Adenocarcinoma of the Uterine Cervix and its Precursor Lesion

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Adenocarcinoom van de baarmoederhals en het voorstadium

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Non mentulando sed terendo Pulchritudo Divinus molestiam custodit

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1

GENERAL INTRODUCTION

HISTORY

More than 2000 years have elapsed since the first description of cervical cancer by Hippocrates. *Aretaeus*, an ancient Greek physician practicing in the first century before Christ, described uterine cancer as superficial and deep ulcers, which later infiltrate the uterus[1]. In 1812 *John Clarke* described a peculiar degeneration of the cervix, which he called a cauliflower tumor because of it's appearance[2]. *Charles Mansfield Clarke* introduced the term carcinoma uteri in 1821 and *Hooper* identified the cauliflower as a carcinoma of the cervix in 1832[3].

Initially there was no distinction between cervical and endometrial carcinoma. Adenoma malignum was used for highly differentiated glandular carcinomas, without distinction in origin. When it became clear that cervical cancer was a separate entity, different types of cervical cancer were described. In German literature *Portiokarzinom* (karzinom der ektocervix, squamous cell carcinoma originating from the portio vaginalis) and *Zervixhöhlenkarzinom* (karzinom der endocervix, adenocarcinoma arizing from the cervical channel)[4-7] were distinguished.

Ruge and Veit 1881 and later the school of *Schroeder* differentiated between a portio carcinoma, arising from the connective tissue of the cervix or from columnar epithelial erosions and cervical carcinoma arising from de cervical glands or from the connective tissue. *Treub* in 1892 proposed another classification, based on the extension of the different tumor types. He described the carcinoma of the cervix, also called cancroid or epithelioma as a cancer which originated from the squamous epithelium of the portio vaginalis and the 'Zervixcarcinoma'

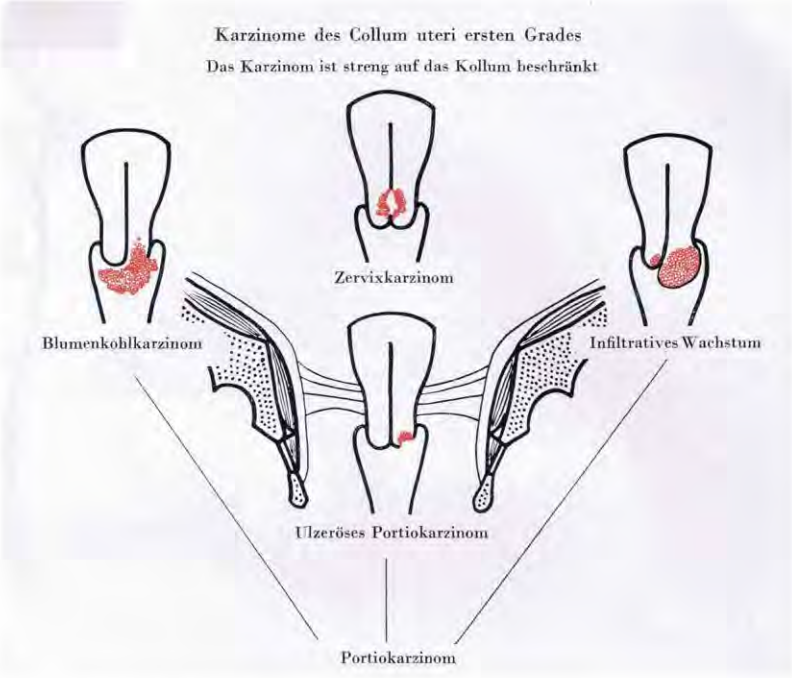

Abb 198 from Lehrbuch der Gynäkologie, prof Guggisberg 1946[5]

(cervical cancer) which originated from the cervical glands[8]. Today, we speak of cervical carcinoma, without differentiating between portio and cervical cancer.

The prognosis of cervical cancer has improved due to improved therapeutic strategies; for many years surgical intervention for cervical cancer was obsolete [*Hippocrates, Celsus*]. *A Morbus contra naturam* as *Galenus* proclaimed. Although it is suspected that in the 15 and 16 century hysterectomies were performed, *CJM Langenbeck* performed the first deliberate and well-planned vaginal hysterectomy for malignancy in 1813[3]. It was performed upon a prolapsed uterus and afterwards the diagnosis of cancer was doubted. French authors stated that the Italian surgeon *Paletta* accidentally performed the first vaginal hysterectomy in 1812. *Sauter* did the second complete vaginal hysterectomy for cervical cancer with an unprolapsed uterus in Konstanz, 28 januari 1822[3]. The operation had no effect on survival, as mortality was almost 100%. On the 30th January of 1878 *W.A. Freund* performed the first abdominal hysterectomy for cervical cancer. Although conditions of asepsis and Trendelenburg position were available, primary mortality was still as high as 70%. *Czerney* in 1878 introduced a new vaginal technique combined with narcosis, asepsis and anatomical hemostasis, which reduced mortality, after which surgical procedures developed rapidly. *Dührssen* and *Schuchhardt* refined this procedure, with their modified incision, making hysterectomy possible in very narrow vaginas. Bleeding which was previously controlled, by leaving clamps in situ for 48 hours, was replaced by carefully laid ligatures. By dissection of the ureters, it was possible to remove the parametrium and with this refinement *Schauta* has discovered the radical vaginal hysterectomy in 1901[9]. *Rumpf* performed the first abdominal radical hysterectomy in 1895, which enabled the removal of the regional lymph nodes. Shortly after that, in 1898, *Wertheim* perfected this technique in 1898 and developed the Wertheim Operation which included removal of uterus, tubes, ovaries, parametria, much of the vagina and paravaginal tissues as well as enlarged pelvic nodes[9]. This procedure had a primary mortality of 15-20% and a 5 year survival of 18.4%. *Bonney* modified the radical abdominal hysterectomy by removing all pelvic lymph nodes[10].

The discovery of radium in 1898 offered a low-risk alternative to surgery, dominating the scene for three decades[11]. Margaret Cleaves is credited with having been the first to treat cancer of the cervix with interstitial application of radium (brachytherapy) in 1903[12]. Soon after sulfa became available, *Meigs* combined bilateral pelvic lymph node dissection with the standard Wertheim operation in 1944. This procedure had no primary mortality[13]. In 1957 *Sindram* succeeded in developing a technique, which routinely combines vaginal hysterectomy according to *Schauta*, with abdominal transperitoneal lymphadenectomy according to Taussig (AVRUEL)[14,15]. In 1982, *Lammes* introduced the Japanese modification of the Wertheim radical hysterectomy, the Wertheim Okabayashi operation, which lead to an improved survival and decrease in morbidity[16,17]. 1994 *Dargent* introduced a conservative radical therapy with preservation of fertility, the radical vaginal trachelectomy[18].

Nowadays in the Netherlands, standard treatment for early stage cervical cancer consists of a radical hysterectomy with pelvic lymphadenectomy or primary radiotherapy. The choice is made on the basis of co-morbidity and age. In patients without contraindication for an operation is usually chosen as primary surgery in view of the effect of radiotherapy on the

ovaries and / or sexual function. For patients who wish to preserve fertility, with stage IB1 cervical carcinoma smaller than 2 cm tumor a radical trachelectomy (vaginal or abdominal) with pelvic lymph node dissection is preferable. Adenocarcinoma is treated as squamous cell carcinoma[19].

AIS, A PRECURSOR OF ADENOCARCINOMA

The word "dysplasia" is derived from the Greek word *dys* for "bad" and *plasia* for "molding" and has been used in many fields of medicine, usually to describe a nonmalignant process.

Cervix, the Latin word for *the neck*, is a narrow cilindric segment of the uterus, positioned between the uterus and vagina. In the average patient it measures 2-4 cm in length. The uterine cervix consists of two parts; the ectocervix (outer part), which is lined by stratified non-keratizing squamous epithelium and the endocervix (inner part) which is covered by a single layer of mucus-secreting columnar epithelium with focally underlying reservecells. Squamous cell carcinoma (SCC) usually originates in the transitionzone between squamous and columnar epithelium. It is preceded by dysplastic precursor lesions characterized by a disturbed epithelial architecture and cellular atypia. In the late 1960s the concept cervical intraepithelial neoplasia (CIN) was introduced[20]. CIN is graded from 1 to 3 (CIN1, CIN2 and CIN3). CIN1, low grade, shows dysplasia in less than one third of the epithelium. CIN2, moderate dysplasia, in two third of the epithelium and CIN 3, severe dysplasia or carcinoma in situ in more than two third of the epithelium. Most CIN lesions will regress even without treatment, however the higher the CIN grade, the less often regressions occurs. Approximately two third of CIN 1 lesions will regress, but only one third of CIN 3 revert and untreated CIN III will result in more than 50% to an invasive cancer on long-term[21-23].

In analogy to premalignant lesions of squamous type in the cervix (CIN), the concept of premalignant lesions of endocervical type epithelium has also been developed. Adenocarcinoma in situ (AIS) was first described by Hepler[24] in 1952. One year later Friedell and McKay[25] published 2 case reports on AIS proposing that AIS was the precursory premalignent lesion for adenocarcinoma of the uterine cervix (AC). AIS has consistently been characterized by the following histological features; preservation of normal glandular architecture coupled with cellular alterations of part or all of the surface and /or glandular epithelium lining the endocervix. These consist of nuclear enlargement, coarse chromatin, small single or multiple nucleoli, increased mitotic activity and variable stratification of nuclei[26]. Cytoplasmic mucin may be either reduced in quantity or be abundant.

There is reasonable evidence that AIS is the precursor of adenocarcinoma of the uterine cervix[25,27-30]. This based on the follow observations 1) AIS is frequently found adjacent to invasive adenocarcinoma[31], 2) the cytologic and histologic features of AIS resemble AC, only stroma-invasion is absent, 3) the mean age of patients with AIS (35 yrs) is 10-20 years younger than of patients with AC (51 yr) underlying its precursory role[32] and 4) the same type hrHPV is found in both AIS and invasive adenocarcinoma. [33]. Furthermore there are published case reports which describe AIS progressing into an AC and the recurrence of AIS as an AC[34-40].

Glandular lesions of lesser severity than AIS represent a heterogeneous group of poorly defined lesions with uncertain biological behavior. The ability to recognize these lesions in a

reproducible fashion is also questionable. Therefore, it has been argued that, irrespective of the number of glands involved, lesions containing columnar cells with enlarged nuclei, coarse chromatin, increased nuclear cytoplasmic ratio and mitotic activity are best classified and treated as AIS[41]. Therefore as opposed to SCC, the precursor lesion of AC, AIS is not further subdivided into conditions with less severe changes. The diagnosis of AIS is a challenge, as AIS has no pathognomonic clinical or colposcopic features.

EPIDEMIOLOGY AND PREVALENCE OF CERVICAL CANCER AND AIS

Cervical cancer is the fourth most common cancer in women worldwide, and the seventh overall, with an estimated 528,000 new cases in 2012 [42-44]. More than 85% of these cases and 88% of the cervical cancer related deaths occur in developing countries. Over the last decades the incidence of cervical carcinoma in industrialized countries has decreased. This disproportional incidence and mortality of cervical cancer is due to the lack of screening in developing countries.

The Netherlands has a low incidence of cervical cancer, 8.0/100.000, compared to a mean of 11.3/100.000 in Europe[43] and one of the lowest mortality rates in Europe for cervical cancer (2.1/100.000). The screening programs for cervical cancer by cytology started in the mid 1960's. However a nationwide population-based screening program was not introduced until 1988. This program was restructured in 1996 and has remained so until the present day: Women aged 30-60 are invited every five years for cytological testing of the cervix[45]. The incidence and mortality of cervical cancer decreased during the seventies and from 2000 on it has remained stable, with 605-722 new patients a year[46]. The decrease in cervical cancer is however restricted to cervical squamous cell carcinoma, while the incidence of other types of cervical cancer like adenocarcinoma (AC) and its precursor AIS has remained stable or has increased[47-55]. The registration for the mortality of cervical cancer in the Netherlands is not divided for histological subtypes as SCC and AC separately, in contrast to the registration for incidence of cervical cancer.

In 1952 4.5% of all cervical cancers were adenocarcinomas[24], this percentage has risen to 20% today[46,53]. Reasons for this phenomenon are the use of oral contraceptives, an increasing prevalence of HPV-infection and the relative inefficiency of screening programs in detecting glandular abnormalities, partially explain the striking increase in cervical adenocarcinoma in women who were in their 20s and 30s during the early 1960s in developed countries[47]. Furthermore, improved performance of pathologists with regard to the subclassification of cervical carcinoma is also thought to play a role[56].

Like adenocarcinoma, the incidence of AIS has also increased significantly since the seventies in the USA, from 0.2 per 100,000 to 1.8 per 100,000 women per year[57-59]. In Korea, the incidence of AC remained stable but the incidence of AIS increased, by 13,2% per year [54]. In contrast to incidence in other countries, in the Netherlands, a decrease was found in the incidence of AIS between 1989-2003 although the incidence of AC remained stable in the same period[60]. The authors explained this decrease by pointing out that many lesions being detected were combined and harbored an AIS and squamous component, the latter of which was being detected during routine screening.

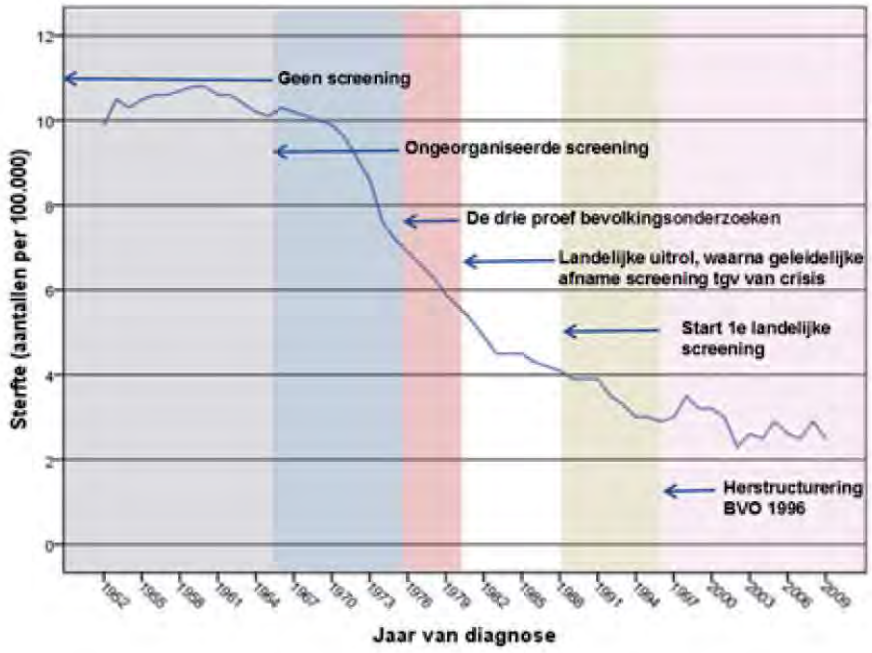

Fig. Mortality of cervical cancer in the Netherlands in relation to screeningsprogram[45]

DIAGNOSIS AND THERAPY

Adenocarcinoma in situ

AIS is generally asymptomatic. In 50% of cases, AIS and CIN are both present and identifying and treating the CIN lesion facilitates the diagnosis[61]. AIS is usually diagnosed in a conization specimen. Only 38 - 69% AIS cases will be discovered in cervixcytology, with or without kolposcopic biopsies[62-65]. The combination of cytology, biopsy and endocervix curretage (ECC) has increased the sensitivity of detecting a glandular abnormality before a conization up to 85%[64]. There is no consensus on the diagnostic colposcopic features of AIS[63,66]. The presence of white fused villi after application of acetic acid has been described and also large gland duct openings, papillary abnormalities, epithelial budding, abnormal vasculair pattern[61]. These changes have also been noted in benign diseases.

Treatment is complicated on the one hand by the location of the disease high in the endocervical canal and its possible multifocality and on the other hand by the fact that the patient population often wishes to undergo fertility-preserving therapy.

Microinvasive adenocarcinoma

The optimal treatment for microinvasive cervical AC is controversial. Although curative therapy is pivotal, preservation of fertility is an important issue and therefore influences the choice of the therapeutic strategy. The different strategies vary between radical hysterectomy(RH) with pelvic lymph node dissection (PLND) to conization of the cervix.

Early stage Adenocarcinoma

The outcome in early squamous cell carcinoma of the uterine cervix is similar after either primary surgery or primary radiotherapy. There are reports that this is not the case for early adenocarcinoma (AC) of the uterine cervix: some studies have reported that the outcome is better after primary surgery. It remains controversial whether or not patients with AC have a worse prognosis.

HPV AND CERVICAL CANCER

The Human Papillomavirus (HPV) is a non-enveloped, double-stranded DNA virus that belongs to the Papillomaviridae family[67]. The HPV virion contains an 8-kb circular genome that is enclosed in a capsid shell compromised of major (L1) and minor capsid protein (L2). The genome not only encodes for late structural genes (L1 and L2), but also for several early genes (E1, E2, E4, E5, E6 and E7) that enable viral transcription and replication and interact with the host genome[68]. Papillomavirus genomes can be subdivided into three main regions. HPV strains can be practically classified by their risk of causing cervical cancer into low-risk (e.g. HPV-6 and -11) and high-risk (e.g. HPV-16 and -18) types. Since the nineteenth century it was known that cervical cancer was associated with sexual activity. *Harald zur Hausen* identified HPV as the causal factor in cervical cancer in 1970. Cervical carcinomas are associated with specific high-risk human papilloma virus (HPV) types, mainly HPV-16 and HPV-18[69-71]. All squamous cell cervical carcinomas are HPV positive however, HPV prevalence in cervical adenocarcinomas is variable and generally lower than reported for squamous cell carcinoma[72]. The difference in prevalence may reflect technical factors related to sampling and DNA detection or histologic misclassification i.e. endometrial adenocarcinomas previously being classified as cervical adenocarcinomas. The addition of hrHPV testing in cervical screening programs will lead to a reduction in the incidence of cervical cancer and precursor lesions[73,74].

With the introduction of prophylactic HPV vaccines a new path in the field of primary prevention of cervix cancer has started. The current prophylactic vaccines provide protection against persistent infection with HPV types 16 and 18. These two HPV types together account for over 85% of all adenocarcinomas and for 90% of the adenocarcinoma in situ of the cervix uteri[71]. In cervical squamous cell carcinoma these figures are 75% and for the premalignent disease (CIN) 50%. If the assumed potential of these vaccines can be met, a put big step in the fight against this insidious disease.

AIM AND OUTLINE OF THIS THESIS

A great deal is known about the etiology and treatment of squamous cell carcinoma of the cervix, however a lot less about adenocarcinoma of the cervix. Essential questions such as etiology, relation with HPV, diagnosis, treatment and survival have not been extensively studied.

The central theme of this investigation was to investigate the opinion that adenocarcinoma of the uterine cervix (AC) carries a worse prognosis than squamous cell carcinoma (SCC). Although the incidence of AC and its precursor lesion adenocarcinoma in situ (AIS) is a fraction of mammacarcinoma, and the incidence is declining, it is a disease particularly found in younger

women and it can be fatal. Radical therapy in these young women, when fertility preservation is an issue, is not appropriate anymore but the question is: is conservative therapy justified in early AC and AIS? This is outlined in chapter 2 in a systematic review on AIS, in a retrospective study in 132 patients with AIS and in chapter 5.2 in a retrospective study in 59 cases of microinvasive adenocarcinoma.

Chapter 3 is a retrospective investigation in prognostic factors for survival in adenocarcinoma.

To reduce the incidence of cervical cancer and its precursory conditions, HPV vaccination has been introduced. In chapter 4 in we investigated whether AC and Clear cell carcinoma are HPV related and if HPV-type is of prognostic significance.

In Chapter 5 we investigated whether early stage and microinvasive AC should be treated as early stage and microinvasive SCC; for early stage cancer by a systematic review according to Cochrane guidelines and for microinvasive cancer by a retrospective study in 59 cases.

The general discussion, in chapter 6, provides an overview of our main results. We finally conclude that AIS and early stage AC, rare but increasingly existing, especially in young women, harbor the same prognosis as CIN and early SCC and therefor should be treated as such.

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2

PREMALIGNANT ADENOCARCINOMA

2.1

ADENOCARCINOMA IN SITU OF THE UTERINE CERVIX - A SYSTEMATIC REVIEW

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ABSTRACT

Objective

This study aimed to review literature if therapeutic strategies in AIS could lead to more conservative approach.

Methods

A review of the literature was conducted using a Medline search for articles published between 1966–2013.

Results

35 studies showed that after a radical cone 16,5% residual disease in the re-cone or uterus was found. After cone with positive margins residual abnormalities were found in 49,3%. 37 studies showed after conservative therapy (LLETZ-CKC) 5% recurrence rate. After conization with negative margins the risk of recurrence was 3%.

Conclusions

AIS is a relatively rare premalignant but increasingly frequent lesion of the cervix. Although there is a risk of relapse (3%) with chance of malignancy (<1%), this risk is so small that conservative treatment with negative margins by LLETZ or CKC is justified and justifiable not only for women to have children.

INTRODUCTION

Adenocarcinoma in situ of the cervix (AIS), first described by Hepler[1] in 1952, is the precursory condition for adenocarcinoma. Over the last decades the incidence of cervical carcinoma in industrialized countries has decreased. This is due to the success of organized cytology-based cervical screening programmes. This decrease is, however, restricted to cervical squamous cell carcinoma, while the incidence of adenocarcinoma (AC) and its precursor AIS has remained stable or increased[2-4]. In 1952 4.5% of all cervical cancers were adenocarcinomas[1], nowadays this percentage has risen to 20%[5]. The proper management of AIS is complicated by on one side the location of the disease high endocervical and the potential for multifocal disease and on the other side the patient population who wish to undergo fertility-preserving therapy.

The aim of this study was to review literature if therapeutic strategies in AIS could lead to more conservative approach.

MATERIALS AND METHODS

A review of the literature was conducted using a Medline search for articles published between 1966 – 2013. Search strategies have been carried out with a combination of the following MeSH headings: adenocarcinoma *in situ* of the cervix, adenocarcinoma, AIS and glandular dysplasia of the cervix. In addition, the references of the selected studies were checked. Studies were excluded if information was missing about treatment. We therefore enrolled patients with an adenocarcinoma *in situ* of the cervix, which were treated with a Cold Knife Conization (CKC) or Large Loop Excision Transformation Zone (LLETZ) or Loop Electrocautery Excision Biopsy (LEEP) or hysterectomy. The study parameters were residual lesions, cutting edges and disease recurrence. Studies describing residual disease in subsequent surgical specimen in relation to margin status in initial cone were evaluated. Studies describing patients treated conservatively with cervical conization (CKC or LLETZ) alone were evaluated for recurrent disease.

Case reports were evaluated for the study but not included in the analysis.

Studies were excluded if more recent reports of the same series of patients were published.

RESULTS

The MEDLINE search using the described search strategy identified 740 hits. The reference lists were checked and the hand searching of congress abstracts did not add any studies (only abstracts).

Going through all the abstracts of the studies/hits has produced 104 possible eligible studies, which were retrieved for more detailed information. We have found no RCT. Of the remaining abstracts obtained, 63 studies were excluded for the following reasons: invasive carcinoma, case reports, review article, about cytology, no abstract, russian-chinese language, not about therapy and if more recent reports of the same series of patients were published. 41 studies were left for analysis; 3 prospective studies[6 7-10], all other studies were retrospective.

Residual disease

A total of 35 studies showed that after a radical cone 75/454=16,5% residual disease in the re-cone or uterus was found (table I). A radical cone is a conization by cold knife, LLETZ or Laser in which the margins are without AIS, also called negative margins. After cone with positive margins (AIS in margins) residual abnormalities were found in 252/511=49,3%. Furthermore 3 invasive carcinomas were found after a radical cone (0,6%) and after a cone with positive margins, 31 carcinomas were found (5,9%).

Recurrence

37 studies showed after conservative therapy (LLETZ-CKC) 64/1277=5% recurrence rate with 17 carcinomas (table II). In most studies, a subdivision of recurrence after positive and negative margin was indicated. This shows that after conization with negative margins the risk of recurrence was 26/870=3% while in positive margins, the recurrence rate rises to 17% (23/135). Mean/median follow up varied between 12 and 120 months.

The manner and duration of follow up was different in the various studies, as it was already mentioned, the precise histology of the recurrence was also not described in every study.

Conization vs. Large Loop Excision Transformation Zone (LLETZ)

Several studies compared the conization (with a surgical knife, in the English literature as cold knife cone) with the LLETZ and found a clinically significantly higher rate (average 51%) of incomplete excision at the LLETZ in comparison with the conization (average 30%). However, there are no prospective studies between the different treatments. Recurrence rate after LLETZ is 9-29% compared to 6-11% after CKC[20 22 29 57](table III).

DISCUSSION

Nowadays AIS is a well-described entity in pathology. Argument supporting the hypothesis that AIS is the precursor of invasive adenocarcinoma of the uterine cervix[23 47-50] are, *1)* the presence of AIS next to AC, 2) the cytologic and histologic resembles of AIS to AC, except for the missing stroma-invasion, 3) the mean age of patients with AIS (37 yr) is 6-13 years younger than of patients with AC (51 yr) and 4) the same type hrHPV. Furthermore there are case reports, which showed how AIS progressed into an AC and the fact of recurrences of AIS as an AC[51-55].

Therapy

The treatment of AIS is controversial. In the past, a hysterectomy, even radical hysterectomy was recommended as treatment because AIS was considered as a multifocal disease, because negative margins had a limited predictive value for the presence of residual lesions and because of the risk of occult carcinoma. Since women in who AIS is established, usually are in the fertile phase of life are, this is not acceptable treatment. Contrary to what was adopted, AIS is less than 15% multifocal[16 19]. In the last few years there is a trend to more conservative treatment. The main reason to abject conservative treatment is the high incidence of residual disease after various forms of conization.

Table I. Residual AIS lesions in re-conus or uterus in relation to margin of the cone

				proportion with residual disease	
	year	n	age	positive margin	negative margin
Qizilbash[11]	1975	7	38,4		0/7
Luesley[12]	1987	19	35,0	4/8	1/2
Andersen[13]	1989	28	33,6	2/4	0/4
Hopkins[14]	1988	18	37,0	4/5	1/7
Nicklin[15]	1991	37	36,4	5/11	2/11
Cullimore[6]	1992	51	35,7	1/8	0/7
Muntz[16]	1992	40		7/10 (2ca)	1/12
Im[17]	1995	18	35,0	4/6	4/9
Poynor[18]	1995	28	37,0	4/8 (1ca)	4/10
Denehy[19]	1997	42	37,0	8/10	4/7
Houghton[20]	1997	19	31,0	0/3	0/2
Goldstein[21]	1998	61	43,0	8/18	13/43
Maini[22]	1998	50	37,1	8/16 (1 ca)	1/2
Azodi[23]	1999	40	37,0	9/16 (2ca)	5/16
Tay[24]	1999	21	44,2	3/6	0/5
Kuohung[25]	2000	48	35,8	6/18 (3ca)	0/3
Ostor[26]	2000	100	35,0	9/12	2/8
Shin[8]	2000	132	29,0	13/21	1/16
McHale[27]	2001	42	36,7	10/14	1/6
Soutter[28]	2001	84	37,3	11/27 (2ca)	0/4
Kennedy[29]	2002	98	37,0	14/21 (3 ca)	0/6
Bryson[30]	2004	22		0/6	0/2
Hwang[31]	2004	95	35,8	10/24	1/11
Bull[32]	2007	101	29,0	3/24	
Young[33]	2007	74	34,3	11/18 (3ca)	1/13
Dalrymple[34]	2008	82	34,0	4/11(1ca)	2/13
Dedecker[35]	2008	115	37,5	12/26	2/11
Kim[36]	2009	78	42,0	14/29(5ca)	5/30 (1ca)
Kim 2011[37]	2011	99	40,0	3/10	2/45
Desimone[38]	2011	43	34,0	13/19	5/11
Kietpeerakool[39]	2012	60	45,1	17/26	0/26
Hanegem[40]	2012	112	25,0	6/25	0/15
Costales[41]	2013	180	33,8	3/13 (1ca)	7/52 (1ca)
Hiramatsu[42]	2013	10	44,0	3/4 (2ca)	0/5
Tierney[43]	2013	78	40,0	23/34(4ca)	10/44(1ca)
TOTAL		**2125**		**252/511=49,3%**	**75/454=16,5%**
				30 ca=5,9%	**3ca=0,6%**

Table II. AIS recurrence after conservative treatment

	year	n conservative recurrence (%)	positive margin	negative margin
Luesley[12]	1987	1/6	nm	nm
Andersen[13]	1989	0/23	0/1	0/22
Hopkins[14]	1988	0/3	nm	0/3
Nicklin[15]	1991	0/12	0/1	0/11
Cullimore[6]	1992	0/35	na	0/35
Muntz[16]	1992	0/18	na	0/18
Im[17]	1995	0/3	0/1	0/2
Poynor[18]	1995	7/15 (2 ca)	4/8 (1ca)	3/7(1ca)
Denehy[19]	1997	1/19 (1 ca)	1/3 (1ca)	0/14
Houghton[20]	1997	0/15	0/6	0/9
Maini[22]	1998	3/32 (1ca)	3/18 (1ca)	0/14
Azodi[23]	1999	2/13 (1 ca)	na	2/13(1ca)
Tay[24]	1999	0/10	0/1	0/7
Kuohung[25]	2000	1/12	na	1/12
Ostor[26]	2000	0/53	0/6	0/47
Shin[8]	2000	0/95	0/3	0/92
McHale[27]	2001	3/20 (1 ca)	3/5 (1ca)	0/15
Soutter[28]	2001	4/59	2/26	2/33
Kennedy[29]	2002	9/61 (1ca)	5/17 (1ca)	4/42
Andersen[7]	2002	4/60	1/15	3/43
Schorge[9]	2003	0/7		0/7
Omnes[44]	2003	0/9		0/9
Bryson[30]	2004	0/17	0/2	0/15
Hwang[31]	2004	3/67	3/9	0/35
Akiba[45]	2005	0/15		0/15
Bull[32]	2007	0/101		
Young[33]	2007	6/40(1ca)	1/4	5/27 (1ca)
Dedecker[35]	2008	3/61 (1 ca)	0	3/61 (1 ca)
Kim[36]	2009	0/19	0/2	0/17
Costa[46]	2012	15/119 (8ca)		
Desimone[38]	2011	0/11	0/1	0/10
kim 2011[37]	2011	0/28	0/5	1/23
Hanegem[40]	2012	0/109		0/103
Kietpeerakool[39]	2012	0/6	0/1	0/5
Hiramatsu[42]	2013	0/3	0	0/3
Costales[41]	2013	2/101	0	2/101
TOTAL		**64/1277=5%**	**23/135=17%**	**26/870=3%**
		17 ca (1,3%)	5 ca (3,7%)	4 ca (<1%)

Our review of 35 studies showed that after a radical cone 16,5% residual disease in the re-cone or uterus was found (table I). After cone with positive margins residual abnormalities were found in 49%. Furthermore 3 invasive carcinomas were found after a radical cone (0,6%) and after a cone with positive margins, 30 carcinomas were found (5,9%).

Explanations for the percentage of residual lesions after cone with negative margins:

- Multifocal disease, but research shows that this occurs in about 15%, which is lower than the percentage of residual lesions after radical cone, namely 23%.
- AIS is located beyond the proximal end of the endocervical cutting edge of the cone. This is missed by the long and tortuous elongated invaginations and turns of the endocervical mucosa. Goldstein et al found in a margin of more than 10 mm no residual lesions in uterus[21].
- Inadequate histo-pathological examination of the cone specimen.

