Pergamon

European Journal of Cancer Vol. 32A, No. 8, pp. 1349–1353, 1996

Printed in Great Britain
0959–8049/96 $15.00+0.00

PII: S0959-8049(96)00089-5

Original Paper

Human Papilloma Virus Has No Prognostic Significance in Cervical Carcinoma

G.B. Kristensen,[1] F. Karlsen,[2] A. Jenkins,[3] J. Kærn,[1] V.M. Abeler[2] and C.G. Tropé[1]

Departments of [1]Gynaecologic Oncology and [2]Pathology, The Norwegian Radium Hospital, N-0310 Oslo; and [3]Telelab Ltd, N-3701 Skien, Norway

The prognostic significance of the detection of HPV (human papilloma virus) DNA in cervical carcinoma was evaluated in 223 cases treated from January 1988 to November 1989. HPV DNA was detected by PCR (polymerase chain reaction) on fresh tumour specimens obtained before therapy was started. HPV DNA of any type was detected in 93.3% of all tumours, HPV16 was the predominant type and was detected in 69% of cases. HPV18 was more frequent in adeno- and adenosquamous carcinoma than in squamous cell carcinoma and occurred more often in poorly differentiated tumours than in more highly differentiated tumours. Patients with HPV negative tumours were on average older than patients with tumours containing HPV. Neither presence of HPV DNA nor HPV type had prognostic significance. In 63 women with early stage tumours submitted to surgery, no difference was found in the frequency of lymph node metastasis, vessel invasion or prognosis related to HPV type. We conclude that neither the presence nor the type of HPV DNA had any prognostic significance in cervical carcinoma.

Key words: HPV, papilloma, cancer, cervical, prognosis
Eur J Cancer, Vol. 32A, No. 8, pp. 1349–1353, 1996

INTRODUCTION

THERE IS considerable evidence from both epidemiological and experimental studies that human papilloma virus (HPV) is an aetiological agent of cervical cancer [1]. Some studies have suggested that the presence of nucleic acids from HPV of specific types in the tumour constitutes a prognostic marker of disease severity and outcome [2–4]. These studies have found that women with HPV negative tumours have a poorer prognosis than those with HPV-positive tumours. However, the evidence remains conflicting as some studies have found HPV detection to be unrelated to prognosis [5–8] and still others have reported reduced survival among women with HPV18 positive tumours [9, 10].

The aim of the present study was to evaluate the significance of the presence of nucleic acids from HPV on disease severity and outcome in cervical cancer using PCR on fresh tumour specimens from a large group of patients.

Correspondence to G. B. Kristensen.
Received 30 Aug. 1995; revised 15 Jan. 1996; accepted 16 Jan. 1996.

PATIENTS AND METHODS

Study population

From January 1988 to November 1989, biopsies were obtained for this study from 233 patients with invasive cervical cancer treated at our department. All patients were staged according to FIGO criteria [11]. In patients submitted to surgery, the diameter of the tumour was measured by inspection and palpation. Before treatment, biopsies were selected from viable parts of the tumour and transected. One specimen was sent for histopathological confirmation of the diagnosis and another was stored immediately in liquid nitrogen for HPV analysis. Only patients with a histological diagnosis of squamous cell, adenosquamous or adenocarcinoma were included in the study. 10 cases had to be excluded because the amount of extracted DNA did not allow for complete HPV testing, leaving 223 cases for the study. The clinical and histopathological characteristics of patients in the study group are presented in Table 1.

Treatment consisted of conisation or abdominal hysterectomy for stage IA with stromal invasion of 3 mm or less without vessel invasion, and radical hysterectomy according to Wertheim with pelvic lymphadenectomy in all other stage IA, in stage IB and IIA. In 13 cases, pre-operative intracavitary

Table 1. Univariate analysis of prognostic factors in the total group of patients

