology N (%)	
Number of samples N (%)		punch biopsy	98 (81.7)
0	81 (67.5)	LEEP	15 (12.5)
1	29 (24.2	conisation	2 (1.7)
2	4 (3.3)	endocervical curettage	4 (3.3)
3	4 (3.3)	endometrium sampling	1 (0.8)
4	1 (0.8)	Result first histology N (%)	
6	1 (0.8)	normal	5 (4.2)
Ever had normal histology N(%)	6 (5.0)	infection	1 (0.8)
Worst histology N/39 (%)		inadequate	1 (0.8)
normal	3 (7.7)	CIN nos	1 (0.8)
inadequate	1 (2.6)	CIN I	1 (0.8)
CIN NOS	1 (2.6)	CIN II	2 (1.7)
CIN I	1 (2.6)	CIN III	24 (20.0)
CIN II	2 (5.1)	AIS	4 (3.3)
CIN III	26 (66.7)	SCC	65 (54.2)
AIS	5 (12.8)	ACC	10 (8,3)
Last histology N/39 (%)		ASCC	3 (2.5)
normal	5 (12.8)	small cell carcinoma	3 (2.5)
inadequate	1 (2.6)	Type of specimen cancer diagnosis	
CIN NOS	1 (2.6)	punch biopsy	77 (64.2)
CIN I	1 (2.6)	LEEP	24 (20.0)
CIN II	1 (2.6)	conisation	11 (9.2)
CIN III	24 (61.5)	endocervical curettage	5 (4.2)
AIS	6 (15.4)	hysterectomy	3 (2.5)

Discussion

The national incidence of cervical cancer was 706 in 2008, 720 in 2009 and 731 in 2010. Thus, during our study period around 1731 women had been diagnosed with cervical cancer in The Netherlands. The University Medical Center Utrecht is one of the 8 gynecological oncology referral centers that provide treatment for patients with cervical cancer in The Netherlands. Thus, in this study we describe 120 cases, corresponding to aroung 7% of all newly diagnosed patients in The Netherlands during this period.

Previous studies have shown that performing cervical cancer screening is an effective procedure. In the United Kingdom a 35% decrease in cervical cancer incidence has been observed between 1980 and 1995[128]. Similarly, in the Amsterdam screening region (The Netherlands) the incidence has decreased from 9.2/100.000 in 1988 to 5.9/100.000 in 2000[129].

Cancer 'not detected by screening' can be defined in various ways, and therefore proportions of such cancers vary between studies[130-132]. In our view, non-participants are those women that are in the age range of screening, are invited but do not attend screening. Previous studies have included women outside the age range of screening in their non-participant group, yielding a larger proportion of 'non screen-detected' cancers. In our study 32 (26.7%) of all patients with cervical cancer were non-participants. If this percentage is recalculated by removing pre- and post-screening from our total population there were 32 (40%) non-participants and 47 (60%) screen- or interval detected cancers. These percentages are comparable to previous studies: 55% in the study of Bos et al[130] (with 35 years as starting age of the screening program, as opposed to 30 years in our study) and 53% in the study of of Sung et al[131]. If Table 1 is recalculated and non-responders are defined by adding non-participants, pre- and post-screening patients we show a percentage of 60.9, in line with a recent study[132]. In this study it was shown that half of the cervical cancer patients were not screened, either due to non-attendance, or because they fell outside the age range for screening. Until 1996 the age at which patients were invited for the screening program in The Netherlands was 35 years, thereafter the age was lowered to 30 years. The incidence of cervical cancer in the pre-screening age had been predicted to reduce from 12 to 5% by lowering the screening age[130]. Although our numbers are small, our data correspond with this prediction; we observed 6 (5%) of patients diagnosed with cervical cancer in the pre-screening age. 35 patients were diagnosed after 60 years of age, and 26.7% had never participated in the screening program. The remaining 15.8% had had a normal cytology result within 5.5 years before diagnosis of cervical cancer. Extension of the screening age to <30 and >60-years of age might help to

further reduce the incidence of cervical cancer until the effect of HPV vaccination is observed.

Interestingly, 98 (83.1%) of the patients reported complaints related to cervical cancer at time of diagnosis. Possibly creating more cervical cancer awareness amongst patients and physicians could be an effective method to improve cervical cancer recognition.

In the present study we show that the average time from abnormal cytology to diagnosis of cervical cancer was short (median 5.5 months). There were, however, cases with a delay in diagnosis of up to 333 months (this patient was outside the screening age and had a history of recurrent abnormal cytology years before, and one normal cytology smear before she was excluded from the screening program, we could not revise this cytology smear, and are not sure whether it was a slowly developing or newly derived tumor).

Unfortunately information on colposcopic impression could not be retrieved for all patients, therefore we could not investigate the difference in diagnosis by colposcopy or by speculum examination. Less than 50% of patients had a colposcopy, and the majority of cases were diagnosed by gynecological speculum examination and histological sampling. The majority (98, 81.7%) of patients had a biopsy taken at the first cervical examination and 17 (14.2%) had a direct treatment by loop electrosurgical excision procedure (LEEP) or conisation. Of those 17 'see-and-treat' cases 15 showed cancer and two showed a premalignant lesion (CIN2 and AIS respectively). The final diagnosis of cervical cancer was made based on a punch biopsy in 77 (64.2%) of the patients, and an excision procedure in 35 (29.2%) patients. Of those 35 patients with a diagnosis based on an excision procedure, 15 (43%) were see-and-treat as described above, and the remaining 20 (57%) women had a secondary treatment after a biopsy, LEEP or curettage showing a premalignant lesion.

As more and more younger patients develop cervical cancer, one would like to avoid aggressive and relatively extensive diagnostic surgery, since this might delay and/or complicate fertility sparing surgery. However, in 57% of the cases in the present study it was inevitable to perform a diagnostic excision procedure, because the initial histologic examination showed a premalignant lesion.

Conclusion

Cytological cervical cancer screening has undoubtedly and significantly reduced the incidence of cervical cancer in countries, such as The Netherlands, were screening programs have been implemented. Screening is effective in preventing cervical cancer through the early detection of premalignant lesions, whereas the identification of cancer cases still remains a problem. We identified two major factors related to the failure to prevent cervical cancer: non-participation in the screening program, and failure to clinically recognize (often extended) cancer in women with complaints indicative for cervical cancer or abnormal cytology. As a consequence, one third of the patients needed aggressive (normally avoidable) diagnostic surgery (loop or cold knife conisation) before final treatment of cancer. Better coverage of the screening program that includes a wider age range and measures to improve compliance should enable prevention or earlier detection of cancer. Furthermore strategies to improve physician and patient awareness about complaints related to cervical cancer might lead to earlier detection. Importantly, improving quality and documentation of colposcopy might lead to better detection of cancer and less radical diagnostic procedures, which might delay and/or complicate fertility sparing cancer treatment.

Details of ethics approval

The ethic board of the University Medical Center Utrecht provided consent for this study.

Acknowledgements

We would like to thank D.M.D.S. (Daisy) Sie-Go, Marieke Soesbergen, Leslie J.M. Beks and W.F.J.M. (Miranda) van den Oetelaar for their help with retreiving the clinical and pathological history of the patiens in this study.

Supplementary Table 1. FIGO stage according to type of histological specimen on which the diagnosis of cervical cancer was made

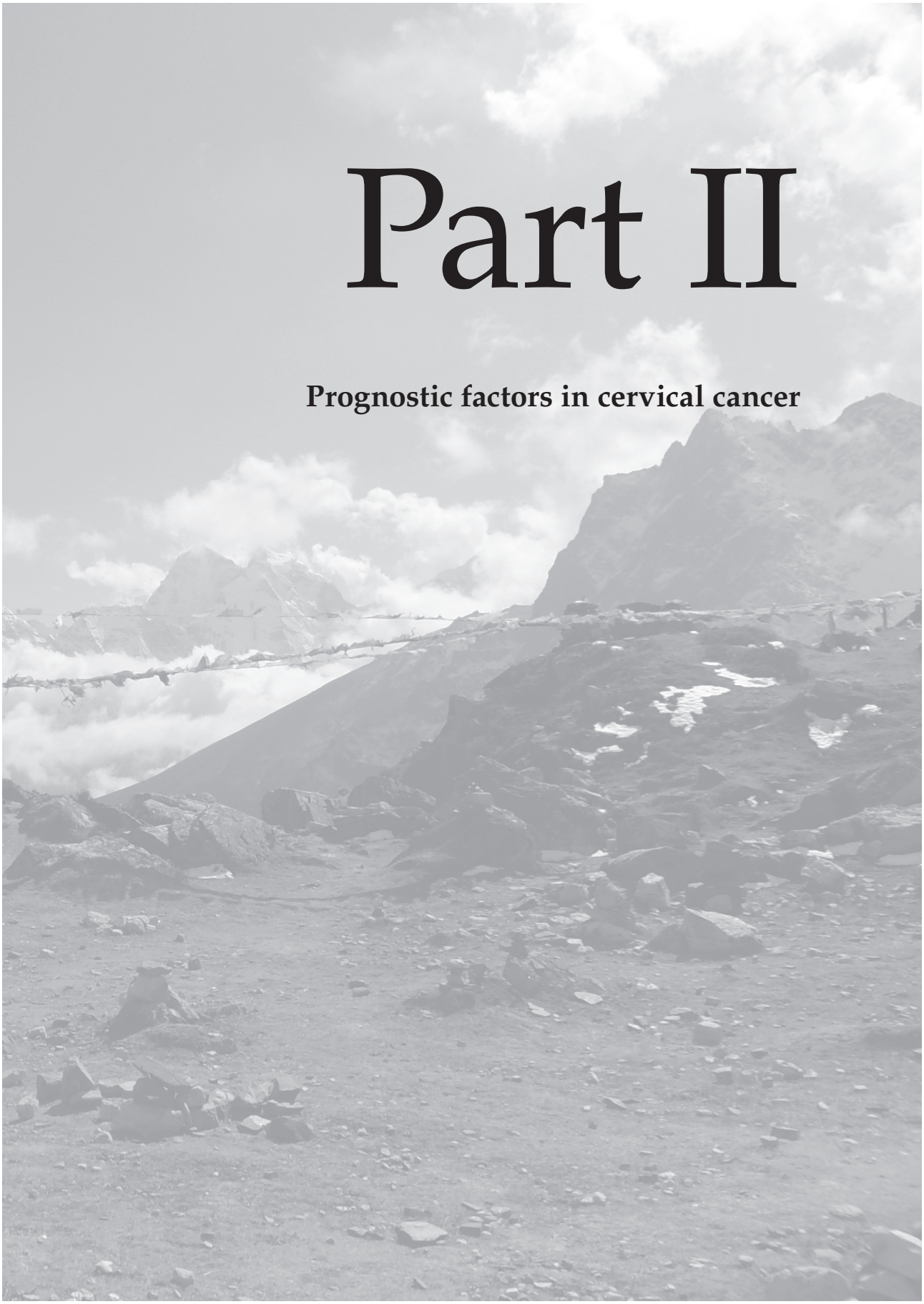
FIGO stage						
	punch biopsy	*LLETZ*	*knife cone*	*hysterectomy*	*ecc*	*total*
IA1	2	3	1	2	1	9
	22%	33%	11%	22%	11%	
IA2	0	1	0	0	0	1
	0%	100%	0%	0%	0%	
IB1	16	15	8	1	1	41
	39%	37%	20%	2%	2%	
IB2	13	3	1	0	0	17
	77%	18%	6%	0%	0%	
IIA1	3	0	1	0	0	4
	75%	0%	25%	0%	0%	
IIA2	4	0	0	0	0	4
	100%	0%	0%	0%	0%	
IIB	17	2	0	0	1	20
	85%	10%	0%	0%	5%	
IIIA	1	0	0	0	0	1
	100%	0%	0%	0%	0%	
IIIB	13	0	0	0	1	14
	93%	0%	0%	0%	7%	
IVA	6	0	0	0	1	7
	86%	0%	0%	0%	14%	
IVB	2	0	0	0	0	2
	100%	0%	0%	0%	0%	
Total	77	24	11	3	5	120
	64%	20%	9%	3%	4%	

Supplementary Table 2. Treatment and outcome details

Treatment	
neo adjuvant N (%)	5/116 (4.3)
Primary tumor N/118 (%)	
surgery	51 (43.2)
radiotherapy	21 (17.8)
chemoradiation	44 (37.3)
chemotherapy	1 (0.8)
HPV vaccination	1 (0.8)
Type of surgery N/51 (%)	
consiation	4 (7.8)
trachelectomy	11 (21.6)
hysterectomy	4 (7.8)
Wertheim Meigs	32 (62.7)
adjuvant N (%)	10/113 (8.8)
Outcome	
Died of disease N (%)	30/119 (25.2)
Age at death median [range]	50.9 [27.8-89.8]
OS in years median [range]	3.5 [0.3-5.5]
Recurrence N (%)	34 (28.3)
RFS median [range]	3.2 [0.0-5.5]

Supplementary Table 3. Colposcopic impression according to type of histological specimen on which the diagnosis of cervical cancer was made

Impression first colposcopy	**Type of specimen on which cancer diagnosis was made**					
	punch biopsy	*LLETZ*	*knife cone*	*hysterectomy*	*ecc*	*Total*
unknown	9	9	7	1	1	27
	33%	33%	26%	4%	4%	100%
normal	0	1	0	0	1	2
	0%	50%	0%	0%	50%	100%
high-grade	3	8	0	1	0	12
	25%	67%	0%	8%	0%	100%
invasive cancer	12	0	2	0	0	14
	86%	0%	14%	0%	0%	100%
NA	53	6	2	1	3	65
	82%	9%	3%	2%	5%	100%
Total	77	24	11	3	5	120
	64%	20%	9%	3%	4%	100%



Part II

Prognostic factors in cervical cancer

6

Pelvic lymphadenectomy improves survival in cervical cancer patients with low-volume disease in the sentinel node: a retrospective multicenter cohort study

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Abstract

Objective: In this study we aim to describe the value of pelvic lymph node dissection (LND) following sentinel lymph node biopsy (SN) in early-stage cervical cancer.

Methods: We performed a retrospective multicenter cohort study in 8 gynecological oncology departments. In total, 645 women with International Federation of Gynaecology and Obstetrics stage IA to IIB cervical cancer of squamous, adeno or adenosquamous histological type who underwent SN biopsy followed by pelvic LND were enrolled in this study. Radioisotope tracers and blue dye were used to localize the sentinel node, and pathologic ultrastaging was performed.