The discrepancies found in the literature can be explained by different pathological interpretation of AIS. There is also a difference between a patient with a small focus AIS in a superficial gland and a patient with multifocal disease extending into deeper layers, both types are defeated as "AIS cone with negative margins". Young patients often have smaller lesions and less frequently positive margins, therefore they are a candidate for a more conservative treatment with conization than older women. If strict follow up is not possible due to for example cervical stenosis or if there are other complains like dysmenorrhoea this justifies a hysterectomy.

Recurrence

In literature, the risk of recurrence is between 0-47% after conservative treatment. Several authors have attempted to determine (prognostic) factors which predict residual lesions or recurrence after conservative treatment, as the depth and length of the cone[56], volume deviation, number of quadrants in which AIS existed[57], age, endocervix curettage. None of these factors, however, has a significant meaning.

36 studies showed after conservative therapy (LLETZ-CKC) 64/1277=5% recurrence rate with 17 carcinomas. After conization with negative margins the risk of recurrence was 26/870=3% while in positive margins, the recurrence rate rises to 17% (23/135). The margin of the cone appears to be a predictor for the risk of recurrence. The manner and duration of follow up was different in the various studies, as it was already mentioned, the precise histology of the recurrence was also not described in every study.

The discrepancy between the high residual disease (17-49%) and the low change of recurrence (3-17%) is explained by the fact that residual AIS in conservatively treated patients following conization is often eradicated, possibly from postsurgical inflammation and granulation tissue reaction, tissue devascularization, necrosis and reepithelialization by benign columnar mucosa. This might be similar to processes in breast that showed that 50% of breast reexcision specimens are devoid of invasive carcinoma despite positive margins on initial excision[58].

Conization vs. Large Loop Excision Transformation Zone (LLETZ)

Recurrence rate after LLETZ is 9-29% compared to 6-11% after CKC[20 22 29 57]. The safety of conservative treatment with LLETZ is comparable to CKC when negative margins are achieved[30].

Table III. Margins in cold knife conization versus LLETZ versus Laser-conization

	conus	pos margin	LLETZ	pos margin	Laser	pos margin
Wolf[49]	43	18	7	5		
Denehy[19]	24	8	13	9		
Kuohung[25]	39	11	9	6		
Azodi[23]	25	6	8	6	7	4
Soutter[28]	10	4	43	25	24	14
Houghton[20]			19	8		
Maini[22]			50	34		
Kennedy[29]	37	10	49	28	4	0
Widrich[57]	18	6	14	7		
Bryson[30]			22	8		
Hwang[31]	20	9	23	9	41	11
Akiba[45]					15	0
Bull[32 61]	69	13	32	11		
Young[33]	52	15	9	4		
Dalrymple[34]	38	8			44	6
Dedecker[35]	38	6	64	31	11	2
Costa[46]	74	31	60	33	21	11
Desimone[38]	26	12	17	8		
Hanegem[40]	58	11	54	14		
Kietpeerakool[39]	23	6	37	21		
Costales[41]	110	35	54	30		
Hiramatsu[42]			10	4		
TOTAL	704	209 (30%)	594	301 (51%)	167	48 (28%)

LLETZ as treatment will lead to better obstetric outcome (preterme partus OR CKC 2.8; LLETZ 1.7[59]. Every additional excised mm will increase risk preterm partus with 6%[60].

Conclusion

AIS is a relatively rare premalignant but increasingly frequent lesion of the cervix. It is considered a pre-invasive disease of invasive adenocarcinoma. Although there is a risk of relapse (3%) with chance of malignancy (<1%), this risk is so small that this review encourage to consider conservative treatment if AIS is completely resected by LLETZ or CKC and the patient is well informed about the follow-up. Nowadays patients should chose whether they want a strict follow up with the small change of recurrence of definite therapy with hysterectomy.

Follow up after conservative treatment should preferably be done by endocervixcytologie and HPV and deviations hereby further histological examination should be performed.

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2.2

CONSERVATIVE TREATMENT IS JUSTIFIED IN ADENOCARCINOMA IN SITU OF THE CERVIX UTERI

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Submitted

ABSTRACT

Objective

We studied diagnostic and therapeutic strategies and follow-up in a large population of women with adenocarcinoma (AIS) *in situ* of the uterine cervix. We further investigated whether Human Papilloma Virus (HPV) typing in previous cytology, classified as normal, would have helped with early AIS detection.

Materials and Methods

Records of 132 AIS cases diagnosed between 1989 and 2012 were retrieved. Clinical and pathological data were reviewed and analyzed.

Results

Mean age at diagnosis was 37.2 years (95% CI 37.2 ± 1.4). Seventy-two percent (n=95) of all patients were asymptomatic, so diagnosis was established by cytology and biopsy. Primary treatment for 124 patents was Cold Knife Cone (CKC) or Loop Electrosurgical Excision Procedure (LEEP). Positive margins were found in 18% of those women treated with CKC versus 40% in those treated with LEEP. The median follow-up time was 55.5 months (range 2-217 months). Three recurrences were found after conservative treatment in 86 patients. High Risk HPV (HrHPV) positivity was detected in 96%, with HPV-18 being the most commonly occurring type (51%). Negative cytology taken 5 years prior to the diagnosis turned out to be positive for hrHPV in 71% of cases, with 88% exhibiting the same type of HPV.

Conclusions

There is a small risk of relapse after conservative therapy with CKC or LEEP when resection margins are negative in women with AIS. Patients should be given the options of hysterectomy or conservative therapy with strict follow up. HPV typing of normal smears could enable early detection of AIS.

INTRODUCTION

Adenocarcinoma *in situ* of the cervix (AIS), first described by Hepler[1] in 1952, is known to be the precursory condition to most adenocarcinoma of the cervix (AC). In contrast to the precursory conditions of squamous cell carcinoma (SCC), cervical intraepithelial neoplasia (CIN), there is no gradation of the adenocarcinoma precursors.

Over the last few decades, the incidence of cervical carcinoma in industrialized countries has decreased. This decrease is, however, restricted to cervical squamous cell carcinoma, while the incidence of AC and its precursor AIS has increased[2-4] from 4.5% in 1952 [1] to 20% in the nineties[5]. In the Netherlands, the incidence rate (European Standardized Rates - ESR) of cervical cancer in the period 1989-2012 decreased from 9.16 to 7.91 per 100.000. This is due primarily to the decreasing incidence of SCC (ESR 7.07 to 5.86), whereas the incidence of AC has actually increased from 1.22 to 1.50 [6]. In 2012, 140 of 735 new patients with cervical cancers were AC (19%). The incidence of AIS has also grown significantly in the USA from 0.2 per 100,000 in the seventies[7] to 1.8 per 100,000 women per year in the late nineties[8]. In the Netherlands, the incidence of AIS compared to AC is 1,1: 1 [9].

Early detection of AIS can prevent the occurrence of AC. Diagnosis and treatment of AIS, however, remains a challenge. This is because AIS has no pathognomonic clinical or colposcopic features and treatment is complicated, on one hand, by the location of the disease high in the endocervical canal and the fact that it maybe multifocal and, on the other hand, by the patient population who wish to undergo fertility-preserving therapy. Preferred therapy for women diagnosed with AIS who have completed having a family is hysterectomy. Conservative management is generally more acceptable if future fertility is desired[10].

The aim of this study was to analyze diagnostic and therapeutic strategies for AIS through clinical evaluation and pathologic review including HPV typing, in order to investigate whether conservative therapy is justified and if HPV typing in previous cytology, previously classified as normal, would have helped detect AIS earlier.

MATERIALS AND METHODS

Patient material

One hundred ad seventy cases of women diagnosed with AIS between 1989 and 2012 in Erasmus Medical Center in Rotterdam and the Reinier de Graaf Hospital in Delft were retrieved from the Dutch National Pathology Archive (PALGA), a central computer, in which coded information from all reports regarding cervical smears and histologic specimens from all pathology and cytology laboratories in the Netherlands are registered. Case notes were retrieved from the Erasmus MC University Hospital Rotterdam and the Reinier de Graaf Hospital. Formalin-fixed, paraffin - embedded samples were available for HPV typing from all patients. Hematoxylin and eosin (H&E) stained slides, supplemented with mucin-stained slides, were used for review. A pathologist (FS) reviewed the histology of all cases. Thirty-eight cases were excluded when there was clinical or pathologic doubt with regard to the diagnosis of cervical AIS were excluded from the study, as were patients in whom clinical follow-up was inadequate or unavailable.

The project was approved by the Medical Ethics Committee of the Erasmus MC University Hospital Rotterdam (nr.211.651/2002/48) and the Reinier de Graaf Hospital.

HrHPV testing

Samples with a confirmed histopathological diagnosis of AIS underwent HPV DNA typing using SPF_{10}PCR-DEIA-$LiPA_{25}$ Version 1 System (Labo Biomedical Products, The Netherlands). Total DNA was isolated and extracted using a proteinase-K lysis procedure. DNA was amplified by S-phase promoting Factor $(SPF)_{10}$ and generic amplification products were detected by DNA probe hybridization and DNA enzyme immune assay (DEIA). HPV-positive specimens were typed by reverse hybridization line probe assay using 25 type-specific hybridization probes ($LiPA_{25}$), which detected 14 high-risk (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68/73) and 11 low-risk (HPV6, 11, 34, 40, 42, 43, 44, 53, 54, 70, 74) types, as previously described[11]. Samples that were DEIA-positive and $LiPA_{25}$ negative were classified as HPV non-typeable. Positive and negative controls were used to monitor DNA isolation, polymerase chain reaction (PCR) amplification, HPV detection and typing procedures.

Statistics

Baseline characteristics of the patients were evaluated using a commercially available package (IBM Statistical package of the Social Science/Predictive Analytic Software version 22). Mann-Whitney-U test en chi square test were performed to compare baseline characteristics of the patients. A p-value < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

We were able to analyze 132 patients with cervical AIS between 1989-2012 for this study. The mean age at diagnosis of this group was 37.2 years (range 22-60 years; 95% CI 37.2 ± 1.4). Nine percent were younger than 30 years (the age at which screening for cervical cancer starts in The Netherlands) and were noticed due to external symptoms (post-coital bleeding, spotting, vaginal discharge) or to abnormal cytology done for non-screening reasons. Seventy-five percent of patients were younger than 41 years and 35% of were nullipara. The majority of patients (72%) were asymptomatic at time of diagnosis (Table I).

55 of the 132 (42%) cases were diagnosed with cytological as atypical glandular cells (AGC) favoring neoplasia or endocervical AIS, and 39 (30%) were diagnosed with biopsy. In 130 patients, previous cytology was available (Figure 1), where 7 (5%) were normal, 36 (28%) showed no abnormality of glandular cells or showed AGC-NOS (atypical glanudular cells not otherwise specified) and in 78 (60%) AGC- favoring neoplasia or AIS was diagnosed. Ninety-three percent (n=123) of patients had colposcopy, which was adequate in 71% of the cases (95/123). Colposcopic impression was normal in 15 (11%) patients, was characteristic for CIN in 52 (39%), and showed signs of glandular pathology in 44 patients (33%). In 12 cases (9%), the colposcopic impression was not mentioned in the file.

Table I. Patient characteristics

	No of patients	percentage
Major presenting symptoms		
None	95	72
Postcoital bleeding/spotting	27	21
Vaginal discharge	2	1
Other	8	6
Diagnosis made by		
Cytology	55	42
Biopsy	39	30
Endocervix curettage	6	4
Loop Electrosurgical Excision Procedure	19	14
Conization	10	8
Hysterectomy	3	2
Colposcopic impression		
Cervical Intraepithelial Neoplasia	52	39
Signs of glandular pathology	44	33
Normal	15	11
Not mentioned	12	9
No colposcopy	9	7
Primary therapy		
LEEP-laser conization	55	42
Cold Knife Conization	69	52
Hysterectomy	8	6
Definite therapy		
Loop Electrosurgical Excision Procedure	26	20
Cold Knife Conization	60	45
Hysterectomy	46	35

Treatment

Primary treatment included Cold Knife Cone (CKC) (n=69, 52%), Loop Electrosurgical Excision Procedure (LEEP) (n= 52, 39%), laser conization (n=3, 2%) and hysterectomy (n=8, 6%) (Table I).

Margin status was available in 65 of the 69 patients who underwent CKC as primary treatment. Twelve (18%) had positive and 53 (82%) had negative margins. Margin status was also available in 45 of the 52 patients who had LEEP as primary treatment. Eighteen (40%) had positive and 27 (60%) had negative margins. The 3 patients treated by laser conization had negative margins.

A co-existing squamous lesion was present in 57 of the 132 patients (43%). Of the 129 patients for whom information regarding focallity of the lesion was available, AIS was noted

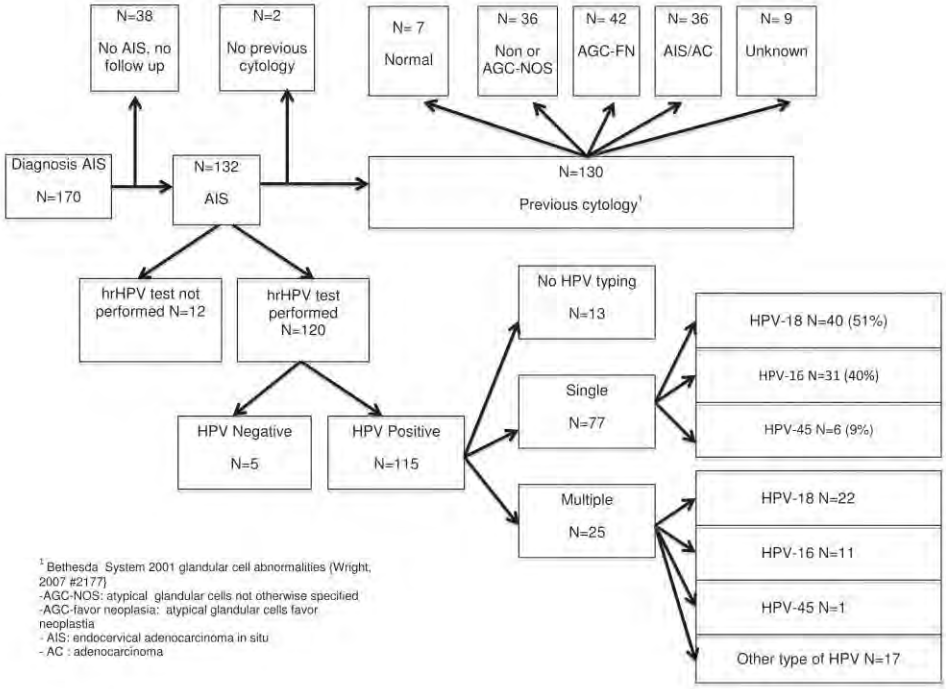

Figure 1. Flowchart cytology and hrHPV testing in the study group

to be multifocal in 9 patients (7%). Multifocal disease was not associated with higher rates of positive cone margins (11%) versus 24% in unifocal disease.

Fifty-three of the 124 patients initially treated with CKC, LEEP or laser conization underwent further therapy within 9 months after diagnosis of AIS (Figure 2), with 1 patient having a re-LEEP, 1 patient having a CKC with positive margins followed by hysterectomy, 14 patients having a CKC and 37 patients having a hysterectomy of whom 5 were radical. These 5 patients had radical hysterectomies because of microinvasive AC. Three had no residual disease and 2 had AIS in the hysterectomy specimen. No lymph node metastases were found.

Final specimens showed residual disease in 33 patients. In 45 of the 53 patients, margin status was available. In 12 of the 24 (50%) patients with positive margins in CKC or LEEP, residual disease (AIS, carcinoma) was found. In patients with negative margins this result was lower (5/21 - 24%).

Because there is a tendency towards more conservative treatment, we compared therapy choices from 1990-2000 and 2001-2012. These 2 groups were comparable for number of cases (71 versus 61) and mean age (37.0 (range 31.0-42.0) versus 37.4 (range 32.0-40.5) years) (Table II). Diagnosis of AIS was mostly cytological, being 40.8 and 42.6% for the two groups, respectively. LEEP was performed as definite therapy in 19.7% for both groups. Hysterectomy, as definite therapy, was done in 38.0% of cases in the first period versus 31.1% in the most recent period. No significant difference was observed.

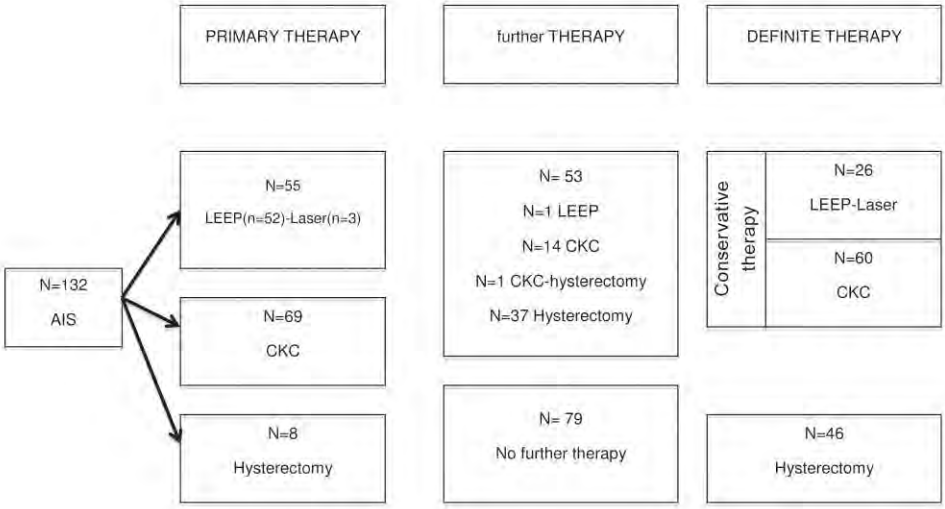

Figure 2. Flowchart primary therapy and definite therapy in the study group

Follow-up

In 116 patients (88%), follow-up was available, with a mean time frame of 59.8 months (range 1.5-217 months; median 46.3 months). Follow-up was performed by cytology, only, in 57% of cases, by cytology plus endocervical curettage (ECC) in 2%, by cytology plus HPV testing in 24% and by cytology plus ECC-HPV in 3%. No follow-up procedure was reported in 14% of cases. Of the 86 conservatively treated patients, those with a median follow up of 55.5 months (range 2-217 months), 3 had recurrences, with 2 cases of AIS after CKC, 1 with negative margins and 1 where margin status could not be evaluated. One patient treated with a LEEP, in which margins were negative, had recurrence of AIS detected by biopsy, which was treated by hysterectomy. Histology of the uterus showed AIS with early invasion. All recurrences occurred within 24 month after therapy.

Of the 86 conservatively treated patients of reproductive age, 24 (27.9%) became pregnant, of which 18 cases had incomplete follow-up was. Of the 46 patients treated by hysterectomy, 31 had follow-up, with a median of 21 months (range 2-180 months) and no recurrences were found.

HPV status

In 120 patients, hrHPV at time of diagnosis was determined by cytology or histology. One hundred and fifteen patients (95.8%) were positive and 5 (4.2%) tested negative. In 102 of the 115, HPV-typing could be performed (88.7% - Figure 2). Seventy-five percent (n=77) of patients had a single HPV type and 25% (n=25) had multiple types of HPV, the majority of which had co-infection with HPV type 18 and 16. The prevalent HPV types were HPV-18 (51%), HPV-16 (40%) and HPV-45 (9%).

Table II. Diagnosis and therapy 1990-2000 versus 2000-2010

	1990-2000 N=71	2001-2012 N=61	p-value
Age (years)	37.0 (31.0-42.0)	37.4 (32.0-40.5)	0.58
Diagnosis n (%)			0.08
Cytology	29 (40.8)	26 (42.6)	
Biopsy	28 (39.4)	17 (27.9)	
LEEP/CKC	11 (15.5)	18 (29.5)	
Uterus	3 (4.2)	0	
Definite Therapy n (%)			0.67
LEEP	14 (19.7)	12 (19.7)	
CKC	30 (42.3)	30 (49.2)	
Hysterectomy	27 (38.0)	19 (31.1)	

Baseline characteristics are presented in means and interquartile range (IQR) for continuous variables and numbers and percentages (%) for categorical variables. A p-value < 0.05 was considered statistically significant.
LEEP Loop Electrosurgical Excision Procedure; CKC Cold Knife Conization

In 24 patients, cervical smears diagnosed within normal limits taken 5 years before the diagnosis of AIS, were available and could be tested for hrHPV. In 17 (71%) of the cases, hrHPV was present. In 15 (88%), the same type of HPV was present in the smear at time of diagnosis indicating a persistent HPV infection.

DISCUSSION

Diagnosis

AIS is generally asymptomatic. This was the case with 72% of our patients, who had no symptoms. AIS in these patients was eventually detected by Pap smear screening. Cytology showed a glandular abnormality (AGC-FN, AIS, AC) in 60%. AIS in 42% of our cases was detected by cytology alone, which is the same as reported in the literature[12,13]. Although the previous diagnosis of AIS was often made based on a conization[14], in our study, 76% of cases were diagnosed before CKC or LEEP. In 19 of 132 (14%), patients were diagnosed with AIS based on a CKC or LEEP, which was performed for high grade squamous dysplasia.

HPV in AIS detection

The most important cause of cervical cancer is a persistent hrHPV infection of the cervical epithelium[15]. Analogous to CIN and SCC, different studies have proved the connection between hrHPV and the presence of AIS and AC[8,16,17]. In particular HPV type 16, 18 and 45 are important in AIS and AC[18,19]. Generally, type 18 is more commonly found[8,20,21]. Our findings support this conclusion.

In our study, negative cytology taken 5 years before the diagnosis of AIS was positive for hrHPV in 74% of cases and in 88% the same type of HPV was found at the time of diagnosis,

implying a persistent HPV infection. In the Dutch nationwide cervical cancer-screening program, women between 30 and 60 years are tested by cytology every 5 years. If HPV testing had added to the Pap smear, patients testing positive for hrHPV would be rescheduled sooner than the normal 5 years. This supports the theory that HPV testing might be superior to cytology in screening for AIS and AC[22].

Therapy

The treatment of AIS is still controversial. In the past[23], a hysterectomy, even radical hysterectomy, was recommended as a treatment. This aggressive approach was advocated because, firstly, AIS was considered to be a multifocal disease, secondly because negative margins were thought to have limited predictive value for the presence of residual disease and thirdly because of the risk of occult carcinoma. Research has however shown that all these arguments are incorrect[17,24]. Because AIS is usually diagnosed in women in the fertile phase of their lives, radical therapy is not acceptable and should be reevaluated. Contrary to what was initially thought, AIS is multifocal in less than 15% of patients[25,26]. In our series, this number was even lower (7%).

With regards to the incidence of residual disease after various forms of conization in women with AIS, a meta-analysis of 33 studies[24] with 1278 patients was published in 2009. Since then another 10 studies have been published. A total of 44 studies (including our own study) showed that after a radical cone (negative margins - 90/556=16%) residual disease in the re-cone or uterus was found (Table III). After cone with positive margins, residual abnormalities were found in 303/586 (52%). Furthermore, 7 invasive carcinomas were found after a radical cone (1.3%) and after a cone with positive margins, 30 carcinomas were found (5.4%).

CKC versus LEEP

The goal of theses procedure is to remove not only the lesion with a 3 - 4 mm margin, but also, whenever possible, the total transformation zone, in a single pass. In our study, 80% of the patients treated with CKC had negative surgical margins versus only 60% in the group treated by LEEP. Fortunately, the safety of LEEP is comparable to CKC when AIS is unexpectedly found in a loop excision and margins can be judged and negative margins are achieved[27]. Achieving negative margins is important because positive margins are associated with a 3-fold increase in risk of residual disease and a 7-fold increase in risk of recurrence (2.3% versus 16,5% positive versus negative margins, respectively - Table IV).

Recurrence

In the literature, recurrence is reported between 0[28]- 47[29]% after conservative treatment (Table IV). Several authors have attempted to determine (prognostic) factors which predict residual lesions or recurrence after conservative treatment, such as the depth and length of the cone[30], volume deviation, number of quadrants in which AIS is present[31], patient age and endocervix curettage. None of these factors are however, significant.

We found 3% (3/86) recurrence rate after conservative therapy. We added our and 10 recent studies to the meta-analysis of Salani in 2009[24]. Thirty-six studies showed 4.6% (59/1285) recurrence rate, with 20 carcinomas after conservative therapy (CKC or LEEP). Furthermore,

Table III. Residual lesions after conization for AIS

	N	Mean age	proportion with residual disease	
			positive margin	negative margin
Salani-metaanalysis[24]	1278		180/341 (17ca)	54/266 (4ca)
Dedecker[32]	115	37,5	12/26	2/11
Kim[33]	78	42	14/29 (5ca)	5/30 (1ca)
Desimone[34]	43	34	13/19	5/11
Kim 2011[35]	99	40	3/10	2/45
Hanegem[28]	112	25	6/25	0/15
Kietpeerakool[36]	60	45,1	17/26	0/26
Costales[37]	180	33,8	3/13 (1ca)	7/52 (1ca)
Hiramatsu[38]	10	44	3/4 (2ca)	0/5
Tierney[39]	78	40	23/34 (4ca)	10/44 (1ca)
Li[40]	136	35	17/35	0/30
Baalbergen (present study)	132	37,2	12/24 (1ca)	5/21
TOTAL	2321		**303/586=52%**	**90/556=16%**
			30ca (5,4%)	7 ca (1,3%)

ca carcinoma

in most studies, a subdivision of recurrence after positive and negative margins was reported. These results show that after conization with negative margins the risk of recurrence was 2.3% (24/1030), while with positive margins, the recurrence rate rose to 16.5% (20/121). The resection margin of the cone, therefore, appears to be a predictor for the risk of recurrence. These results support the feasibility of a conservative approach in women with AIS.

CONCLUSION

In our study, most patients with AIS (72%) were asymptomatic and diagnosed only after cytology or histologic biopsy at colposcopy. We are convinced that the etiologic agent in AIS is hrHPV, and that the majority of lesions are positive for HPV-18 (51%) and HPV-16 (40%). The high percentage hrHPV positive cases in normal cytology 5 years before the diagnosis of AIS, with a continuing HPV persistence of 88%, supports the hypothesis that HPV testing may be superior to cytological screening for the detection of AIS and AC. Although there is a risk of relapse and residual disease, with a chance of malignancy, this risk is very low (2.3%) and fortifies the concept that conservative treatment by a CKC or LEEP with negative surgical margins is a feasible approach for all women and not only in those who wish to bear children in the future. Patients should have a choice whether they want a strict follow-up, with the small chance of recurrence or definite therapy with hysterectomy.

Table IV. AIS/AC recurrence after conservative therapy only

	n	Median follow up (month)	Recurrence AIS(%) Conservative therapy	positive margin	negative margin
Salani-metaanalysis[24]	671		34/671 (8ca)	19/98 (6ca)	15/573 (2ca)
Dedecker[32]	61	37,5	3/61 (1ca)	0	3/61 (1ca)
Kim[33]	19	42	0/19	0/2	0/17
kim 2011[35]	99	23,5	0/28	0/5	1/23
Costa[41]	119	42	15/119 (8ca)	nm	nm
Desimone[34]	11	40	0/11	0/1	0/10
Hanegem[28]	109	37*	0/109		0/103
Kietpeerakool[36]	6	60	0/6	0/1	0/5
Costales[37]	101	43,7	2/101	0	2/101
Hiramatsu[38]	3	12	0/3	0	0/3
Li[40]	71	40	2/71 (2ca)	1/1 (1ca)	1/70 (1ca)
Baalbergen (present study)	86	55,5	3/86 (1ca)	0/13	2/64 (1ca)
TOTAL	**1356**		**59/1285=4,6%**	**20/121=16,5%**	**24/1030=2,3%**
			20 ca (1,6%)	7 ca (5,8%)	5 ca (<1%)

ca carcinoma
nm not mentioned
* mean follow up

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3

PROGNOSTIC FACTORS IN ADENOCARCINOMA OF UTERINE CERVIX

3.1

PROGNOSTIC FACTORS IN ADENOCARCINOMA OF THE UTERINE CERVIX

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SUMMARY

Introduction

To determine the behaviour of adenocarcinomas of the uterine cervix, during the last 10 years in the South-West region of the Netherlands, and to determine prognostic factors.

Methods

Three hundred and five cases of primary cervical adenocarcinomas diagnosed between 1989-1999 in the region of Rotterdam, The Netherlands, were retrieved. Clinical and pathological data were reviewed and analysed.

Results

Mean age at presentation was 52 years. The mean follow-up time for surviving patients was 61 months. The overall survival was 60% at 5 years. The 5-year survival rates for stage I and II were respectively 79 and 37%. The 5-yr survival rates for stage III and IV were less than 9%. Using univariate analysis stage, grade, age <35 years and histological type were significant prognostic factors. In the group of patients who underwent surgery (n=200), stage I-IIA, lymph node metastases, lymph-vascular-space-invasion and depth of stromal invasion were significant for survival. For patients with stage I and II-A disease survival was significantly better where the primary treatment was surgical as opposed to primary radiotherapy, p=0.002. Using multivariate analysis only stage, grade and lymph node metastases remained significant independent predictors for survival.

Conclusions

This report about cervical adenocarcinoma in the South-West region of The Netherlands shows similar results for survival to previous reports. Longest survival was for patients with early stage disease, younger patients and after primary surgery. We found FIGO-stage, grade and lymph node metastases of significant prognostic value for survival in cervical adenocarcinoma.

INTRODUCTION

Carcinoma of the uterine cervix is one of the most common malignancies in women worldwide. Although the incidence of cervical cancer has decreased over the past 40 years, the relative proportion and absolute incidence of cervical adenocarcinoma (AC) compared with squamous cell carcinoma (SCC) has increased. In the 1950s and 1960s 5% of all cervical carcinomas were adenocarcinoma. This proportion increased to 25% in the 1990s[1,2,3]. The overall incidence of invasive cervical cancer has decreased in countries with a national screening program. This declining incidence is accounted for by a decrease in cervical squamous cell carcinoma, whereas the incidence of adenocarcinoma has raised or remained stable[4,5,6,7].

It remains controversial whether or not patients with adenocarcinoma have a worse prognosis. The literature is inconsistent. Questions remain about whether cervical adenocarcinoma metastasizes earlier or is detected later, or whether a poorer response to radiotherapy, or the inclusion of special subtypes such as clear cell carcinoma could account for an apparent poorer prognosis[3].

This retrospective study was undertaken to determine the clinical outcome for patients with cervical adenocarcinoma in the Rotterdam region over a ten year period, and to attempt to determine prognostic factors.

PATIENTS AND METHODS

Patient material

All primary cervical adenocarcinomas diagnosed between 1989 and 1999 in the Rotterdam area were retrieved from local cancer registries: IKR cancer registration, Palga pathology registration and oncology registration Daniel den Hoed Cancer Clinic. Case notes were retrieved from the Erasmus MC University Hospital Rotterdam, the Daniel den Hoed Cancer Clinic and the affiliated hospitals in the region. The patients were staged according to the FIGO system.

One pathologist (P.C. E.-G.) reviewed the available haematoxylin and eosin slides (n = 98 cases). The tumours were graded as well (grade I), moderately (grade II) or poorly (grade III) differentiated using architectural and nuclear features. The proportion of the tumour showing glandular and tubular architecture was assessed (>90% well, 50 - 90% moderately and <50% poorly differentiated). Where nuclear atypia was marked this led to the tumour being allocated to a less differentiated category. Those tumours categorised as adenosquamous showed both invasive adenocarcinomatous and squamous elements. Clear cell carcinomas were not graded.

Any cases in which there was clinical or pathological doubt concerning the primary site were excluded from the study, as were patients for whom clinical follow-up was inadequate or unavailable.