	Number of patients			
	Total $n = 223$	HPV positive $n = 208$	Relative hazard	95% confidence interval
FIGO stage				
I	77	74	1.0	(reference)
II	85	77	2.7	1.4–5.0
III	50	47	8.6	4.7–15.9
IV	11	10	20.1	8.8–45.9
	Likelihood ratio statistic on 3 df = 76.469, $P < 0.001$			
Histological type				
Squamous cell	188	174	1.0	(reference)
Adenocarcinoma	19	19	0.9	0.4–1.8
Adenosquamous	16	15	0.8	0.3–1.8
	Likelihood ratio statistic on 2 df = 0.497, $P = 0.78$			
Grade of differentiation				
Well	10	9	0.27	0.07–1.04
Moderate	141	133	0.70	0.47–1.05
Poor	72	66	1.00	(reference)
	Likelihood ratio statistic on 2 df = 6.162, $P = 0.046$			
Age group				
<50	88	85	1.0	(reference)
50–69	97	92	1.9	1.2–3.1
≥70	38	31	3.4	2.0–5.8
	Likelihood ratio statistic on 2 df = 19.911, $P < 0.001$			

irradiation was given. Postoperatively, 12 patients with lymph node metastasis, were given external pelvic radiation with 50 Gy in 25 fractions.

Patients not eligible for surgery and patients with cervical cancer stage IIB and IIIA were given both intracavitary and external irradiation. Patients with tumours in stage IIIB and IVA had either combined intracavitary and external irradiation or external irradiation only. In 3 cases, neoadjuvant chemotherapy was given as part of a randomised study evaluating the benefit of chemotherapy given before irradiation [12]. The chemotherapy regimen used was cisplatinum 100 mg/m^2 on day 1 and 5-FU (5-fluorouracil) 1000 mg/m^2 days 1–5 every 21 days for three courses. Patients with cervical cancer stage IVB had palliative treatment with irradiation, in 2 cases supplemented with cisplatinum/5-FU.

After treatment, patients were followed up at our department or at local hospitals. Follow-up information up to 31 December 1994 was available from all patients, either from medical records or from the cancer registry of Norway. In total, 101 (45.3%) patients died of cervical cancer, 3 were alive with disease, 4 died of intercurrent disease and 115 were alive without evidence of disease. Median duration of follow-up for patients still alive was 62 months (range 49–72 months).

Histopathological evaluation

Routine histological evaluation was performed on haematoxylin and eosin stained sections. Staining for mucin was performed in all cases. The tumours were classified according to WHO [13] and graded as well, moderately or poorly differentiated. In cases where surgery was performed, the total specimen was evaluated. The depth of invasion into the cervical wall, presence or absence of direct extension into the parametria or vaginal cuff, vessel invasion and presence or absence of metastasis to pelvic lymph nodes were assessed.

PCR analysis

PCR (polymerase chain reaction) analysis of 133 of the samples has been published previously [14]. Analysis of the remaining samples was conducted using modified primers oli1b (TGY AAA TAT CCW GAT TAT WT) and oli2i (GTA TCI ACI ACA GTA ACA AA) which are generic primers for the *HPV L1* gene [14]. The only significant difference in performance of the modified primers is that, in contrast to the previous primer set described, the use of oli1b/oli2i allows sensitive detection of HPV18 without recourse to specific primers. Sample DNA was prepared for PCR as previously described [14]. Amplifications were performed on a Hybaid TR1 thermal cycler (Hybaid, Cambridge, U.K.), using the following programme: denaturation, 95°C, 30 sec; annealing, 30°C, 30 sec; extension 72°C, 90 sec (40 cycles). Reactions were conducted in a reaction volume of 100 µl containing 5 ng/µl of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl pH 9.0, 1% Triton X-100, 1.5 units Taq polymerase (Promega, Madison, U.S.A.) and 1 µg (10 µl) of sample DNA. Negative controls (DNA from human lymphocytes) and positive controls (10× serial dilution of HPV16 plasmid DNA from 10^4 to 10 copies in 1 µg human chromosomal DNA) were included in each PCR run to control for the reaction's sensitivity and freedom from contamination. To prevent carry-over contamination, reagent preparation, sample treatment/PCR set-up, and analysis of PCR products were rigorously segregated in three separate rooms with dedicated equipment.