Results: Among patients with low-volume disease (micrometastases and isolated tumor cells) in the sentinel node the overall survival was significantly better (p=0.046) if more than 16 non-SNs were removed. No such significant difference in survival was detected in patients with negative or macrometastatic sentinel nodes.

Conclusion: Our findings indicate that in patients with negative or macrometastatic disease in the sentinel nodes, an additional LND did not alter survival. Conversely, our data suggest that the survival of patients with low-volume disease is improved when more than 16 additional lymph nodes are removed. If in a prospective trial our data is confirmed we would suggest a two stage operation.

Introduction

Cervical cancer is the third most common type of cancer in women worldwide, with an estimated age standardised incidence rate of 14 per 100.000 and a mortality rate of 6.8 per 100.000 women (updated after publication[22]). In the European Union, it is the sixth most common type of cancer in women with an age standardised incidence rate of 9.6 per 100.000 and a mortality rate of 2.8 per 100.000 women[22]. Cervical cancer is clinically staged according to definitions set by the International Federation of Gynaecology and Obstetrics (FIGO)[61]. As opposed to the staging of other gynecological tumors, lymph node metastases are not included in the staging of cervical cancer. However, it is important to assess the lymph node status in this disease, as it is a negative prognostic factor for survival[62] and it determines the choice of initial therapy, as well as the need for adjuvant treatment[63].

Cervical cancer is known to spread to the pelvic lymphatic system via the first draining lymph node, the sentinel lymph node (SN)[68]. If this node is tumor free, the other draining lymph nodes (non-SNs [nSNs]) are assumed not to contain tumor. Currently, the criterion standard for assessing the nodal status in cervical cancer is systematic pelvic lymph node dissection (LND)[64]. Such an extensive lymphadenectomy leads to lymphocyst formation in about 20% and to lymphedema in approximately 10% of patients with FIGO stage IB to IIA disease[65-67]. Morbidity rates have decreased due to implementation of laparsocopic lymphadenectomy[69]. To further minimise these complications, the SN biopsy is currently being evaluated for adoption as the standard of care in early-stage cervical cancer[70,71]. This procedure entails detection and excision of the SN after submucosal injection of a radioisotope tracer and/or blue dye around the primary tumor[72]. Essential in this procedure is the optimal histopathologic evaluation of the SN by serial sectioning and immunohistochemistry (IHC)[73,74]. This technique assures reliable detection of low-volume disease (LVD; micrometastasis [0.2-2 mm] or isolated tumor cells [ITC; <0.2 mm])[75] Compared with pelvic LND, SN biopsy increases the detection rate of metastases up to 2.8 fold[79].

Before pelvic LND can be abandoned in favor of performing SN biopsy alone, the sensitivity of the latter to detect metastases needs to be similar (or higher) than that of pelvic LND. Furthermore, it needs to be clarified whether the assessment of nodal status by pelvic LND, as well as its extent, is solely diagnostic, or whether it also affects survival[133-135]. With SN ultrastaging, information is now provided on LVD that hitherto was not available. This new information can be used to study the effect of extended surgical treatment of pelvic lymph nodes on outcome of the disease.

Therefore, in this study we aimed to clarify whether the extent of pelvic LND affects survival in patients with a negative SN, in patients with LVD and in patients with macrometastasis in the SN.

Materials and Methods

Patients

Our study population consisted of 645 patients from 8 centers (Ostrava and Prague in Czech Republic, Amsterdam and Utrecht in The Netherlands, New York in the United States, Paris and Toulouse in France, and Krakow in Poland). In this study population of 645 patients we previously described the clinical significance of micrometastasis in the lymph nodes and the false-negative rate of 2.8% (whole group) and 1.3% for patients with optimal bilateral mapping[136,137]. Patients with FIGO stage IA to IIB cervical cancer of squamous, adeno or adenosquamous histologic type without clinical or radiologic signs of lymphadenopathy were included. In patients where no SN ultrastaging was performed and/or survival end points were not adequately documented, were excluded from the study.

Therapeutic procedures and pathologic evaluation

Radioisotope and blue dye were injected preoperatively and intraoperatively, respectively, around the primary tumor. Sentinel lymph nodes were detected at laparoscopy or laparotomy by visual inspection and a gamma probe. Fresh frozen analysis of the excised SN with subsequent paraffin embedding and pathologic ultrastaging was performed as previously described[136]. The total surgical specimen was palpated for lymph nodes. Nodes were dissected out and were counted manually. If additional smaller nodes were seen on microscopy, they were added to the total. All SN negative for metastasis on the initial routine section stained by hematoxylin and eosin (H&E) were further examined according to the pathologic ultrastaging protocol of the respective institutions. The entire node was cut at regular intervals which varied at individual centers between 150–500 µm; in 7 out of 8 centers and 98% of patients the intervals measured 250 µm or less. Three consecutive sections (5 µm thick) were obtained at each level. The first slide was stained with H&E while the second was used for immunohistochemical staining for cytokeratin. Pelvic nSNs were processed identically in all institutions by single section of each node examined by a routine H&E staining.

Lymph node involvement was defined as ITC or clusters (smaller than 0.2 mm in greatest diameter), micrometastasis (smaller than 2 mm in greatest diameter), or macrometastasis (equal to or larger than 2 mm)[75].

After the SN biopsy, all patients underwent full pelvic LND. Consequently, simple hysterectomy (N = 3), radical hysterectomy (N = 532), simple trachelectomy (N = 22), or radical trachelectomy (N = 88) was performed. The surgicalspecimens of the latter procedures were evaluated according to common histopathologic practice.

Adjuvant therapy (radiotherapy, chemotherapy, or both) was administered according to national or institutional guidelines in 213 (33.0%) of the 645 patients. Adjuvant therapy was administered to 116 (85.3%) of patients with macrometastasis, 38 (82.6%) patients with micrometastasis, 13 (52%) patients with ITC, and 46 (10.5%) patients with negative lymph nodes (Table 1). In the patients with negative SN, 8 (7.8%) of the patients with 16 or less nodes and 52 (14.7%) of the patients with more than 16 nodes removed received adjuvant therapy (P = 0.062). Among all patients with LVD in the SN, 5 (45.5%) patients with 16 or less nodes and 67 (80.7%) of the patients with more than 16 nodes removed received adjuvant therapy (P = 0.026). In patients with macrometastasis in the SN, 13 (76.5%) of the patientswith 16 or less nodes and 68 (87.2%) of the patients with more than 16 nodes removed received adjuvant therapy (P = 0.283).

Statistical analyses

Standard summary statistics were used to describe primary data, that is, frequency tables and median supplied with fifth to 95th percentile range. Maximum likelihood (ML) and chi-square (χ^2) testing was performed to compare categorical variables, and Kruskal-Wallis followed by Mann-Whitney U testing was applied for mutual comparisons of variants in continuous variables. Kaplan-Meier survival probability estimates with log-rank testing were used to describe and compare variants in time-to-event end points, that is, overall survival (OS) and relapse-free survival (RFS). Time-to-event end point was calculated from time of surgery. We were not able to correct for start and duration of adjuvant therapy because of unavailability. Univariate and multivariate proportional hazards Cox regression models were applied to quantify the association of potential risk factors and survival. First, estimates of hazards ratio (HR; with 95% confidence intervals [CIs]) were tested using Wald χ^2 test. Subsequently, parameters with potential risk power (P<0.10 in univariate Cox regression)were subjected to stepwise selection algorithminmultivariate Cox regression. For all statistical tests, a 2-tailed P value of less than 0.05 was considered significant. Statistical power to detect differences within groups was limited, mainly in the stratified analysis.

Table 1. Baseline characteristics of the patients included in this study stratified according to the final diagnosis based only on the SN ultrastaging

	SN ultrastaging result*				
Factor n (%)	*Negative*	*ITC*	*Micro*	*Macro*	*p**
Age					
median [range]	45 [30 - 70]	47 [32 - 64]	50 [33 - 70]	40 [33 - 74]	0.009
≤ 50 yrs	301 (73.6)	17 (4.2)	34 (8.3)	57 (13.9)	0.143
> 50 yrs	155 (65.7)	12 (5.1)	31 (13.1)	38 (16.1)	
Histology					
ACC	129 (78.7)	10 (6.1)	7 (4.3)	18 (10.9)	0.174
SCC	312 (67.8)	18 (3.9)	55 (12.0)	75 (16.3)	
ASCC	13 (68.4)	1 (5.3)	3 (15.8)	2 (10.5)	
FIGO stage					
IA	46 (83.6)†	2 (3.6)	3 (5.5)	4 (7.3)	< 0.001
IB1	353 (74.0)	18 (3.8)	41 (8.6)	65 (13.6)	
IB2	35 (60.3)	3 (5.2)	10 (17.2)†	10 (17.2)	
IIAB	22 (40.0)†	6 (10.9)†	11 (20.0)†	16 (29.1)†	
LVSI					
No	348 (73.1)	21 (4.4)	46 (9.7)	61 (12.8)	0.107
Yes	108 (63.9)	8 (4.7)	19 (11.2)	34 (20.1)	
Parametrial involvement					
No	440 (73.5)	28 (4.7)	55 (9.2)	76 (12.7)	< 0.001
Yes	16 (34.8)†	1 (2.2)	10 (21.7)†	19 (41.3)†	
Vaginal involvement					
No	439 (73.3)	23 (3.8)	57 (9.5)	80 (13.4)	< 0.001
Yes	17 (36.9)†	6 (13.0)†	8 (17.4)†	15 (32.6)†	
Pelvic nSN examination					
Negative	438 (78.8)	25 (4.5)	46 (8.3)	47 (8.5)	< 0.001
Positive	18 (20.2)†	4 (4.5)	19 (21.4)†	48 (53.9)†	
Events					
Recurrences	25 (52.1)	1 (2.1)	9 (18.7)	13 (27.1)	–
Deaths	9 (37.5)	0 (0)	6 (25)	9 (37.5)	
Total study population (N = 645)	456 (70.7 %)	29 (4.5 %)	65 (10.1 %)	95 (14.7 %)	

Values are presented as n (%).
* Overall level of statistical significance of association between given factor and results of pathologic ultrastaging (P value of ML-W2 test).
† Significantly lower/higher value in comparison with the other values within this subgroup (ML-χ^2 test; $P < 0.05$).
ACC, adenocarcinoma; ASCC, adenosquamous carcinoma; Macro, macrometastases; Micro, micrometastases; SCC, squamous.

Table 2. Number of positive nSNs in relation to RFS and OS in the total study population (N = 645)

pos nSNs		RFS		OS	
		HR (95% CI) *	*p value*	*HR (95% CI)* *	*p value*
		1.18 (1.11; 1.26)	< 0.001	1.13 (1.02; 1.26)	0.018
categories					
	> 0	3.47 (1.92; 6.26)	< 0.001	5.10 (2.28; 11.39)	< 0.001
	> 1	4.81 (2.47; 9.36)	< 0.001	5.94 (1.93; 18.32)	< 0.001
	> 2	5.59 (2.56; 12.21)	< 0.001	6.21 (2.47; 15.59)	< 0.001
	> 3	6.38 (2.80; 14.54)	< 0.001	7.78 (2.52; 24.02)	< 0.001
	> 4	6.72 (2.61; 17.32)	< 0.001	8.11 (2.29; 28.63)	< 0.001
	> 5	8.80 (3.10; 24.96)	< 0.001	8.98 (2.02; 39.37)	0.008

HR and P values are calculated for the total number of positive nSNs and, consequently, per stratum of more than zero to more than 5 positive lymph nodes, compared with negative nSNs.
*HR (univariate Cox proportional hazards regression).
Pos, positive.

Results

Characteristics of patients and tumors

Patient and tumor characteristics were stratified according to the result of the SN ultrastaging (Table 1). With increasing FIGO stage, there was a significant increase in the size of SN metastases (ML- χ^2, $p < 0.001$). Similarly, vaginal and parametrial involvement and metastasis in the nSNs were postitively associated with the size of metastases in SN ($p < 0.001$). No significant association was found with age, histological subtype, or the presence of lymphovascular space invasion (LVSI).

Factors associated with lymph node involvement and survival

The number of positive nSNs was a significant predictor for the development of recurrence and the risk of death (Table 2), both as a continuous variable and when analyzed in categories. The HR for recurrence and death was 8.80 (95%CI, 3.10-24.96) and 8.89 (95%CI, 2.02-39.37), respectively, if more than 5 lymph nodes were positive.

Table 3. Number of removed nSN in relation to detected positivity

Analyzed group of patients	No. nSLN LN removed: cut-off point[1]	ROC analysis[1]		Detection of nSN LN positivity [2]			OR[3] (95% CI)
		AUC (95% CI)	*p value*	*Sensitivity*	*LN positivity*	*Detection of > 1 positive LNs*	
The whole sample (N=645)	≥17 (N = 515)	0.704 (0.641; 0.772)	0.043	91.0 (82.5; 95.7)	14.7 (11.8; 17.4)	8.0 (5.8; 10.7)	1.83 (1.02; 3.27)

[1]Area Under the Curve with corresponding statistical significance
[2]Sensitivity (%) and LN positivity detection rate (%), in parentheses, 95% confidence interval
[3]Odds ratio associated with detection of positive nSN LNs
AUC, area under the curve; OR, odds ratio; ROC: Receiver Operating Characteristic

Clinical impact of the number of removed pelvic lymph nodes

Receiver operating characteristic analysis was performed to establish a cutoff for number of removed lymph nodes (Table 3). On the basis of these small numbers in our study, a cut of 17 is defined (P = 0.043). We confirmed this finding by logistic regression analysis.

To relate the number of removed nodes to outcome, Kaplan-Meier analysis was performed with OS and RFS as end points. No statistically significant difference in RFS or OS in relation to the number of nSNs removed was observed among patientswith FIGOstage IA to IB1 disease (Fig. 1A and B). However, in patients with FIGO stage IB2 to II, both RFS (P = 0.032) and OS (P = 0.014) were significantly better in patients withmore than 16 nodes removed (Fig. 1C and D) than in patients with 16 or less nSNs removed.

The previously mentioned findings were tested using univariate andmultivariateCox proportional hazards regression analysis to exclude a possible confounding effect of other parameters (Table 4). Bothmodels confirmed that removingmore than 16 nSNs significantly reduced the risk of recurrence and the risk of death in patients with FIGO stage IB2 to II disease. Adjuvant treatment was used as a covariate in multivariate models, but no significant multivariate-adjusted effect on the time-to-event end points was found.