Treatment methods

Patients with early disease underwent radical hysterectomy and pelvic lymph node dissection, unless their clinical condition did not permit this. The criteria for postoperative radiotherapy were positive lymph node involvement, compromised surgical margin or extension of tumour into the parametrium. Patients with stage II-B disease or higher were treated with

external irradiation and brachytherapy. In 1999 hyperthermia was added to this regimen to improve survival[8].

Patients were followed-up every three-months during the first 2 years, then every 6 months until the fifth year.

Statistical analysis

Patient disease-specific survival distribution was calculated using the Kaplan-Meier method. Patients who died of intercurrent disease or who were lost to follow-up, were censored at the time of last known follow-up. The significance of the survival was tested by log-rank test. A value of $P < 0.05$ was considered statistically significant. Multivariate analysis was performed using the Cox proportional hazard regression analysis in a forward stepwise manner with a p-value of 0.05 as inclusion.

RESULTS

Between 1989 - 1999 1424 women with cervical carcinoma (of all types) were registered by the IKR cancer registration system. Histological slides from 98 patients were available for review. Twenty-three patients were excluded for the following reasons: clinical doubt concerning the primary site (n=10), pathological doubt concerning primary site (n=3) and inadequate or unavailable clinical follow up (n=10). We analysed the clinical and pathological data of 305 patients with an invasive adenocarcinoma of the cervix. The clinicopathologic characteristics of the patients are summarized in table 1.

Almost half of the patients (45%) had stage IB1 disease at the time of diagnosis while only 25% were found to have stage IIB or more. Age at presentation ranged from 19 to 92, mean 51.6 years, with bimodal distribution; one peak in the mid-thirties and another at 70 years. 19% of the patients were nullipara.

The most predominant presenting symptom was recorded as: vaginal discharge, dysfunctional bleeding/postmenopausal bleeding, postcoital bleeding, asymptomatic or otherwise. 20% of the patients were asymptomatic at time of presentation, predominantly patients in FIGO stage I. The most frequent symptoms were dysfunctional or postmenopausal bleeding. Over 70% of the patients with Stage II, III or IV disease reported this as the main symptom. 28% of the patients with stage I and 16% with stage II complained of postcoital bleeding as the main symptom. Vaginal discharge or pelvic pain were not important presenting symptoms.

In 74% of cases diagnosis was made on a biopsy whereas in 11 % diagnosis was made after surgery for non-malignant reasons.

67% of the woman had a Papanicolaou (Pap) smear before diagnosis. However most smears were taken just before diagnosis (mean interval 6 months) and only 32 patients had an interval between last Pap-smear and diagnosis of more than 6 months. 40% of the smears showed abnormalities categorized as not more than mild or moderate, with a mean interval of 14 months while 60% showed severe dysplasia or more with a mean interval of 1.8 months.

39% of the patients at some time used oral contraceptives (OC), 39 % had never used OC and for 22% the data were missing.

Table 1. The clinicopathologic characteristics of the 305 patients with adenocarcinoma of the uterine cervix

	No of patients	percentage
Stage		
I	204	67
II	56	18
III	22	7
IV	23	8
Age (years)		
<35	60	20
35-64	160	52
>65	85	28
Histological Subtype		
Adenocarcinoma	230	75
Adenosquamous	56	18
Adenoma Malignum	2	1
Clear cell	16	5
Tumor grade		
I	80	26
II	103	34
III	84	28
unknown	38	12
Primary treatment		
Surgery	201	66
Radiotherapy	77	25
Palliative	27	9
Major presenting symptoms		
None	58	19
Dysfunctional/postmenopausal	156	51
Postcoital	65	21
Vaginal discharge	13	5
Other	13	4
Oral Contraception Use		
Never	119	39
Past/now	120	39
Unknown	66	22
Other Oncology		
None	279	92
Yes	24	8
- breast	10	
- intestinal	4	
- other	10	

24 patients (8 %) had another malignancy; 10 breast carcinoma, 4 colon carcinoma.

The follow-up time for surviving patients ranged from 3-180 months (mean 61 months).

The overall survival was 60% at 5 years. The 5-year survival rates for stage I and II were respectively 79 and 37%. The 5-year survival rates for stage III and IV were less than 9%.

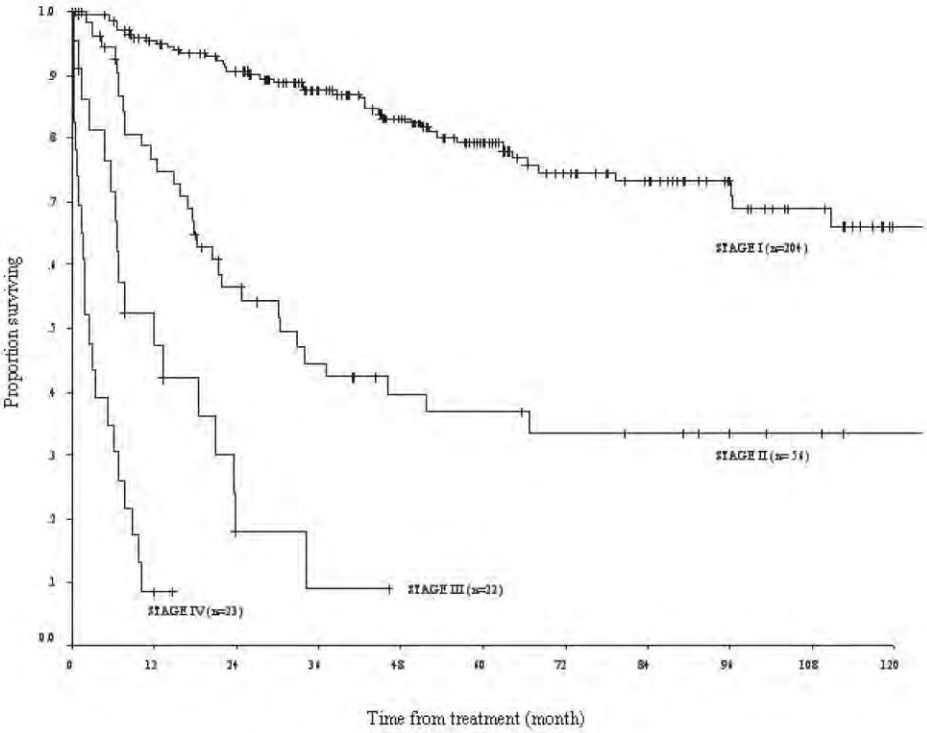

Figure 1. Kaplan-Meier plot for disease specific survival for 305 patients with adenocarcinoma of the cervix uteri according to stage.

The 5-year disease-specific survival related to several clinocopathological variables is presented in table II. For this analysis only treated patients in Stage I-III were used (patients who had no or only palliative therapy were excluded).

Using univariate analysis stage and grade were significant prognostic factors. Younger patients (age < 35 years) had a significantly better survival than older patients (> 65 years) ($p<0.001$). Histological type (adenocarcinoma versus adenosquamous versus clear cell carcinoma) showed significant difference in survival ($p=0.005$).

In the group of patients treated surgically, stage I-IIA (n=200), 17% had lymph node metastases. The patients without lymph node metastases had a 5-year survival of 91% compared to 34% when positive lymph nodes were found. 75% of patients who had lymph node metastases developed recurrent disease while only 13% in the group without lymph node metastases had a recurrence.

Table 2. The 5-year disease-specific survival of treated patients with adenocarcinoma of the uterine cervix related to clinicopathological variables

Factor	No.	5-yr Survival	*p* value univariate	*p* value multivariate
Stage			<0.001	<0.001
I	200	80		
II	55	37		
III	17	<11		
Grade			<0.001	<0.001
I	79	92		
II	94	66		
III	68	53		
Age			<0.001	ns
< 35 year	60	83		
35-65 year	152	69		
> 65 year	60	46		
Histological type			0,005	ns
adenocarcinoma	202	73		
adenosquamous	48	56		
clear cell	16	53		
Lymph node metastases			<0.001	<0.003
no	119	91		
yes	23	34		
Lymphvascular space invasion			<0.001	ns
no	134	89		
yes	46	50		
Depth invasion			<0.001	ns
≤ 10 mm	81	85		
> 10 mm	22	53		

After recurrent disease only 24% survived 5 years or more. 40% of the patients with grade III tumour had lymph node metastases, while only 15% of grade II and 2% of grade I tumours were associated with lymph node metastases. Lymph-vascular-space-invasion (LVSI) was a significant factor for survival. The 5-year survival in the group without LVSI was 89%, whereas in patients with LVSI survival was 50% (p<0.001). When LVSI was found, the chance of positive lymph nodes was 32% whereas, when LVSI was negative, the chance of lymph node metastases was only 5%. The depth of stromal invasion influenced survival significantly; when depth of stromal invasion was ≤ 10 mm the 5-year survival was 85% versus 53% when invasion was > 10 mm (p<0.001).

The survival was significantly better in patients undergoing primary surgical treatment in stage I and IIA when compared to primary radiation therapy, p=0.002. When corrected for stage and grade this significance disappeared (p=0.12).

In the final step, all the significant variables tested in the univariate model were entered in the Cox regression model. Using this approach, only stage and grade remained significant independent predictors for survival. Multivariate analysis of lymph node metastases, depth of infiltration and LVSI showed that the presence of lymph node metastases was the most important prognostic factor. Although depth of invasion and LVSI were significantly related to disease free survival in univariate analysis, adjusted for lymph node metastases both factors lost their significance. This could be explained by the significant ($p<0.003$) relationship between both parameters (depth of invasion and LVSI) and lymph node metastases.

DISCUSSION

This report represents a review of 305 patients with adenocarcinoma of the uterine cervix, presenting between 1989 and 1999 in the region of Rotterdam. The overall survival after 5 years was 60%. The 5-year survival rates for stage I, II, III and IV were 79, 37, <9 and <9 %, respectively. This is consistent with the literature. The reported 5-year survival for stage I varies from 60-99%, for stage II 14-100%, stage III 0-39% and stage IV 0-11%[2,9-18].

The incidence of cervical carcinoma in The Netherlands has decreased during the last decade, this decline representing a fall in squamous cell carcinoma since the incidence of adenocarcinoma has remained stable, approximately 150 new patients a year[19].

The use of oral contraceptives has been repeatedly (albeit inconsistently) associated with the risk of cervical cancer[20], especially adenocarcinoma[21]. Should such an association be confirmed this coupled with an increasing prevalence of HPV-infection and the relative inefficiency of screening programs in detecting glandular abnormalities[22], could offer a partial explanation for the lack of decrease in cervical adenocarcinoma in women who were in their 20s and 30s during the early 1960s in developed countries[4].

Adenocarcinoma is reported 4 times more commonly than squamous carcinoma in cervical stumps. There may be a relationship between ablative treatments and adenocarcinoma. Such a relationship could partly explain the observed increase in the incidence of adenocarcinoma since the 1960s, as this period has coincided with the introduction of screening for cervical cancer and a consequent increase in the number of surgical and ablative procedures performed on the cervix[15]. In our group there were four stump carcinomas. Two were treated with radical surgery and one had a stump resection plus adjuvant radiotherapy. These three patients are without recurrent disease. The fourth patient was treated with radiotherapy (stage I-B2) but died two years later because of recurrent disease.

40% of our patients had no abnormality or abnormalities graded as mild to moderate on cervical smear before diagnosis of cervical adenocarcinoma. Recently several reports have shown that an AGUS smear (atypical glandular cells of uncertain significance) has clinical significance[23]. *Hammoud et al* studied a group of 114 patients with AGUS smears and histological follow up and found 55 significant abnormalities[24]. *Ursin et al* found a protective effect from having had previous cervical smears, which suggests that cervical smears can detect early endocervical lesions[25]. In postmenopausal women, one possible explanation for a lack of screening efficiency is that the transformation zone moves up the cervical canal with age[22].

The mean age of our patients was 51.6 years. Some authors[10,26] have observed a significant decrease in the of age of the patients during the period of their studies. We did not observe this in our study group. In the period 1989-1994, 169 new patients had a mean age of 52.8 years whereas between 1995 and 2000 the mean age for the 139 new patients was 49.9 years.

The significantly better survival for patients younger than 35 years as compared to those over 65 years is the result of different therapy. Radical surgery with or without adjuvant radiotherapy was carried out in 90% of the young patients whereas in the group > 65 years only 41 % underwent radical surgery. 20% of women over 65 had primary radiotherapy, 20% received adjuvant radiotherapy after simple hysterectomy and 6% had palliative therapy only.

In early stage disease we found stage, grade and lymph node metastases to be of prognostic significance. The literature is consistent about stage and lymph node metastases as prognostic factors for survival in cervical adenocarcinoma, but inconsistent about the importance of grade, histological type, LVSI or age. All these studies[2,3,11-18,27-35] are retrospective studies. They include different types of patients and some studies excluded adenosquamous carcinomas. Histology was not always reviewed and statistics were used in different ways. If preoperative factors are of prognostic value, they could be used for decisions about therapy. For example we found LVSI to be predictive of lymph nodes metastases: if LVSI was present 32% had lymph node metastases and thus had an indication for adjuvant radiotherapy. Furthermore, when LVSI was negative the chance of lymph node metastases was only 5%.

Lymph node metastases in early cervical adenocarcinoma is a poor prognostic factor. We found a significantly decreased survival despite adjuvant radiotherapy. The 5-year survival decreased from 90 to 33% where there were positive lymph nodes. *Samlal et al* and *Nakanishi et al* both found that in the absence of lymph node metastases there was no significant difference in 5-year disease-free interval (94% resp. 90%) or survival (97.9% resp. 93.9%) between squamous carcinoma and adenocarcinoma. However, survival and disease-free interval (DFI) were significantly decreased in patients with adenocarcinoma (5-year survival 58%, DFI 59%) compared to squamous carcinoma (5-year survival 84%, DFI 87%), when lymph node metastases were present[34,36].

Standard therapy for cervical carcinoma stage I & IIA is radical surgery, but patients were irradiated when their clinical condition was poor because of old age (mean age 65 years in the radiation group versus 41 years in the surgery group) or coexistent medical problems (obesity, cardiovascular disease). In keeping with a number of earlier reports[11,16,37-40] we found a better survival after primary surgery than after primary radiation therapy, despite the opinion of, for example, *Cuccia et al* who wrote in 1967 "it is widely accepted that the treatment of choice of primary adenocarcinoma of the cervix is radiation therapy"[41]. In our study patients with stage IIA (n=26) had a 5-year disease-specific survival after primary surgery of 77%, whereas after primary radiation therapy this was approximately 15%. When comparing these groups, the mean age was significantly higher in the radiation group and the significant difference in survival disappeared after correcting for age. In stage I we found no difference in survival after primary surgery or radiation therapy.

Like others[12], we found a worse survival in the 27 patients where diagnosis was made after surgery for other conditions. Despite adjuvant radiation therapy survival was worse compared

to the group who received adequate treatment. In stage I the 5-year survival was 60%, in stage II 14%. In patients with cervical squamous cell carcinomas this difference was not found[42].

Despite adjuvant radiotherapy after radical surgery in Stage I and IIA the 5 yr-survival was significant decreased from 92 to 49% in our group. In squamous carcinoma survival is also decreased where adjuvant radiotherapy is required, but not so dramatically[36]. *Peters et al* showed a 4-year progression-free survival of 43% for adenocarcinoma versus 69% for squamous carcinoma in patients initially treated with radical hysterectomy and pelvic lymphadenectomy with adjuvant radiotherapy. This difference in 4-year progression-free survival almost equalised when chemotherapy was added to the adjuvant radiotherapy (80 respectively 85%)[43].

All these studies suggest that cervical adenocarcinoma is less radiosensitive than squamous carcinoma, but hard evidence such as a randomised controlled trial of surgery versus radiation therapy for adenocarcinoma is lacking. In their randomised controlled trial of stage IB-IIA cervical cancer however *Landoni et al* showed in a subgroup of 46 patients with adenocarcinoma that radiotherapy was less effective than surgery for adenocarcinoma of the cervix[39].

CONCLUSION

This report about cervical adenocarcinoma in South-West region of The Netherlands shows a similar pattern of survival to that found in previous reports. The best survival rate was for patients with early-stage disease, younger patients and after primary surgery. We found FIGO-stage, grade and lymph node metastases to be of significant prognostic value for survival in cervical adenocarcinoma.

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3.2

PROGNOSIS OF ADENOCARCINOMA OF THE UTERINE CERVIX: P53 EXPRESSION CORRELATES WITH HIGHER INCIDENCE OF MORTALITY

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ABSTRACT

Introduction

We investigated the significance of prognostic markers-estrogen receptor, progesterone receptor, p53, MIB-1 and bcl-2 - in adenocarcinoma of the uterine cervix.

Methods

In 101 patients with primary cervical adenocarcinoma, treated from 1989 to 2000, we evaluated clinical parameters in relation to these prognostic markers. Estrogen receptor, progesterone receptor, p53 and bcl-2 immunoreactivity was scored as 0 (up to 5% positive cells), 11 (5–25% of cells positive), 21 (26–50% of cells positive), 31 (51–75% of cells positive) or 41 (>76% of cells positive). MIB-1 was scored in 10 categories: 0–10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70, 71–80, 81–90, 91– 100.

Results

Mean age of patients was 45 years. Seventy eight percent of the patients were in FIGO stage I, 16% stage II, 7% stage III and IV. The overall survival rate was 67%. Survival was not influenced by estrogen receptor, progesterone receptor, MIB-1, or bcl-2 strongly positive staining. Only p53 showed significant influence on survival, even when adjusted for stage or tumor grade.

Conclusions

It does not seems useful to determine estrogen receptor, progesterone receptor, MIB-1 or bcl-2 in cervical adenocarcinomas as an indication of prognosis: survival is not influenced by presence or absence. However, if p53 staining is strongly positive survival is significantly worse than in tumors scored as negative or weak positive.

INTRODUCTION

The prevalence of cervical adenocarcinoma has risen worldwide, in recent studies accounting for 25% of all cervical carcinomas.[1] In The Netherlands there are ≈700 new cases of cervical cancer per year, of which 19% are adenocarcinomas.[2] The overall survival rate for cervical adenocarcinoma is worse than for the more common squamous cell carcinoma of the uterine cervix.[3–5] However, for early stage adenocarcinoma survival is equivalent to squamous cell carcinoma.[6,7] Stage is one of the most important factors for survival in cervical adenocarcinoma.[8–10] In breast and uterine corpus carcinoma expression of estrogen receptor and progesterone receptor are associated with better prognosis.[11,12] The prognostic significance of hormone receptor status in adenocarcinoma of the uterine cervix is still unclear. The p53 tumor suppressor gene plays a major role in cell cycle control and growth arrest following DNA damage. Mutations of the p53 tumor suppressor gene are the most common genetic alterations in human cancers. P53 protein overexpression has been found to be a associated with poor prognosis in several malignancies[13–15]; however results of studies in cervical cancer mostly show no association.[16] It has been shown that the aberrant expression of cell cycle regulatory proteins is a potential prognostic indicator within different tumor groups. Ki-67 nuclear antigen is found in proliferating cells. It identifies the growth fraction of normal and neoplastic cells, and is expressed solely in cells in the G1, S, G2 and mitotic phases. It has been reported that the percentage Ki-67 index correlates inversely with the prognosis of various tumors. The MIB-1 murine monoclonal antibody, which reacts with the Ki-67 nuclear antigen,

is a marker for proliferating cells and in breast and ovarian cancer it is a good prognostic marker.[17,18] Bcl-2 is the protein product of a proto-oncogene that inhibits apoptosis, and it has been shown to prevent apoptosis instead of promoting cell proliferation. Together with the proapoptotic protein Bax it forms a dimeer to control progression to apoptosis. Expression of bcl-2 is associated with better survival in patients with solid tumors.[19] The present study evaluates the prognostic significance of estrogen and progesterone receptor, p53, MIB-1, and bcl-2 in adenocarcinoma of the uterine cervix.

MATERIAL AND METHODS

Patient material

All primary invasive cervical adenocarcinomas diagnosed between 1989 and 2000 in the Rotterdam area were retrieved from local cancer registries: IKR regional cancer registration body, Palga pathology registration and oncology registration at the Daniel den Hoed Cancer Clinic. Case notes were retrieved from the Erasmus MC University Hospital Rotterdam, the Daniel den Hoed Cancer Clinic and the affiliated hospitals in the region. The patients were staged according to the FIGO system. From 103 patients formalin-fixed and paraffin-embedded tissue was available for immunohistochemical testing. The haematoxylin and eosin slides (n = 103 cases) were reviewed by an experienced gynaecopathologist (P.C.E-G). Any cases in which there was clinical or pathological doubt concerning the primary site were excluded from the study, as were patients for whom clinical follow-up was inadequate or unavailable. All cases of usual/mucinous type cervix adenocarcinoma were included, along with the variants

of mucinous carcinoma.[20] Where there was a suggestion of endometrioid morphology the case was not included unless clearly located in the cervix only. If a squamous component was present the case was classified separately as adenosquamous. A number of rare tumors were excluded: serous, clearcell and adenoid basal.

The tumors were graded architecturally into well, moderately or poorly differentiated depending on the proportion of solid growth.[21] The project was approved by the Medical Ethical Committee of the ErasmusMC University Hospital Rotterdam (nr.211.651/2002/48).

Marker analysis immunohistochemistry

All immunohistochemical analyzes were carried out by applying the avidin-biotin complex (ABC) method, on 4 lm sections cut from formalin-fixed, paraffin embedded tissues using commercially available mouse- monoclonal antibodies and DAB as color technique. Sections were deparaffinized and then manually incubated in the primary antibodies for 30 min at 20°C Before incubation the sections were placed in a microwave oven with citric acid buffer solution (pH 6.0, 0.1 M) for 15 min to retrieve the antigens. In all cases a microwave pretreatment of the slides was used.

For estrogen and progesterone receptors the antibodies and dilutions used were as follows: oestrogen receptor DAKO M7047, ID5, 1:160, progesterone receptor DAO M3569 PgR 636, 1:40. P53 was analyzed with the mouse monoclonal antibody p53, clone DO-7, isotype IgG2b, which recognizes wildtype and mutant forms of the p53 protein (1:50; DAKO, Glostrup, Denmark). For p53 only cells with a distinct brown stain confined to the nucleus were regarded as immunoreactive. In slides stained for MIB-1 the antibody used was Immunotech 0505 MIB-1, 1:100. Immunohistochemical staining for bcl-2 was with DAKO M0887 124, 1:80 and a semi-quantitative assessment was made of the percentage of cells showing cytoplasmatic staining.

Scoring of immunostaining

Immunostaining was scored semiquantitatively. For estrogen receptor, progesterone receptor, p53 and bcl-2 the percentage of positive cells was graded as follows: 0 (up to 5% positive cells), 11 (5–25% of cells positive), 21 (26– 50% of cells positive), 31 (51–75% of cells positive) or 41 (>76% of cells positive). MIB-1 was scored in 10 categories: 0–10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70, 71–80, 81–90, 91–100. Estrogen receptor, progesterone receptor, p53 and MIB-1 were scored for nuclear staining, whereas bcl-2 was scored for cytoplasmic staining. In the analysis, a cut-off value at ≤50% *versus* >50% was used as it gave the best survival curve separation.

Treatment methods

Patients with early disease underwent radical hysterectomy andpelvic lymph node dissection, unless their clinical condition did not permit this procedure. The criteria for postoperative radiotherapy were: positive lymph node involvement compromised surgical margin or extension of tumor into the parametrium. Patients with stage II-B disease or higher were treated with external irradiation and brachytherapy. In 1999 hyperthermia was added to this regimen to improve survival.[22] Patients were followed-up every three months during the first 2 years, and thereafter every 6 months until the fifth year.

Statistical analysis

Patient disease-specific survival distribution was calculated using the Kaplan-Meier method. Patients who died of intercurrent disease or who were lost to follow-up, were censored at the time of last known follow-up. The significance of the survival was tested by log-rank test. To obtain independent prognostic significance of the variables, Cox's proportional hazards regression analysis was used in multivariate analyzes. A value of $p < 0.05$ was considered statistically significant in all analyzes.

RESULTS

Tumor characteristics such as FIGO stage and tumor grade are presented in Table I. The mean age of the patients (n 5 101) was 45 years (26–81 years). Seventy nine percent of the patients were in FIGO stage I, 14% stage II, 7% stage III and IV. Eight percent had primary surgery,

Table I. The clinicopathologic characteristics of the 101 patients with adenocarcinoma of the uterine cervix

	Number of Patients	Percentage
Stage		
I	80	79
II	14	14
III	2	2
IV	5	5
Age (years)		
< 35	28	28
35-64	60	59
>65	13	13
Histological subtype		
Adenocarcinoma	80	79
Adenosquameus	21	21
Tumor grade		
Well	37	37
Moderate	37	37
Poor	23	23
Unknown	4	3
Primary treatment		
Surgery	85	84
Radiotherapy	13	13
Palliative	3	3
Recurrence		
No	71	70
Yes	30	30

Table II. Immunohistochemical staining results for estrogen receptor, progesterone receptor, p53 and Bcl-2 markers in 101 patients with invasive adenocarcinoma of uterine cervix

Marker	No. of tumors with negative staining (≤50%)	No. of tumors with positive staining by extent of distribution				No. of positive tumors with strong positive staining (>50%)
		1+ (5-25%)	2+ (26-30%)	3+ (51-75%)	4+ (>76%)	
ER	84	4	3	2	8	10
PR	89	3	1	2	6	8
P53	58	21	4	3	8	12
Bcl-2	77	9	5	0	5	5

whereas 13% had primary radiotherapy and 3 patients received palliative therapy. The overall disease-specific survival was 67%.

The results of immunohistochemical staining are shown in Table II. In 7 patients there was insufficient material for all immunohistochemical analyzes. The estrogen receptor was analyzed in 101 patients. In 83% of the patients less than 5% of the tumor cells stained positively for estrogen receptor, leading to a classification as 'negative' for the receptor. In 88% of the 101 patients we found a negative staining for progesterone receptor.

p53 was analyzed in 94 patients. In 62% of the patients less than 5% of the tumor cells stained positively for p53, leading to a classification as 'negative'. 80% of the 96 patients had less than 5% staining for the apoptosis control gene, bcl-2.

The proliferation marker MIB-1 was analyzed in 100 patients. See Figure 1. The mean MIB-1 index was 60%. In univariate analysis using the Kaplan-Meier method only marker P53 was significant

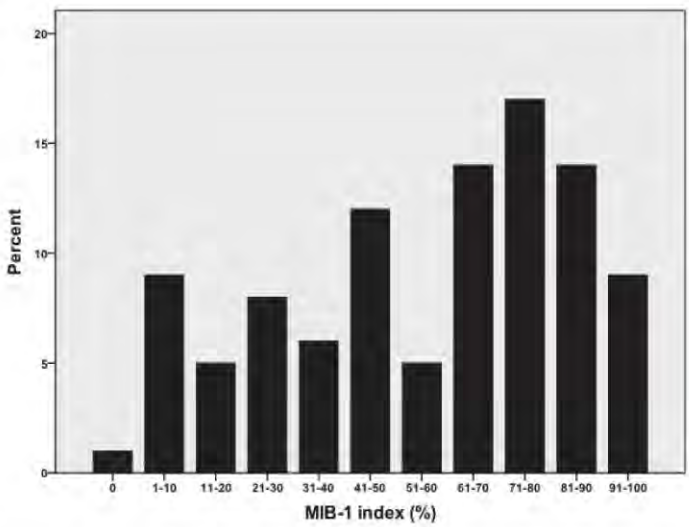

Figure 1. Immunohistochemical results for MIB-1 (n=100)

Table III. Immunohistochemical results and the 5-year disease specific survival based on log rank test in patients with adenocarcinoma of the uterine cervix

	Weak-negative		5-yr survival	Strong positive		5-yr survival	p
ER	91/101	(90%)	68%	10/101	(10%)	64%	0.32
PR	93/101	(92%)	68%	8/101	(8%)	67%	0.26
p53	83/94	(88%)	71%	11/94	(12%)	26%	0.0004
Bcl-2	91/96	(95%)	67%	5/96	(5%)	60%	0.71
MIB-1	41/100	(41%)	61%	59/100	(59%)	72%	0.32

for survival; patients with a higher p53 staining had a worse survival; in the p53 negative group (staining less than 5%) the 5-year survival was 68%, which declined to 40% when staining was 41 ($p < 0.05$). Accordingly, patients were grouped into those with strongly positive tumors (>50% staining) and those with negative or weak immunostaining (≤50%). Ten percent of our patients had a strongly positive staining for ER and 8% of the patients for PR. Only 5% of our patients had a strongly positive staining for bcl-2. Fifty nine percent of patients had a strongly positive staining for MIB-1. Survival was not significantly influenced by strongly positive staining for ER, PR, bcl-2 or MIB-1. Patients with strongly p53 immunoreactive tumors had a significantly worse outcome than patients whose tumors were p53-negative or weakly positive ($p < 0.05$). See Table III and Figure 2.

Strongly positive staining for p53 also was significant in multivariate analysis using the Cox's proportional regression mode, when survival was adjusted for stage and grade ($p < 0.05$). These results are shown in Table IV.

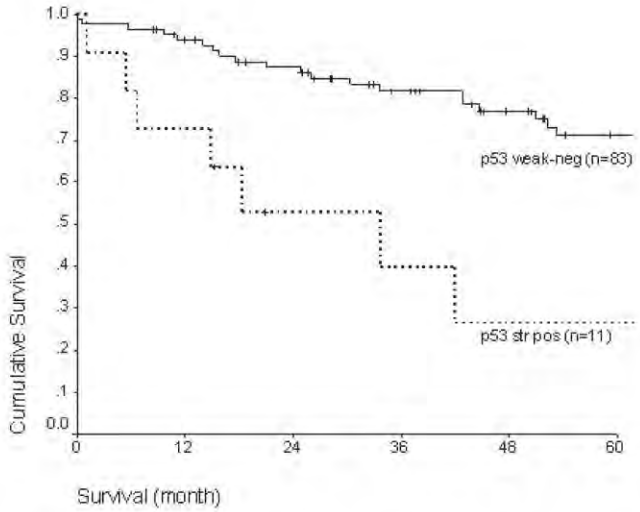

Figure 2. Kaplan-Meier curve for disease specific survival stratified according to p53 status in 94 patients with cervical adenocarcinoma, $p = 0.0004$

Table IV. Results of COX's multivariate analysis for disease free survival in patients with adenocarcinoma of uterine cervix

Variables	Beta (SE)		Relative risk (95% CI)		p-value
P53 expression	1.55	(0.49)	4.7	(1.81-12.34)	0.002
Stage	1.30	(0.31)	3.66	(1.98-6.76)	0.000
Grade	0.54	(0.29)	1.7	(0.96-3.06)	0.068

DISCUSSION

Comparison of different studies in this area is difficult owing to lack of standardized fixation procedures, protocols, heterogeneous tumor stage, differing antibodies and fixation procedures, determination of tumor cell positivity qualitatively or quantitatively, and differing statistical analyses. Another reason for the difference between the immunohistochemical studies are the misclassified cases, for example endometrial cancers that could have been misclassified as being of endocervical origin. There are a number of immunohistochemical reports about adenocarcinoma of the uterine cervix, but there is no consistency among these studies in the definition of positive and negative staining. Used cut-off values are 1%,[23-25] 5%,[26] 10% or 0%.[16,27,28] We showed staining results semi-quantitatively from 0–41 and for analysis used cutoff value off strong positive, which is more that 50% staining. Unlike breast cancer and cancer of the uterine corpus where hormone receptor status is of prognostic significance[12,29,30] and can determine response to endocrine therapy, the significance of hormone receptor status in adenocarcinoma of the uterine cervix still remains unclear. The expression of estrogen receptor in adenocarcinoma of the uterine cervix has been found to vary between 4%[31] and 81%.[32] Ten percent of our patients were classified as having tumors strongly positive for the estrogen receptor. This is lower than in many studies, possibly because of differences in tumor stage distribution or different cut-off values for determining hormone receptor positivity like others[32-34] we found no significant difference in survival between those with positive or negative estrogen receptor status. Some groups have reported better disease free survival where estrogen receptor is positive[35,36] although in some studies this was marginal.[33]

The prevalence of progesterone receptor in adenocarcinomas of the uterine cervix varies between 4%[31] and 54%.[32] In our study 8% of cases were strongly positive for progesterone receptor. As in other studies[32,33,36] we found that survival was not influenced by presence or absence of the progesterone receptor, although Masood et al.[35] and Suzuki et al.[34] found a better survival in patients with positive progesterone receptor. In endometrial cancer high estrogen/progesterone receptor levels are more frequent in well-differentiated adenocarcinomas.[37] We did not detect any relation between tumor grade and immunohistochemical staining for hormone receptors.