PCR products of HPV types 16, 18 and 33 were identified

by hybridisation to type-specific oligonucleotide probes from the HPV region *L1* as described previously [14]. All samples were subjected to this analysis, even where a PCR product was not visible by gel electrophoresis. Samples testing HPV negative and samples with a PCR product of the expected size (323 bp) that did not hybridise to any of the specific probes were further tested using three pairs of consensus primers. The My primers My09 and My11 in *L1* [15] were used with the following final 50 μl PCR solution: 500–2000 ng of the DNA, 50 mM Tris–HCl pH 8.4, 10 mM KCl, 2.0 mM $MgCl_2$, 1% β mercaptoethanol, 0.05% bovine serum albumin, 0.2 mM of each dNTP, 20 pmol of each primer and 2.5 units of Taq polymerase (Boehringer Mannheim). The Gp primers Gp5+ and Gp6+ also in L1 [16], were used under the same conditions as the My primers but with the following exceptions: 1.5 mM $MgCl_2$ and 50 pmol of each primer. The Cp primers in E1 [17] were used under the same conditions as the My primers, but with the following exceptions: 3.0 mM $MgCl_2$ pH 8.8, 17 pmol of Cp-I and 26 pmol of Cp-IIG. The My and Gp+ primers were run with 40 cycles and Cp with 35 cycles. HPV 11, 16, 18, 31 and 33 type-specific primers from the HPV region *E6-E7* were used as described previously [18], i.e. similarly as for My primers but using 1.5 mM $MgCl_2$. The type-specific PCR was performed in sequences: all cases were tested for type 16 and 18, other types in negative cases only.

All the PCR products from My, Cp, Gp and E6-E7 type specific primers were analysed on 7.5% acrylamide gels stained with SYBR Green I. Samples testing HPV negative in all tests were tested for the presence of intact DNA using primers for chromosome 3 [19]. Samples negative by this test were excluded from the study.

Statistics

Difference in proportions were evaluated by the chi-square or Fisher's exact test, whichever appropriate. All *P* values given are two-sided. Cancer related survival was calculated from start of treatment to death of disease or 31 December 1994, using the method of Kaplan and Meier [20]. A Cox proportional hazards regression model [21] was used for both univariate and multivariate evaluation of survival rates. The hazard proportionality was verified by computing the log cumulative hazard against time. The EGRET statistical package (SERC, Seattle, Washington, U.S.A.) was used for the survival statistics. Statistical significance was considered as $P < 0.05$.

RESULTS

Clinical and histopathological characteristics of patients in the study are presented in Table 1 together with HPV status. Using the oli primers, HPV of any type was detected in 181 (77.6%) of all tumours and HPV16 in 58.4%. Out of 52 samples testing negative with the oli primers, HPV of any type was found in 27 with further testing with My, Cp and Gp consensus primers and E6-E7 type-specific primer (14 HPV16, 2 HPV33 and 11 of unidentified HPV type), while 15 remained negative and the amount of available DNA did not allow for further testing in 10 cases. Of 27 samples found positive on further testing, 16, 14 and 9 samples amplified with the My, Cp and Gp primers, respectively, while 5 samples amplified with type-specific primers only. The prevalence of HPV of any type in the 223 fully evaluated cases was 208/223 (93.3%). HPV16 was the predominant type and was detected in 69%, as single infection in 137 cases and as double infection together with HPV18 or HPV33 in an additional 17 cases. HPV18 was detected as single infection in 26 cases and as double infection together with HPV16 or HPV33 in additional 11 cases. HPV33 was detected in 13 cases as single infection and in 9 additional cases together with HPV16 or HPV18. HPV11 was seen in one case and HPV of unidentified type was present in 13 cases.

HPV16 was present in 132/188 (70.2%) squamous cell carcinomas, in 11/16 (68.8%) adenosquamous carcinomas and in 11/19 (57.9%) adenocarcinomas. HPV18 was detected more frequently in adenocarcinoma (8/19 = 42.1%) and adenosquamous carcinoma (7/16 = 43.8%) than in squamous cell carcinoma (22/188 = 11.7%) ($P = 0.0001$, Fisher's exact test). There was a higher frequency of HPV18 in poorly differentiated tumours than in more highly differentiated tumours, 18/72 versus 19/151, respectively, $P = 0.036$, Fisher's exact test). There was no difference in the distribution of FIGO stage for any of the tested HPV types. There was a highly significant correlation between age and FIGO stage, as 58% of women less than 50 years of age had disease in stage I compared to 19% of women of 50 years of age or above ($P < 0.0001$, chi-square).

The median age of the total group of patients was 56.5 years (range 21.7–89 years). Women with HPV negative tumours were older than women with tumours positive for HPV of any type ($P = 0.006$, Wilcoxon test), the median ages being 67.6 and 54.5 years, respectively. Within the group of women with HPV positive tumours, there was no significant difference in age related to HPV type.

The results of univariate analysis of the prognostic significance of FIGO stage, histological type, grade of differentiation and age is shown in Table 1, given as relative hazard (RH) with 95% confidence intervals. FIGO stage was of primary importance as expected. Age and grade of differentiation was significant, while histological type was not.