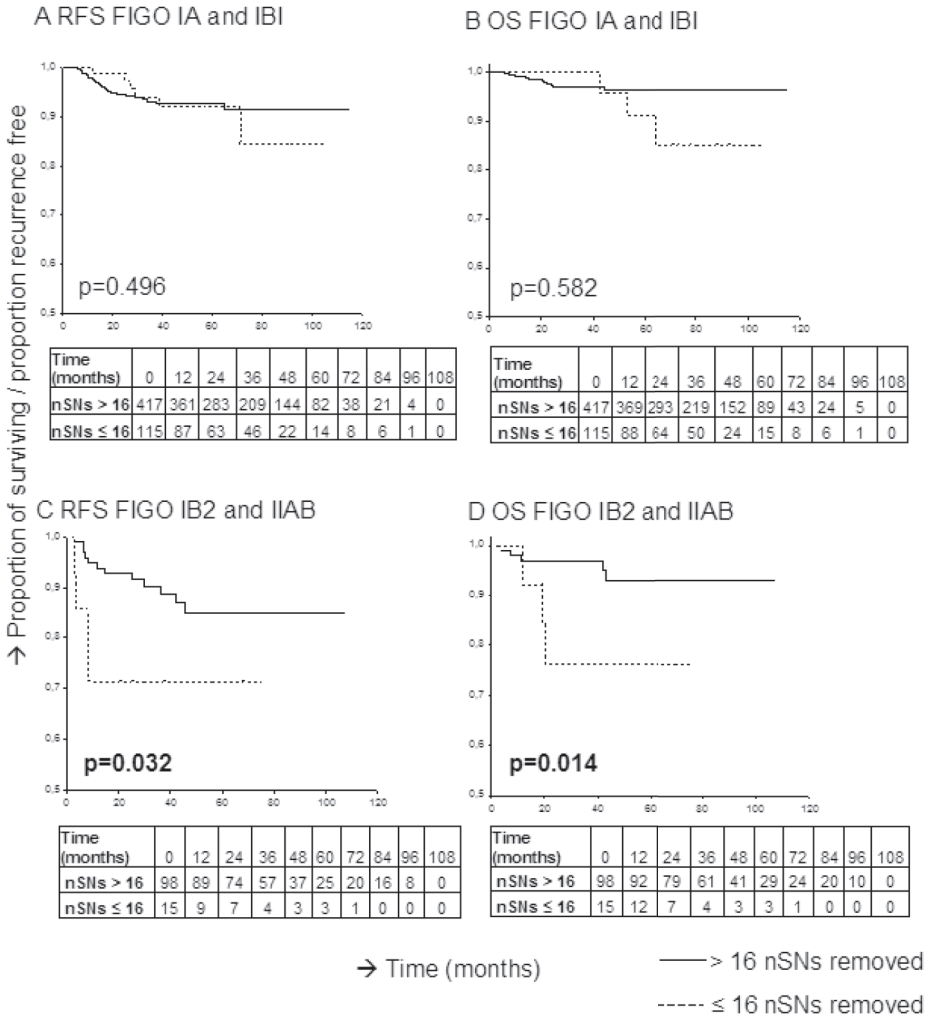


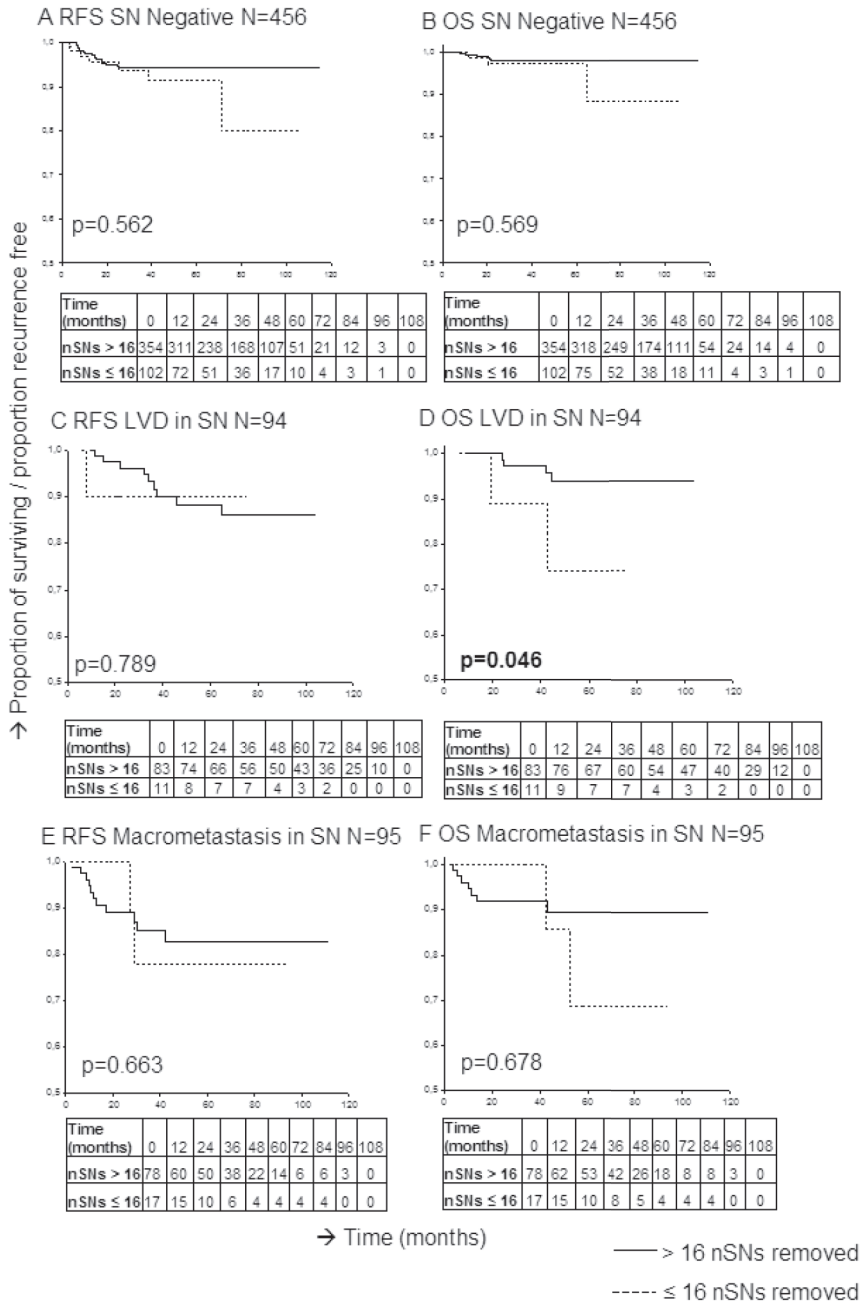
Figure 1. Kaplan-Meier survival probability estimates with log-rank testing were used to describe and compare RFS and OS in months stratified according to the number of removed nSNs. Relapse-free survival in patients with FIGO stage IA and IB1 (A), OS in FIGO stage IA and IB1 (B), RFS in FIGO stage IB2 and IIAB (C), and OS in FIGO stage IB2 and IIAB cervical cancer (D). Number of patients at risk can be seen from the tables below the graphs.

Table 4. Univariate and multivariate-adjusted HR of the threshold of 16 or more removed nSNs as potential predictor of survival in Cox proportional hazard models

	RFS		OS	
	HR (95% CI) [1]	*p value*	*HR (95% CI)* [1]	*p value*
Total population (n=645)				
Stage IA & IB1 (N = 532)				
Univariate HR	1.03 (0.42; 2.50)	0.490	0.67 (0.22; 2.09)	0.580
Multivariate Adjusted HR[2]	0.94 (0.38; 2.94)	0.885	0.66 (0.21; 2.08)	0.473
Stage IB2 & IIAB (N = 113)				
Univariate HR	0.32 (0.10; 0.99)	0.032	0.19 (0.04; 0.79)	0.013
Multivariate Adjusted HR[2]	0.30 (0.11; 0.99)	0.047	0.17 (0.04; 0.79)	0.023
Cases with any positivity in SN or nSNs (N =207)				
Univariate HR	0.63 (0.24; 1.68)	0.358	0.32 (0.12; 0.84)	0.021
Multivariate Adjusted HR[2]	0.58 (0.22; 1.56)	0.281	0.31 (0.11; 0.85)	0.022
Cases with LVD in SN (n=94)				
Univariate HR	0.95 (0.12; 7.50)	0.789	0.19 (0.04; 0.99)	0.047
Multivariate Adjusted HR[2]	0.85 (0.11; 6.92)	0.879	0.17 (0.03; 0.96)	0.044

[1] HR: hazard ratio (univariate Cox proportional hazard regression); CI: confidence interval
[2] Only factors which reached a p value < 0.1 in univariate Cox regression were selected for multivariate analysis from the following list: age, stage, histological subtype, LVSI, vaginal involvement, parametrial involvement, (neo) adjuvant therapy, number positive nSNs (strata 0, 1, 2, 3, 4, ≥ 5).

To determine whether removing more than 16 nSNs has a similar effect on survival in patients with or without metastatic lymph nodes, we stratified patients for SN status (Fig. 2). Three categories were defined; these are as follows: SNnegative (n = 456), LVD(including ITC and micrometastasis, n = 94), and macrometastasis (n = 95). We showed that only among patients with LVDin the SN that theOSwas significantly better (P = 0.046) if more than 16 nSNs were removed. No statistically significant differenceswere observed among patients with negative SN or macrometastasis in the SN. There were too few patients with LVD to further stratify the results according to ITC or micrometastases.



Time (months)	0	12	24	36	48	60	72	84	96	108
nSNs > 16	354	311	238	168	107	51	21	12	3	0
nSNs ≤ 16	102	72	51	36	17	10	4	3	1	0

Time (months)	0	12	24	36	48	60	72	84	96	108
nSNs > 16	354	318	249	174	111	54	24	14	4	0
nSNs ≤ 16	102	75	52	38	18	11	4	3	1	0

Time (months)	0	12	24	36	48	60	72	84	96	108
nSNs > 16	83	74	66	56	50	43	36	25	10	0
nSNs ≤ 16	11	8	7	7	4	3	2	0	0	0

Time (months)	0	12	24	36	48	60	72	84	96	108
nSNs > 16	83	76	67	60	54	47	40	29	12	0
nSNs ≤ 16	11	9	7	7	4	3	2	0	0	0

Time (months)	0	12	24	36	48	60	72	84	96	108
nSNs > 16	78	60	50	38	22	14	6	6	3	0
nSNs ≤ 16	17	15	10	6	4	4	4	4	0	0

Time (months)	0	12	24	36	48	60	72	84	96	108
nSNs > 16	78	62	53	42	26	18	8	8	3	0
nSNs ≤ 16	17	15	10	8	5	4	4	4	0	0

Figure 2. Kaplan-Meier survival probability estimates with log-rank testing were used to describe and compare RFS and OS in months stratified according to the number of removed nSNs. Relapse-free survival in patients with negative SN (A), OS in patients with negative SN (B), RFS in patients with LVD in the SN (C), OS in patients with LVD in the SN (D), RFS in patients with macrometastatic SN (E), and OS in patients with macrometastatic SN (F). Number of patients at risk can be seen from the tables below the graphs.

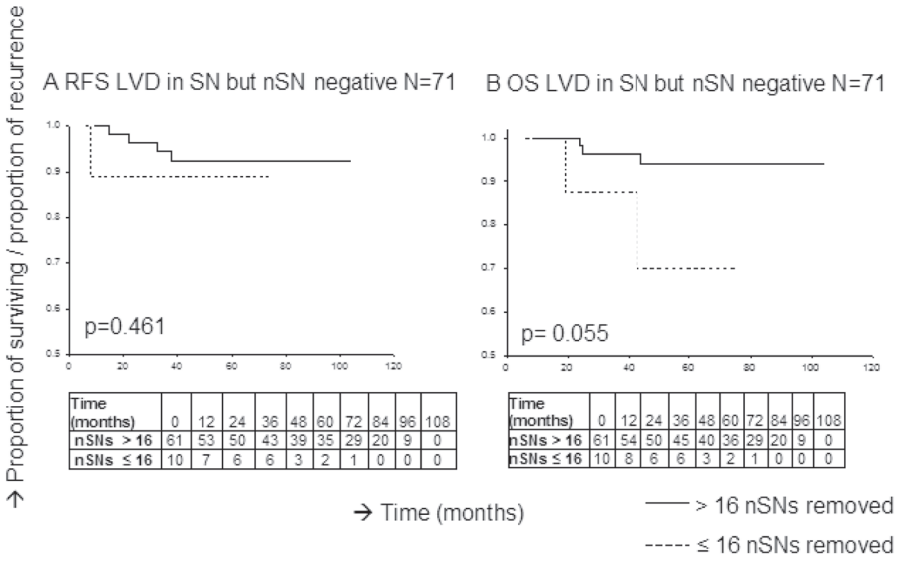


Figure 3. Kaplan-Meier survival probability estimates with log-rank testing were used to describe and compare RFS and OS in months stratified according to the number of removed nSNs. Relapse-free survival in patients with LVD in the SN but with negative nSNs (A) and OS with LVD in the SN but with negative nSNs (B). Number of patients at risk can be seen from the tables below the graphs.

Of the 94 patients with LVDin the SN, 71 (75.5%) had no metastasis detected in the nSNs. The Kaplan-Meier analysis was repeated for this subpopulation of women (Fig. 3) and showed a trend toward better OS in women with more than 16 nSNs removed (P = 0.055) than in patients in whom less than 16 nSNs were removed.

Discussion

In this multicenter cohort study, we studied 645 patients who had undergone an SN biopsy with pathologic ultrastaging and subsequent pelvic LND. This is the largest multicenter retrospective study of its kind to date,which provided sufficient numbers to analyze the effect of LND after SN biopsy in the subset of patients with LVD. This study is limited because of its retrospective study design. There was no randomization for number of removed nodes, treatmentwas not randomized, there were small differences in sentinel node analysis techniques within centers, and some interesting data (location of nodes)were not available. We showed that known risk factors (FIGO stage, vaginal involvement, and parametrial involvement) were

not only related to the occurrence of lymph nodemetastasis but also to the size of metastases. Furthermore, in this study, we confirmed the finding that the number of positive nSNs is associated with survival and recurrence[62,138,139].

For all stages, besides having a diagnostic value, we can conclude that systematic pelvic lymphadenectomy performed in addition to SN was only associated with better survival for patients with LVD in the SN.

To assess whether this effect was due to increased detection of lymph node metastases or this was a true therapeutic effect, we performed survival analysis in patients with LVD in the SN and negative nSNs.We detected a trend toward better OS in the latter group (P = 0.055). This possible therapeutic effect could be explained by removing additional LVDin nSNs, which is not detected by routine pathologic assessment. Unfortunately, we were unable to stratify for both FIGO stage and SN status because of the limited number of events.