When DNA damage occurs, the cell cycle is stopped in G1 and DNA repair is carried out prior to cell division. If DNA damage is irreversible, apoptosis is induced. P53 is involved in the regulation of cell proliferation by stimulating the transcription of other specific cell cycle control genes. Cells with inactivated or mutant p53 cannot delay progression from the G1 to

the S phase of the cell cycle and thereby cannot prevent the replication of abnormal DNA. Mutations result in a conformational change of the protein, which becomes stabilized, thus allowing for immunohistochemical detection.[38] Usually when p53 mutation is present, there is a diffuse intense nuclear positivity. However, lower levels of p53 positivity may occur without mutation, as a result of stabilization of wild-type p53 by nonmutational events.[39] It has been suggested that over-expression of wild type p53 gene products may indicate a poorer prognosis. There are conflicting reports about p53 as a prognostic factor in cervical cancer. Some found no relationship between p53 and survival in cervical adenocarcinoma.[19,40-44] However, in accordance with some other groups,[45-48] we found a significantly worse 5-year disease specific survival if p53 staining was strongly positive. Multivariate analysis showed, adjusted for FIGO stage and grade, a significantly worse survival in patients with strong positive p53 staining.

Ki-67 antibody is found in proliferative cells and is observed during the late G1, S, G2 and M phases of the cell cycle; cells in G0 and early G1 consistently lack reactivity.[17] MIB-1 is a monoclonal antibody, which reacts with the Ki-67 antigen. MIB-1 is a prognostic factor in breast cancer,[49] ovarian cancer,[18] squamous cell cervical carcinomas[50] and endocervical adenocarcinomas.[51] In general a high MIB-1 index reflects a poorer prognosis. Higher proliferative activity of cancer cells is associated with a more aggressive behavior and results in a higher frequency of recurrence or metastases. However, we found no difference in survival with higher MIB-1 index. Suzuki et al.[34] and Nakano et al.[52] showed a better survival in patients with squamous cell carcinoma of the uterine cervix with higher levels of MIB-1. This study group studied a large homogeneous group of patients with long follow up. The primary treatment was radiotherapy, and the authors suggest that this treatment may explain their results. Tumors containing a large numbers of proliferating cells, as indicated by a high Ki-67 growth fraction or MIB-1 index, may be more sensitive to radiation therapy, possibly conferring a better prognosis. In our group most patients (84%) were treated by primary surgery. It may be that growth fraction of the tumor does not correlate directly with its biological behavior. Van der Putte et al. found in a large group of early SCC, no differences in survival, but an inverse relation between Ki-67 and both tumor size and stromal invasion.[28] It is our interpretation that MIB-1 seems not to be of clinical significance for survival in adenocarcinomas of the cervix. The prevalence of bcl-2 positivity in cervical adenocarcinomas varies between 27%[53] and 61%.[42] Using a cutoff value of 50% we found a much lower level of bcl-2 positivity: only 5% of cases. Even when the cutoff was lowered to 1%, the level used by other groups,[15,23] the percentage of bcl-2 positivity remained low, 23%. Unlike other studies where a better[19,44,45] or worse[16,41,54] survival was demonstrated for bcl-2 positive tumors, we found no significant difference in survival.

In summary, our study suggests that it is not of clinical significance to determine estrogen receptor, progesterone receptor, MIB-1 or bcl-2 in cervical adenocarcinomas as an adjunct to determine survival. However, determination of p53 seems useful since p53 staining is a marker for survival. p53 positivity appears to be linked to poorer survival in cervical adenocarcinoma, and adjuvant therapy may need to be adjusted.

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4

HPV IN ADENOCARCINOMA

4.1

HPV-TYPE HAS NO IMPACT ON SURVIVAL OF PATIENTS WITH ADENOCARCINOMA OF THE UTERINE CERVIX

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ABSTRACT

Objective

To review and characterise by clinical evaluation, immunohistochemistry and HPV typing a group of adenocarcinomas initially diagnosed with primary localisation in the cervix. Furthermore, to assess the prevalence and prognostic significance of HPV genotypes in a large series of HPV positive cervical adenocarcinomas (AC).

Methods

One hundred and seventy-one cases of adenocarcinomas (AC) with a primary localisation in the cervix and diagnosed between 1989 and 2008 in the region of Rotterdam, the Netherlands were retrieved. Slides and blocks were reviewed and immunohistochemically stained for CEA and Vimentin. HPV testing for high-risk HPV (hrHPV) by PCR (GP5+/6+) and genotyping by reversed line blot were performed.

Results

In 113 of 171 patients HPV evaluation was possible.101 were HPV-positive (89%) and 11 were HPV-negative (11%). The 5-year disease free survival was 80% in the HPV-positive group versus 74% in the HPV-negative group (ns). The distribution of HPV types was type 18 in 55 patients (54%), type 16 in 37 (37%), type 45 in 7 (7%), types 53 and 39 were found in 2 respective patients. 5-yr overall-survival in patients with HPV-18 was not significantly worse than in patients with HPV-16 (81 versus 87%). Patients with HPV-45 had a worse 5-yr survival, 57%.

Conclusions

AC is hrHPV related in most cases (89%) and HPV-18 is the most frequent type (54%). With the exception of HPV-45, HPV-positivity or type in endocervical AC has no significant influence on survival.

INTRODUCTION

Cervical carcinoma is the third most common type of malignancy in women worldwide[1]. Over the last decades the incidence of cervical carcinoma in industrialized countries has decreased. This is due to the success of organized cytology-based cervical screening programmes. This decrease is, however, restricted to cervical squamous cell carcinoma, while the incidence of adenocarcinoma (AC) has remained stable or increased[2,3]. In the Netherlands this phenomenon has also been observed[4]. The use of oral contraceptives, an increasing prevalence of HPV-infection and the relative inefficiency of screening programs in detecting glandular abnormalities, partially explain the striking increase in cervical adenocarcinoma in women who were in their 20s and 30s during the early 1960s in developed countries[2]. Furthermore, improved performance of pathologists with regard to the subclassification of cervical carcinoma is also thought to play a role[5].

Invasive AC and adenosquamous carcinoma of the cervix detected by screening are found at an earlier stage and associated with lower disease-specific mortality than carcinomas not detected by screening[6]. Therefore, initiatives directed toward improving the efficacy of screening for AC are worthwhile.

Cervical carcinomas are associated with specific high-risk human papillomavirus (hrHPV) types, mainly HPV-16 and HPV-18[7-9]. Almost all cervical squamous cell carcinomas are HPV positive. However, HPV prevalence in cervical adenocarcinomas is variable and generally lower than reported for squamous cell carcinoma[10]. The difference in prevalence may reflect technical factors related to DNA detection or misclassification, i.e. endometrial AC erroneously being classified as cervical AC. The addition of hrHPV testing in cervical screening programs will lead to a reduction in the incidence of cervical cancer and precursor lesions[11,12].

The aim of this study was to characterise by clinical evaluation, pathologic review including immunohistochemistry and HPV typing a group of AC with primary localisation in the cervix and formerly classified as of cervical origin. Moreover, we assessed the HPV type-specific prevalence in undisputable cervical AC compared the survival of respective patients by HPV status.

MATERIALS AND METHODS

Patient material

All primary cervical AC diagnosed between 1989 and 2008 in the Rotterdam area were retrieved from local cancer registries: IKR cancer registration, Palga pathology registration and oncology registration Daniel den Hoed Cancer Clinic. Case notes were retrieved from the Erasmus MC University Hospital Rotterdam, the Daniel den Hoed Cancer Clinic and the affiliated hospitals in the region. Patients had been staged according to the FIGO system. Of 171 patients formalin-fixed paraffin embedded blocks were available for HPV typing. H&E stained slides supplemented with mucin-stained slides were used for review. Any cases in which there was clinical or pathologic doubt with regard to the diagnosis, cervical AC were excluded from the study, as were patients for whom clinical follow-up was inadequate or unavailable.

The project was approved by the Medical Ethics Committee of the Erasmus MC University Hospital Rotterdam (nr.211.651/2002/48).

Revision of histology and additional use of immunohistochemistry

An expert pathologist first reviewed the histology of all cases. Review of the adenocarcinomas included additional immunohistochemistry for carcinoembryonic antigen (CEA) and vimentin to differentiate between cervical and endometrial AC when the origin of the tumour was disputable. AC that stained diffusely or focally positive for CEA but completely negative for vimentin were classified as cervical AC. On the other hand, AC that stained negative for CEA but diffusely or focally positive for vimentin were typed as endometrial AC. Non-mucinous (clear-cell), minimal deviation AC and adenosquamous carcinomas were excluded, from the study.

HrHPV testing

To ensure adequate DNA preparation, all samples were subjected to β-globin PCR. We used the primer combination PCO3 and PCO5 to generate a 209 bp product[13]. Detection of hrHPV was performed by a home-brew PCR-based assay; GP5+/6+-PCR. The clinically validated GP5+/6+-PCR with enzyme-immuno assay read-out uses a cocktail probe for 14 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), according to established protocols[14]. The PCR products of hrHPV-positive women were subsequently genotyped by reverse line blot hybridization.

Treatment methods

Patients with early disease underwent radical hysterectomy and pelvic lymph node dissection, unless their clinical condition did not permit this. The criteria for postoperative radiotherapy were positive lymph node involvement, compromised surgical margins or extension of tumour into the parametrium. Patients with stage II-B disease or higher were treated with external irradiation and brachytherapy. In 1999 chemotherapy or hyperthermia was added to this regimen to improve survival. Patients were followed-up every three-months during the first 2 years, then every 6 months until the fifth year.

Statistical analysis

Statistical comparison was carried out using a Mann-Whitney U Test or the Pearson Chi-Square test to determine significant differences between HPV-positive and HPV-negative AC. Patient disease-specific survival distribution was calculated using the Kaplan-Meier method. Patients who died from intercurrent disease or who were lost to follow-up, were censored at the time of last known follow-up. The significance of survival was tested by log-rank test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Characterization of the study group, Review and Reclassification

We retrieved 171 patients with primary cervical AC diagnosed between 1989 and 2008 in the Rotterdam area. In all cases tissue was available for review and HPV typing.

The results are shown in Figure 1. After clinical evaluation 58 cases were excluded from the study. The reasons for exclusion were: in 23 patients further analysis could not be performed because insufficient pre-treatment tissue was available for the assays. In eleven carcinomas were reclassified as endometrial carcinomas and in twenty-four cases carcinomas were classified as

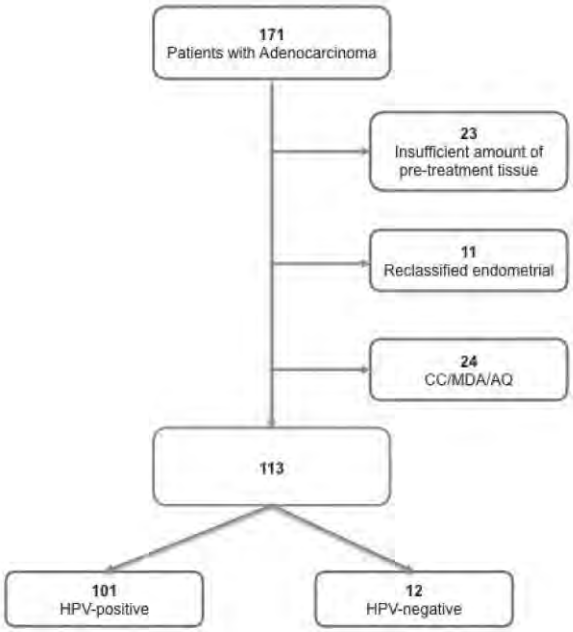

Figure 1. Flowchart of patients
CC clear cell carcinoma
MDA minimal deviation adenocarcinoma
AQ adenosquamous carcinoma

non-mucinous (clear cell, n=5), minimal deviation adenocarcinoma (n=2) and adenosquamous types (n=17).

Characteristics such as FIGO stage and tumour grade of the remaining 113 patients are presented in Table 1. The mean age was 42.8 years (26-74 years). Sixty-nine percent was between 35-65 years old and six percent younger than 30 years (the age when the National screening program in the Netherlands starts). Eighty-eight percent of the patients had FIGO stage I, 11% stage II and 1% stage III and IV. 19% were nullipara. A quarter of the patients had no symptoms at the time of diagnosis. In 82% of patients cervical cytology was available, of which only 24% had revealed a glandular abnormality in the cytology slides. Eighty-nine percent of women were primarily surgically treated and 11% had primary radiotherapy. The mean follow up was 63 months (1-168). The overall disease-specific survival was 80%.

hrHPV detection and survival by hrHPV status

Of the 113 patients with undisputable cervical AC 101 (89%) were HPV positive and 12 (11%) were HPV negative. Figure 1. The 5-year disease free survival tended to be better in HPV-positive patients than in HPV-negative patients; 80% in HPV-positive group versus 74% in HPV-negative group, but did not reach statistical significance. Figure 2.

There was no significant difference in stage, grade, lymph node metastases, primary therapy or recurrence rate between the two groups. Table 2.

Table 1. Patient characteristics, n=113

	No of patients	percentage
Stage		
I	99	88
II	12	11
III & IV	2	1
Age (years)		
<35	30	27
35-64	78	69
>65	5	4
Tumor grade		
I	47	42
II	39	39
III	26	26
LNM		
no	77	87
yes	12	13
not done	24	
LVSI		
no	80	77
yes	24	23
unknown	9	
Primary treatment		
Surgery	100	89
Radiotherapy	13	11
Major presenting symptoms		
None	33	29
Dysfunctional/postmenopausal	38	34
Postcoital	31	27
Vaginal discharge	7	6
Other	4	4
Recurrence		
No	87	77
Yes	26	23

The distribution of viral types amongst the 101 HPV-positive patients was as follows: type 18 in 55 patients (54%), type 16 in 37 (37%), type 45 in 7 (7%), types 53 and 39 were found in 2 respective patients. Table 3.

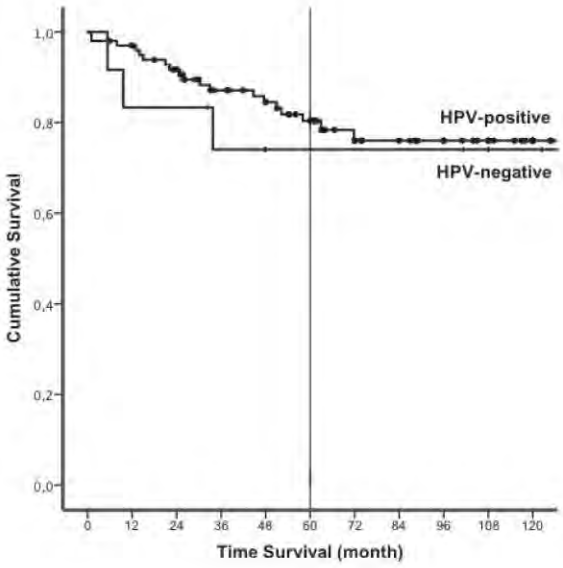

Figure 2. Survival in patients with adenocarcinoma in relation to HPV

5-yr survival of patients with HPV type 18 was not significantly worse than survival of patients with HPV type 16, 81 versus 87%. Patients with HPV type 45 had the worse 5-yr survival, i.e. 57%. Figure 3.

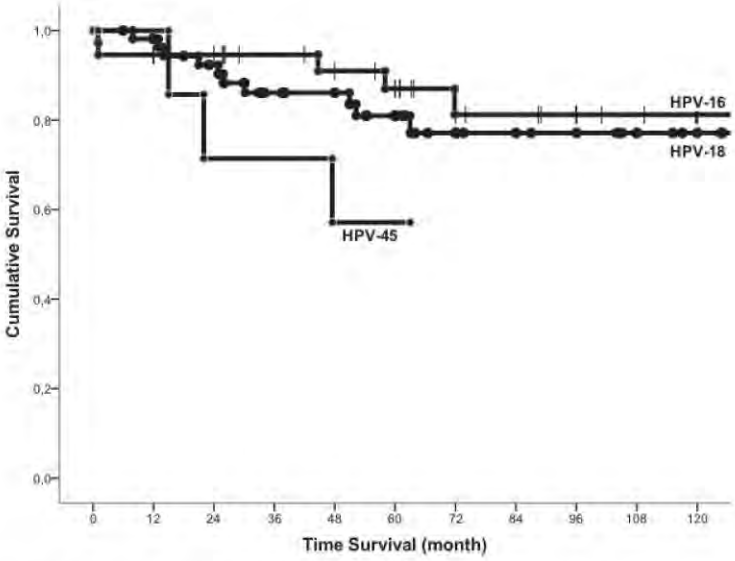

Figure 3. Survival related to type specific HPV

Table 2. HPV-positive versus HPV-negative, n=113

	HPV pos, n=101		HPV neg, n=12		
	n	%	n	%	
Age (mean, yrs)	42,8		42,2		ns[1]
Stage					
I	89	88	10	84	ns[1]
II	11	11	1	8	
III/IV	1	1	1	8	
Grade					
I	42	42	5	42	ns[1]
II	35	35	4	33	
III	23	23	3	25	
LNM					
no	68	67	9	75	ns[2]
yes	9	9	3	25	
not done	24	24	0	0	
LVSI					ns[2]
negative	74	73	6	50	
positive	19	19	5	42	
unknown	8	8	1	8	
Prim therapy					
surgery	89	88	11	92	ns[2]
radiation	12	12	1	8	
Recurrence					
no	77	76	10	83	ns[2]
yes	24	24	2	17	
Survival					
5 yr disease free		80		74	ns[3]

1. Mann-Whitney Test
2. Chi-Square Test
3. Kaplan Meier
LNM Lymph Node Metastases
LVSI Lymph Vascular Space Invasion

DISCUSSION

This study confirms the hypothesis that almost all early stage AC of the cervix are HPV positive and therefore HPV-testing seems to be a more powerful tool in detection of AC than routine cytologic screening, in which only 24% of the cases were detected. The reported prevalence of HPV DNA in AC varies significantly, from 48[15] – 95%[16]. Possible reasons for this extreme variation are differences between the HPV test used either or not combined with lower viral load in glandular lesions as compared to squamous lesions. Recently, it has

Table 3. Literature review (since 2008) of HPV types in adenocarcinoma of the uterine cervix

Author	Clinic	N (AC)	HPV positive (%)	percentage HPV-18	percentage HPV-16	Percentage HPV-45
Dabic *et al.*[23]	Zagreb, Croatia	51	84	42	74	7
Insinga *et al.*[24]	Meta analysis 22 USA studies	413	84	38	39	3
Clifford and Franceschi[10]	Meta analysis worldwide	2521	80	38	35	6
Quint *et al.*[16]	New York, USA.	55	95	42	44	11
Sanjose *et al.*[31]	RIS & HPV TTS study	760	62	32	50	12
Tornesello *et al.*[25]	Milan, Naples. Italy	39	72	18	57	7
Li *et al.*[9]	Meta analysis 243 studies	3538	82	37	36	5
Du *et al.*[32]	Stockholm, Sweden	35	91	48	40	3
Coutlee *et al.*[33]	Canada	70	89	40	45	3
Tjalma *et al.*[27]	Heracles/scale study	321	92	40	54	8
Present study	R'dam NL	113	89	54	37	7

been suggested that new HPV detection methods are more sensitive than those used in our study[17]. Other reasons for variation could be variability in sample size, geographical variation in HPV distribution, and/or inclusion of tumours of endometrial origin in some studies. It is well accepted that AC is HPV associated, with the exception of the rare non-mucinous types such as serous, mesonephrenic and clear cell carcinomas and adenoma malignum (minimal deviation AC)[18, 19]. In DES-related clear cell carcinoma (CC), HPV has recently suggested to be a co-carcinogen[20]

HPV type

In SCC, the frequency of HPV 16 is much greater than of HPV 18 or HPV 45 (HPV 16: 49-70%; HPV18: 6-13%; HPV 45: 2-5%)[9, 21, 22]. Published studies have shown that HPV type 16, 18 and 45 are found in up to approximately 90% of all AC[22]. HPV 16 accounts for 35-74%, HPV 18 for 18-57% and HPV45 for 3-12%[10, 21, 23-25].. HPV-18 is the type most strongly associated with AC. The majority of AC in this study harboured HPV type 18 (n=55; 54%); 37 (37%) were type 16, 7 (7%) were type 45, and type 53 and 39 were found in 1 patient. This compares well with the literature. Table 3.

Age

The prevalence of HPV in AC is age-dependent [26]. We found a similar mean age in HPV-positive tumours (42.2 years) and HPV-negative tumours (42.8 years), suggesting that the patients in the latter group tested false negative. In our group mean age in HPV-type 18 and 16 was the same (42.1 and 43.2 years). However, a younger mean age of patients with HPV type-45 positive carcinomas (40.7 years) was noted in this study. This has also been reported by Seoud et all[22] and Tjalma et all[27].

Prognosis

The prognosis of HPV-18 positive patients was worse than of HPV-16 associated AC. However, this was not statistically significant; 5-year disease free survival was 81% for HPV-18 positives compared to 87% for HPV-16 positives. A worse survival was noted in cases with HPV type 45, 5-year survival 57%. Because this study comprised only 7 patients with this viral type, the reliability of this conclusion is limited. Previous studies have shown that compared to HPV-16, HPV-18 has been associated with a poorer prognosis[28-30]. However, respective studies have included AC, adenosquamous carcinoma and SCC and the authors were not able to obtain significant results for HPV-18 in pure AC due to small numbers of patients with this cancer type in these studies. Studies investigating survival in solely AC[23] have also not shown a worse survival in HPV-18 positive adenocarcinomas, which is in line with our observations.

CONCLUSIONS

Despite the limitations of analyzing retrospective data, the current large study shows that the large majority of AC of the cervix are hrHPV associated. Except for HPV 45 presence, HPV genotyping does not seem to have prognostic impact on patient survival. However, this should be confirmed in a larger study.

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4.2

HIGH-RISK HUMAN PAPILLOMAVIRUS SEEMS NOT INVOLVED IN DES-RELATED AND OF LIMITED IMPORTANCE IN NONDES RELATED CLEAR-CELL CARCINOMA OF THE CERVIX

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ABSTRACT

Introduction

Over 90% of all cervical adenocarcinoma are caused by a transforming infection with a high-risk type human papillomavirus (hrHPV). Previous studies demonstrated that the association between hrHPV positivity and cervical clear-cell adenocarcinoma (CCAC) varies between 0% and 100%. As approximately 60% of all CCAC are associated with intra-uterine diethylstilbestrol (DES) exposure, we determined in a cohort of both DES-exposed and DES-unexposed women the prevalence of hrHPV infections, and the potential etiological role of hrHPV by additional analysis of p16INK4a and p53 expression.

Methods

Representative slides of 28 women diagnosed with CCAC were tested for hrHPV by two PCR methods (the clinically validated GP5+/6+ PCR and the very sensitive SPF10PCR/LiPA25). Fifteen women were DES-exposed, 10 unexposed and of 3 women DES-exposure was unknown. Twenty-one cases with sufficient material were immuno-histochemically stained for p16INK4a and p53.

Results

Seven tumors, of which four DES-exposed and two unexposed tested positive for hrHPV withGP5+/6+ PCR. Thirteen tumors, of which five DES-exposed and seven unexposed, tested positive with SPF10PCR/LiPA25. In one women with unknown exposure, a CCAC tested positive in both assays. Only three cases, none in DES-exposed women, and all positive with both hrHPV assays, revealed diffuse p16INK4a immuno-staining and weak p53 staining as well, supporting indisputable hrHPV involvement.

Conclusions

Although the prevalence of hrHPV was high, only two DES-unrelated CCAC (25%) and one tumor in a woman with unknown exposure could be attributed to hrHPV.

INTRODUCTION

Clear cell adenocarcinomas of the cervix (CCAC) are relatively rare tumors of the lower genital tract and are characterized by abundant clear cytoplasm and hobnail cells[1,2]. CCAC have a bimodal age distribution, with one peak in the early twenties and another after menopause[3,4]. In 1971, intrauterine exposure to the non-steroid estrogen diethylstilbestrol (DES), used between 1938 and 1978 to prevent miscarriage and other pregnancy-related problems[5], was found to be associated with CCAC[6]. DES-exposed women have a 40-fold increased risk of developing

CCAC, resulting in a cumulative incidence of 0.1–0.2%[7,8]. As this tumor is still very rare in DES-exposed women, DES is suggested to be an incomplete carcinogen[7]. Most CCAC are found at a relatively low stage and therefore have a good prognosis with a five-year survival rate of 90%[3,4,9]. Although 60% of CCAC are detected in DES-exposed women, 40% develop in unexposed women, indicating the involvement of alternative etiological factors[2,4,7,10].

A factor of interest might be a transforming infection with a high-risk human papillomavirus (hrHPV) type, the key causative factor in almost all cervical squamous cell- and adenocarcinomas[11-13]. Transformation is provoked by inactivation of tumor-suppressor proteins by viral oncoproteins E6 and E7[14,15]. The E6-oncoprotein degrades p53 and thereby can block p53-mediated apoptosis. The E7-oncoprotein interferes with cell cycle control by blocking retinoblastoma (Rb) (Fig. 1), ultimately leading to immortalization and invasive growth[14,15]. As a consequence, hrHPV-induced cancers are generally characterized by absence of p53 whereas cancers without hrHPV often display an increase in p53 protein reflecting stabilization caused by mutations in this gene[13-16]. In addition, hrHPVinduced cancers are characterized by over-expression of $p16^{INK4a}$[13-15,17] most likely reflecting an oncogenes senescence-like response triggered by E7[18], but functionally ineffective because Rb is blocked downstream in the pathway (Fig. 1). HrHPV-positive tumors without these characteristics reflect transient, sometimes productive, infections which are commonly found in the general population. Only few studies have explored the association between hrHPV and CCAC (Table I). In these small studies hrHPV positivity varied between 0% and 100%, thereby hampering any conclusion to be made about its potential causal role[1,16,19-28]. Only two studies provided information about immuno-histochemical staining. In one study the inverse relation between hrHPV presence and p53 presence was displayed in 11 CCAC[16], the other showed that extensive $p16^{INK4a}$ staining was absent in 3 CCAC[24]. The aim of this study was to determine in both DES-exposed and DES-unexposed women whether hrHPV infections present in CCAC could be etiologically involved, or rather represent non-transforming infections. Therefore, we studied tissue specimens for the presence of hrHPV DNA and for the expression of $p16^{INK4a}$ and p53.

MATERIALS AND METHODS

Tumor specimens

Twenty-eight paraffin-embedded CCAC samples registered in the Central Netherlands Registry (CNR) for CCAC were collected from four university medical centers and reviewed by an expert in gynecologic pathology (JB). Of these samples, diagnosed between 1975 and 2005, fifteen were FIGO stage 1, 12 stage 2, and one stage 3. Follow-up varied between 14 and 405 months

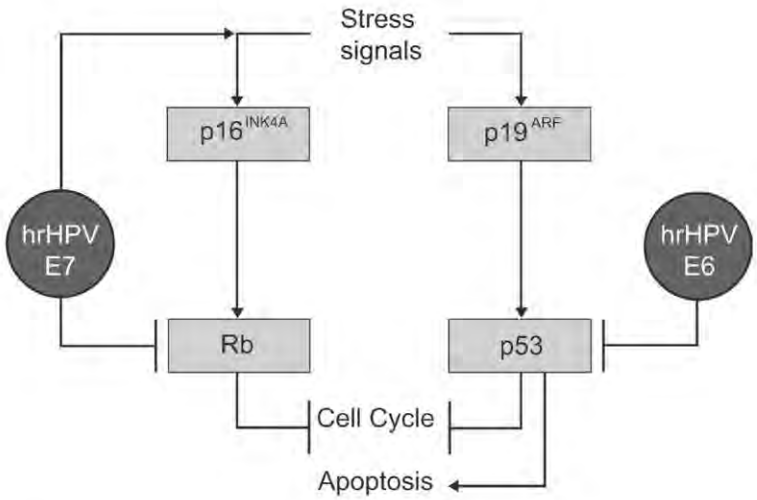

Figure 1. Simplified scheme of hrHPV-mediated carcinogenesis effecting Rb and p53 activity
Abbreviations: hrHPV, high-risk human papillomavirus; Rb, retinoblastoma
HrHPV-E7 degrades Rb, which results in inhibiting the cell cycle arrest, and triggers over-expression of $p16^{INK4a}$.[14,15,18] HrHPV E6 degrades p53 leading to a block of p53-mediated apoptosis and cell cycle arrest.[14,15]

and depended on date of diagnosis and date of death, or last known visit to the outpatient clinic. The total number of women years in our study was 350. During follow-up, five women developed recurrent disease, four of them died of progression within 32 months. None of these women had a history of DES-exposure. Another two women died of unrelated disease, respectively 81 and 220 months after diagnosis. Considering the similarities between the total CNR-cohort and our sample, we believe the latter was representative (Table II)[29]. Series of 4-µm sections were cut using a new blade for each tissue sample to prevent contamination. Outer sections were used for histological confirmation and immuno-histochemical assays, while inner sections were collected for DNA extraction and hrHPV analyses. Ethical approval was waived, since study material was anonymized according to Dutch regulation[30].

DES exposure

Previously, DES exposure was not specified uniformly and varied between a statement concerning exposure by mother, daughter, or physician, and confirmation of exposure by hospital birth records[3,8-10,29]. We collected information regarding intra-uterine DES exposure from CNR patient files[29]. Three categories were distinguished: (1) exposed (confirmation: (a) in medical record; or (b) by mother/daughter and clinical signs), (2) unknown (no data available), and (3) unexposed ((a) stated in medical record; or (b) DES denial by mother/daughter).