Corrected survival related to HPV type is shown in Figure 1, and to presence/absence of HPV in Figure 2. There was no significant difference related to HPV type or between patients with HPV positive or negative tumours. In multivariate analysis, only FIGO stage and grade of differentiation had independent prognostic significance (Table 2). When evaluated on the results obtained by the oli primers and type-specific primers, women with HPV negative tumours had a 1.6-fold increased risk of dying of cervical cancer compared to women

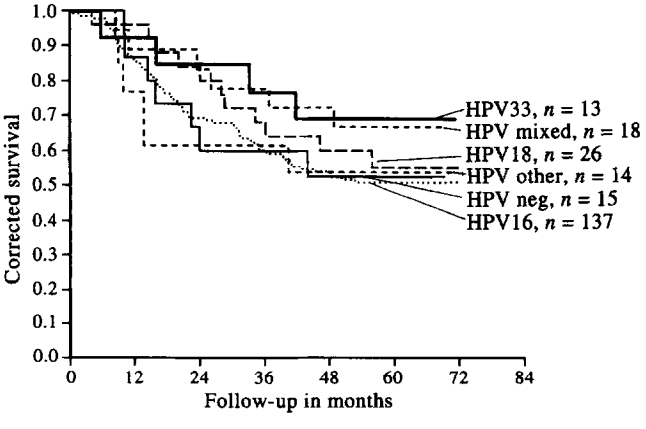

Figure 1. Corrected survival related to HPV type. HPV mixed includes cases with HPV DNA of more than one of the types 16/18/33. HPV other includes cases with HPV DNA different from type 16, 18 or 33.

Table 2. Multivariate analysis of factors of independent prognostic significance

	P value	Relative hazard	95% confidence limits
Total group of patients			
FIGO stage	<0.00001	1.71	1.49–1.95
Grade of differentiation	0.041	1.45	1.02–2.07
Patients submitted to radical hysterectomy			
Tumour size	0.009	4.79	1.49–15.40
Invasion into parametria	0.056	6.00	0.95–37.80
Grade of differentiation	0.068	3.60	0.91–14.26

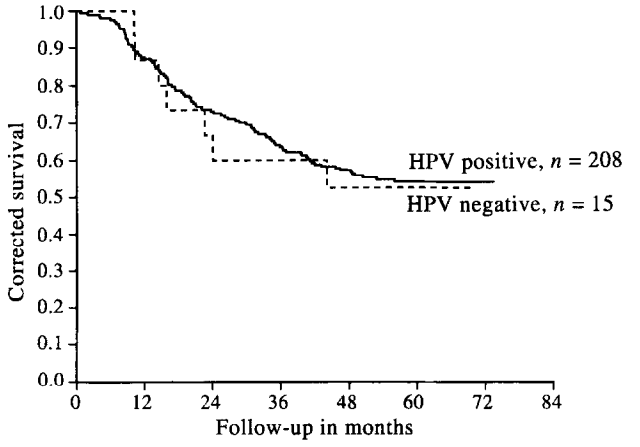

Figure 2. Corrected survival related to presence/absence of HPV DNA.

with tumours containing any type of HPV ($P = 0.035$, RH = 1.6, 95% confidence limits 1.03–2.38) in multivariate analysis including stage, grade of differentiation and age. Based on the complete HPV analysis, no difference existed.

A separate analysis was performed on 63 patients submitted to Wertheim hysterectomy with pelvic lymphadenectomy. 2 patients had tumours in stage IA, 56 in stage IB and 5 in stage IIA. There were no differences in tumour size, vessel invasion or lymph node metastasis between patients with tumours containing HPV18 or HPV of any other type (data not shown). In univariate analysis, tumour size ($P = 0.007$) and grade of differentiation ($P = 0.03$) had prognostic significance, while HPV status did not, neither when categorised as presence/absence of HPV DNA of any type nor when different HPV types were evaluated. Invasion into parametria was at the margin of significance ($P = 0.056$). In multivariate analysis (Table 2), tumour size ($P = 0.009$) obtained independent prognostic significance, while invasion into parametria ($P = 0.056$) and grade of differentiation ($P = 0.068$) were of marginal significance.