Previous studies have shown a therapeutic impact of LNDin patientswith cervical cancerwho underwent full pelvic LND without SN biopsy[140,141]. Excising at least 15 lymph nodes was associated with better survival (p=0.01) compared to patients in whom less than 15 lymph nodes were removed[142]. Whether this effect on survival is related to lymph node status has thus far only been investigated by 2 research groups who have provided conflicting data. Kenter et al.[133] showed that completing LND resulted in a longer DFS in 63 patients with lymph node positive cervical cancer. In addition, they showed a longer DFS in 136 patients with lymph node-positive, whereas completing LND in 331 patients with lymph node negative had no effect on survival[133,134]. Contrastingly, Shah et al.[135] analyzed the SEER database and showed no effect of completing LND in 873 patients with lymph node positive FIGO stage IA2 to IIA cervical cancer. However, survival was improved in 4648 lymph node negatieve patients with more than 20 lymph nodes removed.

Our finding, that completion of LND showed better survival in case of LVD and not in patients with negative lymph nodes seems to contradict the outcome of the SEER study. However, SN biopsy, as used in our analysis, provides a more sensitive procedure to detect metastases than what was used in the SEER study. The beneficial effect of full LND in patients with negative lymph nodes might therefore be due to treatment of LVD, who were analyzed in the SEER study as node negative patients. In contrast with 2 previous studies[133,134] and in line with the SEER data, we could not find evidence that performing LND showed better survival in patients with macrometastatic lymph nodes. This difference may be explained by the fact that we excluded patients with evidently involved nodes, whereas the 2 studies that found a beneficial effect of LND also included bulky enlarged nodes[140,141].

Whether our resultswarrant clinical implementation of full LND as a second operation after the sentinel node procedure should be further validated, preferably in a randomized controlled trial. In such prospective study, it should be assessed whether patients in whom a SN procedure is performed and who are found to have LVD do indeed benefit from additional lymphadenectomy and/or radiotherapy, as this retrospective cohort analysis suggests.

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Disclosure of Interests

The authors have no conflicts of interest to declare.

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7

Detection of cervical cancer recurrence during follow-up: a multivariable comparison of 9 frequently investigated serum biomarkers

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Abstract

Objective: To assess the diagnostic accuracy and model the optimal combination of commonly studied serum biomarkers aimed at identifying recurrence in cervical cancer patients.

Methods: From a systematic literature search, nine biomarkers (CA-15.3, CA-125, CEA, CYFRA 21-1, hsCRP, IL-6, SCC-Ag, TNF-α and VEGF) were selected for a serum analysis. Samples were derived from a historical cervical cancer cohort. Subjects with serum samples stored in a biobank were included when quality criteria were met, and one sample preceding and at least one following primary treatment was available. In case of recurrence, two additional post-recurrence samples were analyzed. Biomarker serum levels were quantified by enzyme linked or chemiluminescence microparticle immunoassays. Logistic regression and receiver operating curve analysis were employed for selection, modeling and comparison on the diagnostic accuracy of the tested biomarkers.

Results: 205 samples were analyzed from 75 subjects, of whom 19 (25.3%) had a recurrence. The area under the curve (AUC) of CA-15.3, CA-125, CEA, CYFRA 21-1, IL-6, TNF-α and VEGF were all <0.750. Only SCC-Ag and hsCRP were included in the final model with an AUC of 0.822 (95%CI: 0.744-0.900) and 0.831 (95%CI: 0.758-0.905) respectively. Combined AUC was 0.870 (95%CI: 0.805-0.935). Rises in SCC-Ag and hsCRP significantly increased the odds for recurrence. Each ng/ml of SCC-Ag increase, related to an odds ratio (OR) of 1.117 (95%CI: 1.039-1.200). Comparably, the OR for hsCRP (in mg/ml) was 1.025 (95%CI: 1.012-1.038).

Conclusion: Combined testing of SCC-Ag and hsCRP yields the highest detection rate of disease recurrence during cervical cancer follow-up.

Introduction

In cervical cancer, disease recurrence during follow-up is still a frequent and important cause of cervical cancer mortality and morbidity[80]. The risk of recurrence is stage dependent and ranges from 10-20% for stage IB1/IIA up to 72% for stage IVA[81,82]. Early detection of recurrence is desired since curation may still be possible in selected cases with local recurrence[80,83,84].

Follow-up surveillance schemes commonly entail 5 years of ambulant visits with increasing time intermissions. The basis of each follow-up visit is an interval history and a gynecological physical examination, supplemented by advanced diagnostics when indicated[143,144]. This may include magnetic resonance imaging (MRI), positron emission tomography - computed tomography (PET-CT) or (imaging guided) histology sampling, in order to establish disease recurrence[145]. While cancer follow-up programs serve multiple purposes, an increasing number of studies show only a limited added value for recurrence detection in asymptomatic patients, in particular for the last three follow-up years[146,147].

To improve these follow-up programs, a range of serum biomarkers has been assessed. In these predictive studies, multiple outcome variables have been studied, including detection of recurrence, presence of biomarker elevation prior to clinical manifestation, disease specific and overall survival[85-96]. Besides differences in study objectives, often only a single marker is tested with an inherent heterogeneity between studies in design, populations, data collection and analysis. This hinders inter-study comparisons on combined or overlapping diagnostic accuracy.

We aimed to analyze, within one cohort of cervical cancer patients, the most frequently investigated serum biomarkers for their respective individual, combined and overlapping diagnostic accuracy for detecting recurrent disease. Hereof, we aimed to identify the optimal combination of biomarkers for detecting a recurrence. Preceding this analysis, biomarkers of interest were selected from a structured search of the medical literature.

Methods and Materials

Design

We assessed serum biomarkers in a retrospective cervical cancer cohort derived from a single institutional biobank. Sampling occurred consecutively between January 1988 and January 2000. A longitudinal design was adopted in which multiple samples were analyzed within a single subject to allow recurrence detection. Individual information on diagnosis, staging, treatment and follow-up were retrieved observationally from medical records and the municipal personal records

database. The institutional review board approved the initial serum biobanking, and separately, a second approval was granted for this specific study.

Marker selection

Markers relevant for assessment were derived from existing peer-reviewed literature. A Medline/Pubmed and Cochrane database search was performed using the generic terms 'cervical cancer' and 'serum', and medical subject heading (MeSH) 'uterine cervical neoplasm' in the title/abstract field. Limits were set to English or Dutch language and subjects of the female gender. This yielded 1157 unique articles, which were screened on title/abstract, or when inconclusive on full text. Two hundred articles met our inclusion criteria of 1) minimally one biomarker was tested, 2) a population of ≥50 subjects was studied, and 3) disease status conversion was considered. Two independent reviewers (AZ, ER) critically appraised these articles with quality scoring based on the REMARK (REporting recommendations for tumor MARKer prognostic studies) criteria from McShane and colleagues[148]. A total of 53 articles (41 biomarkers) were deemed of sufficient quality (score ≥4) and subsequently read in full text. From these articles we selected, concordant to our research aim, those biomarkers which were numerically most often studied or promising for detection of disease status conversion. Due to differences in marker class, (epi)genetic markers (e.g. DNA methylation markers) were excluded and nine biomarkers meeting the above criteria remained for serum analysis in this study. Five were classical tumor markers: squamous cell carcinoma antigen (SCC-Ag), cytokeratin fragment 21-1 (CYFRA 21-1), carcinoembryonic antigen (CEA), cancer antigen 15.3 (CA-15.3) and 125 (CA-125). Additionally, three inflammatory response markers, namely; high sensitivity C-reactive protein (hsCRP), tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and one angiogenesis marker – vascular endothelial growth factor (VEGF) – were included.

Subject and sample selection

Subjects were eligible for serum testing when minimally one sample preceding and one following primary treatment were available. Cases were excluded when a minimal volume of 1 ml for each sample was not available, or when clinical follow-up was insufficiently retrievable. Samples with any prior defrosting, regardless of duration, were excluded from this study to safeguard sample quality. To prevent selectively favoring any biomarker (i.e. selection bias), no subject selection on baseline characteristics (e.g. stage, histology) was performed.

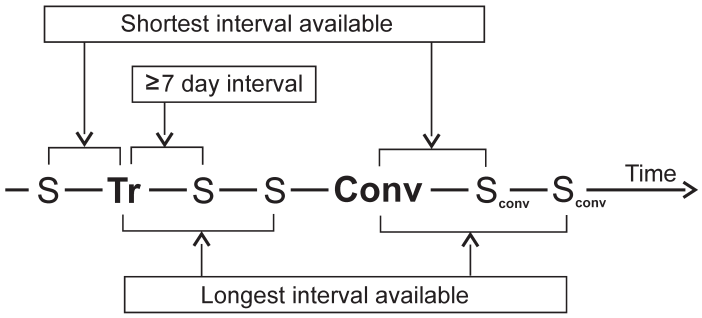

Figure 1. The timeline representing serum sampling timepoints (S) respective to the primary treatment (Tr) on an individual patient level. When a conversion in disease status (Conv) occured two post-conversion samples (S_{conv}) were added, when available. The boxed text below and above the timeline depicts the criteria for sample timepoint intervals.

Since sampling, for ethical reasons, occurred concomitantly with clinically indicated blood draws, the number and intervals between serum collections were non-uniform. For the pre-treatment sample, only the most recent sample prior to primary treatment was selected. From multiple follow-up samples a maximum of two were analyzed which were selected on predetermined criteria. These criteria comprised; the first follow-up sample was taken minimally seven days (i.e. 'wash-out period') after the end of primary treatment and the second sample tested had the longest follow-up available. In subjects whom had a conversion, two post-conversion samples were added. These consisted of the first sample taken after diagnosing a recurrence and the one available with the longest follow-up. In these subjects, non-availability of the sample prior to primary treatment did not exclude the subject from the study (Figure 1).

Disease status was scored at each follow-up visit concurrent to serum sampling. This was concluded on an interval history, gynecological pelvic examination and when indicated, imaging studies and histology sampling. Conversion (i.e. recurrence) was defined as a multidisciplinary agreement on a disease status category change from 'no evidence of disease' (NED).

Sample analysis

Biobanked samples containing 1.0 ml serum were stored in 2 ml polypropylene vials (screw cap microtube 45 x 10.8 mm, Sarstedt AG & Co, Nümbrecht, Germany) at minus 80 degrees Celsius. All transport, from refrigerated storage to the laboratory, was on dry ice and confined in an isolation container. Analyzes were performed by trained staff and in adherence to good laboratory practice. In a 200 µl portion, serum levels of VEGF, TNF-α, CYFRA 21-1 and IL-6 were quantitatively measured by an enzyme-linked immunosorbent assay (ELISA) with commercially available kits (Research & Diagnostics Systems Inc., Minneapolis, MN, USA). Serum values returned as outside the measurable range were conservatively coded to their corresponding upper or lower test limit. The SCC-Ag, CEA, CA-15.3, CA-125 and hsCRP were tested in the remaining 800 µl portion using a chemiluminescence microparticle immunoassay on an automated platform (Architect, Abbott Diagnostics, Lake Forrest, IL, USA). To prevent false positively increased levels of SCC-Ag by contamination during manual handling, open vials were handled using a full facial mask, long gloves, and hair covers.

Statistical analysis

Statistical calculations were performed with the 'Statistical Package for the Social Sciences' version 20.0.0 (International Business Machines, New York, USA). Manually performed (stepwise) multivariable logistic regression was used for biomarker selection and followed a forward modeling principle. Presence of recurrence was the modeled outcome variable. Comparison between and reduction of models was based on the likelihood ratio test and adhered to the 'Occam's razor' principle. Corresponding odds ratios (OR) of modeled biomarkers were calculated, and adjusted for histological tumor type, differentiation grade, primary treatment outcome and the interval length between end of treatment and the moment of sampling. The saved multivariable probabilities, derived from the selected regression model(s), were added to the receiver operating curve (ROC) analysis[149]. Areas under the curve (AUC) were calculated, including a nonparametric 95% confidence interval (95%CI). An identical strategy was adopted during an analysis of elevated biomarkers preceding clinical recurrence detection. Statistical significance was preset at $p<0.05$, model inclusion and exclusion limits for explanatory variables were set accordingly.

Table 1. Population characteristics

Population characteristics	N	%
N (total)	75	
N with recurrence	19	
Median age (range)	50 (25 – 83) years	
FIGO Stage		
IA1-2	4	5,3
IB1	28	37,3
IB2	7	9,3
IIA1-2	8	10,6
IIB	13	17,3
IIIA	1	1,3
IIIB	10	13,3
IVA	3	4,0
IVB	1	1,3
Histological type		
SCC	63	84,0
AC	9	12,0
Other	3	4,0
Differentiation		
Grade I	1	1,3
Grade II	18	24,0
Grade III	52	69,3
Undefined	4	5,3
Primary treatment		
Surgery	38	50,7
(chemo)Radiotherapy	35	46,7
NAC and surgery	2	2,7

Abbreviations: FIGO: International Federation of Gynecology and Obstetrics, SCC: squamous cell carcinoma, AC: adenocarcinoma, NAC: neo-adjuvant chemotherapy.

Results

Study population

In 75 subjects, 205 samples were analyzed. Patient characteristics are described in detail in table 1. Follow-up samples ranged from seven to 1812 days (5.0 years) after the end of primary treatment. Samples were available to detect a proven disease recurrence in 19 subjects (25.3%) of which 7 were a local recurrence. The dominant histological type and stage were a squamous cell carcinoma (n=63; 84.0%) and stage IB1 (n=28; 37.3%) respectively. Clinical follow-up data until five years after treatment or death was present in 72 subjects (96.0%); the remaining three cases were censored. The 5-year disease specific mortality was 33.3%, the overall mortality was 41.3%.