HrHPV testing

To ensure adequate DNA preparation, all samples were subjected to β-globin PCR. We used the primer combination PCO3 and PCO5 to generate a 209 bp product[31]. Detection of hrHPV

Table I. Summary of hrHPV detection in CCAC

					β-globin	hrHPV				
Study	**Year**	**Country**	**Primer**	**n**	**present**	**present**	**16**	**18**	**Other hrHPV types**	**DES**
Milde-Langosch[25]	1993	Germany	MY09/MY11	1	1	0				ns
Waggoner[16]	1994	USA	L1-concensus	14	11	3	0	0	HPV31 (3x)	9
Duggan[21]	1995	Canada	DBH, L1	1	1	1	1	0		ns
Tenti[28]	1996	Italy	PCR	3	3	2	0	2		ns
Pirog[1]	2000	USA	SPF10	4	4	0				ns
Ding[20]	2004	Taiwan	ns	1	1	0				0
Stewart[27]	2006	USA	ISH	1	1	0				0
Hadzisejdic[23]	2007	Croatia	E6/E7 consensus	5	5	5	0	2	HPV33, HPVX, HPV16/18/33	ns
Chen[19]	2007	Taiwan	ns	1	1	1	0	1		0
Guo[22]	2009	China	Nested PCR	1	1	1	0	1		ns
Nofech-Mozes[26]	2010	Canada	L1	3	3	2	ns	ns		0
Houghton[24]	2010	Ireland	PCR	4	3	0				ns
Total				*39*	*35*	*15*	1	6		
This study	2011	Netherlands	SPF10, GP5+/6+	28	28	13	7	2	HPV31, HPV45, HPV 51, HPVX	15

Table II. Baseline characteristics

	Study cohort (n=28)		**CNR cohort (n=144)[29]**	
Characteristics	**median**	**range**	**median**	**range**
Age at diagnosis *(years)*	29.0	17-54	25.0	8 -54
Follow-up *(months)*	151	14-405	161	
	n	%	n	%
Recurrence	5/28	17.9	34/123	27.6
Deceased	6/28	21.4	32/127	25.2
DES-exposure	15/25*	60.0	76/122	62.3
Tumour FIGO stage 1	15/28	53.6	55/123	44.7
10-years survival (95%CI)		81.2 (66.2-96.2)		77.6 (69.8-85.0)

Abbreviations: CNR, Central Netherlands Registry for clear-cell adenocarcinoma; DES, diethylstilbestrol
* Exclusion of three women with unknown DES-exposure

was performed by two PCR-based assays; GP5+/6+-PCR and the ultrasensitive SPF_{10}-PCR/$LiPA_{25}$[32]. The clinically validated GP5+/6+-PCR with enzymeimmuno assay read out uses a cocktail probe for 14 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), according to established protocols[33,34]. The PCR products of hrHPV-positive women were subsequently genotyped by reverse line blot hybridization. The SPF^{10}PCR/$LiPA_{25}$ (version 1) was performed according to specifications of the manufacturer (Labo Bio-Medical Products, Rijswijk, Netherlands) to detect and genotype 25 HPV genotypes[35]. For both HPV detection assays, samples that were positive in the enzyme-immunoassay format, but negative for any specific probe in the genotyping format were considered positive for uncharacterized HPV (sub) types or variants (HPVX).

Immuno-histochemistry (IHC)

Immuno-histochemical staining was performed according to manufacturer's instructions: $p16^{INK4a}$ (E6H4, MTM-Laboratories, Heidelberg, Germany) and p53 (BP53-12, BioGenex-Laboratories, San Ramon, USA).

Sections were deparaffinised and incubated with the primary mouse monoclonal antibodies against $p16^{INK4a}$ or p53 after which they were incubated with a secondary biotinylated rabbitanti-mouse bridging antibody followed by incubation with streptavidin-biotinylated peroxidase coupled with horse radish peroxidase conjugate. The peroxidase activity was detected with DAB (diaminobenzidine; Fluka, Sigma Aldrich, Buchs SG, Switzerland). They were then were counterstained, washed, dehydrated and coverslipped. For positive controls sections from a breast carcinoma were used for p53 and sections from a CIN3+ lesion for $p16^{INK4a}$. The negative controls were provided by performing the standard procedure replacing the primary antibody with BSA (1% bovine serum albumen).

The immuno-reactivity of $p16^{INK4a}$ and p53 was scored according to the percentage of tumor cells that stained positive as follows: no (≤10% cells), weak (>10% but ≤25% cells), moderate (>25% but ≤50% cells) and extensive (>50% cells) staining. Intensity of staining was not taken into account. All light-microscopic evaluations were scored blinded by two pathologists (CM/FS). In cases of discrepancy, slides were reviewed until consensus was reached.

Statistics

The main outcome of this retrospective cohort study was the number of hrHPV-positive CCAC and the number of tumors staining positive for p16INK4a, and/or p53. The relationships between various parameters and the outcomes in women with and without intrauterine DES-exposure were evaluated with 2×2 tables, Fisher-Exact,

Cox-regression and Mann-Whitney analysis. All calculations were performed using SPSS Version 17.0 (SPSS Inc, Chicago Illinois, USA). For all tests, the level of significance was set at 0.05.

RESULTS

DES-exposure

Fifteen women were DES-exposed in utero, 10 were unexposed and of three women DES-exposition was unknown (Table III). Exposed women developed CCAC at a younger median age

Table III. Characteristics of study population: age at diagnosis and status of human papillomavirus, $p16^{INK4A}$ and p53.

				hrHPV		Immuno-histochemistry**		
Nr	Age* (years)	Year*	DES	GP5+/6+ (type)	SPF_{10} (type)	$p16^{INK4a}$	p53	Putative aetiology
1	20	1975	+	+ (16)	+ (16)	n.m.	n.m.	DES
2	21	1981	+	-	-	n.m.	n.m.	DES
3	21	1982	+	-	+ (18)	n.m.	n.m.	DES
4	19	1983	+	+ (16)	+ (16)	n.m.	n.m.	DES
5	19	1983	+	-	-	25	10	DES
6	17	1984	+	+ (16)	+ (16)	n.m.	n.m.	DES
7	20	1989	+	+ (16)	+ (16)	<5	<1	DES
8	21	1989	+	-	-	25	0	DES
9	19	1990	+	-	-	50	0	DES
10	27	1990	+	-	-	10	<5	DES
11	24	1991	+	-	-	0	<5	DES
12	37	1993	+	-	-	50	0	DES
13	27	1993	+	-	-	45	60	DES
14	38	1995	+	-	-	60	35	DES
15	29	2001	+	-	-	20	40	DES
16	33	1985	U	+ (45)	+ (45)	100	10	hrHPV
17	44	1997	U	-	-	90	5	unknown
18	41	1997	U	-	-	90	5	unknown
19	29	1982	-	-	+ (51)	30	0	unknown
20	41	1997	-	-	+ (X)	10	10	unknown
21	36	2000	-	-	+ (16)	n.m.	n.m.	unknown
22	54	2002	-	-	+ (31)	50	60	unknown
23	28	2003	-	-	+ (16)	30	60	unknown
24	34	2004	-	+ (16)	+ (16)	100	5	hrHPV
25	30	2005	-	+ (18)	+ (18)	100	5	hrHPV
26	33	2000	-	-	-	50	50	unknown
27	44	2000	-	-	-	n.m.	n.m.	unknown
28	48	2001	-	-	-	5	60	unknown

Abbreviations: DES, diethylstilbestrol exposure; hrHPV, high-risk type of the human papillomavirus; SPF_{10}, SPF_{10}PCR/$LiPA_{25}$; n.m, no material available; U, DES-exposition unknown; X, HPV infection, unable to type.

* at diagnosis

** indicated are the percentages of immuno-positive tumour cells

than unexposed women (21 versus 35 years, p<0.001). Although no difference in tumor stage (p=0.23), growth pattern (p=0.09), nuclear atypia (p=0.83), or lymph-vascular invasion (p=0.67) could be demonstrated, DES-unexposed women had a worse overall survival (p=0.04, Hazard Ratio 0.10, 95%CI 0.01–0.86).

HrHPV presence

DNA quality was sufficient for all samples. With GP5+/6+ PCR testing seven specimens tested hrHPV-positive. Six more tested positive by SPF_{10}PCR/$LiPA_{25}$, resulting in 13 (46.4%) positive tumors for either or both assays. Among hrHPV-positives, HPV16 was the most prevalent type (7/13, 53.8%), followed by HPV18 (2/13, 15.4%). The remaining four tumors all contained a different hrHPV type:

HPV31, HPV45, HPV51 and HPVX (Table III). Multiple infections were not found.

Immuno-histochemistry (IHC)

Of 21 tumors sufficient material remained for additional IHC. These included specimens of 10/15 DES-exposed women, of 8/10 unexposed women, and of 3/3 women with unknown DES-exposure (Fig. 2).

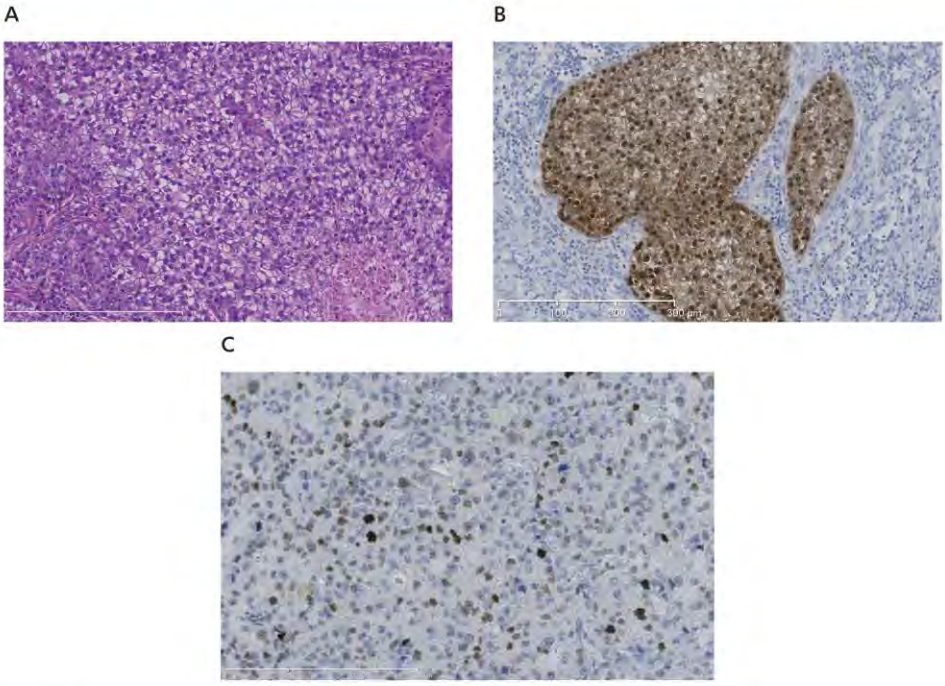

Figure 2. Expression of $p16^{INK4a}$ and p53 in cervical clear-cell adenocarcinoma
A) shows the typical features of a clear cell adenocarcinoma, composed of polygonal cells with distinct cell membranes and clear cytoplasm (H-E staining)
B) clear-cell adenocarcinoma after staining with $p16^{INK4a}$, note both nuclear and cytoplasmatic staining
C) p53 staining showing distinct nuclear staining in approximately 60% of the nuclei

Two CCAC of DES-unexposed women (Table III, nr 24, 25) and one CCAC of a woman with unknown exposure (Table III, nr 16) displayed characteristics supporting a causal hrHPV involvement, i.e. extensive diffuse $p16^{INK4a}$ immuno-staining in all tumor cells, and only weak, focal p53 staining. All these cases were positive by GP5+/6+ PCR and SPF_{10}PCR/$LiPA_{25}$. These three tumors all had a high nuclear mitotic activity and a mainly solid growth pattern (data not shown). All other hrHPV-positive cases (Table III, nr 7, 19, 20, 22, 23) displayed a wide variation in p53 expression (0%–60% of tumor cells) and at maximum moderate $p16^{INK4a}$ staining (<50% of tumor cells). None of the tumors found in DES-exposed women that were analyzed with all parameters, showed both hrHPV presence and extensive, diffuse $p16^{INK4a}$ immuno-staining in combination with no, or weak, focal p53 staining.

In three other tumors, one of a DES-exposed woman (Table III, nr 14) and 2 of women with unknown exposure (Table III, nr 17,18) extensive $p16^{INK4a}$ staining in more than 50% of all tumor cells was found. However, none of these CCAC tested positive for hrHPV. None of the hrHPV assays or immuno-histochemical profiles was significantly associated with tumor stage, age at diagnosis, or survival rate. Table III lists the putative etiology for each tumor.

DISCUSSION

In a relatively large group of CCAC we showed that hrHPV has a limited role in the carcinogenesis of CCAC. Taking into account that in hrHPV-positive women diffuse $p16^{INK4a}$ staining and absence or weak p53 immuno-staining can be seen as a cellular correlate to E6/E7 mRNA expression of hrHPV and thus as functional involvement of hrHPV[13,14,16], only three of 28 tumors could be attributed to a transforming hrHPV infection. None of these were found in DES-exposed women. Interestingly, all these three tumors tested positive in both hrHPV assays (Table III). The fact that 3 out of 4 fully analyzable GP5+/6+ PCR positive tumors versus 3 out of 8 fully analyzable SPF_{10}PCR/$LiPA_{25}$ positives fulfilled the criterion of a clinically

meaningful infection is in line with the higher specificity of a clinically validated PCR (i.e. GP5+/6+ PCR) for relevant disease caused by hrHPV[32,36]. Hence, hrHPV positivity detected solely by SPF_{10}PCR/$LiPA_{25}$ most likely reflects non-transforming, transient hrHPV infections, which are also characterized by the presence of more diverse hrHPV types.

Overall, 60% (15/25) of all analyzed CCAC developed in DES-exposed women[4,7,16]. The estrogenic effects of DES interfere with fetal development resulting in adenosis. This tissue is thought to be more susceptible to malignant transformation[4]. In DES-exposed women CCAC were diagnosed at a younger age than in unexposed women[3,4]. Furthermore, these women had a better five-year survival[4,10].

In our study hrHPV was detected in 46.4% (13/28) of all CCAC, similar to the overall percentage of 43% (15/35) found in literature (Table I). When limited to DES-unexposed women the prevalence increased to 70% (7/10). Although similar to other reported frequencies in CCAC[26,28], this is lower than the prevalence found in common cervical adenocarcinoma[1,12]. It is unlikely that this reflects deletion of sequences targeted by our PCR assays because of viral integration in the host DNA, since in most tumors no sign of viral activity reflected by diffuse $p16^{INK4a}$ immunostaining was found. $P16^{INK4a}$ immuno-staining is now widely considered a cellular correlate of the oncogenic expression of E6/E7 mRNA[14,17,24,37]. Extensive $p16^{INK4a}$ immuno-staining

was only found in three hrHPV-negative tumors. This may also reflect undetectable hrHPV with L1-based PCR assays applied[37], however, it is more likely to reflect an hrHPV independent mechanism triggering p16^{INK4a}[24].

As can be seen from Table I, most previously described CCAC were positive for HPV18[19,22,23,28], followed by HPV31[16]. In contrast, HPV16 was most commonly found (7/13) in our cohort. HPV18 was only found in two CCAC, which was surprising as in most cervical adenocarcinoma HPV18 is more[13] or equally[1,11] often found as HPV16. However, 14/28 women in our cohort were younger than 30 years at time of diagnosis. Because HPV DNA testing is not very specific under the age of 30[38], the frequency of hrHPV-types might have been distorted due to the detection of transient hrHPV infections. Indeed, when we considered only the three CCAC with a likely hrHPV etiology, HPV16 and HPV18 both occurred in one tumor.

A limitation of our study is that only 21/28 samples enclosed enough material to perform immuno-histochemical assays, hampering to draw conclusions about 7 tumors remaining. Five of these tumors were positive for hrHPV of which three in both assays (Table III, nr 1, 4, 6). As IHC could not be performed in these three DES-exposed CCAC, a causal role of hrHPV in DES-exposed tumors might have been missed.

A second limitation is the young median age in our cohort. Although consistent with previously published data[3,8,29] we can only comment on hrHPV-related carcinogenesis concerning the first peak in the bimodal age distribution[3,4].

CONCLUSIONS

In summary, we limited our conclusions to the 21 of 28 fully analyzed CCAC. In none of the 10 DES-related tumors a causal role of hrHPV could be identified. Overall, three tumors were likely caused by a transforming hrHPV infection. Two were found in DES-unexposed women (2/8) and one in a women of whom the DES-exposition was unknown (1/3). In the remaining 8 tumors (6 in DES-unexposed women and 2 in women with an unknown exposure) the etiology remains unclear, leaving room for other, unexplored factors in its carcinogenesis.

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5

THERAPY IN ADENOCARCINOMA

5.1

PRIMARY SURGERY VERSUS PRIMARY RADIOTHERAPY WITH OR WITHOUT CHEMOTHERAPY FOR EARLY ADENOCARCINOMA OF THE UTERINE CERVIX (REVIEW)

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ABSTRACT

Background

For early squamous cell carcinoma of the uterine cervix, the outcome is similar after either primary surgery or primary radiotherapy. There are reports that this is not the case for early adenocarcinoma (AC) of the uterine cervix: some studies have reported that the outcome is better after primary surgery. There are no systematic reviews about surgery versus chemoradiation in the treatment of cervical cancer. This is an updated version of the original Cochrane review published in Cochrane Database of Systematic Reviews 2006, Issue 4. Art. No.: CD006248. DOI:10.1002/14651858.CD006248.

Objectives

The objectives of this review were to compare the effectiveness and safety of primary surgery for early stage AC of the uterine cervix with primary radiotherapy or chemoradiation.

Search methods

We searched Cochrane Central Register of Controlled Trials (CENTRAL) Issue 3, 2009, MEDLINE (1950 to July week 5, 2009), EMBASE (1980 to week 32, 2009) and we also searched the related articles feature of PubMed and the Web of Science. We also checked the reference lists of articles. For this update, the searches were re-run in June 2012: MEDLINE 2009 to June week 2, 2012, EMBASE 2009 to 2012 week 24, CENTRAL Issue 6, 2012, Cochrane Gynaecological Specialised Register June 2012.

Selection criteria

Studies of treatment of patients with early AC of the uterine cervix were included. Treatment included surgery, surgery followed by radiotherapy, radiotherapy and chemoradiation.

Data collection and analysis

Forty-three studies were selected by the search strategy and 30 studies were excluded. Twelve studies were considered for inclusion. Except for one randomised controlled trial (RCT), all other studies were retrospective cohort studies with variable methodological quality and had limitations of a retrospective study. Comparing the results from these retrospective studies was not possible due to diverging treatment strategies.

Main results

Analysis of a subgroup of one RCT showed that surgery for early cervical AC was better than radiotherapy. However, the majority of operated patients required adjuvant radiotherapy, which is associated with greater morbidity. Furthermore, the radiotherapy in this study was not optimal, and surgery was not compared to chemoradiation, which is currently recommended in most centres. Finally, modern imaging techniques (i.e.magnetic resonance imaging (MRI) and positive emission tomography - computed tomography (PETCT) scanning) allow better selection of patients and node-negative patients can now be more easily identified for surgery, thereby reducing the risk of 'double trouble' caused by surgery and adjuvant radiotherapy.

Authors' conclusions

We recommend surgery for early-stage AC of the uterine cervix in carefully staged patients. Primary chemoradiation remains a second best alternative for patients unfit for surgery; chemoradiation is probably first choice in patients with (MRI or PET-CT-suspected) positive lymph nodes. Since the last version of this review no new studies were found.

PLAIN LANGUAGE SUMMARY

Surgery or radiotherapy for early cervical cancer of the adenocarcinoma type

Early-stage cervical cancer of the common type, squamous cell carcinoma, has the same prognosis after primary surgery or radiotherapy. For cervical cancer of the glandular cell type (adenocarcinoma) we recommend surgery. Second best alternative for patients unfit for surgery is chemoradiation. For patients with suspected positive lymph nodes, chemoradiation is probably the first choice.

BACKGROUND

Description of the condition

This review is an update of a previously published review in the Cochrane Database of Systematic Reviews 2006, Issue 4. Art.No.:CD006248. DOI:10.1002 /14651858.CD006248.

Cervical cancer is the second most common cancer among women worldwide (Ferlay 2004). The prognosis of patients with cervical cancer depends on FIGO (International Federation of Gynecologists and Obstetricians) (Benedet 2001) stage at time of diagnosis, presence of lymph node metastases, tumour size and histological type (Baalbergen 2004; Chen 1998; Kasamatsu 2009). The three major histological types of invasive cervical cancer are squamous cell carcinomas (SCC), adenocarcinomas (AC) and adenosquamous carcinoma (ASC). SCC comprises 80% of cases, and AC and ASC comprise approximately 15% (ACOG 2002). Over the past 40 years the relative proportion and absolute incidence of AC compared to SCC has increased, especially in women younger than 35 years (Alfsen 2000; Chan 2003; Krane 2001; Liu 2001;Schoolland 2002; Vizcaino 1998). Screening for SCC has effectively reduced both incidence and mortality of invasive squamous cancer by early detection and treatment of pre-invasive lesions (Smith 2000). Although screening reduces mortality from cervical AC, the incidence remains unaltered (Nieminen 1995). It remains controversial whether or not patients with AC have a worse prognosis. The literature is inconsistent; some studies report a similar prognosis for ACof the uterine cervix and SCC (Grisaru 2001; Ishikawa 1999; Kilgore 1988) whereas others report a poorer prognosis for AC (Bulk 2003; Eifel 1995;Hopkins 1991). Questions remain about what factors account for this apparent poorer prognosis. Cervical AC may metastasize earlier (Lea 2002) or may be detected later (Drescher 1989; Hurt 1977). It may respond less well to radiotherapy (Hong 2000;Hurt1977), have a higher incidence of relapse and the treatment of recurrent disease less successful (Kasamatsu 2002; Lai 1999) or possibly the inclusion of special subtypes such as clear cell carcinoma could account for this difference in prognosis (Look 1996).

Description of the intervention

Treatment protocols used for SCC and AC are similar and therapy is based on clinical staging according to FIGO (Benedet 2001). Due to recent developments in imaging such as magnetic resonance imaging (MRI) and developments of surgical techniques such as endoscopy, the current FIGO classification for cervical cancer has been revised (Pecorelli 2009). Micro-invasive disease managed by cone biopsy or hysterectomy. Radical hysterectomy (removal of the uterus with adjacent tissue and draining pelvic lymph nodes) has become standard management for the majority of early cervical cancers, but external beam-irradiation along with a vaginal application of brachytherapy to the cervix has been increasingly employed for bulky stage I and II disease (tumour diameter of more than four centimetres). Both external beam irradiation and brachytherapy have undergone rapid developments, of which the therapeutic consequences are not yet clear. Intensity-modulated radiotherapy (IMRT) allows more conformal external beam dose delivery to the clinical target (uterine cervix and regional pelvic lymph nodes) thereby sparing critical organs (bladder and intestines). IMRT requires an accurate definition and delineation of clinical target (Small 2008; Taylor 2005; Taylor 2007;

Vizcaino 1998). Paradoxically, in clinical practice, compared to 'old fashioned' four-field box-technique defined by osseous anatomical structures (Fletcher 1973), image-guided target definition has increased rather than decreased the irradiated volumes for radiotherapy of pelvic tumours. The historical low dose rate (LDR) brachytherapy techniques using radium and caesium have largely been replaced by iridium as the radioactive source. Iridium allows high dose rate (HDR) and pulsed dose rate (PDR), which both have decreased irradiation time and patient burden. These techniques, particularly if combined with intraoperative MRI, have reduced the risk of misplacement of the brachytherapy applicator, and allow image-guided brachytherapy, thereby increasing local control whilst reducing toxicity (Georg 2009). After primary surgery, it may be useful to add radiotherapy (in up to 50% of operated patients depending on the selection criteria of the series). In primary radiotherapy in selected cases, adjuvant surgery (salvage hysterectomy) may be performed if the tumour recurs locally (Weiner 1975). The use of both surgery and radiotherapy leads to more severe morbidity (Barter 1989; Landoni 1997) than either used alone. Complications of radical hysterectomy are chronic bladder dysfunction (3% to 13%), ureterovaginal or vesicovaginal fistula (1%to 2%), pulmonary embolism (1% to 2%), small bowel obstruction (1%), lymphocoele formation (5% to 8%) and hydroureter nephrosis (3%). Complications of radiotherapy arise later but are often permanent: proctitis (7.6%), radiation colitis, early menopause, sexual dysfunction, shortening and fibrosis of the vagina, oedema of the legs (0.6%), hydroureter nephrosis (5%) and vesicovaginal fistula (1.4%). The combination of radical surgery followed by radiotherapy carries the worst morbidity: hydroureter nephrosis (10%), severe oedema of the legs (9%), lymphocoele formation (15%), ureterovaginal or vesicovaginal fistula (7.4%) and vesical complications and bowel morbidity (Boronow 1971; Kucera 1998 Landoni 1997; Waggoner 2003).

Why it is important to do this review

In 1999, after the publication of four randomised controlled trials (RCTs) on this issue (Keys 1999; Morris 1999; Rose 1999; Whitney 1999) the US National Cancer Institute (NCI) issued an alert indicating that combined chemoradiation should be considered for all patients with cervical cancer who previously would be treated with radiotherapy. In 2001, a Cochrane review showed concomitant chemotherapy and radiotherapy improved overall survival (OS) and progression-free survival (PFS) in locally advanced cancer (Green 2001; Green 2005). For early SCC, the outcome is similar after either primary surgery or primary radiotherapy (Hopkins 1991; Landoni 1997). There are reports that this is not the case for early AC of the uterine cervix and some studies have reported that the outcome is better after primary surgery (Chen 1999; Kucera 1998). Currently there are no systematic reviews comparing surgery versus chemoradiation in the treatment of cervical cancer.

OBJECTIVES

To compare the effectiveness and safety of primary surgery for early-stage AC of the uterine cervix with primary radiotherapy or chemoradiation.

METHODS

Criteria for considering studies for this review

Types of studies

It was anticipated that only a very small number of RCTs, the preferred type of study, would have been conducted on cervical cancer treatment. Therefore, observational studies, non-randomised studies with concurrent controls and studies with historical controls were also considered for incorporation in this review. The methodological quality of non-RCTs was assessed on the basis of comparability of treatment groups at baseline, adjustment for potential confounders and allocation of the treatment.

Types of participants

Patients with histological confirmed early-stage AC of the uterine cervix were included. For the purpose of this review early-stage AC was defined as cancer in which the primary tumour was confined to the cervix and upper two-thirds of the vagina or the parametrium (FIGO stage IA to IIB). For FIGO staging see Appendix 1.

Types of interventions

The following surgical interventions were studied:

- extrafascial hysterectomy or Rutledge class I hysterectomy, which is defined as removal of all cervical tissue by incision of the pubocervical ligament allowing reflection and retraction of the ureters laterally without actual dissection from the ureteral bed;
- Rutledge class II extended hysterectomy, which is defined as the removal of the medial half of the cardinal and uterosacral ligaments and upper third of the vagina. It is usually combined with a pelvic lymphadenectomy;
- radical hysterectomy or Rutledge class III extended hysterectomy, which can be defined as the removal of the entire cardinal and uterosacral ligaments and removal of the upper third of the vagina and a pelvic lymphadenectomy (Piver 1974).

The following radiotherapy interventions were studied:

- whole pelvis radiotherapy, defined as external beam radiation in which the clinical target volume (CTV) encompasses the cervix, the uterus, the upper two-thirds of the
- vagina, the parametria and the draining lymph nodes at risk, up to the level of lumbar spine 5 and sacral spine 1;
- vaginal application of a radioactive source to the cervix (brachytherapy). There are different brachytherapy techniques that apply the radioactive source for short periods of time or for several days;
- chemoradiation, which is defined as concomitant radiotherapy and cytotoxic chemotherapy.

Any comparison of a surgical intervention with a radiotherapy intervention was considered.

Types of outcome measures

Primary outcomes

The primary outcomes were OS and disease-free survival (DFS).

Secondary outcomes

Secondary outcomes of interest were adverse effects of treatment as intestinal, urogenital and premature menopausal complications and quality of life (QoL).

Search methods for identification of studies

Electronic searches

The literature search was carried out according to the criteria set by the Cochrane Gynaecological Cancer Review Group. There were no language restrictions. Searches of Cochrane Central Register of Controlled Trials (CENTRAL Issue 3, 2009), MEDLINE (1950 to July week 5 2009) and EMBASE (1980 to week 32 2009). Searches of the Group's Specialised Register and Non-Trials Database was devised using the groups coding system, was carried out on 6 July 2009.

Subsequent searches were run in June 2012 (MEDLINE 2009 to June week 2, 2012, EMBASE 2009 to 2012 week 24, CENTRAL Issue 6, 2012, Specialised Register June 2012).

For the search strategy we used a combination of free text and indexed terms and included an extended RCT filter to include cohort and case control studies (which also picked up follow-up, retrospective and prospective studies). See Appendix 2; Appendix 3; Appendix 4; Appendix 5.

The Web of Science and the register of ongoing controlled trials were checked (www.controlled-trials.com). The reference lists of the selected publications were searched. All relevant articles found, were identified on PubMed, and using the 'related articles' feature, a further search was carried out for newly published articles.

Searching other resources

A handsearch of publications on the treatment of cervical cancer in the following journals was carried out: CME Journal of Gynecologic Oncology (from 1995), International Journal of Gynecologic Cancer (from 1993). Abstracts from conferences on gynaecological cancer (IGCS, SGO) and the British Library's Inside Conferences were checked.

Data collection and analysis

Selection of studies

All titles and abstracts retrieved by electronic searching were downloaded to a reference management database (Reference Manager 11), duplicates were removed and the remaining references were examined by two review authors (AB, YV) independently. Those studies that clearly did not meet the inclusion criteria were excluded and copies of the full text of potentially relevant references were obtained. The eligibility of retrieved papers was assessed independently by two review authors (AB, YV). Reasons for exclusion were documented. The number of references excluded is reported in a QUOROM flow chart (Figure 1 and Figure 2).