DISCUSSION

HPV of any type was detected in 93.3% and HPV16 in 69% of 223 cervical cancer cases using a PCR technique utilising a single set of primers as a first line testing, and then using a broader spectrum of primers on those cases testing negative in the first line testing. This is in accordance with our experience in a separate study on 146 cervical carcinoma cases, using three different pairs of consensus primers [22] where HPV DNA was detected in 96% of cases. Using the oli consensus primers, we detected HPV DNA in 75% of cases, a detection rate in accordance to most published reports [4, 7, 23, 28] using a single set of PCR primers. The new PCR technique utilising multiple primers directed against different regions of the HPV genome allowed for a substantial increase in the HPV detection rate. This increase was mostly due to a higher detection rate for HPV16, but also to detection of rarer HPV types. This was possibly accomplished by identification of cases with deletions in *L1*, as these cases may be missed if the deletion is located to the target area of a single primer pair. Similar results have been reported by others [24].

We found women with HPV negative tumours to be on average older than women with tumours containing HPV. Age has been proposed as a prognostic factor in cervical cancer, with younger women having a poorer prognosis [25]. A similar trend was found in a large, population-based Norwegian study, but the difference was only minor, and statistically non-significant [26]. In univariate analysis, we found the relative hazard of dying of disease increased with age. This was a reflection of increasing disease stage with increasing age as age was non-significant in multivariate analysis when stage was included.

Consistent with other reports [2, 3, 8, 9, 28], HPV18 was more common in adenocarcinoma and adenosquamous carcinoma than in squamous cell carcinoma. In survival analysis, histological type was non-significant in both univariate and multivariate analysis, as also was the presence of HPV18.

HPV18 has been associated with indicators of poor prognosis [9, 10, 27]. We found tumours containing HPV18 to be associated with a higher frequency of poor differentiation, a prognostic indicator that did obtain independent significance in the multivariate analysis. We did not find HPV18 associated with other indicators of poor prognosis, such as invasion into lymph/vascular spaces or lymph node metastasis, and the survival for patients with tumours containing HPV18 did not differ significantly from that of other patients, neither in the total group nor in the subgroup submitted to radical hysterectomy with pelvic lymphadenectomy. This is in accordance with the findings of Riou and associates [2] and Higgins and associates [3]. In a study on 433 cases in stage I–II, treated with radical hysterectomy and bilateral pelvic lymphadenectomy, Chen and colleagues [28] found HPV in 79% of cases and found a higher incidence of lymph node metastasis in HPV positive than in HPV negative cases in squamous cell carcinomas but not in adenocarcinomas, with no difference between HPV16 and HPV18 positive cases. They thought it unlikely that the presence of HPV would be of prognostic significance in cervical cancer, but did not report follow-up data. In a series of 107 invasive squamous carcinomas of the cervix from Sweden, we found patients with

HPV18 or HPV33 to have a poorer prognosis than patients with tumours containing other HPV types [29]. This study employed a different HPV detection methodology and paraffin-embedded specimens. The present study could not reconfirm an adverse outcome for patients with tumours containing HPV18 or HPV33.

We did not find HPV status of the carcinomas to be of any prognostic significance, either when evaluated as positive/negative nor when HPV type was considered. This is in accordance with the findings of von Bommel and coworkers [23] in a study of 64 cases matched for lymph node metastasis, and several other reports [5–8] comprising relatively few patients. In contrast, Riou and associates [2], Higgins and associates [3] and DeBritton and associates [4], reported that HPV negative cervical cancer cases had a 2.6-fold [2], 3.5-fold [3] and 1.9-fold [4] excess risk of dying from cervical cancer in studies containing 106, 212 and 200 patients, respectively. These studies had detection rates for HPV of any type of 75 to 81%, and for HPV16 of 53 to 55%, similar to our test results obtained with the oli primers. Based on these results, HPV negative tumours seem to be associated with a 1.6-fold increased risk of death of disease, but after further testing with multiple PCR primers, this difference disappeared. These results and the comparison of our results with those of Riou and colleagues [2], Higgins and colleagues [3] and DeBritton and colleagues [4] suggest that it might be precisely those circumstances where HPV detection is most difficult (e.g. highly deleted genomes, low HPV copy numbers or the presence of certain rare HPV types) where HPV DNA is of negative prognostic significance.

In conclusion, HPV DNA was detected in 93.3% of cervical cancer cases using multiple PCR consensus primers directed against *L1* and *E1* and type-specific PCR primers directed against *E6–E7*. The presence and the type of HPV in the tumour had no prognostic significance.