Table 2. Median biomarker serum levels

	Recurrence absent	Recurrence present	
	Median (range)	*Median (range)*	*AUC (95% CI)*
CA-15.3 (IU/ml)	16,5 (7,8 – 60,2)	28,8 (9,6 – 835,5)	0.707 (0.607 – 0.807)
CA-125 (IU/ml)	12,4 (3,3 – 117,5)	21,1 (5,6 – 6286,2)	0.730 (0.642 – 0.818)
CEA (ng/ml)	1,8 (0,4 – 52,7)	2,8 (0,5 – 130,8)	0.623 (0.521 – 0.726)
CYFRA 21-1 (ng/ml)	7,3 (0,4 – 140,1)	3,8 (0,2 – 149,8)	0.462 (0.354 – 0.571)
SCC-Ag (ng/ml)	1,0 (0.4 - 29.1)	4,4 (0,6 – 129,5)	0.822 (0.744 – 0.900)
hsCRP (mg/ml)	3,8 (0,3 –165,2)	33,0 (1,2 – 513.6)	0.831 (0.758 – 0.905)
IL-6 (pg/ml)	1,2 (1,2 – 180,0)	1,8 (1,2 – 900,0)	0.570 (0.471 – 0.668)
TNF-α (pg/ml)	2,8 (2,8 – 196,0)	2,8 (2,8 – 57,0)	0.560 (0.457 – 0.663)
VEGF (pg/ml)	398 (156 – 2980)	687 (156 – 3580)	0.624 (0.513 – 0.734)

Abbreviations: AUC: area under the curve, CI: confidence interval, IU: international units, ml: milliliter, ng: nanogram, mg: milligram, pg: picogram.

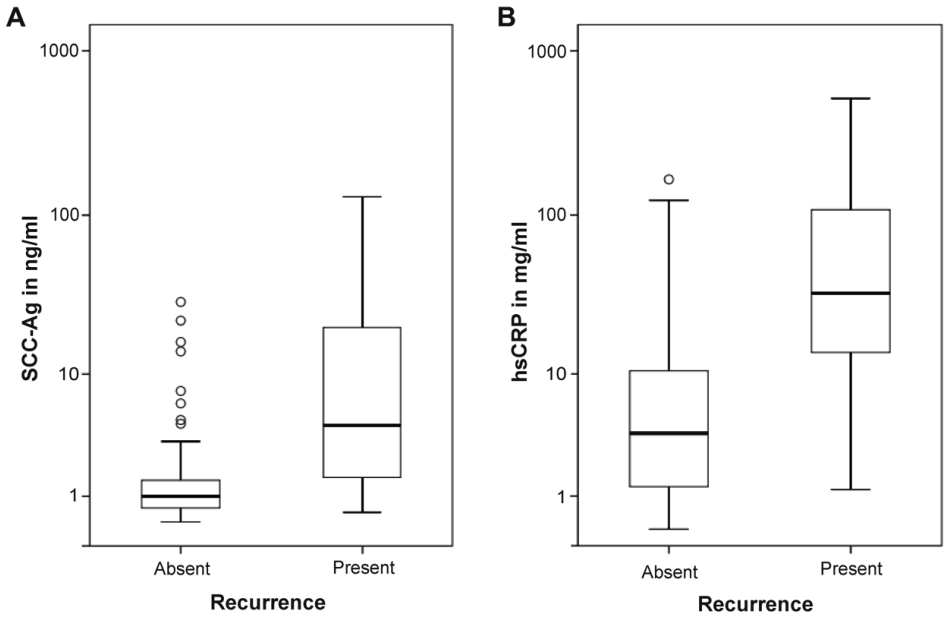

Figure 2. Box-whisker plots of SCC-Ag (A) and hsCRP (B) serum values respective to the presence or absence of recurrent disease. Both biomarkers were significantly ($p<0.001$, Mann-Whitney test) elevated when a recurrence was detected.

Diagnostic accuracy for detecting disease recurrence

Five serum biomarkers, namely CEA, CYFRA 21-1, IL-6, TNF-α, and VEGF, all yielded a univariable AUC below 0.650. Both CA-125 and CA-15.3 showed an intermediate diagnostic accuracy with an AUC of 0.730 (95%CI: 0.642-0.818) and 0.707 (95%CI: 0.607-0.807) respectively (Table 2).

Of all biomarkers, only SCC-Ag and hsCRP were included in the final model. Median SCC-Ag and hsCRP values in samples from patients with NED were 1.0 ng/ml (range: 0.4-29.1 ng/ml) and 3.8 mg/ml (range: 0.3-165.2 mg/ml), respectively. Sera sampled after recurrence showed significantly (both $p<0.001$) elevated median serum levels of 4.4 ng/ml (range: 0.6-129.5 ng/ml) for SCC-Ag and 33.0 mg/ml (range: 1.2-513.6 mg/ml) for hsCRP (Figure 2). Regarding diagnostic accuracy, the univariable AUC of SCC-Ag was 0.822 (95%CI: 0.744-0.900), for hsCRP 0.831 (95%CI: 0.758-0.905) and when combined a bivariable AUC of 0.870 (95%CI: 0.805-0.935) was found (Figure 3). Rises in both SCC-Ag and hsCRP serum levels significantly ($p=0.003$ and $p<0.001$, respectively) increased the odds of having a disease recurrence. Each base unit rise in serum SCC-Ag (i.e. ng/ml) related to an OR of 1.117 (95%CI: 1.039-1.200), and an OR of 1.025 (95%CI: 1.012-1.038) for hsCRP (in mg/ml). There was no significant statistical interaction between SCC-Ag and hsCRP ($p=0.322$).

Subgroup analysis with only histology proven squamous cell carcinoma cases increased the diagnostic accuracy of these two biomarkers. The AUC's for detecting recurrence increased to 0.879 (95%CI: 0.798-0.960) for SCC-Ag, 0.859 (95%CI: 0.786-0.933) for hsCRP and 0.911 (95%CI: 0.855-0.966) for the two markers combined. Corresponding OR's were 1.141 (95%CI: 1.054-1.234) for SCC-Ag (in ng/ml), and 1.024 (95%CI: 1.010-1.038) for hsCRP (in mg/ml). Figure 3. Multivariable adjusted OR's showed minimal changes, the adjusted OR for SCC-Ag was 1.140 (95%CI: 1.051-1.235), and 1.027 (95%CI: 1.013-1.040) for hsCRP in all histological types. The adjusted OR specific to the subgroup with only squamous cell carcinoma cases were highly similar at 1.150 (95%CI: 1.058-1.251) for SCC-Ag and 1.025 (95%CI: 1.010-1.039) for hsCRP.

Detailed reviewing yielded 9 samples (7 subjects) in which SCC-Ag serum levels were elevated above a 2.5 ng/ml threshold at a NED timepoint. In 8 samples (6 recurrence subjects; 31.6%) clinical recurrence was detected after a median 3 months (range: 1-14 months). After outcome status reclassification of these samples, the AUC of SCC-Ag increased to 0.886 (95%CI: 0.823-0.949) in all histological types and 0.934 (95%CI: 0.872-0.996) for squamous cell carcinoma cases only. Comparable, for hsCRP 12 samples (10 subjects; 52.6%) were elevated above 10 mg/ml with a median lead time of 2 months (range: 1-8 months) to recurrence detection. After reclassification, the AUC of hsCRP changed to 0.891 (95%CI: 0.835-0.947) in all histological types.

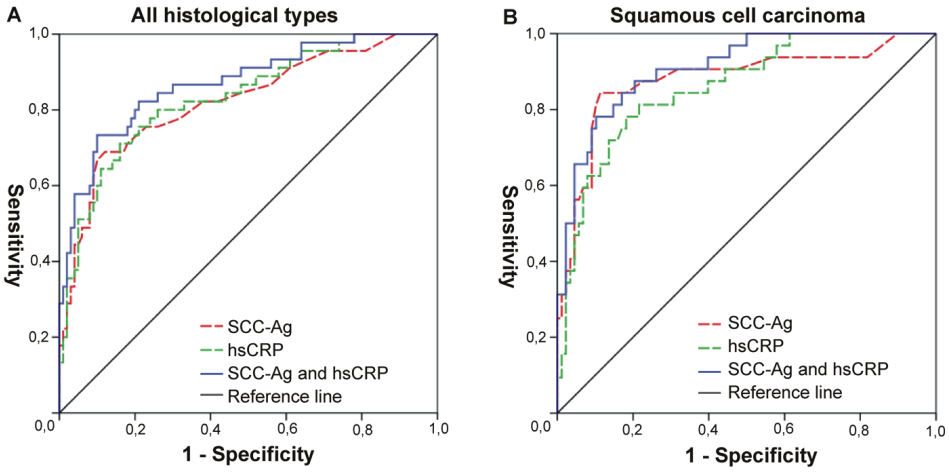

Figure 3. A) Receiver operating characteristics (ROC) curves of the study population for SCC-Ag, hsCRP and both biomarkers combined. The area under the curve (AUC) of SCC-Ag, hsCRP and both combined was 0.822 (95%CI: 0.744-0.900), 0.831 (95%CI: 0.758-0.905) and 0.870 (95%CI: 0.805-0.935) respectively. B) ROC curves of the subgroup with squamous cell carcinoma cases, yielding an AUC of 0.879 (95%CI: 0.798-0.960) for SCC-Ag, 0.859 (95%CI: 0.786-0.933) for hsCRP and 0.911 (95%CI: 0.855-0.966) when combined.

Discussion

To directly compare their diagnostic accuracy, we assessed nine common serum biomarkers within a single cohort. The results indicate that elevated serum levels of SCC-Ag and hsCRP accurately detect cervical cancer recurrence during follow-up. In some cases these elevated levels preceded clinical manifestation. The remaining seven biomarkers that we tested yielded a low AUC and did not add significant information to the model once SCC-Ag and hsCRP were included.

Routine biomarker testing during follow-up could provide more individually tailored surveillance programs and possibly improve the detection of asymptomatic recurrence. In such individualized programs, definitive proof of recurrence should be provided by additional diagnostics, such as histology or imaging studies (e.g. PET-CT). These could be particularly beneficial in patients presenting without symptoms but raised serum levels of SCC-Ag and/or hsCRP caused by local and thus potentially curable recurrences[85,90].

Additional benefits of marker testing during follow-up are its possible independence of clinic visits, the minimal effort it demands from patients and the easy implementation due to the wide availability and low costs of these two generic biomarkers.

Early reports indicated a clinical value of SCC-Ag, over CYFRA 21-1, during cervical cancer follow-up [22]. More recently, Pras et al. showed that elevated SCC-Ag levels were present in 33 (70%) of their recurrence cases (n=47, all histological types) and preceded clinical manifestation in 20 subjects by 1 to 20 months[95]. Comparably, Forni et al. published a 79% sensitivity for detection of recurrences (n=43, histological distribution unknown) with all of these cases showing SCC-Ag elevation prior to clinical symptoms by a mean lead time of 5 months[88]. Equally in line with our study, Yoon et al. reported a sensitivity and specificity of 61% and 98% for the detection of 18 recurrences in their population, composed of all histological tumor types. Eleven recurrence cases showed elevated SCC-Ag serum levels a median 2 months (range 1 to 15 months) before clinical manifestation[96]. In an earlier study by Esajas et al., a comparable sensitivity (74%) was noted, yet the relatively low proportion of early detected recurrences (14%) and their poor outcomes at salvage therapy translated in no significant survival benefit[87].

We preferred a ROC analysis to present the interdependency between sensitivity and specificity, instead of two select values corresponding to an 'optimal' cutoff. The definition of 'optimal' depends on the implementation goals of the test[150]. A high sensitivity, achieved by low cut-off values, is generally favored for detecting disease recurrence, yet sacrifices specificity. Also, such choices influence false-positive and negative rates, with both being a potential cause of emotional distress in patients. Furthermore, careful subject selection may further improve the diagnostic accuracy. For example, cases with eczema, dermatitis or psoriasis as comorbidities are known for false positively elevated SCC-Ag levels and could be deliberately excluded[87,151,152].

Chan and colleagues performed a cost-effectiveness evaluation for routine serum SCC-Ag screening during follow-up. It was calculated that for each recurrence detected 4750 US dollars (2001 equivalent) needed to be spent in diagnostic work-up. They stated that SCC-Ag monitoring never altered clinical management and was therefore unable to achieve cost-effectiveness[153]. However, their analysis was questioned on several key issues and believed of being overly disadvantageous for SCC-Ag monitoring[88,154]. None of the recurrences (n=55) were treated by curatively aimed salvage therapy (e.g. laterally extended endopelvic resection[155]) which does not seem to represent current clinical practice. [29]. Additionally, a single serum SCC-Ag measurement was priced at 51 dollars (2001 equivalent) in their analysis, a striking fivefold increase of the 11 dollars (2013 equivalent) we paid in our study.

Certain limitations of the presented research merit further clarification. The sample size in our study, in terms of subjects and samples, is only of intermediate proportion. A larger study population can generate more precision in the estimates (e.g. smaller confidence intervals) and a lower risk of model overfitting. However, the fact that

statistical significance is already achieved in this sample size, in itself substantiates the strength of the effect measures under study.
Our study was designed to compare serum biomarkers on their diagnostic performance for recurrence detection. In general, results like these should always be validated in an external dataset or, preferably, a prospective study. Secondly, an improved disease specific survival rate – caused by early recurrence detection – is the clinically desired outcome measure. Future research which prospectively investigates this endpoint is needed, for instance by randomizing between regular follow-up and a biomarker enhanced surveillance scheme. An exemplary study has already been successfully executed for CA-125 and detection of relapsing epithelial ovarian cancer[156]. An analogous cervical cancer trial may provide definitive evidence on whether and how serum biomarkers, like SCC-Ag, could be optimally incorporated into follow-up schemes. Unfortunately, however, searching the 'clinicaltrials.gov' register yielded no active or planned trials focused on SCC-Ag evaluation during cervical cancer follow-up[157].

In conclusion, serum SCC-Ag and hsCRP testing during cervical cancer follow-up appears promising for detection of disease recurrence. While the combination of both biomarkers is superior to either one on a single basis, the associated increase in diagnostic accuracy is limited. Careful consideration regarding desired cutoffs and which subjects to test, may further improve their diagnostic value and clinical usability. Any inference on increased survival rates due to potential early detection of recurrence needs to be validated in a prospectively designed study. Such a trial is urgently needed in order to conclusively end the ongoing biomarker debate, in particular revolving around SCC-Ag, which spans the past three decades.

Conflict of interest

All authors of the presented manuscript declare that they have no relevant conflicts of interest.

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8

General discussion and future perspectives

In this thesis the detection of (pre)malignant cervical lesions is studied in detail, in order to gain a better insight into possible pitfalls in the diagnostic pathway and to identify areas in need of improvement. In addition, a number of important risk factors for cervical cancer have been studied. The relevance and clinical implications of these findings and the future perspectives for improvement of diagnosis, treatment and follow-up are discussed in this chapter.

Introduction of the cervical cancer screening program in The Netherlands, as in other countries, has significantly reduced the cervical cancer incidence. However, the program's effectiveness is influenced by the test characteristics of the pap smear and the compliance rate, which now seems to have reached its maximum. Cervical cancer incidence and mortality rates have been stationary over the last decades, and the distribution of FIGO stages has not changed. The future perspective is positive, since hrHPV vaccination and hrHPV-based cervical cancer screening are expected to lead to a decrease in cancer incidence. However, these effects are expected decades from now. In the meantime cervical cancer screening should be optimized where possible.