1. QUOROM statement flow diagram

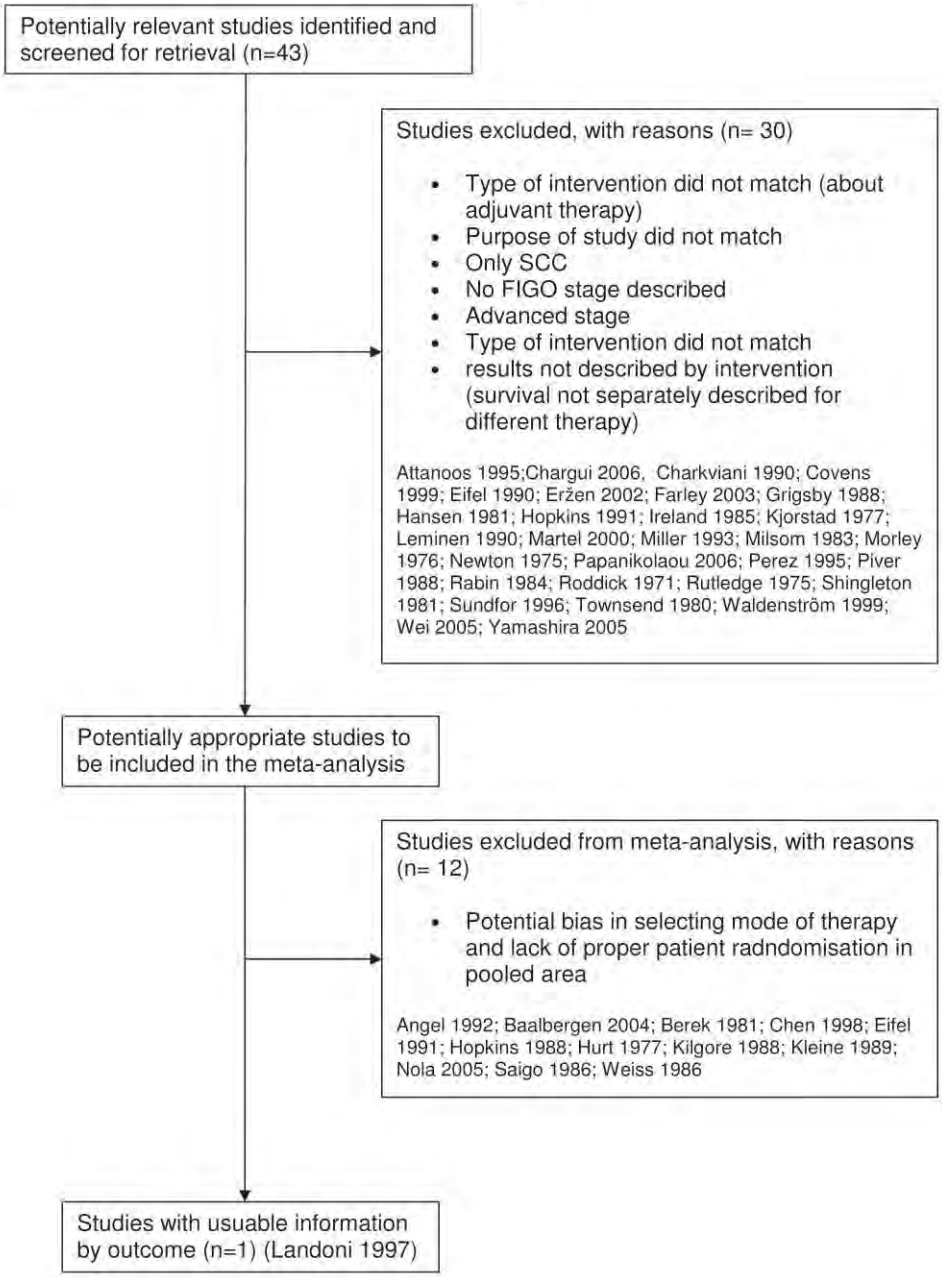

Figure 1. Quorum statement flow diagram

2. QUOROM statement flow diagram review-update

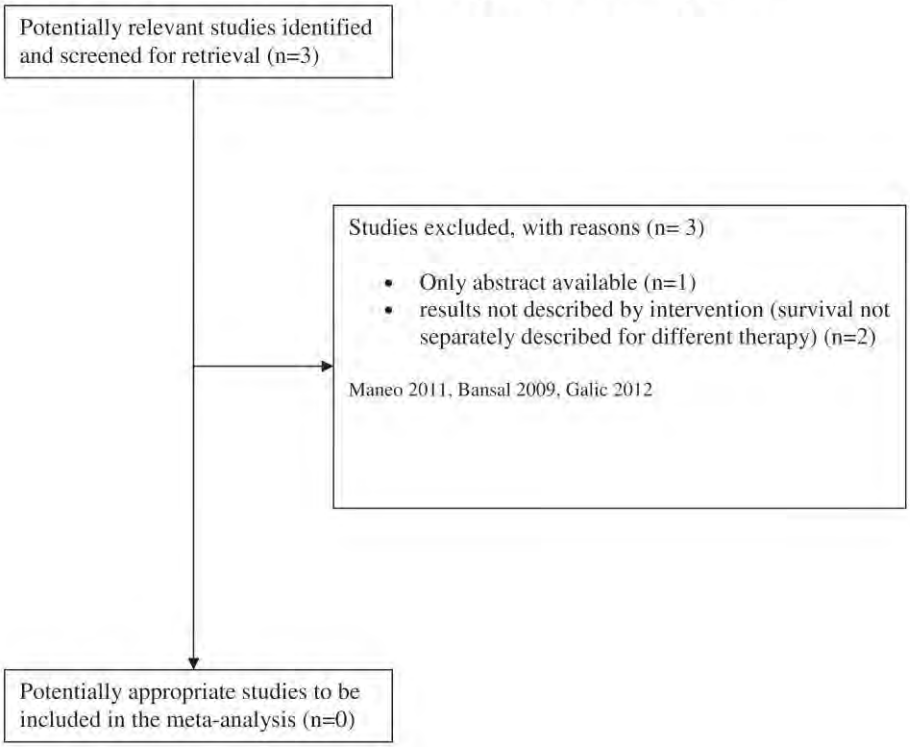

Figure 2. QUORUM statement flow diagram for review-update

Data extraction and management

For included studies, data on characteristics of patients and interventions (surgery, radiotherapy, chemotherapy), study quality and end points were abstracted independently by two review authors (AB and YV) onto data abstraction forms (Table 1; Table 2; Table 3; Table 4) that were developed for the review. Differences between review authors were resolved by discussion or by appeal to a third review author (AA) if necessary. No effort was made to blind the review authors of names of investigators, institutions, journals, etc. The data abstraction forms were designed a priori and were filled out independently.

Participants

For each trial, data on the number of patients assigned to each treatment, analysed and excluded from the investigators' analyses was extracted independently. The distribution of patients by age, stage, histology, grade and performance status was abstracted where available.

Interventions

Data on the type of surgery was being collected. Details of dose and fractionation of external beam radiotherapy and details of the brachytherapy dose, insertions and dose rate were collected. Details of any chemotherapy given concomitantly with radiotherapy were recorded. Details on duration or follow-up and ascertainment of long-term toxicity were also recorded.

Outcomes

For time to event (OS and recurrence-free survival) data, we extracted the log of the hazard ratio [log(HR)] and its standard error from trial reports; if these were not reported, we attempted to estimate them from other reported statistics using the methods of Parmar 1998. For dichotomous outcomes (e.g. adverse events or deaths) if it was not possible to use an HR, we extracted the number of patients in each treatment arm who experienced the outcome of interest and the number of patients assessed at end point, in order to estimate a risk ratio (RR). For continuous outcomes (e.g. QoL), we extracted the final value and standard deviation (SD) of the outcome of interest and the number of patients assessed at end point in each treatment arm at the end of follow-up, in order to estimate the mean difference (MD) (if trials measured outcomes on the same scale) or standardized mean differences (SMD) (if trials measured outcomes on different scales) between treatment arms and its standard error. If reported, both unadjusted and adjusted statistics were extracted. Where possible, all data extracted were those relevant to an intention-to-treat (ITT) analysis, in which participants were analysed in groups to which they were assigned.

The time points at which outcomes were collected and reported were noted.

Assessment of risk of bias in included studies

An assessment of the risk of bias of included RCTs was assessed using the following criteria.

Blinding

We coded separately the blinding of patients, treatment providers and outcome assessors as:

- yes;
- no;
- unclear.

Randomisation

We coded the randomisation of participants to intervention groups as:

- adequate, for example a computer-generated random sequence or a table of random numbers;
- inadequate, for example date of birth, clinic identification number or surname;
- unclear, for example not reported.

Allocation concealment

We coded the concealment of allocation sequence from treatment providers and participants as:

- adequate, for example where the allocation sequence could not be foretold (A);
- unclear, for example not reported (B);

- inadequate, for example the computer-generated random sequence was displayed so treatment providers could see which arm of the trial the next participant was assigned to, or kept in a sealed opaque envelope (C).

Loss to follow-up

We recorded the number of participants in each intervention arm whose outcomes were not reported at the end of the study; we noted if loss to follow-up was not reported.

Risk of bias were assessed as above with the exception of randomisation and additionally assessed on the basis of:

- Comparability of treatment groups at baseline:
  - yes;
  - no;
  - unclear.
- Adjustment for potential confounders:
  - yes;
  - no;
  - unclear.

Assessment of heterogeneity

Heterogeneity between studies was assessed by visual inspection of forest plots, by estimation of the percentage heterogeneity between trials that could not be ascribed to sampling variation (Higgins 2003) and by a Chi^2 test of the significance of the heterogeneity (Deeks 2001), irrespective of whether HRs or odds ratios (ORs) were calculated. If there was evidence of substantial heterogeneity, the possible reasons for this were investigated and reported.

Data synthesis

For meta-analysis of the time-to-event outcomes (OS and PFS), the most appropriate statistic is the HR. If provided in a trial report, the HR and associated variance were used directly in the meta-analysis. Alternatively, using the methods described in Parmar 1998, they were estimated indirectly from other summary statistics (95% confidence intervals (CI), P values, total number of events) or from data extracted from published Kaplan-Meier curves (Parmar 1998). Where feasible, a number of methods were used to estimate the trial HR indirectly, to check its reliability. The estimated HRs were then combined across all trials using the generic inverse variance facility in RevMan 5 software to give a pooled HR (RevMan 2011). This represents the overall risk of an event with surgery versus radiotherapy.

In some papers only overall rates of local and distant recurrence were presented rather than a time-to-event analysis of these events. Therefore, only an OR of the rates of recurrence could be calculated, with no account being taken of the time to recurrence or any censoring. Data for recurrence were extracted from the text and the OR calculated from the total number of patients and the observed number of recurrences in each arm. The ORs for individual trials were then combined across all trials. These ORs indicate the odds of a local or distant recurrence in the surgery arm versus the radiotherapy arm.

Chi² tests were also used to assess the consistency of effect across different subsets of trials and were referred to as Chi² test for interaction. Pooling of data was only done if there was no clinical heterogeneity and if there were outcomes that could be combined. In the absence of statistical heterogeneity, a fixed-effect model was used; if there was statistical heterogeneity a random-effects model was used. Where poolingwas not appropriate, the results of eligible trials was discussed in a narrative form. Ideally the analysis was on an ITT basis. In all tests of significance a two-sided P value is given.

Sensitivity analysis

If there was a major variation in the quality of studies, it was examined in a sensitivity analysis.

RESULTS

Description of studies

See: Characteristics of included studies; Characteristics of excluded studies.

Results of the search

A MEDLINE search (Appendix 3) identified 453 hits. A similar EMBASE search was carried out (Appendix 4), which identified 174 studies and a CENTRAL search (Appendix 2) revealed 153 hits. Search of Group's Specialised Register and Non-Trials Database revealed 81 and 40 studies, respectively. Searches of the Web of Science did not add any studies. The reference lists were checked and the handsearching of journals and congress abstracts did not add any studies.

As it was known to us that only a small number, if any, of RCTs had been published, we also incorporated other types of studies in this review, that is prospective observational studies, case-control studies and studies with historic controls.

Forty-three possible eligible studies were retrieved for more detailed information. We found five RCTs (Landoni 1997; Morley 1976; Newton 1975; Piver 1988; Roddick 1971). Reasons for excluding were description of histology was not provided, short follow-up time (Roddick 1971), survival of patients with AC was not described separately (Morley 1976;Newton 1975), the studies were identified as not being RCTs (Morley 1976; Piver 1988). One RCT was found to meet the inclusion criteria (Landoni 1997).

Of the remaining 38 abstracts obtained, 25 studies were excluded for the following reasons: not AC, wrong FIGO stage, duplicate report about same study, only abstract available (Rabin 1984;Wei 2005), FIGO stage not described, different type of intervention, no detailed result information. This left a total of 12 non-RCTs that were considered for inclusion (Angel 1992; Baalbergen 2004; Berek 1981; Chen 1998; Eifel 1991; Hopkins 1988; Hurt 1977; Kilgore 1988; Kleine 1989; Nola 2005; Saigo 1986;Weiss 1986). Two studies reported data from the same department, but from different time periods. Eifel et al reported from 1965 to 1985 and Rutledge et al from 1947 to 1971, which overlapped by five years (Eifel 1991; Rutledge 1975). The five-year survival after surgery in stage IB in the Rutledge study was 33.3%, which is not in accordance to literature. Therefore we excluded the Rutledge study. After primary surgery, patients were irradiated in case of positive lymph nodes, compromised surgical margins, extension to parametrium. The indication for adjuvant therapy was not well described in some studies

(Berek 1981;Hurt 1977;Nola 2005; Saigo 1986) as well as the percentage of patients who received adjuvant radiotherapy in Angel 1992 (12%), Baalbergen 2004 (21%),Chen 1998 (13%), Eifel 1991 (14%), Hopkins 1988 (14%), Hurt 1977 (0%), Kilgore 1988 (18%), Landoni 1997 (64%), Nola 2005 (not reported), Saigo 1986 (11%) and Weiss 1986 (55%). All studies apart from the RCT (Landoni 1997) were retrospective and with a long time span of between nine (Weiss 1986) and 32 (Saigo 1986) years. The studies of Baalbergen 2004 and Saigo 1986 were multicentric but therapy was uniform. All the other studies were single centre.

Except for the RCT study (Landoni 1997), all other studies were retrospective cohort studies with variable methodological quality and limitations of a retrospective study. Comparing the results from these retrospective studies was not possible due to diverging treatment strategies. See QUOROM statement flow diagram (Table 1).

Subsequent searches (2012) identified in EMBASE 135 hits and in CENTRAL 172 hits. Search of Group's Specialised Register and Non-Trials Database revealed no new studies. Searches of the Web of Science did not add any studies. The reference lists were checked and the handsearching of journals and congress abstracts did not add any studies. Three studies seemed potentially relevant; of one only the abstract was available (Maneo 2011) and in two the results were not described by intervention (Bansal 2009; Galic 2012) (Figure 2).

Included studies

We found only one RCT (Landoni 1997), which is described in detail in Characteristics of included studies. This study was a prospective RCT of radiotherapy versus surgery in stage IB-IIA cervical cancer from 1986 to 1991, in patients referred to the Department of Obstetrics and Gynecology and Radiation Oncology at the Istituto di Scienze Biomediche S Gerardo, University of Milan. Of the 468 eligible patients, a high percentage, 27% (N=125) were excluded because of age (N=43), medical illness (N=54), former or concurrent malignancy (N=21), or doctors or patients preference for a primary therapy (N = 7). Women under 30 years of age were excluded, the mean age in the study was 50 years.

This study included 46 patients with AC. Twenty-six patients had primary surgery and 20 had primary radiotherapy. A relatively high percentage of the primary surgery patients had adjuvant radiotherapy (64%).

Primary surgery was uniform. Surgery consisted of a class III radical hysterectomy as described by Piver 1974. Adjuvant radiotherapy was given as a precaution for the following pathological risk factors: stage was greater than FIGO stage IIA, less than 3 mm of uninvolved cervical stroma, cut through or lymph node metastases. Adjuvant radiotherapy consisted of external pelvic irradiation, with a total dose of 50.4 Gy over five to six weeks. Sixty-four per cent (108 out of 170) of the surgery group received adjuvant radiotherapy, which is high compared to the percentages of 9% to 38%cited in literature (Morris 1994). For the 26 AC patients who had primary surgery and received adjuvant radiotherapy similar details were not provided.

Primary radiotherapy included external pelvic irradiation with 18 MV photon beam by a multi-portal technique. The median total dose was 47 Gy (range 40 to 53). After two weeks one caesium-137 LDR insertion was given. The median total dose at point A (external beam plus brachytherapy) was 76 Gy (range70 to 90). When lymphangiography showed common iliac or para aortic metastases, para aortic lymph nodes were treated with a radiotherapy dose of 45 Gy

over five weeks. A boost of 5 to 10 Gy was given to the positive lymph nodes. In the surgery group, lymphangiography revealed positive nodes in 24 patients (14%). Six of these 24 patients showed no lymph-node metastases in the surgical specimen. Whereas 27 of the 145 patients in the lymphangiography negative surgery group also had nodal metastases. If nodal tumour metastases were discovered at the time of an attempted radical hysterectomy, some surgeons completed the radical hysterectomy while other surgeons abandoned it and patients were treated by radiotherapy. It was not described in this study how these patients were allocated, to the primary surgery or the primary radiotherapy group.

Median follow-up was 87 months (range 57 to 120). No patient was lost to follow-up. The outcomes assessed were the five-year survival, rate and pattern of complications, and recurrences associated with each primary therapy.

Excluded studies

We had planned to incorporate observational studies, case-control studies, non-randomised studies with concurrent controls and studies with historical controls in this review. We found 42 possible eligible studies but all these studies were of insufficient methodological quality, therefore we excluded all these 42 non-RCTs. See Characteristics of excluded studies.

Risk of bias in included studies

Allocation

In the Landoni study patients were randomly assigned radical surgery or radical radiotherapy (Landoni 1997). Patients were also stratified by cervical diameter. There was adequate sequence generation and allocation concealment (block randomisation from a computer-generated table in clusters of 10 cases of each stratum of cervical diameter).

Blinding

There was no blinding during treatment or follow-up surveillance.

Incomplete outcome data

After randomisation there were six protocol violations: two in the surgery group and four in the radiotherapy group. In 10 patients a treatment cross-over occurred. A total of 327patients received the scheduled treatment, 169 primary surgery and 158 primary radiotherapy. The median follow-up was 87 (range 57 to 120) months. No patient was lost to follow-up.

Selective reporting

To describe survival all patients with ITT were analysed. For the analysis of complications, 10 patients who had a treatment crossover were excluded. A high percentage of patients (27%, N = 125) were excluded before randomisation due to age or medical illness.

Other potential sources of bias

The current staging procedure for cervical cancer (FIGO clinical staging system including imaging) is under discussion as it is a clinical pre-treatment staging. However, at the time of performing this study, it was, and still is, the standard tool of staging cervical cancer.

Effects of interventions

There was no survival benefit for either arm for all cervical cancer patients, but the multivariate (subgroup) analysis showed a marginally significant advantage in OS in the 46 AC patients after primary surgery compared to primary radiotherapy (OR 0.67; 95% CI 0.2 to 2.26; P = 0.05) (Analysis 1.1). OS was only just significantly better after primary surgery (70%) versus primary radiotherapy (59%). It is not clear if this minor difference could be explained by the average higher age of the radiotherapy group. The DFS was 66% after primary surgery and 47% after primary radiotherapy (OR 0.43; 95% CI 0.13 to 1.43; P = 0.02) (Analysis 2.1).

Most complications were described after combination therapy. In the surgery group (surgery only and surgery plus radiotherapy), 48 (28%) patients showed severe (grade 2 to 3) morbidity that required medical or surgical treatment, compared with 19 (12%) patients in the radiotherapy group (OR 3.32; 95% CI 0.61 to 18.12) (Analysis 1.2). After surgery only 16% of the patients had short-term morbidity and 24% had long-term morbidity. After surgery and adjuvant radiotherapy these percentages were 20% and 29%, respectively, and after radiotherapy alone were 7% and 16%, respectively. Owing to the high percentage of adjuvant radiotherapy after surgery, and as a result of combining treatment, the morbidity was relatively high in the surgery arm. The study gave the complication data for the whole group but not for AC separately.

DISCUSSION

Summary of main results

For early-stage AC surgery was better than radiotherapy. The majority of operated patients required adjuvant radiotherapy. Combined therapy (surgery and adjuvant radiotherapy) gave the highest complications and morbidity. The radiotherapy used in this study was not optimal.

Overall completeness and applicability of evidence

We have found only one RCT for this review. It included 46 patients with AC. The mean age of patients in the study was high (50 years) compared to that in other studies (43 to 47 years) (Chen 1998; Eifel 1991; Kilgore 1988; Nola 2005; Saigo 1986). Because of the high percentage of patients excluded before randomisation due to age or medical illness, the results for this study apply only for relatively healthy patients in the age range 30 to 70 years.

The patients received a relatively low radiation dose (median dose: 76 Gy; range 70 to 90). According to the recommendation of the American Brachytherapy Society, the total dose to 'point A' in stage IB-IIA diseases should be in the range of 80 to 85 Gy (Nag 2002).

The study was performed from 1986 to 1991. At that time, it was not standard practice to combine chemotherapy with radiotherapy in the treatment cervical cancer patients. Since then, concurrent chemoradiation in either definitive or postoperative setting has been shown to be superior to radiotherapy alone (Green 2001; Green 2005; Peters 2000).

Quality of the evidence

The quantity and quality of the evidence was scarce and only one RCT was found (Landoni 1997), which included only 46 patients with AC from 337 cervical cancer patients.

AUTHORS' CONCLUSIONS

Implications for practice

Analysis of a subgroup of the single RCT showed that surgery for early-stage AC was better than radiotherapy. However, the majority of the surgery group patients required adjuvant radiotherapy, which was associated with greater morbidity. Furthermore, radiotherapy was not optimised and surgery was not compared to chemoradiation, which is currently recommended inmost centres. Finally, modern imaging techniques (MRI, PET-CT), allow for better patient selection enabling node-negative patients to be more easily identified for surgery, thereby reducing the risk of morbidity associated with surgery and adjuvant radiotherapy.

In conclusion, we recommend surgery for early-stage AC of the uterine cervix in carefully staged patients. Whereas primary chemoradiation remains a second best alternative for patients unfit for surgery and chemoradiation probably is first choice in patients with (MRI or PET-CT-suspected) positive lymph nodes.

Since the last version of this review no new studies were found.

Implications for research

There is a need for well-designed RCTs comparing primary surgery versus primary radiotherapy plus concurrent chemotherapy for early AC. This can only be carried out in women who do not need fertility-sparing treatment.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Landoni 1997		
Methods	Randomised controlled trial 1986 to 1991; Milan, Italy	
Participants	337 patients with stage IB or IIA cervical cancer: 46 with AC	
Interventions	Surgery consisted of a Class III radical abdominal hysterectomy. Adjuvant radiotherapy was given if at least 1 pathological risk factor (stage > pT2a, less than 3 mm uninvolved stroma, cut through, lymph-node metastases) Radiotherapy included external beam pelvic irradiation plus brachytherapy. Total dose at point A ranged 70 to 90 Gy (median 76 Gy)	
Outcomes	5-year overall survival: 70% after primary surgery (N = 26) versus 59% after primary radiotherapy (N = 20). No evidence of disease at 5 years: 66% after surgery versus 47% after radiotherapy Complications surgery-related 28%, radiation-related 12%	
Notes	-	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	
Allocation concealment (selection bias)	Low risk	
Blinding (performance bias and detection bias) 5 yr survival	High risk	In the follow-up primary therapy was obvious
Blinding (performance bias and detection bias) complications	High risk	
Incomplete outcome data (attrition bias) All outcomes	Low risk	
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Angel 1992	Retrospective study, 1966 to 1990, New York USA. 89 patients with stage I. Treatment prior to 1980 consisted mainly of radiotherapy and pre-operative radiotherapy, after 1980 the primary therapeutic approach was radical surgery
Attanoos 1995	Retrospective study, 1971 to 1990, Cardiff UK.55 patients. Survival was not described separately for stage and therapy
Baalbergen 2004	Retrospective study, 1989 to 1999, Rotterdam, Netherlands. 200 stage I and IIA patients. Patients had primary radiotherapy when their clinical condition was poor or because of old age
Bansal 2009	Retrospective study, 1988 to 2005, SEER database USA. Survival for different therapies for adenocarcinoma alone was not given separately
Berek 1981	Retrospective study, 1953 to 1978, UCLA USA. 48 stage IB patients. Reason for choice of primary therapy not given
Chargui 2006	Retrospective study, 1990 to 1999 Tunis. Patients with stage I and IIA had pre-operative radiotherapy 45 Gy followed by radical surgery (51 patients) or surgery and radiotherapy (1 patient)
Charkviani 1990	Retrospective study, 1964 to 1989, USSR. 98 patients. Survival not separately mentioned for AC
Chen 1998	Retrospective study, 1977 to 1994, Taipei Taiwan. 240 patients. Patients were encouraged to undergo surgical treatment instead of radiotherapy
Covens 1999	Retrospective study, 1984 to 1995, Toronto Canada. Study was only about surgery in early stage I AC
Eifel 1990	Retrospective study, 1965 to 1985, MD Anderson, USA. Different treatment for early stage was precisely described but survival was not given separately for primary surgery versus primary radiotherapy
Eifel 1991	Retrospective study, 1965 to 1985, MD Anderseon USA. 160 patients with an abnormal lymphography were treated with radiotherapy. Patients determined to have positive nodes at explorative surgery did not undergo planned hysterectomy but were given radiotherapy
Erzen 2002	Retrospective study, 1995 to 1999, Slovenia. Therapy (surgery versus radiotherapy) and outcome were not described separately
Farley 2003	Retrospective study, 1988 to 1999, Military Health Care System USA. Survival for different therapies was not given separately
Galic 2012	Retrospective study, 1988 to 2005, SEER database. Survival for different therapies was not given separately
Grigsby 1988	Retrospective study, 1959 to 1982, Washington USA, only about radiation
Hansen 1981	Prospective non-randomised study, 1974 to 1977, Odense, Denmark. Histology was not mentioned. Standard therapy was pre-operative radiotherapy followed by surgery. When a contraindication to operation was found patients had radiotherapy only
Hopkins 1988	Retrospective study, 1970 to 1985, Michigan USA. 125 stage I AC patients. Allocation for primary therapy not given
Hopkins 1991	Retrospective study, 1970 to 1985, Michigan USA. Only description of P value in a Cox Model Multiple Proportion Hazard Analysis for patients with stage IB AC according to treatment
Hurt 1977	Retrospective study, 1954 to 1974, Virginia USA. 20 stage I AC patients. Choice for primary therapy not described, only 3 had primary surgery

Characteristics of excluded studies *[ordered by study ID]* *(Continued)*

Study	Reason for exclusion
Ireland 1985	Retrospective study, 1969 to 1983, Gateshead, UK. Survival was not given separately for different treatment
Kilgore 1988	Retrospective study, 1963 to 1985, Alabama USA. 130 stage I AC patients. Selection of treatment was not described
Kjorstad 1977	Retrospective study, 1963 to 1968, Oslo Norway. All patients had intracavitary radium treatment followed by surgery or radiotherapy
Kleine 1989	Retrospective study, 1964 to 1985, Freiburg Germany. 64 stage I patients. Clinical stage differentiation inadequate
Leminen 1990	Retrospective study, 1976 to 1980, Helsinki Finland. 63 patients. Surgery was pre-treated with a single intracavitary irradiation
Martel 2000	Case-control study, 1978 to 1992, Toulouse, France. Small numbers, survival was not separately given for different therapy per stage
Miller 1993	Retrospective study, 1964 to 1989, Memphis USA. Survival was not described for different therapies
Milsom 1983	Retrospective study, 1965 to 1974, Göteborg Sweden. Primary therapy consisted of intracavitary radiation followed by surgery or intracavitary plus external irradiation
Morley 1976	Retrospective study, 1945 to 1975, Michigan USA. Survival of patients with AC was not separately described
Newton 1975	Prospective study of surgery versus radiotherapy in cervical cancer, 1956 to 1966, Chicago USA. Survival of 7 patients with AC was not described separately
Nola 2005	Retrospective study, 1978 to 1998, Zagreb Croatia. 36 AC stage I-IV patients. Survival after primary surgery versus primary radiation was not subdivided for stage
Papanikolaou 2006	Retrospective study, 1993 to 2000, Greece. Therapy and survival for AC (only 11 patients) not separately described
Perez 1995	Retrospective study, 1966 to 1995, Missouri USA. Irradiation versus irradiation plus surgery in cervical cancer. Survival of AC patients is not separately described
Piver 1988	Retrospective study, 1974 to 1983, Buffalo USA. Treatment and survival of patients with AC was not separately described
Rabin 1984	South-African article from 1984. Study about radiotherapy plus surgery versus surgery in cervical cancer. In abstract no description of AC histology. Article could not be obtained
Roddick 1971	Randomised study, Kentucky USA, Surgery versus radiotherapy in cervical cancer. But no description of histology, no AC described, short follow-up
Rutledge 1975	Retrospective observational study, 1947 to 1971, MD Anderson USA. 61 stage I and IIA patients. 5-year survival after surgery in stage IB was 33.3%; this is not according to literature
Saigo 1986	Retrospective study, 1949 to 1981, New York USA. 102 stage IB and IIA patients. Allocation for primary treatment not described. Wide variation in radiation treatment during the interval of this study
Shingleton 1981	Retrospective study, 1969 to 1980, Alabama USA. Survival is not separately described for different therapies. Same clinic as Kilgore 1988
Sundfor 1996	Randomised study, 1968 to 1980, Oslo Norway. Radiotherapy versus radiotherapy plus surgery in SCC
Townsend 1980	Randomised study, Melbourne. Intracavity radon followed by radical hysterectomy and pelvic lymph nodes versus intracavitary radon plus external megavoltage irradiation followed by extended hysterectomy in cancer of the cervix. Histology AC not described

Characteristics of excluded studies *[ordered by study ID]* *(Continued)*

Study	Reason for exclusion
Waldenström 1999	Retrospective study, 1987 to 1994, Göteborg Sweden. Survival was not separately described after primary surgery versus primary radiotherapy
Wei 2005	Retrospective study, 1970 to 2002, China. 105 AC patients. 5 year-survival for stage I 58%, which is not in accordance with literature. Only abstract available
Weiss 1986	Retrospective study, 1970 to 1979, San Diego USA. 28 AC stage IB and IIA patients, < 4 cm. Treatment was based on stage of the lesion and the general medical condition of the patient
Yamashita 2005	Retrospective study, 1991 to 2004, Tokyo Japan. Surgery versus radiotherapy in cervical cancer. Survival of 24 patients with AC was not described separately

DATA AND ANALYSES

Comparison 1. Survival

Outcome or Subgroup title	No. of Studies	No. of Participants	Statistical Method	Effect Estimate
1 5-year survival	1	46	Odds Ratio (M-H, Fixed, 95% CI)	0.67 [0.20, 2.26]
2 Complications	1	46	Odds Ratio (M-H, Fixed, 95% CI)	3.32 [0.61, 18.12]

Comparison 2. Disease-free survival

Outcome or Subgroup title	No. of Studies	No. of Participants	Statistical Method	Effect Estimate
1 Disease-free survival	1	46	Odds Ratio (M-H, Fixed, 95% CI)	0.43 [0.13 , 1.43]

Analysis 1.1. Comparison I survival, Outcome I 5-year survival

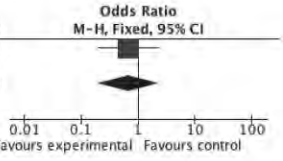
Comparison: I Survival

Outcome: I 5-year survival

Study or Subgroup	Primary surgery Events	Total	primary radiotherapy Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Landoni 1997	8	26	8	20	100.0%	0.67 [0.20, 2.26]
Total (95% CI)		**26**		**20**	**100.0%**	**0.67 [0.20, 2.26]**
Total events	8		8			

Heterogeneity: Not applicable

Test for overall effect: Z = 0.65 (P = 0.52)



Analysis 1.2. Comparison 1 Survival, Outcome 2 Complications.

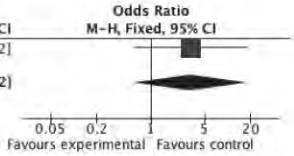
Comparison: 1 Survival

Outcome: 2 Complications

Study or Subgroup	Primary surgery Events	Total	Primary radiotherapy Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Landoni 1997	7	26	2	20	100.0%	3.32 [0.61, 18.12]
Total (95% CI)		**26**		**20**	**100.0%**	**3.32 [0.61, 18.12]**
Total events	7		2			

Heterogeneity: Not applicable

Test for overall effect: Z = 1.38 (P = 0.17)



Analysis 2.1. Comparison 2 Disease-free survival, Outcome 1 Disease free survival.