Current cervical cancer diagnosis

Our retrospective evaluation of colposcopy in five hospitals in The Netherlands (chapter 2) shows that performance indicators are achievable but score poorly if held against current standards of care. In a multicentre series time-to-colposcopy proved to be long, and the follow-up after treatment to be inadequate. Of note, the diagnosis of cancer was suspected in less than 3 out of 10 cases who actually had cancer at the time of colposcopy. Probably as a result of this, and equally disturbing even with a two-step approach still 6 out of 14 cancer cases had undergone unnecessary excisional treatment. It has not been investigated or reported in the literature, but our clinical experience suggest that such local exisional treatment might hamper, or might delay proper staging and the ability to determine the exact tumor size. Further, it may impair tailored surgical treatment (especially minimally invasive and fertility-sparing treatment options). Reassuring is the low rate of overtreatment in patients who underwent a see-and-treat regimen. Furthermore, we found a higher sensitivity of colposcopy than generally reported in the literature, most likely because this was not a randomized trial, and only those cases with documented impression were analyzed. As expected, if a colposcopic impression is explicitly stated and documented, the colposcopist will be more certain about the diagnosis and therefore be more accurate.

Our retrospective analysis of the total diagnostic pathway of women with cervical cancer (chapter 5), shows additional interesting information. At the same time, there is less information in this study about the initial colposcopy, since not all data could be retrieved for these patients. In this study it was again shown that half of the cervical cancer patients were not screened, either due to non-attendance, or because they fell outside the age range for screening[130,132]. The majority of patients had an adequate and prompt diagnosis, after reporting complaints or establishment of an abnormal smear, but in a subset of patients a very long period between first signs or symptoms to diagnosis of cervical cancer was observed. Better coverage of the screening program by measures to improve compliance should enable prevention or earlier detection of cancer. Furthermore, strategies to improve physician and patient awareness about complaints related to cervical cancer might lead to earlier detection. Importantly, improving quality and documentation of colposcopy might lead to better detection of cancer and less radical diagnostic procedures, which cause delay and impede fertility sparing cancer treatment.

The future of cervical cancer diagnostics

Colposcopic mapping is essential for reliable histologic sampling and adequate treatment of (pre) malignant cervical lesions. However, from the data presented in this thesis, the question arises whether we should abandon colposcopy altogether, and should rely on novel markers in cervical smears to stratify patients that need immediate LEEP, need referral to a gynecological oncologist, or need follow-up only. Nonetheless, for the moment colposcopy remains the best available tool to assess women considered at high risk for developing cervical cancer. Fortunately, there are a number of novel discoveries, which may aid the gynecologist, and strategies that may improve cancer recognition.

Technical improvements in colposcopy

There have been numerous developments over the years in digital colposcopy. Several of those techniques have been investigated in the field of ophthalmology and dermatology as well. These range from optical coherence tomography (OCT), a non-invasive technique that can be used to image tissue structures slice by slice with high resolution[158,159] to confocal microscopy which can reconstruct three-dimensional images[160-162]. Furthermore, there is the development of spectroscopy of which the DySIS colposcope has been studied in this thesis.

We have shown (chapter 3) that the addition of digital colposcopy (DySIS) may improve the sensitivity of colposcopy to detect high-grade CIN lesions up to 88% (95%

CI 82-95). There are a number of factors that may hamper the use of DySIS in clinical practice, for example, when only a part of the cervix can be visualised, or when there is blood or mucus obscuring the cervix. Even in these situations, addition of DySIS shows a higher sensitivity (80%, 95% CI 72-87) than that of regular colposcopy. This effect can be partly explained by DySIS detection of smaller lesions, which are more often missed by the colposcopist.

It is reassuring that DySIS picks up HPV 16 positive cervical lesions better than lesions caused by other HPV types, possibly because of the specific acetowhitening effect present on these particular lesions (chapter 4). These and other clinical studies with DySIS have contributed to the decision of the British National Institution for Health and Care Excellence (NICE) to accept DySIS as adjunctive colposcopy technology[163].

Further research has to investigate the 'optical biopsy' function of DySIS. With this option the colposcopist would fully rely on the DySIS color coded map, and would only take a biopsy if a high-grade lesion is suspected. In case DySIS predicts a low-grade lesion only, the colposcopist would be able to refrain from histological sampling, a very interesting feature for women in which preferably no biopsies are taken, such as pregnant women.

Diagnosis in the era of hrHPV-based cervical screening

In 2016, an hrHPV-based cervical screening program will be implemented in The Netherlands, with the opportunity of self-sampling for non-responders[31]. This program aims at accrual of an extra 6% participants (due to self-sampling), and is expected to detect 30% more high-grade CIN lesions[32-41]. The drawback of hrHPV testing is the lower specificity for high-grade CIN lesions than cytology. This may lead to unnecessary referral for colposcopy and possible overtreatment. To diminish this effect a triage test with cytology will be implemented.

In this regimen, it has been calculated that there will be 5200 instead of the current 3900 referrals from the screening program to the colpsocopist per year[31]. Caution has to be taken not to over-treat these women. More treatment may lead to more side effects of LEEP, as cervical stenosis, although a recent meta-analysis has shown LEEP itself does not increase the risk of preterm birth[164]. Furthermore, time to colposcopy is already too long; measures need to be implemented at colposcopy clinics, so that the novel screening program and the expected increase in referred patients does not further increase waiting times.

To what extent should histology be sampled during colposcopy?

A recent meta-analysis has defined the sensitivity of biopsy, which might be an overestimate because of ascertainment bias, and demonstrates that the pooled

sensitivity for a single punch biopsy is 90%, if one or more punch biopsies are performed this increases to 93%[165]. Pretorius et al have investigated the role of random histology sampling, in order to increase colposcopic performance[58]. They have shown that the yield of CIN2+ per endocervical curettage, was 38% for high-grade and 15.6% for women with low-grade cytology. Furthermore, the yield of CIN2+ lesions per random biopsy was 17.6% in women with high-grade cytology as opposed to 3.6% in women with low-grade referral cytology. These data have been confirmed by other research groups[166,167] and recently also by our group (van der Marel, personal communicaion) showing that a second colposcopy guided biopsy inreases the CIN2+ detection rate, irrespective of referral cytology, colposcopic impression or hrHPV status. The effectiveness of a random biopsy depends, in their opinion, on the threshold for abnormal colposcopic impression, which differs greatly across studies. In the study of Pretorius et al[58] and Massad et al[168,169], this threshold was high, and therefore there was an overrepresentation of CIN2+ in their 'random biopsies'. Van der Marel and Wentzensen advocate to lower this treshold and take at least two colposcopy guided biopsies, of any lesion or acetowhite area. Van der Marel furthermore shows that performing an ECC was the most effective in women with HSIL referral cytology and a normal colposcopic impression. In this group performing an ECC was more effective than a random biopsy.

The 'gold standard' of histology and role of new biomarkers

To increase the quality of our gold standard of histology, several issues need to be addressed. Firstly, there is the obvious risk of sampling error, which has been discussed and is related to the poor sensitivity of colposcopy.

Secondly, there may be an issue with providing sufficient clinical information. From studies in radiology we know that clinical information will help the radiologist to effectively diagnose patients[170,171]. Similarly, it has been shown in a clinical trial on interpretation of bronchial brush specimens that absence of history leads to lower diagnostic accuracy[172]. Although this has not been examined, a similar pattern is to be expected in the evaluation of cervical cytology and histology samples. Thus documentation of colposcopic impression may be important for all involved, the patient, pathologist and gynecologist for final evaluation of the case, and opportunities for further examination in case of discrepancies between cytology and colposcopic findings.

A third issue is the diagnostic interpretation of conventional stained microscopic slides, which has a poor inter-observer variability between pathologists. Especially the CIN2 category is difficult to reproduce. Certain research groups suggest abolishing CIN2 as a category, and only report on low- (CIN1) or high-grade lesions

(CIN2 and 3) as used in international grading[173]. Further efforts have been made to optimize pathological examination of cervical specimens. Immunohistochemical analysis of histologic specimens for p16 has been shown to be a good option[59,164,174-179]. In addition, much effort has been put into identifying novel prognostic biomarkers in cytology and histology samples to be able to distinguishwomen with a transient hrHPV infection from those likely to progress to cervical cancer. Promoter methylation, which is an epigenetic event that plays an important role in tumor cells, has been shown to be a promising biomarker for development of cervical cancer[180]. Methylation of CpG islands within the promoter regions can lead to silencing of tumor suppressor genes, which in turn leads to loss of cell cycle control and induces proliferation[181]. Tumor suppressor genes showing frequent promoter hypermethylation in cervical cancer include CDH1, DAPK, FHIT, HIC-1, p16, RAR-beta, RASSF1A, TIMP-2, SPARC, TFPI2, CADM1 and MAL. Thus far, silencing of the tumor suppressor genes CADM1 and MAL was found to be promising as clinical biomarkers for development of cervical cancer[182-185].

Further research aims at other biomarkers as hrHPV viral load measurements and hrHPV typing. If these molecular markers could be detected in the cervical smear and are more sensitive and specific than colposcopy, one could argue to only use colposcopy to exclude cancer, and perform a LEEP in all other cases where the markers indicate high-grade disease. However, thus far, this level of efficacy has not been reached yet.

In all, the performance of colposcopy relies on adequate tissue sampling, providing sufficient clinical information and performing a high quality pathologic evaluation in order to decide on follow-up, treatment or referral to a gynecological oncologist.

Simplified follow-up post LEEP

In The Netherlands, as in many other countries, women with high-grade CIN lesions are treated by excision (mostly LEEP) in order to prevent progression to cervical cancer[101,105]. Despite treatment, there is a 15% (range 5-25%) recurrence rate[186-188]. From our study (chapter 2) and previous research we know that only around 50% of women complete the recommended three cytology smears after treatment. This follow-up protocol could benefit from simplification. Recently, it has been shown that the risk for recurrence with combined testing for cytology and hrHPV at 6 and 24 months after treatment was similar to the conventional cytology smears at 6, 12 and 24 months[189,190].

Will all cervical cancer be prevented in the future?

Vaccination against hr-HPV type 16 and 18 as implemented in 2009 in The Netherlands, is expected to provide 70% lifelong protection against cervical cancer[191]. Calculations have been made estimating the impact of hrHPV vaccination, in various uptake levels, on cervical cancer incidence[192]. As per January 2014, the compliance rate of vaccination in The Netherlands was only 60.7%[193]. Thus the expected decrease in cervical cancer as a result of vaccination will be around 40 %. A first effect on the cervical cancer incidence is expected in 2027, when the vaccinated cohort of 12 year olds will reach the screening age of 30. Given the low incidence of cervical cancer in the 30-34 age group, this will result in around 30 less cancer cases.

A further decrease is expected from hrHPV-based screening, which is expected to result in a decrease in incidence of CIN3 lesions of 30%[31]. It is expected that (depending on which lead time to cancer is used) around 2035, there will be a decrease of cervical cancer incidence of around 30% as well, bringing down the total number of cervical cancers to around 500. The full effects of screening will be reached around 2054 when all screening-eligible women will have been offered vaccination.

Unfortunately, with the current measures, cervical incidence will never reach extinction in the future. No screening or vaccination program can provide 100% protection against cervical cancer, there will be cervical cancer related to other hrHPV types and there will unfortunately be women not responding to calls for vaccination or screening as well.

From the diagnosis of cervical cancer onward

In The Netherlands, treatment options have greatly improved and individualized over the last years, and fertility sparing surgery combined with sentinel lymph node sampling can be offered. This sentinel node sampling has not only brought improvements but also unanswered questions. Furthermore, the role of novel biomarkers in the follow-up remains open for further research.

How to deal with a microscopically involved sentinel lymph node

The detection rate of sentinel nodes has proven to be adequate in cervical cancer. A large international study has shown a false-negative rate of 1.3% in patients with optimal bilateral mapping using radioisotope and blue dye with pathologic ultrastaging[136,137]. We are still awaiting survival data from SENTICOL-2 study, a prospective multicenter randomised trial comparing pelvic lymphadenectomy versus sentinel node biopsy in cervical cancer.

In chapter 6 we have addressed the issue whether the time has now come to refrain from complete lymphadenectomy in case of a negative SN. For all stages, besides having a diagnostic value, we can conclude that systematic pelvic lymphadenectomy performed in addition to the SN was only associated with better survival in a small subset of patients with LVD in the SN. This possible therapeutic effect could be explained by removing additional low-volume disease in non SNs, which is not detected by routine pathologic assessment. Whether our results warrant clinical implementation of full LND in a subgroup should be further validated, preferably in a randomized controlled trial. In such a prospective study, it should be assessed whether patients in whom a SN procedure is performed and who are found to have low-volume disease do indeed benefit from additional lymphadenectomy and/or radiotherapy, as this retrospective cohort analysis suggests.

Is it time to implement serum markers in the follow-up of cervical cancer?

Cervical cancer follow-up surveillance schemes have not changed over the last decade. They commonly entail 5 years of ambulant visits with increasing time intermissions. Each follow-up visit comprises an interval history and a gynecological physical examination, supplemented by advanced diagnostics when indicated[143,144]. To improve these follow-up programs, a range of serum biomarkers has been assessed in chapter 5.

The results indicate that elevated serum levels of SCC-Ag and hsCRP accurately detect cervical cancer recurrence during follow-up. In some cases these elevated levels precede clinical manifestation. Routine biomarker testing during follow-up could provide more individually tailored surveillance programs and possibly improve the detection of asymptomatic recurrence. In such individualized programs, definitive proof of recurrence should be provided by additional diagnostics, such as histology or imaging studies (e.g. PET-CT). Early detection of (loco-regional) cervical cancer recurrence and early radical treatment of these patients is important as curative treatment may still be possible in selected cases[80,83,84]. Additional benefits of marker testing during follow-up are its possible independence of clinic visits, the minimal effort it demands from patients and the easy implementation due to the wide availability and low costs of these two generic biomarkers. Any inference on increased survival rates due to a potential early detection of recurrence and treatment needs to be validated in a prospectively designed study. Such a trial is urgently needed in order to conclusively end the ongoing biomarker debate, in particular revolving around SCC-Ag, which spans the past three decades.