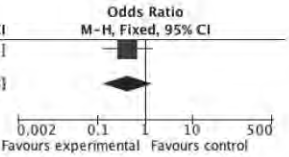
Comparison: 2 disease-free survival

Outcome: 1 Disease-free survival

Study or Subgroup	Primary surgery Events	Total	Primary radiotherapy Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Landoni 1997	9	26	11	20	100.0%	0.43 [0.13, 1.43]
Total (95% CI)		**26**		**20**	**100.0%**	**0.43 [0.13, 1.43]**
Total events	9		11			

Heterogeneity: Not applicable

Test for overall effect: Z = 1.37 (P = 0.17)



ADDITIONAL TABLES

Table 1. Data collection form

Intervention A	Intervention B
Study identification: Form filled in by:	
Reference checked by:	
Date completing form:	
Name study:	
1st author, journal, year:	
Study properties	
RCT, non-randomised controlled study, CCT, observational	
study prospective/retrospective	
Time of inclusion:	
Purpose of the study, as stated by authors:	
Selection bias	
Performance bias	
Attribution bias	
Detection bias	
Analysis (statistics)	

Table 1. Data collection form (*Continued*)

Intervention A	Intervention B
Study eligible for review: yes / no	
If not, why not:	
Types of participants: Intervention A Intervention B	
Number of patients:	
Age:	
Mean:	
Median:	
SD:	
Ranges:	
Primary tumours:	
FIGOstage IA	
IB-IIA	
IIBHistological	
type	
Adenocarcinoma	
Adenosquamous	
Other (specify)	
Grade: I	
II	
III	
unknown	
Performance Status: WHO	
Types of intervention:	
Surgery planned	
Conservative surgery	
Radical surgery	
Protocol violations	
Radiationtherapy planned	
-External & brachytherapy:	
total Gy: fractions: frequency: field:	
-Chemoradiation	
total Gy: fractions: frequency: field:	
CT agent(s) doses: frequency	
Protocol violations	
Surgery & Radiation therapy	
-reason:	
Outcome A B	
Total patients entering the study	
Declared ineligible	
Removed from study for other reasons	

Table 1. Data collection form (*Continued*)

Intervention A	Intervention B
Included in analysis	
Completed prescribed treatment plan	
(and available for response)	
Follow up: A B	
Known of .. patients.	
Time of f.u. median:	
SD:	
Range:	
Alive (5-yr survival)	
Without evidence of disease	
With disease	
Death:	
DOD	
Treatment complications	
Not related death	
Unknown	
Recurrence: yes / no	
If yes time-interval (month)	
If yes: local, distant, both	
Complications:	
-radiation-related	
-surgery-related	
-death	

Table 2. Critical review form; randomized studies

Yes – no
Did study population meet our criteria?
or: is it possible to analyse patients that meet our criteria separately?
Was assignment of patients to treatment randomised?
Were patients analysed in the groups to which they were randomised?
Were the groups similar at the start of the trial?
Aside from the experimental intervention, were the groups treated equally?
Were all patients who entered the trial accounted for at its conclusion?
How long was follow up? (Median and range)
Were interventions defined adequately?
Were all clinically important outcomes considered?
-disease free survival
-complications

Table 3. Critical review form; studies with non- randomized controls

Yes – no
Did study population meet our criteria?
or: is it possible to analyse patients that meet our criteria separately?
Is the study adjusted for confounders?
Were patients analysed in the groups to which they were assigned?
Were the groups similar before treatment?
Aside from the experimental intervention, were the groups treated equally?
Are controls concurrent or retrospective?
Were all patients accounted for at the end of follow up?
How long was follow up?
Were interventions defined adequately?
How precise was the estimate of the treatment effect?
-disease free survival
-complications
Were all clinically important outcomes considered?
-disease free survival
-complications

Table 4. Critical review form; observational studies

Yes – no
Did study population meet our criteria?
or: is it possible to analyse patients that meet our criteria separately?
Were all observed patients accounted for at the end of follow up?
How long was follow up?
Were interventions defined adequately?
Is the study cohort defined temporally?
Is the study cohort defined geographically?
Percentage of defined patient population who are included in the study?
Were all clinically important outcomes considered?
-disease free survival
-complications

APPENDICES

Appendix 1. FIGO staging

FIGO Stage I

Carcinoma is strictly confined to the cervix (extension to the corpus should be disregarded). Invasive carcinoma that can be diagnosed only by microscopy. All macroscopically visible lesions, even with superficial invasion, are allotted to Stage IB carcinomas. The involvement of vascular spaces, venous or lymphatic, should not change the stage allotment.

- IA1 Measured stromal invasion of not more than 3.0 mm in depth and width of not more than 7.0 mm.
- IA2 Measured stromal invasion of more than 3.0 mm and not more than 5.0 mm with a width of not more than 7.0 mm.
- IB1 Clinically visible lesions not more than 4.0 cm, or pre-clinical lesions greater than IA2.
- IB2 Clinically visible lesions more than 4.0 cm.

FIGO Stage II

Cervical carcinoma invades beyond the uterus, but not to the pelvic sidewall or to the lower third of the vagina.

- IIA1 No obvious parametrial involvement and tumour size of 4 cm or less with involvement of less than the upper two-thirds of the vagina.
- IIA2 No obvious parametrial involvement and tumour size of more than 4 cm with involvement of less than the upper two-thirds of the vagina (Pecorelli 2009).
- IIB Obvious parametrial involvement (Benedet 2001).

Appendix 2. CENTRAL search strategy

CENTRAL Issue 3 2009
#1 MeSH descriptor Uterine Cervical Neoplasms explode all trees
#2 MeSH descriptor Cervix Uteri explode all trees
#3 cervi*
#4 (#2 OR #3)
#5 cancer* or tumor* or tumour* or neoplas* or malignan* or carcinom* or adenocarcinom*
#6 MeSH descriptor Adenocarcinoma explode all trees
#7 MeSH descriptor Carcinoma, Adenosquamous explode all trees
#8 (#5 OR #6 OR #7)
#9 (#4 AND #8)
#10 (#1 OR #9)
#11 MeSH descriptor Gynecologic Surgical Procedures explode all trees
#12 surg*
#13 Any MeSH descriptor with qualifier: SU
#14 MeSH descriptor Hysterectomy explode all trees
#15 hysterectomy
#16 (#11 OR #12 OR #13 OR #14 OR #15)
#17 MeSH descriptor Radiotherapy explode all trees

#18 Any MeSH descriptor with qualifier: RT
#19 radiation
#20 brachytherapy
#21 chemoradi*
#22 radiochemo*
#23 (#17 OR #18 OR #19 OR #20 OR #21 OR #22)
#24 (#10 AND #16 AND #23)

Appendix 3. MEDLINE search strategy

MEDLINE Ovid 1950 to July week 5 2009
1 exp Uterine Cervical Neoplasms/
2 exp Cervix Uteri/ or cervi*.mp.
3 1 or 2
4 exp Adenocarcinoma/
5 adenocarcinoma*.mp.
6 exp Carcinoma, Adenosquamous/
7 adenosquamous carcinoma*.mp.
8 4 or 5 or 6 or 7
9 3 and 8
10 exp Gynecologic Surgical Procedures/
11 surg*.mp.
12 surgery.fs.
13 exp Hysterectomy/
14 hysterectomy.mp.
15 10 or 11 or 12 or 13 or 14
16 exp Radiotherapy/
17 radiotherap*.mp.
18 radiotherapy.fs.
19 radiation.mp.
20 brachytherapy.mp.
21 chemoradi*.mp.
22 radiochemo*.mp.
23 16 or 17 or 18 or 19 or 20 or 21 or 22
24 9 and 15 and 23
25 randomized controlled trial.pt.
26 controlled clinical trial.pt.
27 randomized.ab.
28 clinical trials as topic.sh.
29 randomly.ab.
30 trial.ti.
31 exp Cohort Studies/
32 cohort*.mp.

33 exp Case-Control Studies/
34 (case* and control*).mp.
35 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34
36 24 and 35
37 (animals not (humans and animals)).sh.
38 36 not 37
key:
mp=title, original title, abstract, name of substance word, subject heading word
fs=floating subheading
pt=publication type
ab=abstract
sh=subject heading

Appendix 4. EMBASE search strategy

EMBASE 1980 to 2009 week 32
1 exp uterine cervix tumor/
2 exp uterine cervix/ or cervi*.mp.
3 1 or 2
4 exp adenocarcinoma/
5 adenocarcinoma*.mp.
6 exp adenosquamous carcinoma/
7 adenosquamous carcinoma*.mp.
8 4 or 5 or 6 or 7
9 3 and 8
10 exp gynecologic surgery/
11 surg*.mp.
12 su.fs.
13 exp hysterectomy/
14 hysterectomy.mp.
15 10 or 11 or 12 or 13 or 14
16 exp radiotherapy/
17 radiotherap*.mp.
18 rt.fs.
19 radiation.mp.
20 brachytherapy.mp.
21 chemoradi*.mp.
22 radiochemo*.mp.
23 16 or 17 or 18 or 19 or 20 or 21 or 22
24 9 and 15 and 23
25 exp controlled clinical trial/
26 randomized.ab.
27 randomly.ab.

28 trial.ab.
29 groups.ab.
30 exp cohort analysis/
31 cohort*.mp.
32 exp case control study/
33 (case* and control*).mp.
34 exp retrospective study/
35 exp prospective study/
36 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35
37 24 and 36
key:
mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer name
fs=floating subheading
ab=abstract

Appendix 5. Cochrane Gynaecological Cancer Group's Specialised Register and Non-Trials Database

#8=CVX AND#11=SU AND#11=RT AND#12=TRT AND#4 <>ADVANCED AND#4 <>RECURRENT AND#4 <>REFRACTORY

WHAT'S NEW

Last assessed as up-to-date: 19 October 2009.

Date	Event	Description
14 November 2012	Amended	Contact details amended
7 November 2012	New citation required but conclusions have not changed	No new studies were identified for inclusion
7 November 2012	New search has been performed	A new search has been performed. The literature searches as described in the search strategy section were updated in June 2012

CONTRIBUTIONS OF AUTHORS

AA and AB wrote the protocol. AB and YV did the search strategy, with help from Anne Oestmann and Jane Hayes of the Cochrane Gynaecological Cancer Review Group. AB and YV assessed eligibility of retrieved papers. AB prepared the initial text. AA advised on the methodology content and edited the text. LS searched for background material with special emphases on the radiotherapeutic subject and edited the text.

DECLARATIONS OF INTEREST

None known.

SOURCES OF SUPPORT

Internal sources

- None, Not specified.

External sources

- None, Not specified.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In the Methods under the Types of studies we added *"The methodological quality of non-RCTs was assessed on the basis of comparability of treatment groups at baseline, adjustment for potential confounders and allocation of treatment"*. We had not clearly stated this in the protocol. When we encountered the non-RCTs we found them on methodologically grounds (mainly due to selection of primary treatment) not qualified for our review, so that we excluded the non-RCTs.

As only one RCT was found to be suitable for inclusion the methods described in the Assessment of heterogeneity, Data synthesis and Sensitivity analysis were not used.

5.2

CONSERVATIVE THERAPY IN MICROINVASIVE ADENOCARCINOMA OF THE UTERINE CERVIX IS JUSTIFIED. AN ANALYSIS OF 59 CASES AND A REVIEW OF THE LITERATURE

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Frank Smedts
Theo J.M. Helmerhorst

International journal of gynecological cancer. 2011;21: 1640-1645

ABSTRACT

Introduction

An evaluation of treatment and follow up in a large series of women with early cervical adenocarcinoma (AC), stages 1A1 and 1A2 and an extensive literature review, in an effort to ascertain weather conservative therapy is justified.

Methods

Fifty-nine cases of microinvasive AC diagnosed between 1987 and 2006 in the Rotterdam district, The Netherlands were retrieved. Clinical and pathological data were reviewed and analysed. A mesh review of all relevant literature concerning stage IA1 and IA2 was performed.

Results

Thirty-three patients had stage 1A1 and 26 stage 1A2 cervical AC. 42 patients were treated conservatively (i.e. conization or simple hysterectomy) and 17 patients were treated radically (i.e. radical hysterectomy /trachelectomy with lymph node dissection). One recurrence (1.7%) in a patient with stage IA1disease (grade 1 adenocarcinoma, depth 1.4 mm and width 3.8 mm, with lymph vasculair space invasion (LVSI) treated by vaginal hysterectomy. The mean follow-up was 79.9 months. From the literature, pooling all data from patients with stage IA1 and IA2 AC, the risk of recurrent disease after conservative therapy was 1.5%, and after radical therapy 2.0%.

Conclusions

Extensive treatment such as radical hysterectomy with pelvic lymph node dissection or trachelectomy does not prevent recurrent disease. Patients with microinvasive AC should be treated identically to patients with squamous cell carcinoma. In stage IA1 and IA2 AC we recommend conservative therapy (by conization). In cases with LVSI, an additional lymphadenectomy is advised. For patients with stage IA2 AC with LVSI a trachelectomy / radical hysterectomy with lymph node dissection should be considered.

INTRODUCTION

Although the incidence of cervical squamous cell carcinoma (SCC) is decreasing in the Netherlands, the incidence of adenocarcinoma (AC) is increasing, especially in women of childbearing age. There has been a 16% increase in women diagnosed with AC between 15-29 years of age and a 2.5% increase in women between 30-44 years of age, in the period between 1989-1998 [1]. The mean age at birth of the first child is 29.4 years in the Netherlands[2], obviating the need for as conservative a therapeutic approach as possible in women with cervical carcinoma.

The optimal treatment for microinvasive cervical AC is controversial. Although curative therapy is pivotal, preservation of fertility is an important issue and therefore influences the choice of therapeutic strategy. The different strategies vary between radical hysterectomy (RH) with pelvic lymph node dissection (PLND) to conization of the cervix. There are several reasons for the fact that there is no international consensus regarding treatment in early cervical carcinoma. Firstly, because the International Federation of Gynecology and Obstetrics (FIGO) and Society of Gynaecologic Oncologists (SGO) definitions of early cervical carcinoma are different, secondly the suggestion that cervical AC behaves more aggressively than SCC and therefore AC should be treated in a different way[3,4]. And thirdly because prospective randomized trials comparing conservative versus radical treatment in cervical adenocarcinoma are lacking, due to the rarity of this carcinoma type, the optimal therapeutic strategy is not known.

In the Netherlands, in patients with stage 1A1 AC a hysterectomy is performed. When fertility is an issue, treatment is conservative e.g. by conization. If lymph vascular space involvement (LVSI) is present, a lymphadenectomy is advised. In patients with stage IA2 AC without LSVI and who desire to maintain fertility, a conization is sufficient however if LVSI has been demonstrated a lymphadenectomy is indicated {http://www.oncoline.nl}.

The purpose of this study is to evaluate the treatment and follow up in a large series of women with early cervical AC, stages 1A1 and 1A2 in an effort to ascertain weather conservative treatment is justified. Furthermore to review relevant literature concerning the outcome of therapy in early stage cervical adenocarcinoma in an effort to come to a more unifying conclusion as to the most optimal therapeutic approach in the treatment of this carcinoma type.

MATERIALS AND METHODS

From the Rotterdam cancer registries: IKR regional cancer registration body, National pathology registration and oncology registry of the Daniel den Hoed Cancer Clinic Rotterdam, all recorded patients with stage IA1 and IA2 invasive cervical adenocarcinomas diagnosed during the period between 1987 and 2006 were requested. Cases were retrieved from the Erasmus MC University Hospital Rotterdam, the Daniel den Hoed Cancer Clinic and the affiliated hospitals in the region. From each case, case files and all available slides were requested and revised. Slides were reevaluated according to FIGO criteria i.e.: 1A1 lesions with a depth of invasion ≤3mm and width ≤7mm, and 1A2 lesions with a depth >3mm but ≤5mm and width ≤7mm.

The following information was taken from the medical records: age, parity, type of treatment, recurrence, number of pregnancies after conization, pregnancy outcomes and

other follow-up data. All slides were reviewed by an experienced gynaecopathologist and classified according to WHO. Cases of usual/endocervical type cervix AC were included, along with the variants of cervical adenocarcinoma. In cases of endometrioid type carcinoma the case was only included when the carcinoma was clearly located in the cervix. If a squamous component was present the case was classified separately as adenosquamous. The following parameters were determined; histologic type, depth of stromal invasion, horizontal extent of the tumor, presence/absence of LVSI, presence/absence of metastasis to the pelvic lymph nodes and ovary, treatment, and recurrence.

Patients were excluded from the study if tumor location was not obvious and/or clinical data were incomplete.

The project was approved by the Medical Ethics Committee of the ErasmusMC University Hospital Rotterdam (nr.211.651/2002/48).

Furthermore a review of the English literature on microinvasive adenocarcinoma of the uterine cervix was performed using Pubmed (1952-2010) and EMBASE (OVID: 1950-2010) using combined disease-specific terms (uterine cervix neoplasms/ AND adenocarcinoma AND microinvasive) with treatment-specific terms (conservative therapy, radical therapy, hysterectomy, conization). The reference lists were reviewed. Case reports were excluded and also studies reporting the same patient group more than once. Our search particularly focused on conservative or radical therapy in relation to recurrent disease. We designated treatment consisting of a conization or simple hysterectomy as conservative. Radical treatment consisted of a radical hysterectomy or trachelectomy.

RESULTS

Fifty-nine patients were included in the study; 33 women had FIGO stage 1A1 AC and 26 FIGO stage 1A2 AC. In all 59 patients formalin-fixed and paraffin-embedded tissue was retrieved. 52 women had an adenocarcinoma of endocervical type. Six patients had an adenosquamous carcinoma and in one a clear cell carcinoma was diagnosed. The mean age at diagnosis in stage 1A1 carcinoma was 37.8 (range 26-66) years and for stage 1A2 carcinoma it was 42.04 (range 28-66) years. The clinicopathologic characteristics of the patients are summarized in Table 1.

Initial treatment consisted of a radical hysterectomy in 17 women, 22 women underwent conization, and in 20 a simple hysterectomy was performed.

Of the 22 patients treated by conization, 15 had a stage 1A1 and 7 had stage 1A2 disease. Eleven of these women had eighteen pregnancies with thirteen live births and four spontaneous abortions. In two women follow-up data were not available, due to emigration. One of these women was three months pregnant on her last visit to the outpatient clinic. Two women had primary cerclage of the cervix and both delivered by a planned caesarean section.

Only one recurrence (1.7%) was reported. This patient had a vaginal hysterectomy for prolaps uteri. During routine histopathology a grade 1 adenocarcinoma was diagnosed. Tumor depth was 1.4 mm and width 3.8 mm, there was LVSI. She developed lymph node metastasis 30 months after initial therapy and was treated by a course of chemo-radiation. Twelve months after therapy there is no evidence of disease.

Table 1. The clinicopathological characteristics of the 59 patients with microinvasive adenocarcinoma of the uterine cervix

	No of patients	percentage	No of patients	percentage
Stage	IA1		IA2	
	33	56	26	44
Age (years) mean	37.8		42.0	
<35	13	39	8	31
35-64	19	58	17	65
>65	1	3	1	4
Para				
0	11	33	10	39
>=1	22	67	16	61
Major presenting symptoms				
None	17	52	12	46
Dysfunctional/postmenopausal	6	18	6	23
Postcoital	8	24	7	27
Vaginal discharge	2	6	1	4
Primary treatment				
conization	15	46	7	27
radical surgery	4	12	13	50
simple hysterectomy	14	42	6	23
Histological Subtype				
Adenocarcinoma	29	88	23	89
Adenosquamous	4	12	2	7
Clear cell			1	4
Tumor grade				
I	26	90	13	52
II	2	7	8	32
III	1	3	4	16
unknown	4		1	
LVSI				
yes	2	6	4	16
no	29	94	21	84
unknown	2		1	
LNM				
no	4		13	
not done	29	88	13	50
recurrence				
No	32	97	26	100
yes	1	3	0	0
pregnancy				
yes	6	18	5	8
no	7	21	2	19
na	20	61	19	73

LVSI lymph vascular space involvement
LNM lymph node metastases
NA not applicable

The mean follow-up period after initial therapy in all patients is 79,9 months (range 10-131 month).

17 women were treated by RH with PLND; 4 patients with stage 1A1 and 13 patients with stage 1A2 disease. No lymph node metastases were found. In only 6 of the 59 women (10%) LVSI was reported. Four of these patients were treated by radical surgery. Positive lymph nodes were not found in these patients. Two had a simple hysterectomy, of which one developed lymph node metastasis 30 month after therapy and follow up in the other patient has been uneventful (5 years).

DISCUSSION

To determine the optimal therapy in women with microinvasive cervical AC, a number of aspects must be considered. In the past, a radical hysterectomy was recommended in all patients with any degree of invasive cervical cancer. In an effort to reduce morbidity without sacrificing efficacy, the radicality of surgery has decreased over the last decades. In 1973 the SGO accepted a new definition for microinvasive cervical cancer, the criteria for which were depth of invasion less than 3 mm and the absence of LVSI. Conservative treatment was advised in cervical carcinomas complying with this criterium[5]. In 1985, FIGO introduced the histologic definition of stage IA cervical cancer, which was refined in 1994; the FIGO stage was based on invasion depth and horizontal extention[6]. Stage IA1 was defined as a tumor, which invades to a depth of 3 mm or less with 7 mm or less horizontal spread. Stage IA2 was defined as stromal invasion of more than 3 mm and less than 5 mm with a horizontal spread of 7 mm or less. From 1994 the two different classification systems were used to define and treat microinvasive carcinoma. In 2009 the new FIGO staging system was published [7]. It states that a stage IA carcinoma can only by diagnosed microscopically. The involvement of vascular spaces–venous or lymphatic- does not change the stage. Microinvasive AC of the uterine cervix should be staged using the same criteria as are used in microinvasive SCC of the uterine cervix. In our study we applied the FIGO staging classification criteria, combined with WHO grading.

During revision of the cases we were regularly confronted by difficulties in evaluating early invasion. These are well described in textbooks, never the less application can be difficult. The main problems noted were the delineation of an AIS-like gland structure, from adenocarcinoma. Also frank invasion was sometimes difficult to call because inflammation and a desmoplastic tissue response were not always present. Bulky outgrowth of tumor tissue was also noted in several cases and invasion in these cases was based on a deeply expansile growth pattern.

According to available literature, in stage IA1 SCC a conization is the treatment of choice if fertility is desired [8], otherwise a simple hysterectomy is sufficient. The risk for nodal metastases is <1%[9].

If a woman has a stage IA2 SCC, the risk of lymph node metastases is estimated to be between 2%[9] and 7.4% [10]. Treatment consists of radical hysterectomy with pelvic lymph node dissection but if there is desire to remain fertile, a large cone biopsy plus extra-peritoneal or laparoscopic pelvic lymphadenectomy, or radical trachelectomy and extra peritoneal or laparoscopic pelvic lymphadenectomy is considered adequate [11], although the necessity of a lymph node dissection is questionable [12].

Although one could argue that microinvasive AC should be staged and treated in the same way as microinvasive SCC, in clinical practice, AC tends to be managed more aggressively than its squamous counterpart. There is a uniform consensus concerning treatment in patients with stage IA1 AC, which are treated with conization or simple hysterectomy. In stage IA2 this is not the case and there are different and diverse approaches. Many studies report stage IA1 and IA2 carcinomas together as one group.

Reviewing literature concerning the optimal treatment of microinvasive AC highlights this diverse approach, see table 2 and 3. This is attributable to the fact that substantial trials comparing conservative versus radical treatment are lacking, due to the rarity of this condition. Besides that, all retrospective reports lack a centralized pathology review. And it is not always clear whether the guidelines for staging as reflected by FIGO staging committee have been strictly followed by the investigators, e.g. some studies included tumors with horizontal spread of more than 7 mm (in a study of Kaku et al [13]50% of the 30 patients had a width of more than 7 mm) and other studies did not specifically note this.

The general consensus is that stage IA1 AC can be treated conservatively. After reviewing literature and including our own data we found 19 studies on stage IA1 AC. 8 recurrences were reported in 733 cases of IA1 disease (1.1%). Pooling the percentages of recurrences for type of therapy, we found 1.4% recurrence after conservative therapy versus 1.0% after radical surgery. See table 2. The review by Östör et al[14] comprising 26 papers on microinvasive AC totalling 436 cases showed that when invasion is less than 5 mm, LVSI is absent, and the conization margins are free, conservative surgery (including conization) may be acceptable and, even if radical surgery is performed, a cure is not guaranteed. In our study 4 of 33 patients with stage IA1had radical surgery, none had LNM and there have been no recurrences. Smith et al[15] found an excellent survival in patients with stage IA1 and IA2 AC of the uterine cervix as defined by the Surveillance, Epidemiology and End-Results Cancer Incidence Public-Use database (SEER). They included SEER and all other available data and found that the risk of positive lymph nodes, recurrence and death in stage IA1 versus IA2 were not statistically different. 98 cases were managed conservatively (conization) and no recurrences were reported, but up to 10% of these patients received adjuvant radiation therapy or underwent lymph node dissection. In a study by Ceballos et al[16] it could not be concluded whether the favourable prognosis of the 29 patients with stage IA1 AC was the product of a favourable stage or the aggressive surgical treatment received. Most of the patients (24/29) in this study had a radical hysterectomy with lymph node dissection. Bisseling et al[17], reviewed 38 patients with early AC and reviewed literature observing that conization is an effective treatment in stage IA1 but when LVSI is present a lymphadenectomy should be performed. Recently more studies propose a conservative therapy in stage IA1. Yahata et al found no LVSI, no LNM and no recurrent disease in a serie of 27 patients with stage IA1 treated conservatively (44%) or radically[18]. Reynolds et al reported an identical outcome in their study of 52 patients with stage IA1 AC in which 44% of patients were treated conservatively (conization, simple hysterectomy). This contrasts with our study in which 1 recurrence was noted after conservative therapy, which had LVSI. The explanation may be that we treated 29 of our 33 patients (88%) with stage IA1 conservatively, which is a higher proportion than described in most of the studies outlined above.

Table 2. Review of literature Stage IA1 adenocarcinoma

				conservative therapy			radical therapy		
Author	Year	Clinic	n	cone	SH	rec	RH	RT	rec
Jones 1993[39]	1977-1990	NY, USA	9	2			7		
Matthews 1993[40]	1975-1988	Texas, USA	24				24		
Kaku 1997[13]	1972-1994	Fukuoka, Japan	21	0					
Ostor 1997[41]	1971-1995	Melbourne, Australia	43	12	21		10		
Covens 1999[42]	1984-1995	Toronto-Ontario, Canada	46	0	0		46		
Nicklin 1999[43]	1986-1998	Queensland, Australia	26	1	10		15		
Schorge 1999[44]	1982-1996	Boston, USA	21	1	4		16		
Elliott 2000[19]	1953-1992	Sydney, Australia	48			1			1
Schorge 2000[45]	1998-	Boston, USA	6	5			1		
McHale 2001[46]	1985-1996	California, USA	20	4	2		14		
Kasamatsu 2002[47]	1969-1997	NCCH, Tokyo, Japan	24		3		21		1
Smith 2002[15]	1983-1997	new mexico, usa	200	31	93	2	76		1
Hirai 2003[48]	1977-1998	CIH, Tokyo, Japan	22				22		1
Balega 2004[22]	1987-1998	Chicago, USA	32	0	0		32	0	
Poynor 2006[49]	1992-1999	NY, USA	21	2	4		14	1	
Ceballos 2006[16]	1985-2002	Ontario, Canada	29	1	4		22	2	
Bisseling 2007[17]	1987-2004	Nijmegen, NL	29	16	9		4		
Reynolds 2010[24]	1993-2007	Rochester, USA	52	7	16		29		
Yahata 2010[18]	1990-2004	Niigata, Japan	27	10			17		
Baalbergen present study	1987-2006	Rotterdam, NL	33	15	14	1	4		0
TOTAL			733	107	180	4	374	3	4

Conservative therapy (conization, simple hysterectomy)
SH simple hysterectomy
RH radical hysterectomy
RT radical trachelectomy
Rec recurrence

In the literature we found 17 studies on stage IA2 adenocarcinoma. Pooling the data, including our own data, we found 12 recurrences in 466 cases (2,5%). After conservative therapy, the recurrence percentage was 1,5% and after radical therapy it was 3.5%. The risk for nodal metastases in stage IA2 AC is about 1.7%[15] and therefore a lymphadenectomy is recommended[19-21]. But there is ample evidence in the published literature today to cast significant doubt on the wholesale application of lymphadenectomy in true stage IA2 disease[22-24].

The question arises weather a conization is not sufficient, instead of parametrial resection as performed in a trachelectomy or radical hysterectomy. In our study, 15 of the 33 (45%) patients with stage IA1 and 7 of the 26 (27%) patients with stage IA2 were treated with conization only, no recurrence was noted. Dargent et al did not find it logical to perform a pelvic lymphadenectomy without removing the paracervical tissues, because of the strong

Table 3. Review of literature Stage IA2 adenocarcinoma

				conservative therapy			radical therapy		
Author	year	clinic	n	cone	SH	rec	RH	RT	rec
Jones 1993[39]	1977-1990	NY, USA	3				3		
Matthews 1993[40]	1975-1988	Texas, USA	15				15		3
Kaku 1997[13]	1972-1994	Fukuoka, Japan	9	0					2
Ostor 1997[41]	1971-1995	Melbourne, Australia	34	4	14		16		1
Nicklin 1999[43]	1986-1998	Queensland, Australia	4				4		
Elliott 2000[19]	1953-1992	Sydney, Australia	10			1			0
Kasamatsu 2002[47]	1969-1997	NCCH, Tokyo, Japan	4				4		
Smith 2002[15]	1983-1997	New Mexico, USA	286	29	123	2	134		2
Hirai 2003[48]	1977-1998	CIH, Tokyo, Japan	6				6		1
Schlaerth 2003[50]	1995-1999	Pasadena, USA	4					4	
Balega 2004[22]	1987-1998	Chicago, USA	16	0	0		16	0	
Poynor 2006[49]	1992-1999	NY, USA	12	1	0		11	0	
Ceballos 2006[16]	1985-2002	Ontario, Canada	3	0	0		3	0	
Bisseling 2007[17]	1987-2004	Nijmegen, NL	9	2	3		4		
Meurs 2009[51]	1994-2006	AMC, NL	3		1		2		
Reynolds 2010[24]	1983-2008	Rochester & LA, USA	14	1	2		9	2	
Park 2010[52]	1989-2006	Seoel, Korea	8				8		
Baalbergen present study	1987-2006	Rotterdam, NL	26	7	6		13		
TOTAL			466	44	149	3	248	6	9

Conservative therapy (conization, simple hysterectomy)
SH simple hysterectomy
RH radical hysterectomy
RT radical trachelectomy
Rec recurrence

correlation between the risk of pelvic lymph node involvement and the risk of paracervical involvement [25]. In contrast to Covens et al[26], Stegeman et al[27], Frumovitz et al[28], Smith et al [29] and Reynolds et all [24] all advocated a less radical surgery in selected patients with early-stage cervical cancer, because of the absence of paracervical/metrial involvement and because trachelectomy is associated with considerable morbidity and obstetrical outcomes following this procedure are not always ideal[30]. A prospective study evaluating the safety and feasibility of conservative surgery (conization or simple hysterectomy) plus PLND in stage IA2 and IB1 is ongoing[31]. Patients with LVSI are excluded.

The question remains whether LVSI is a prognostic factor in microinvasive AC. In the past the SGO staging system excluded lesions with these characteristics based on the hypothesis that SCC lesions with LVSI have a greater propensity to metastasize, which was recently confirmed in a study by Milan et [32]. Hou et al [33] found in their recently published thorough review on

microinvasive adenocarcinoma in stage IA1 with LVSI no LNM, but LVSI was a significant risk factor for recurrence irrespective of lymph node metastases. None of the AC cases with LVSI reported in the literature had lymph node metastases[17]. In our group of 26 patients with stage IA2 16% had LVSI, but with no lymph nodes metastases or recurrent disease. We had one recurrence, in a stage IA1 with LVSI treated with vaginal hysterectomy. Admittedly, with such low numbers, it remains difficult to definitively state the risk of lymphatic metastasis based on presence of LVSI in microinvasive adenocarcinoma of the cervix; however, the combined data suggest it is quite low and radical hysterectomy with or without lymph node dissection does not always cure the patient [9].

In our study of almost 60 patients with microinvasive adenocarcinoma with a long follow up we demonstrate that a conservative approach is safe in microinvasive AC. Although we had 1 recurrence after conservative therapy. This patient had a grade 1 endocervical adenocarcinoma, stage IA1 disease and was treated by simple hysterectomy. Therefore histologic tumor characteristics do not seem capable of explaining why the recurrence rate is higher than could be expected. The recurrence occurred more than one year after primary therapy. Case reports underline that recurrences occur not only in stage IA2 but also in IA1 irrespective of therapeutic modality[34-38]. Therefore we agree with other retrospective studies that a more conservative fertility sparing approach is justified for stage IA1 and IA2 AC. Pooling all data from patients with stage IA1 and IA2, the risk of recurrent disease after conservative therapy was 1.5%, and after radical therapy 2.0%. No difference was found in recurrence after conservative or radical treatment, underlining the fact that conservative therapy is justified. However the question remains, is the excellent survival due to the low stage or due to the aggressive treatment? Even though all studies are retrospective, with relatively small numbers of patients seen over a long time span, we think there is enough evidence to advise not to treat microinvasive AC radically.