In conclusion, although hrHPV vaccination and hrHPV-based screening will hopefully lead to a major reduction in the incidence of cervical cancer in the years to come, we still have decades to bridge until then, years in which implementation of the measures mentioned above may contribute to women's health. We anticipate a future where the physician is assisted by various biomarkers and digital imaging enhancements techniques to facilitate the detection of cervical cancer and its precursor lesions. This is necessary, because the exposure of the individual caretaker to cervical pathology is expected to decrease as a result of implementation of hrHPV-based screening and hrHPV vaccination. If, however, a women still develops cervical cancer (hopefully a fraction from the number who do today) adequate staging and follow-up will lead to less overtreatment and less recurrence.

9

Summary

As outlined in the introduction (**Chapter 1**) it is expected that cervical cancer incidence will decrease in The Netherlands over the next decades, as a result of hrHPV vaccination and hrHPV-based screening. Untill then, quality of care could need some improvements as suggested by the work described in this thesis. Novel tools are being indicated to improve care and information is discussed on prognostic factors once cervical cancer has been diagnosed.

In **Chapter 2** we described the quality of colposcopy with regard to recognition of cervical cancer, hospital access time and adherence to follow-up in a retrospective cohort of 532 women from five Dutch colposcopy clinics. We also compared the outcome of women who underwent direct treatment by excision of the transformation zone during the first colposcopy ('see-and-treat'), with those who had treatment following a cervical biopsy (two-step approach). The majority (88.3%) of patients had a colposcopy within 10 weeks after index cytology. In total, 18 (3.6%) women were diagnosed with invasive cervical cancer, of which only 5 (28%) were recognized at colposcopy. This leads us to conclude that cancer recognition is poor and needs improvement. The sensitivity of 74% for the colposcopic impression to identify clinically significant high grade lesions was high in this particular series compared to other published series. This might be due to a registration bias, as obviously only the 71% documented impressions could be analysed. Treatment was performed in 338 (63.5%) of the patients, being 'see-and-treat' in 115 (21.6%) and two-step approach in 223 (41.9%) patients. Overtreatment rate, incidence of invasive cervical cancer and the number of recurrences or residual disease were similar for 'see-and-treat' and two-step approach. These data suggest that 'see-and-treat' could be considered for women with a suspected high-grade lesion and no wish to conceive. Follow up after treatment in this clinical cohort was very low (34.2% completed total follow-up), and warrants simplified post treatment monitoring.

Novel colposcopic techniques have been developed to improve the performance of colposcopy. In **Chapter 3** we describe a prospective comparative study to validate the use of a dynamic spectral imaging system (DSI, DySIS™) in the clinical practice in three Dutch colposcopy clinics. In this study the DySIS colposcope was first used as a regular video colposcope. Thereafter a computer generated color-coded map was displayed representing localisation and severity of the cervical lesions. Biopsies were taken from all abnormal areas as well as from a random (normal) site. In total 275 women were included in the study: 183 women were analyzed in the 'according-to-protocol' (ATP) cohort and 239 women in the 'intention-to-treat' (ITT) cohort. In the ATP cohort, the sensitivity of DySIS colposcopy to detect high-grade (CIN2+)

lesions was 79% (95% CI 70- 88) and the sensitivity of conventional colposcopy was 55% (95% CI 44-65) (P = 0.0006, asymptotic McNemar test). When the DySIS color-coded map was combined with conventional colposcopy, the sensitivity reached 88% (95% CI 82-95). These data show that the addition of DySIS to the colposcopic examination by the colposcopist can yield a high senstitivity for the detection of high-grade cervcial lesions.

In addition, in **Chapter 4** we further analysed the performance of both the colposcopist as well as DySIS in detecting lesions in HPV16 positive women. Previous research showed that lesions in these women are more easily detected than cervical lesions in non HPV16 infected women. We show that the sensitivity of the DySIS colposcope for detecting CIN2+ lesions was higher in HPV16+ women than in non-16 hrHPV+ women (97% versus 74%, P = 0.009). This is a reassuring finding, since hrHPV16 is the most frequently associated with cervial cancer. In addition, as expected, we show that mainly the smaller cervical lesions are missed by the colposcopist.

In the prologue of **Chapter 5** we illustrated the difficulties and challenges in diagnosing cervical cancer by describing the diagnostic pathway in several clinical examples. We have included cases in which one or more suboptimal factors could be identified. This prologue is followed by **Chapter 5,** which describes a retrospective in-depth analysis of the diagnostic process of 120 patients diagnosed with cervical cancer between 2008-2010 in the Utrecht region. Only 39.1% of the women diagnosed with cervical cancer were screen or interval detected; the other 60.9% of patients had not been screened, either due to non-attendance or because they fell outside the age range for screening. Less than 50% of cancer cases were recognized at the first gynecological referral examination. The final diagnosis of cervical cancer was established by biopsy in 77 (64.2%) and by excision of the cervical transformation zone in 35 (29.2%) women. Fifteen (43%) of the excisions could have been avoided if 'no see-and-treat' had been performed, whereas the other 20 patients (57%) had a premalignant histology result prior to the excision procedure. This in-depth analysis suggests that improvement of quality of care is to be expected from correctly recognizing cervical cancer by physicians. Furthermore, great care should be taken with the use of diagnostic excisional procedures, particularly in young women, as they might impede fertility sparing surgery.

In **Chapter 6** we described the value of pelvic lymph node dissection (LND) following sentinel lymph node biopsy (SN) in early-stage cervical cancer. This retrospective multicenter cohort study was conducted in seven European and one American gynecological oncology department. In total, 645 women with FIGO stage IA to IIB cervical cancer of squamous, adeno or adenosquamous histological type who

underwent sentinel lymph node biopsy followed by pelvic lymph node dissection were enrolled. Radioisotope tracers and blue dye were used to localize the sentinel node, and pathologic ultrastaging was performed. In this study we show that among patients with low-volume disease (micrometastases and isolated tumor cells) in the sentinel node, the overall survival was significantly better (p=0.046) if more than 16 non-sentinel lymph nodes were removed. No such significant difference in survival was detected in patients with negative or macrometastatic sentinel nodes. Our findings indicate that in patients with negative or macrometastatic disease in the sentinel nodes, an additional lymph node dissection did not alter survival. Conversely, our data suggest that the survival of patients with low-volume disease is improved when more than 16 additional lymph nodes are removed. If in a prospective trial our data would be confirmed we would suggest a two step approach with sentinel node sampling during the first surgery and a second operation according to the ultrastaging results of the sentinel node procedure.

The diagnostic accuracy and the optimal combination of commonly studied serum biomarkers aimed at identifying recurrence in cervical cancer patients was studied in **Chapter 7**. From a systematic literature search, nine biomarkers (CA-15.3, CA-125, CEA, CYFRA 21-1, hsCRP, IL-6, SCC-Ag, TNF-α and VEGF) were selected for a serum analysis. Samples were derived from an historical cervical cancer cohort and were selected if one sample preceding and at least one sample following primary treatment was available. In case of recurrence, two additional post-recurrence samples were analyzed. We analyzed 205 samples from 75 subjects, of whom 19 (25.3%) had a recurrence. The area under the curve was studied for all individual markers. Only SCC-Ag and hsCRP were included in the final model with an AUC of 0.822 (95%CI: 0.744-0.900) and 0.831 (95%CI: 0.758-0.905) respectively. Combined AUC was 0.870 (95%CI: 0.805-0.935). Rises in SCC-Ag and hsCRP significantly increased the odds for recurrence. We hereby show that combined testing of SCC-Ag and hsCRP yields the highest detection rate of disease recurrence during cervical cancer follow-up. These data should be validated in a prospective setting in order to obtain information on the potential survival benefit of early detecion of recurrence.

Finally, in **Chapter 8** we provided a general discussion of the results presented in this thesis and the future perspectives. We anticipate a future where the physician is assisted by various biomarkers and digital imaging enhancements techniques to facilitate the detection of cervical cancer and its precursor lesions. Despite an expected decrease in cervical cancer incidence as a result of implementation of hrHPV vaccination and hrHPV-based screening, cervical cancer will still be diagnosed 30 years from now and requires adequate and early recognition. Further

improvement of the therapeuic options for early-stage cervial cancer is expected when large international trials on comparing sentinel lymph node sampling with full lymphadenectomy have been published. If these studies would show the same effects as found in our retrospective data, the time has come to refrain from full lymphadenectomy in case of a negative sentinel node. SCC-Ag and hsCRP could be interesting serum markers to predict recurrence in the follow-up after treatment. However, the clinical siginificance of these markers has to be validated in a prospective trial, in order to decide whether they should be included in the regular follow-up protocols after cervical cancer treatment.

9

Nederlandse samenvatting

Zoals uiteengezet in de inleiding (**Hoofdstuk 1**) verwacht men dat de incidentie van baarmoederhalskanker in Nederland in de komende decennia zal verminderen ten gevolge van hrHPV vaccinatie en het op hrHPV gebaseerde bevolkingsonderzoek. Tot die tijd zou de kwaliteit van zorg gebaat zijn bij een aantal verbeteringen zoals beschreven in dit proefschrift. Dit proefschrift beslaat het geheel van nieuwe instrumenten die de detectie van (pre)maligne afwijkingen kunnen verbeteren tot prognostische factoren die bruikbaar zijn vanaf het moment dat er baarmoederhalskanker is vastgesteld.

In **Hoofdstuk 2** beschrijven we de kwaliteit van colposcopie met betrekking tot de herkenning van baarmoederhalskanker, toegangstijd tot het ziekenhuis en de naleving van de nacontrole in een retrospectief cohort van 532 vrouwen uit vijf Nederlandse colposcopie klinieken. We vergeleken in dit hoofdstuk eveneens de uitkomst van de vrouwen die een excisie van de transformatiezone ondergingen tijdens de eerste colposcopie (directe behandeling), met vrouwen bij wie eerst een biopt is afgenomen (twee staps methode). De meerderheid (88,3%) van de patiënten onderging een colposcopie binnen 10 weken na het maken van het uitstrijkje. In totaal werden er 18 (3,6%) vrouwen gediagnosticeerd met baarmoederhalskanker, van deze tumoren werden er slechts 5 (28%) herkend bij colposcopie. Hieruit hebben wij geconcludeerd dat de herkenning van baarmoederhalskanker tijdens colposcopie niet optimaal is, en verbetering behoeft. In vergelijking met de bestaande literatuur vonden wij een hogere sensitiviteit (74%) voor de colposcopische impressie voor significante hooggradige laesies. Dit valt waarschijnlijk te verklaren doordat slechts in 71% een colposocpische impressie was vastgelegd. Waarschijnlijk komt de colposcopische impressie beter overeen met de uiteindelijke histologie als de colposocopist heeft besloten om deze te documenteren. Er werden 338 (63,5 %) vrouwen behandeld, van wie 115 (21,6%) direct en 223 (41,9 %) per twee staps methode. Het bleek dat het percentage overbehandeling en recidief, alsmede de incidentie van baamoederhalskanker gelijk was in deze twee groepen. Deze bevindingen suggereren dat directe behandeling zou kunnen worden overwogen voor vrouwen zonder kinderwens met verdenking op een hooggradige laesie. De opkomst voor nacontrole was zeer laag in deze patientengroep (34,2% voltooide nacontrole bezoeken). Deze laatste bevinding geeft aan dat er wellicht noodzaak is tot vereenvoudigen, danwel inkorten van de huidige nacontrole.

Er zijn nieuwe colposcopische technieken ontwikkeld om de kwaliteit van het huidige colposcopisch onderzoek te verbeteren. In **Hoofdstuk 3** beschrijven we een prospectief vergelijkend onderzoek ter validatie van een systeem dat werkt door

middel van dynamische spectrale analyse (DSI, DySIS ™) in de klinische praktijk in drie Nederlandse colposcopie klinieken. In deze studie werd de DySIS colposcoop eerst gebruikt als een gewone videocolposcoop. Daarna werd een computer gegenereerde kleurenkaart weergegeven die de lokalisatie en de ernst van de cervicale laesies weergaf. Vervolgens werden er biopten genomen van alle abnormale gebieden en van een willekeurige plaats op de cervix. In totaal werden 275 vrouwen geincludeerd in deze studie: 183 vrouwen werden volgens protocol behandeld (according-to-protocol, ATP), en 239 vrouwen werden met intentie tot behandelen geanalyseerd ('intention-to -treat', ITT). In het ATP cohort was de gevoeligheid van DySIS colposcopie voor detectie van hooggradige laesies (CIN2 +) 79% (95 % CI 70-88), in tegenstelling tot de gevoeligheid van conventionele colposcopie van 55% (95 % CI 44-65) (P=0.0006, asymptotische McNemar test). Wanneer de DySIS kleurenkaart werd gecombineerd met het oordeel van de colposcopist werd de sensitiviteit 88% (95 % CI 82-95). Deze gegevens tonen aan dat de toevoeging van DySIS een meerwaarde heeft in de gevoeligheid van het colposcopie onderzoek.

In aanvulling hierop werd in **Hoofdstuk 4** nader geanalyseerd hoe effectief de colposcopist en DySIS waren, in het detecteren van laesies bij vrouwen die geïnfecteerd waren met HPV16. Eerder onderzoek heeft aangetoond dat cervicale laesies makkelijker te detecteren waren bij deze vrouwen in vergelijking met vrouwen die niet waren geïnfecteerd met HPV type 16. Wij laten in dit proefschrift zien dat de gevoeligheid van de DySIS colposcoop voor het detecteren van CIN2+ laesies beter was bij HPV16 positieve vrouwen in vergelijking met vrouwen die niet geïnfecteerd waren met HPV type 16 (97 % versus 74 %, p = 0.009). Dit is een geruststellende bevinding, aangezien een infectie met HPV type 16 het vaakst is geassocieerd met baarmoederhalskanker. Verder tonen wij aan dat, zoals verwacht, met name de kleinere laesies wel door DySIS maar niet door de colposcopist worden herkend.

Het proloog van **Hoofdstuk 5** bestaat uit een beschrijving van het diagnostische traject van een aantal individuele patiënten, om te illustreren waar mogelijke aandachtpunten te vinden zijn. We beschrijven bewust het beloop van een aantal geselecteerde patiënten waarin een of meerdere suboptimale factoren kunnen worden benoemd. Deze proloog wordt gevolgd door **Hoofdstuk 5**, een gedetailleerde retrospectieve analyse van het diagnostisch proces van 120 patiënten met baarmoederhalskanker, gediagnostiseerd tussen 2008 en 2010 in de regio Utrecht. Slechts in 39,1% werd de diagnose gesteld naar aanleiding van een uitstrijkje uit het bevolkingsonderzoek. De overige 60,9% van de patiënten was niet gescreend, hetzij omdat ze geen uitstrijkje hadden laten maken of omdat ze buiten de leeftijdscategorie van het bevolkingsonderzoek vielen. Minder dan de helft van de diagnoses werd gesteld tijdens het eerste bezoek aan de gynaecoloog. De diagnose baarmoederhalskanker werd gesteld door

middel van biopsie bij 77 (64,2%) en door middel van excisie van de cervicale transformatiezone bij 35 (29,2%) vrouwen. Vijftien (43%) van de excisies had voorkomen kunnen worden als deze niet direct waren uitgevoerd maar als er eerst een biopt zou zijn afgenomen. De overige 20 patiënten (57%) had een biopt of excisie preparaat met daarin een premaligne afwijking voorafgaand aan de excisie procedure. Dit onderzoek suggereert dat er verbetering van de kwaliteit van de zorg is te verwachten van als artsen beter worden getraind in het herkennen van baarmoederhalskanker. Bovendien zou er zeer zorgvuldig moeten worden omgegaan met excisie procedures zonder voorafgaande histologie, vooral bij jonge vrouwen, omdat deze excisie het eventuele uitvoeren van een vruchtbaarheidssparende operatie zouden kunnen vertragen of bemoeilijken.

In **Hoofdstuk 6** beschrijven we de waarde van het verrichten van een volledige lymfeklierdissectie in aanvulling op een schildwachtklier (SN) procedure bij vrouwen met een vroeg stadium van baarmoederhalskanker. Deze retrospectieve studie werd uitgevoerd in zeven Europese en een Amerikaanse afdeling gynaecologische oncologie. In totaal werden 645 vrouwen geïncludeerd met baarmoederhalskanker FIGO stadium IA tot IIB, van plaveisel-, adeno- of adenosquameuze origine. Alle patiënten hadden zowel een schildwachtklier procedure als een reguliere pelviene lymfeklierdissectie ondergaan. Radioactief geladen vloeistof en blauwe kleurstof werden gebruikt om de schildwachtklier te lokaliseren, en er werd een zeer zorgvuldige analyse van het pathologisch preparaat verricht (ultrastagering). In deze studie tonen we aan dat bij patiënten met microscopische metastasen in de schildwachtklier (micrometastasen en geïsoleerde tumorcellen), de totale overleving significant beter was ($p = 0.046$) als er meer dan 16 lymfeklieren werden verwijderd naast de schildwachtklier. Een dergelijk verschil in overleving werd niet waargenomen bij patiënten zonder metastasen of met macroscopische metastasen in de schildwachtklier. Onze bevindingen wijzen erop dat het verrichten van een volledige lymfeklierdissecte bij patiënten met een negatieve schildwachtklier of met macroscopische ziekte in de schildwachtklier geen effect op de overleving heeft. Omgekeerd suggereren onze gegevens dat de overleving van patiënten met microscopische metastasering in de schildwachtklier verbetert wanneer er meer dan 16 aanvullende klieren worden verwijderd. Als deze bevindingen in een prospectieve studie zouden worden bevestigd dan zouden wij opteren voor een aanpak in twee stappen met een schildwachtklier procedure tijdens de eerste operatie en een tweede operatie afhankelijk van de uitslagen van het pathologisch onderzoek van de schildwachtklier.

De diagnostische nauwkeurigheid en de optimale combinatie van de meest bestudeerde serumbiomarkers gericht op het identificeren van een recidief bij patiënten met baarmoederhalskanker werd bestudeerd in **Hoofdstuk 7**. Via een systematisch literatuuronderzoek werden negen potentiële biomarkers geselecteerd voor een serum analyse (CA-15.3, CA-125, CEA, CYFRA 21-1, hsCRP, IL-6, SCC-Ag, TNF-α en VEGF). Serum werd verkregen uit een historisch baarmoederhalskanker cohort en werd geselecteerd als er ten minste een monster voorafgaande en ten minste twee monsters na behandeling aanwezig waren. Indien er meerdere buisjes beschikbaar waren na vaststellen van het recidief werden er maxi maal twee monsters van na het recidief gemeten. We analyseerden 205 monsters van 75 patiënten, van wie er 19 (25.3 %) een recidief hadden ontwikkeld. Het gebied onder de curve (AUC) werd onderzocht voor alle afzonderlijke markers. Alleen SCC-Ag en hsCRP werden opgenomen in het uiteindelijke model met een AUC van 0,822 (95% BI : 0,744-0,900) en 0.831 (0,758-0,905 95% BI). Het gecombineerde AUC was 0,870 (95% BI : 0,805-0,935). Stijgingen van SCC-Ag en hsCRP gaven een sterk toegenomen recidiefkans. We zagen hierbij dat het gecombineerd testen van SCC-Ag en hsCRP de beste detectie van recidieven gaf tijdens de controles na behandeling voor baarmoederhalskanker. Deze gegevens zouden moeten worden gevalideerd in een prospectieve studie om de klinische consequenties en het mogelijke overlevingsvoordeel van vroege detectie van een recidief te kunnen beoordelen.

Tenslotte wordt in **Hoofdstuk 8** een algemene discussie beschreven over de bevindingen in dit proefschrift en de toekomstperspectieven. We anticiperen op een toekomst waarin de arts wordt geholpen door diverse biomarkers en digitale beeldvormingstechnieken, ter verbetering van het herkennen van baarmoederhalskanker. Ondanks een afname in de incidentie van baarmoederhalskanker als gevolg van de implementatie van hrHPV-vaccinatie en hrHPV-screening, zal er over 30 jaar nog steeds baarmoederhalskanker zijn. Verdere verbetering van de behandeling van vroeg stadium baarmoederhalskanker wordt verwacht wanneer grote internationale studies naar het vergelijken van de schildwachtklier procedure met volledige lymfeklierdissectie zijn gepubliceerd. Indien deze studies resultaten tonen die vergelijkbaar zijn met onze retrospectieve gegevens, dan is de tijd aangebroken om af te zien van een volledige lymfeklierdissectie als de schildwachtklier negatief is. Verder zouden SCC-Ag en hsCRP interessante biomarkers kunnen zijn om het recidiveren van kanker na behandeling te kunnen voorspellen. Echter, de klinische significantie van deze markers moet worden gevalideerd in een prospectieve studie, om te concluderen of ze moeten worden opgenomen in de reguliere protocollen voor controle na behandeling.

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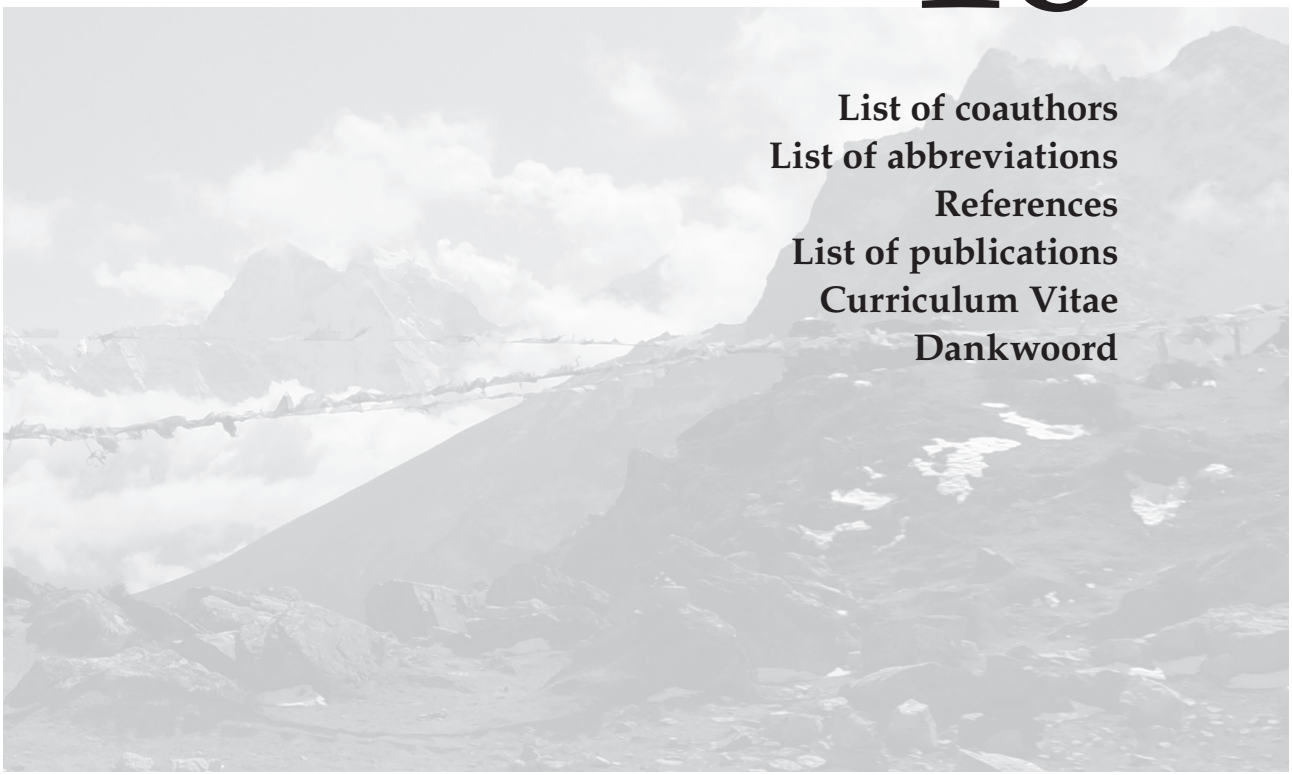

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List of abbreviations
References
List of publications
Curriculum Vitae
Dankwoord

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List of abbreviations

AdCA/ACC/AC	Adenocarcinoma
AGC	Atypical glandular cells
AIS	Adenocarcinoma in situ
ASCC	Adenosquamous carcinoma
ASC-H	Atypical squamous cells cannot exclude HSIL
ASCUS	Atypical squamous cells of undetermined significance
ASR	Age-standardized incidence rate
ATP	According to protocol
AUC	Area under the curve
BMD	Borderline or mild dyskaryosis
CADM1	Cell adhesion molecule 1
cGIN	Cervical glandular intraepithelial neoplasia
chi-2 or χ^2	Chi-square test
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
DFS	Disease free survival
DySIS	Dynamic spectral imaging system
ECC	Endocervical curettage
FIGO	International Federation of Gynecology and Obstetrics
HIV	Human immunodeficiency virus
HG	High-grade
HPV	Human papillomavirus
HR	Hazard ratio
hrHPV	High-risk human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
ITC	Isolated tumor cells
ITT	Intention to treat
IU	International units
LBC	Liquid based cytology
LEEP	Loop electrosurgical excision procedure
LG	Low-grade
LND	Lymph node dissection
lrHPV	Low-risk human papillomavirus
LSIL	Low-grade squamous intraepithelial lesion
LVD	Low-volume disease
LVI/LVSI	Lymphovascular invasion

MAL	T-lymphocyte maturation associated protein
mg	milligram
ml	milliliter
ML	Maximum likelyhood
NA	Not applicable
NAC	Neo-adjuvant chemotherapy
NED	No evidence of disease
NEG	Negative
ng	nanogram
NOS	Not otherwise specified
NPV	Negative predictive value
OS	Overall survival
OR	Odds ratio
PA	Pathological examination
PCR	Polymerase chain reaction
pg	picogram
POS	positive
PPROM	Preterm, premature rupture of membranes
PPV	Positive predictive value
qMSP	Quantitative methylation-specific PCR
Rb	Retinoblastoma
RFS	Recurrence free survival
ROC	Receiver operating characteristic
SN	sentinel lymph node
SCC	Squamous cell carcinoma
SCJ	Squamocolumnar junction
WHO	World Health Organisation

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List of publications

Pelvic lymphadenectomy improves survival in patients with cervical cancer with low-volume disease in the sentinel node: a retrospective multicenter cohort study. **Zaal A**, Zweemer RP, Zikán M, Dusek L, Querleu D, Lécuru F, Bats AS, Jach R, Sevcik L, Graf P, Klát J, Dyduch G, von Mensdorff-Pouilly S, Kenter GG, Verheijen RHM, Cibula D. *Int J Gynecol Cancer*. 2014 Feb;24(2):303-11.

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Curriculum Vitae

Afra werd op 6 maart 1980 geboren in Amsterdam en groeide samen met haar broertje op in een liefdevol gezin in Castricum. Het VWO diploma behaalde zij in 1998 aan het Jac P Thijsse college aldaar. Zij heeft twee jaar Biologie gestudeerd aan de Universiteit van Amsterdam, en is in 2000 gestart met de studie Geneeskunde, eveneens aan de Universiteit van Amsterdam. Tijdens haar studie werkte Afra als laborante voor het klinisch chemisch laboratorium en de afdeling cardiologie, bij de hyperbare geneeskunde en als verpleeghulp. Haar wetenschappelijke stage deed zij bij het DNAX research institute, Palo Alto, California, Verenigde Staten. Dit onderzoek was een voortzetting van haar onderzoek bij het Center for Experimental and Molecular Medicine, AMC Amsterdam. Voor haar klinische stage is zij naar het Turiani Missieziekenhuis in Tanzia vertrokken, alwaar haar liefde voor het vak der gynaecologie bevestigd werd. Na haar coschap verloskunde en gynaecologie in het Onze Lieve Vrouwe Ziekenhuis, Amsterdam, kwam zij via prof.dr. J. van Lith bij prof. dr. R.H.M. Verheijen in het VU Medisch Centrum. Zij startte als arts-onderzoeker met onderzoek naar Farletuzumab, een therapie voor het ovariumcarcinoom en vervolgens werkte zij voor de HumaVac onderzoeksgroep aan HPV gerelateerde cervicale (pre) maligniteiten en HPV vaccinatie. Zij is mee verhuisd met haar promotor naar de Universiteit Utrecht alwaar ze haar promotieonderzoek voltooide. Afra heeft bijgedragen aan de tot standkoming van landelijke richtlijnen en implementatie van screening en diagnose van baarmoederhlaskanker (NVOG 'herziening richtlijn CIN', RIVM uitvoeringstoets nieuwe bevolkingsonderzoek, Zichtbare Zorg kwaliteitsindicatoren baarmoederhalsafwijkingen). Sinds 2012 is zij bestuurslid van de NVOG Werkgroep Cervix Uteri. In augustus 2012 is zij gestart als arts in opleiding tot specialist in de verloskunde en gynaecologie in het Tweesteden Ziekenhuis Tilburg (Opleider dr. H.J.H.M. van Dessel) en sinds april 2014 zet zij haar opleiding voort in het WKZ te Utrecht (Opleider prof.dr. A. Franx). Afra is gelukkig getrouwd met Maarten en samen met hun zoon Jonas wonen zij in Utrecht.

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