Patients with microinvasive AC should be treated in the same way as patients with SCC; in stage IA1 and IA2 AC we recommend conservative therapy (conization). The patient can choose for a hysterectomy. In cases with LVSI, an additional lymphadenectomy is advised. For patients with stage IA2 AC with LVSI a trachelectomy / radical hysterectomy with lymph node dissection could be considered.

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6

GENERAL DISCUSSION

INTRODUCTION

Although the incidence of cervical cancer is decreasing, the relative and absolute incidence of adenocarcinoma of the uterine cervix (AC) en adenocarcinoma in situ (AIS) is increasing. As they are becoming more common, they are being recognized more frequently. A great deal is known about squamous cell carcinoma of the uterine cervix (SCC) but there are still many questions pertaining to AC and AIS. AC is said to have a worse survival, AC and AIS are recognized with difficulty in screening, they are less often hrHPV positive, radical therapy for AIS and early stage AC is recommended at present, but this is open to debate as reliable figures are lacking.

In chapter 1 the aim of the study is presented. Chapter 2 is an outline on premalignant adenocarcinoma of the uterine cervix with an overview of the literature including optimal therapy. In chapter 3 prognostic factors and markers in adenocarcinoma are described. In chapter 4 hrHPV and its prognostic value in adenocarcinoma and in clear cell carcinoma are discussed. Chapter 5 describes the optimal therapy in early stage and microinvasive adenocarcinoma and proposes the best approach.

CHAPTER 2 – ADENOCARCINOMA IN SITU (AIS) CAN BE TREATED CONSERVATIVELY

AIS is a relatively rare, but increasingly more common precursor of invasive adenocarcinoma of the uterine cervix. In the nineties the incidence of squamous carcinoma in situ of the cervix in the USA was 41.4 per 100.000, whereas the incidence of AIS was only 1.25 per 100.000[1]. Although the overal incidence of AIS remains low, the incidence of AIS has been increasing; this is attributable to improved detection and also an absolute increase. The incidence of AIS increased from 0.2 in the seventies to 1.8/100.000 in the late nineties in the USA[1-3]. There are not many studies investigating the incidence of AIS in the Netherlands. A national study showed a decrease in the incidence of AIS between 1989-2003[4]. We studied the incidence of AIS and AC in the Netherlands by using data collected via PALGA, the nationwide Dutch database for histo- and cytopathology and the national cancer registration IKNL[5]. 2093 cytologic and histologic cases of AIS/severe atypia were registered between 1 January 2003 and 31 December 2012 in the Netherlands. In total, 619 were excluded: 445 cases that developed carcinoma and another 174 cases the cytologic diagnosis of AIS was not confirmed histologically. 1474 cases of AIS remained. The population data for the Netherlands used to calculate European standardized rates (ESRs) were obtained from the database of Statistics Netherlands (https://www.cbs.nl). We found a decreasing incidence for cervical cancer, with its lowest point in 2001-2003. This extra decrease was compensated by a following increase in cadence, probably resulting from a reorganization of the Dutch screening programme for cervical carcinoma[6]. This decrease is attributable to a decrease in the frequency of SCC, the incidence for AC has remained stable with a slight increase after 2009. The incidence for AIS decreased between 1992 and 2004. From 2009 the incidence has been increasing (unpublished data).

Most patients with AIS are asymptomatic and are found by cytologic screening. 42% of our cases were detected by diagnosing glandular abnormalities in cytology (AGC-FN, AIS,

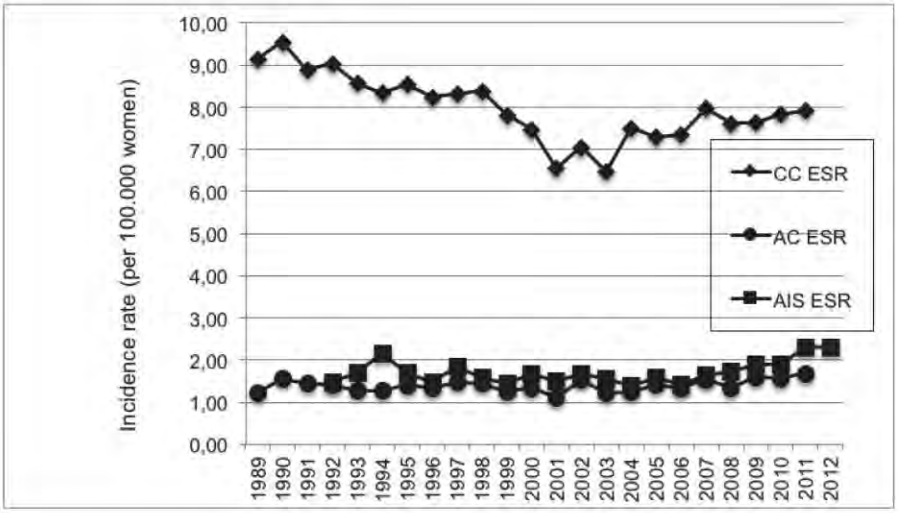

Figure 1. Incidence rate (ESR) of cervical cancer (CC), Adenocarcinoma (AC) and Adenocarcinoma in situ (AIS) in the Netherlands, 1989-2012.

AC). 76% of cases were diagnosed before cold knife conization (CKC) or large loop excision transformation zone (LLETZ) / loop electrosurgical excision procedure (LEEP). hrHPV is the etiologic agent in AIS and in CIN. In particular HPV type 16, 18 and 45 are important in AIS and AC[7,8]. Type 18 is in general more commonly found[2,9,10]. This is confirmed in our study in which 51% of cases harbored this type HPV. Cases with normal cytology taken 5 years before the diagnosis of AIS were hrHPV positive in 70% of cases and in 88% the same type of HPV was present as was found in the AIS, indicating a persistent HPV infection.

In the last few years there has been a trend to more conservative treatment. The main reason to abject a conservative treatment is the high incidence of residual disease after various forms of conization. Achieving negative margins is important in this respect because positive margins are associated with a 3-fold increase in risk of residual disease and a 6-fold increase in risk of recurrence, 3% versus 17%. Previous studies[11] have shown that patients who undergo an LEEP are more likely to have positive margins than those who undergo CKC. As achieving negative margins is so important, a LEEP should not consist of fragments of cervical tissue (chips) but the entire transformation zone and the lesion should be removed in a single pass.

Although after this uterine sparing approach there is a risk of relapse and residual disease with a chance of malignancy, this risk is so low that conservative treatment by a CKC or LEEP with negative cutting edges is justified and justifiable not only for women who wish to have children. These days patients should have the choice between conservative therapy and strict follow up with the small chance of recurrence or definite therapy with hysterectomy.

Follow up after conservative treatment should preferably consist of endocervical cytology and HPV-typing. If abnormalities are detected further colposcopic and histologic examination should be performed.

CHAPTER 3 PROGNOSTIC FACTORS IN AC

3.1 FIGO stage, grade and lymph node metastases are significant prognostic factors in adenocarcinoma of the uterine cervix

Our study on cervical adenocarcinoma in southwest region of The Netherlands shows a similar pattern of survival to that found in previous reports. Survival rates were the highest in patients with early-stage disease, in younger patients and after primary surgery. In early stage disease, we found stage, grade and lymph node status to be of prognostic significance. The literature is consistent with regards to stage and lymph node status as prognostic factors for survival in cervical adenocarcinoma, but inconsistent when it comes to factors such as of grade, histological type, LVSI and age. All studies investigating these characteristics are retrospective. They include different types of patients and some studies excluded adenosquamous carcinomas. Histology was not always reviewed and statistical analysis was not uniform.

The significantly better survival noted in patients younger than 35 years as compared to those over 65 years is the result of a different approach. Radical surgery with or without adjuvant radiotherapy was carried out in 90% of the young patients, whereas in the group >65 years, only 41% underwent radical surgery.

If preoperative factors were of prognostic value, they could be used when deciding on the most optimal therapy. Lymph node status in early cervical adenocarcinoma is a poor prognostic factor. For example, we found LVSI to be predictive of lymph nodes metastases: if LVSI was present, 32% of patients had lymph node metastases and thus had an indication for adjuvant radiotherapy. Furthermore, when LVSI was not found, the chance of lymph node metastases was only 5%.

We found a significantly decreased survival despite adjuvant radiotherapy. The 5-year survival decreased from 90% to 33% when there were positive lymph nodes.

The standard therapy for cervical carcinoma stages I and IIA is radical surgery, but patients were irradiated when their clinical condition was poor due to old age (mean age 65 years in the radiation group vs. 41 years in the surgery group) or coexistent medical problems (obesity, cardiovascular disease). We found that survival was better after primary surgery than after primary radiation therapy. In our study, patients with stage IIA (n = 26) had a 5-year disease-specific survival after primary surgery of 77%, whereas after primary radiation therapy, this was approximately 15%. When comparing these groups, the mean age was significantly higher in the radiation group and the significant difference in survival disappeared after correcting for age. In stage I, we found no difference in survival after primary surgery or radiation therapy.

We found a worse survival in 27 patients in which cervical adenocarcinoma was diagnosed after surgery for other conditions. Despite adjuvant radiation therapy, survival was worse compared to the group who received adequate treatment. This was not seen in patients with SCC.

Since our report in 2004, 9 other studies have been published on prognostic factors in cervical adenocarcinoma. All studies [12-20] confirmed the prognostic value of lymph node metastases on survival and 7 studies also showed prognostic significance of tumor stage, size, parametrial invasion. Tumor grade was only found to be of significant prognostic value in

1 study[15]. Our conclusion that FIGO stage, grade and lymph node metastases are of significant importance for disease free survival in cervical adenocarcinoma, still stands.

3.2 P53 is of significance for survival in cervical adenocarcinoma

Unlike breast cancer and cancer of the uterine corpus where hormone receptor status is of prognostic significance and can determine response to endocrine therapy, the significance of hormone receptor status in adenocarcinoma of the uterine cervix still remains unclear. Our study suggests that it is not of clinical significance to determine estrogen receptor, progesterone receptor, MIB-1 or bcl-2 in cervical adenocarcinomas as an adjunct to determine survival. However, determination of p53 seems useful as p53 positivity appears to be linked to poorer survival in cervical adenocarcinoma, and adjuvant therapy may need to be adjusted. Since our study in 2007 no further studies about prognostic markers as estrogen receptor, progesterone receptor, p53, MIB-1or bcl-2 and AC were published.

CHAPTER 4 HPV IN CERVICAL ADENOCARCINOMA

4.1 HPV-type has no impact on survival of patients with adenocarcinoma of the uterine cervix

This study confirms the hypothesis that almost all early stage AC of the cervix are HPV positive and therefore HPV-testing seems to be a more powerful tool in detection of AC than routine cytologic screening. The majority of AC in our study harboured HPV type 18 (n=55; 54%); 37 (37%) were type 16, 7 (7%) were type 45, and types 53 and 39 were found in 1 patient. This compares well with the literature.

Although the prevalence of HPV in AC is age-dependent, we found a similar mean age in HPV-positive tumours (42.2 years) and HPV-negative tumours (42.8 years), suggesting that the patients in the latter group tested false negative. In our group mean age of women harboring HPV-types 18 or HPV-16 was the same (42.1 and 43.2 years). However, a younger mean age of patients with HPV type-45 positive carcinomas (40.7 years) was noted in this study. We found a worse prognosis in women with AC harboring HPV-18 than in women who's AC were associated with HPV-16. However, this was not statistically significant. A worse survival was noted in cases with HPV type 45, 5-year survival 57%.

Despite the limitations of analysing retrospective data, the current large study shows that the great majority of AC of the cervix is hrHPV associated. Except for HPV-45, HPV type does not seem to have a prognostic impact on patient survival. However, this conclusion should be verified in a larger study.

4.2 High-risk human papillomavirus seems not involved in DES-related and of limited importance in non-DES-related clear-cell carcinoma of the cervix (CCAC).

In a relatively large group of CCAC we showed that hrHPV has a limited carcinogenic role. In summary, we limited our conclusions to the 21 of 28 fully analyzed CCAC. A causal role of hrHPV could not be identified in any of the 10 DES-related tumors. Overall, three tumors were

propably caused by a transforming hrHPV infection. Two were found in women not exposed to DES (2/8) and in one woman no information about DES-exposition was available (1/3). In the remaining 8 tumors (6 in DES-unexposed women and 2 in women with an unknown exposure) the etiology remains unclear, leaving room for other, unexplored factors in its carcinogenesis.

CHAPTER 5 THERAPY IN AC

5.1 Early stage adenocarcinoma (Figo stage I and II) should be treated by surgery

We conducted a systematic review conform the Cochrane guidelines to evaluate if there was objective proof for the hypothesis that surgery was a better option than radiation therapy in the treatment of early stage AC. The quantity and quality of the evidence was scarce as only one RCT was found (Landoni 1997).

Analysis of a subgroup of the single RCT showed that surgery for early-stage AC was better than radiotherapy. However, the majority of the surgery group patients required adjuvant radiotherapy, which was associated with greater morbidity. Furthermore, radiotherapy was not optimized and surgery was not compared to chemoradiation, which is currently recommended in most centers. Finally, modern imaging techniques (MRI, PET-CT), allow for better patient selection enabling node-negative patients to be more easily identified for surgery, thereby reducing the risk of morbidity associated with surgery and adjuvant radiotherapy. In conclusion, we recommend surgery for early-stage AC of the uterine cervix in carefully staged patients. Whereas primary chemoradiation remains the second best alternative for patients unfit for surgery, chemoradiation probably is the first choice in patients with (MRI or PET-CT-suspected) positive lymph nodes. Since the last version (2010) of this review no new studies were found (2013).

There is a need for well-designed RCTs comparing primary surgery versus primary radiotherapy plus concurrent chemotherapy for early AC. This can only be carried out in women who do not need fertility-sparing treatment.

5.2 Microinvasive adenocarcinoma (Figo stage IA1 And IA2) should be treated conservatively

In our study of almost 60 patients with microinvasive adenocarcinoma of the cervix a long follow up was available, we demonstrated that a conservative approach is safe. Although we found 1 recurrence after conservative therapy. This patient had a grade 1 endocervical adenocarcinoma, stage IA1 disease and was treated by simple hysterectomy. Therefore histologic tumor characteristics do not seem capable of explaining why the recurrence rate is higher than could be expected. The recurrence occurred more than one year after primary therapy. Case reports underline that recurrences occur not only in stage IA2 but also in IA1 disease irrespective of therapeutic modality[21-25]. Therefore our study is in line with other retrospective studies that demonstrate that a more conservative fertility sparing approach is justified in stage IA1 and IA2 AC. Pooling all data from patients with stage IA1 and IA2, the risk of recurrent disease after conservative therapy was 1.5%, and after radical therapy 2.0%. This means that no difference

was found in recurrence after conservative or radical treatment; underlining the fact that conservative therapy is justified. The remaining question is however; is the excellent survival due to the low stage of the carcinoma or due to the aggressive treatment? Even though all studies are retrospective, with relatively small numbers of patients diagnosed over a long time span, we think that there is enough evidence to conclude that microinvasive AC should not be treated radically with radical hysterectomy with lymph node dissection. Patients with microinvasive AC should be treated in the same way as patients with SCC; in stage IA1 and IA2 AC we recommend conservative therapy (by radical conization in a single pass). The patient should be given the choice between hysterectomy and conization. In cases with LVSI, an additional lymphadenectomy is advised. For patients with stage IA2 AC with LVSI a trachelectomy / radical hysterectomy with lymph node dissection could be considered.

CONCLUSION

As a general conclusion based on these studies we think that although they are relatively uncommon, but increasing in incidence, AIS and early stage adenocarcinoma of the uterine cervix harbour, when they are correctly diagnosed, the same prognosis as CIN and early SCC and therefore should be treated in the same way. In AIS a radial cone by CKC or LEEP is obligatory and should really be radical with negative resection margins for conservative treatment. Microinvasive AC should be treated as microinvasive SCC. For stage IA1 and IA2 this means a radical conization (by CKC or LEEP), except in cases in which LVSI is present. Patients should be allowed to choose between radical therapy and conservative therapy with follow up. Surgery is the treatment of choice in early stage AC.

Stage, grade and LNM are the most important factors for survival in AC. AIS and AC are hrHPV related, especially type 18 and 16 and 45 are implicated. However in DES-related cervical clear-cell carcinomas HPV seems not involved. As more than 90% of AIS and AC are HPV-18 and 16 related, it can be expected that prophylactic HPV-vaccination may lead to an impressive reduction in the incidence of AIS and AC comparable to what can be expected in SCC.

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7

SUMMARY & SAMENVATTING

SUMMARY

The current study was designed to give more insight in adenocarcinoma of the uterine cervix. The more common cervix carcinoma, squamous cell carcinoma, has been thoroughly studied and published; etiology, diagnosis, therapy, prognosis etc. AC used to be a rare cervical tumor, only 5% of all cervical cancers, lately its incidence has been increasing to 20%. AC was said to have a worse survival and therefor more radical therapy was adjusted. The incidence of AC has not declined as the incidence of SCC after starting the screeningsprogramms, which was due to the inability to detect abnormalities in glandular cells in cervical cytology.

In chapter 2 we studied the precursor of AC, adenocarcinoma in situ. AIS is relatively rare but increasingly frequent. We evaluated the diagnostic and therapeutic strategies and follow-up in a large series of women with pre-invasive cervical adenocarcinoma, and we investigated whether HPV typing in previous cytology that has been classified as normal would have helped to detect AIS earlier. Beside, we conducted a review of literature if therapeutic strategies in AIS could lead to more conservative approach. We found that conservative treatment of AIS by cold knife conization (CKC) or Large Loop Excision Transformation Zone (LLETZ) or Loop Electrosurgical excision procedure (LEEP) with negative cutting edges is justified and justifiable not only for women to have children. Nowadays patients should chose whether they want a strict follow up with the small change of recurrence or definite therapy with hysterectomy. Although AIS was in almost ¾ of patients found by pap smear screening, HPV testing might be superior to cytology in screening for AIS.

In chapter 3 we studied prognostic factors for survival in cervical adenocarcinoma. Our report about cervical adenocarcinoma in South-West region of The Netherlands showed a similar pattern of survival to that found in previous international reports. The best survival rate was for patients with early-stage disease, younger patients and after primary surgery. We found FIGO-stage, grade and lymph node metastases to be of significant prognostic value for survival in cervical adenocarcinoma. In some tumors, like mammacarcinoma it has been shown useful to determine immunohistochemical markers in the tumor for survival. Our study suggests that it is not of clinical significance to determine estrogen receptor, progesterone receptor, MIB-1 or bcl-2 in cervical adenocarcinomas as an adjunct to determine survival. However, determination of p53 seems useful since p53 staining is a marker for survival. p53 positivity appears to be linked to poorer survival in cervical adenocarcinoma, and adjuvant therapy may need to be adjusted.

Chapter 4 we described HPV in AC. We found that AC is hrHPV related in most cases (89%). Subtyping showed HPV-18 as the most frequent type (54%), 37% were HPV-16, 7% HPV-45 and 1% were type 53 and 39. With the exception of HPV-45, HPV-positivity or type in endocervical AC has no significant influence on survival. Clear cell carcinoma (CCAC) is a relatively rare cervical tumor. Approximately 60% of all CCAC are associated with intra-uterine diethylstilbestrol (DES) exposure. Previous studies demonstrated that the association between hrHPV positivity and cervical clear-cell adenocarcinoma (CCAC) varies between 0% and 100%. We determined in a cohort of both DES-exposed and DES-unexposed women with CCAC the prevalence of hrHPV

infections, and the potential etiological role of hrHPV by additional analysis of p16INK4a and p53 expression. Although the prevalence of hrHPV was high, only two DES-unrelated CCAC (25%) and one tumor in a woman with unknown exposure could be attributed to hrHPV. In none of the 10 DES-related tumors a causal role of hrHPV could be identified.

Chapter 5 therapy in AC

For early squamous cell carcinoma of the uterine cervix, the outcome is similar after either primary surgery or primary radiotherapy. There are reports that this is not the case for early adenocarcinoma (AC) of the uterine cervix. We conducted a systematic review conform the Cochrane guidelines. We found one randomized controlled study where they compared the effectiveness and safety of primary surgery for early stage AC of the uterine cervix with primary radiotherapy or chemoradiation. We concluded that for early stage cervical cancer of the glandular cell type (adenocarcinoma) surgery is recommended. Second best alternative for patients unfit for surgery is chemoradiation. For patients with suspected positive lymph nodes, chemoradiation is probably the first choice.

Microinvasive adenocarcinoma is divided in stage IA1, which is defined as a tumor, who invades to a depth of 3 mm or less with 7 mm or less horizontal spread and stage IA2, defined as stromal invasion of more than 3 mm and less than 5 mm with a horizontal spread of 7 mm or less. We evaluated the treatment and follow-up in a large series of women with MIC. Extensive treatment such as radical hysterectomy with pelvic lymph node dissection or trachelectomy does not prevent recurrent disease. Patients with microinvasive AC should be treated identically to patients with SCC. In stage IA1 and IA2 AC, we recommend conservative therapy (by conization). In cases with LVSI, an additional lymphadenectomy is advised. For patients with stage IA2 AC with LVSI, a trachelectomy/radical hysterectomy with lymph node dissection should be considered.

Finally, we concluded that AIS and early stage AC, rare but increasingly existing, especially in young women, harbour the same prognosis as CIN and early SCC and therefore should be treated as such.

SAMENVATTING

De huidige studie werd ontworpen om meer inzicht te krijgen in baarmoederhalskanker van het cilinderceltype (adenocarcinoom, AC). Het meest voorkomende carcinoom van de baarmoedermond, het plaveiselcelcarcinoom (SCC), is grondig bestudeerd en gepubliceerd; etiologie, diagnose, therapie, prognose etc. Het AC is een zeldzame tumor, halverwege de vorige eeuw was slechts 5 % van alle gevallen van baarmoederhalskanker een AC. Er werd altijd beweerd dat het AC een slechtere overleving zou hebben dan het plaveiselcelcarcinoom en daarom werd er meer radicale therapie toegepast. Sedert het invoeren van het bevolkingsonderzoek naar baarmoederhalskanker is de incidentie van het AC is niet afgenomen, zoals bij het SCC. Dit komt doordat afwijkingen van cilindercellen niet goed worden vastgesteld in het uitstrijkje. De laatste tijd is de incidentie van het AC toegenomen tot 20 %.

In hoofdstuk 2 beschrijven we de voorloper van het adenocarcinoma, het adenocarcinoom in situ (AIS). AIS is relatief zeldzaam, maar komt steeds vaker voor. We evalueerden de diagnostische en therapeutische strategieën en follow-up in een grote reeks van vrouwen met een AIS en onderzochten we of HPV-typen gevonden in voorgaande jaren afgenomen uitstrijkje dat werd geclassificeerd als normaal, zou hebben geholpen om AIS eerder detecteren. Tevens werd een systematisch literatuuronderzoek uitgevoerd om te beoordelen of therapeutische strategieën in AIS zouden kunnen leiden tot een meer conservatieve aanpak. Wij vonden dat de conservatieve behandeling van AIS door conisatie of LEEP met negatieve snijranden gerechtvaardigd is. Niet alleen voor vrouwen met kinderwens. Tegenwoordig moeten patiënten kunnen kiezen of ze een baarmoedersparende behandeling met strikte follow-up en met een kleine kans van recidief willen of kiezen voor een definitieve behandeling met hysterectomie. Hoewel AIS in bijna driekwart van de patiënten wordt gevonden door middel van een uitstrijkje, zou HPV-testen superieur zijn aan screening door middel van uitstrijkjes voor AIS.

In hoofdstuk 3 onderzochten we prognostische factoren voor overleving in het adenocarcinoom. Ons verslag over adenocarcinoom in Zuidwest Nederland toonde een vergelijkbaar patroon van de overleving met die in eerdere internationale studies. De beste kans op overleving was er voor patiënten met een vroeg stadium van de ziekte, bij jongere patiënten en na primaire chirurgie. We vonden FIGO-stadium, graad en lymfekliermetastasen de belangrijke prognostische factoren voor de overleving bij het adenocarcinoom. Bij de behandeling van sommige tumoren, zoals het mammacarcinoom is het nuttig om immunohistochemische markers te bepalen, die invloed hebben op de overleving. Onze studie suggereert dat het niet zinvol is om oestrogeenreceptor, progesteronreceptor, MIB - 1 of bcl - 2 in het adenocarcinomen te bepalen omdat zij geen significante invloed op de overleving hebben. Echter de bepaling van p53 lijkt wel nuttig omdat p53-positiviteit verband lijkt te houden met een slechtere overleving in het adenocarcinoom, zodat de aanvullende behandeling zou moeten worden aangepast.

In hoofdstuk 4 beschrijven we de bijdrage van hrHPV in het adenocarcinoom. We vonden dat AC meestal hrHPV gerelateerd was (89%). Subtypering toonde HPV-18 als het meest frequent

(54%) , 37% was HPV-16 , 7% HPV-45 en 1% type 53 en 39. Met uitzondering van HPV-45 , heeft HPV-positiviteit in AC heeft geen significante invloed op de overleving .

Het heldercellige adenocarcinoom van de baarmoedermond (CCAC) is een relatief zeldzame tumor. Ongeveer 60 % van alle CCAC zijn geassocieerd met intra - uteriene diethylstilbestrol (DES) blootstelling. DES is een synthetisch vervaardigd oestrogeen dat vroeger (onterecht) werd gebruikt om miskramen te voorkomen. Eerdere studies toonden aan dat de associatie tussen hrHPV-positiviteit en baarmoederhalskanker van het clear cell adenocarcinoom type (CCAC) varieert tussen 0 % en 100 % . We bepaalden in een cohort van zowel DES-gerelateerde en niet-DES gerelateerde vrouwen met CCAC de prevalentie van hrHPV infecties en de mogelijke etiologische rol van hrHPV door aanvullende analyse van $p16^{INK4a}$ en p53 expressie. Hoewel de prevalentie van hrHPV hoog (46,4%) was, toonde onze studie een zeer bescheiden rol van hrHPV in de carcinogenese van CCAC. Geen van de hrHPV geassocieerde tumoren werd aangetroffen in vrouwen die intra-uterien waren blootgesteld aan DES en in de overige vrouwen bleek slechts een minderheid (20-25%) door hrHPV veroorzaakt.

Hoofdstuk 5 beschrijft de therapie van het adenocarcinoom.

Voor het vroegstadium plaveiselcelcarcinoom van de baarmoedermond is het succes van een primaire behandeling (operatie of radiotherapie) gelijk. Er zijn studies dat dit niet het geval is voor het vroegstadium adenocarcinoom (AC) . We verrichtten een systematische review conform de Cochrane richtlijnen. We vonden één gerandomiseerde gecontroleerde studie waarin men de effectiviteit en veiligheid van primaire chirurgie met primaire radiotherapie of chemoradiatie van het AC vergeleek. We concludeerden dat voor een vroegstadium baarmoederhalskanker van het cilinderceltype (adenocarcinoom) chirurgie wordt aanbevolen. Het beste alternatief voor patiënten die niet geopereerd kunnen worden, is bestraling gecombineerd met chemotherapie. Voor patiënten met verdenking op positieve lymfeklieren is chemoradiatie waarschijnlijk de eerste keus .

Het microinvasive adenocarcinoom (MIC) is onderverdeeld in stadium IA1 en IA2. Stadium IA1 is gedefinieerd als een tumor met infiltratiediepte van 3 mm of minder en een horizontale diameter van 7 mm of minder. Stadium IA2 is gedefinieerd als een tumor met infiltratiediepte tussen de 3 mm en 5 mm en met een horizontale diameter van 7 mm of minder . We evalueerden de behandeling en follow - up in een groot aantal van de vrouwen met MIC . Uitgebreide behandeling zoals radicale hysterectomie met verwijdering van de lymfeklieren in het bekken of trachelectomie (verwijdering van de baarmoedermond en –hals) verhindert niet de terugkeer van de ziekte . Patiënten met MIC moeten net zo behandeld worden als patiënten met microinvasief SCC . In stadium IA1 en IA2 AC adviseren wij conservatieve therapie (door middel van conisatie) . In gevallen met een uitzaaiing in de lymfklieren (LVSI) is een lymfadenectomie geadviseerd. Voor patiënten met stadium IA2 AC met LVSI, moet een trachelectomie of een radicale hysterectomie met lymfeklierdissectie overwogen worden.

Tot slot concluderen we dat AIS en het vroeg stadium AC, hoewel zeldzaam doch in toenemende mate voorkomend vooral bij jonge vrouwen, dezelfde prognose als het voorstadium (CIN) en het vroegstadium SCC hebben. Eenzelfde behandeling ligt dan ook voor de hand.

8

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ABOUT THE AUTHOR

Astrid Baalbergen was born on August 26st 1966 in 's Gravenhage, the Netherlands, as the eldest doughter of Elsbeth Wulf and Johannes Pieter Baalbergen. In 1984 she graduated from high school (Christelijk Lyceum) in Gouda. Although only a mean 6,1 she was directly signed by lottery for Medical School at the Erasmus University Rotterdam. During this study she was student-assistant in Otoneurologic research (dr AGM van Vliet). At the end of the study she did a 3-month research in *Thromboxane synthetase inhibitor ridogrel in the normotensive late-pregnant ewe* (AJ Schneider & prof dr HCS Wallenburg). During her co-schappen (professional training) she worked as a weekend-resident at the obstetric department in the Clara Hospital Rotterdam en after graduation from Medical school in 1991, she started her residency at that place. Dr JA Wijnen (# 2012) encouraged her to become a gynecologist and in 1992 she started, in the beginning as a resident-not-in-training in Obstetrics & Gynecology in Erasmus University Hospital Rotterdam (dr WM Huisman, dr F Huikeshoven & dr FKL Lotgering). The training started June the first of 1993 (prof AC Drogendijk & prof HCS Wallenburg). To improve her surgical skills she operated on her day off with dr J Schram in Zwijndrecht. After three years, the training was continued in the St Franciscus Gasthuis Rotterdam (dr ATh Alberda). In 1998 decided to do a differentiation in gynecological oncology in the Daniel den Hoed Oncology Center Rotterdam (prof ThJM Helmerhorst). After obtaining her registration as a consultant in Obstetrics & Gynecology in 1999, she started a fellow ship gynecological Oncology in the Erasmus Medical Center- Daniel den Hoed (prof ThJM Helmerhorst, prof CW Burger & dr A Ansink). Several radical surgery operations were taught there, but also laser of the vulva, internships radiotherapy (AMC) and oncology trainings, attending at international gynecological oncology meetings and EORTC and Cochrane. 2002 she started working as a gynecologist in the Reinier de Graaf Hospital Delft, with a focus on gynecological oncology.

The basis of the scientific cooperation with prof Helmerhorst was laid in 1998 in a patient with adenoma malignum, a particular form of adenocarcinoma of the cervix. This resulted in a lecture for the regional gynecologists and laid the foundation for this thesis about adenocarcinoma of the uterine cervix. Pathological support was initial with Dr S Chadha-Ajwani, followed by dr P Ewing-Graham and later dr Frank Smedts, my co-promotor.

2004 she met the father of her children, Milco Linssen, which was effectuated one year later in the birth of Roderick Maximiliaan (*11.05.2005) and later on in the birth of Maurits Diederick (*23.05.2008). He is a very proud and caring husband (of she is a very proud en caring wife).

During the few hours of leisure time, Astrid loves to go out shopping in European cities such as London and Milano. She loves the music of the Rolling Stones, is a great fan of the

Burberry and last but not least she loves a glass of champagne. She has a lot of good friends, due to her warm, open and generous character.

Knowing Astrid is loving Astrid...
Her paranymphs,
Francine Oldenburg & Mieke Kerkhof

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