1. Franco EL. Viral etiology of cervical cancer: a critique of the evidence. *Rev Infect Dis* 1991, **13**, 1195–1206.
2. Riou G, Favre M, Jeannel D, Bourhis J, Le Doussal V, Orth G. Association between poor prognosis in early-stage invasive cervical carcinomas and non-detection of HPV DNA. *Lancet* 1990, **335**, 1171–1174.
3. Higgins GD, Davy M, Roder D, Uzelin DM, Phillips GE, Burrell CJ. Increased age and mortality associated with cervical carcinoma negative for human papillomavirus RNA. *Lancet* 1991, **338**, 910–913.
4. DeBritton RC, Hildesheim A, De Lao SL, Brinton LA, Sathya P, Reeves WC. Human papillomaviruses and other influences on survival from cervical cancer in Panama. *Obstet Gynecol* 1993, **81**, 19–24.
5. Ji HX, Syrjänen S, Klemi P, Chang F, Tosi P, Syrjänen K. Prognostic significance of human papillomavirus (HPV) type and nuclear DNA content in invasive cervical cancer. *Int J Gynecol Cancer* 1991, **1**, 59–67.
6. Jarrell MA, Heintz N, Howard P, *et al.* Squamous cell carcinoma of the cervix: HPV 16 and DNA ploidy as predictors of survival. *Gynecol Oncol* 1992, **46**, 361–366.
7. Kenter GG, Cornelisse CJ, Jiwa NM, *et al.* Human papillomavirus type 16 in tumor tissue of low-stage squamous carcinoma of the uterine cervix in relation to ploidy grade and prognosis. *Cancer* 1993, **71**, 397–401.
8. King LA, Tase T, Twiggs LB, *et al.* Prognostic significance of the presence of human papillomavirus DNA in patients with invasive carcinoma of the cervix. *Cancer* 1989, **63**, 897–900.
9. Walker J, Bloss JD, Liao S-U, Berman M, Bergen S, Wilczynski SP. Human papillomavirus genotype as a prognostic indicator in carcinoma of the uterine cervix. *Obstet Gynecol* 1989, **74**, 781–785.
10. Kristensen GB, Sebbelov AM, Norrild B, Holund B. HPV types 11, 16 and 18 in squamous cell neoplasia of the lower genital tract. *J Vir Dis* 1993, **1**, 15–20.
11. Pettersson F. Annual report on the results of treatment in gynecologic cancer. FIGO 21, Radiumhemmet, S-104 01 Stockholm, Sweden, 1991.
12. Sundfør K, Bertelsen K, Høgberg T, Onsrud M, Tropé CG, Simonsen EA. Randomised multicenter study of induction chemotherapy in patients with squamous cell carcinoma of the uterine cervix. *Eur J Cancer* 1991, Suppl. 2, abstract 718.
13. Poulsen H, Taylor C. *Histological Typing of Female Genital Tract Tumors*, No 13. Geneva, World Health Organization, 1975.
14. Kristiansen E, Jenkins A, Kristensen G, *et al.* Human papillomavirus infection in Norwegian women with cervical cancer. *APMIS* 1994, **102**, 122–128.
15. Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM. The use of polymerase chain reaction amplification for detection of genital human papillomaviruses. *Cancer Cells* 1989, 7, 209–214.
16. Snijders PJF, Van den Brule AJC, Schrijnemakers HFJ, Snow G, Meijer CJM, Walboomers JMM. The use of general primers in the polymerase chain reaction permits the detection of a broad spectrum of human papillomavirus genotypes. *J Gen Virology* 1990, **71**, 173–181.
17. Tieben LM, Ter Schegget J, Minaar RP, *et al.* Detection of cutaneous and genital HPV types in clinical samples by PCR using consensus primers. *J Virol Meth* 1993, **2**, 265–280.
18. Hagmar B, Johansson B, Kalantari M, Petersson Z, Skyldberg B, Walaas L. The incidence of HPV in a Swedish series of invasive cervical carcinoma. *Med Oncol Tumor Pharmacother* 1992, **9**, 113–117.
19. Karlsen F, Rabbits PH, Sundresan V, Hagmar B. PCR-RFLP studies on chromosome 3p in formaldehyde-fixed, paraffin-embedded cervical cancer tissues. *Int J Cancer* 1994, **58**, 787–792.
20. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958, **53**, 457–481.
21. Cox DR. Regression models and life tables. *J R Stat Soc (B)* 1972, **34**, 187–220.
22. Karlsen F, Kristensen G, Holm R, Chitemerere M, Hagmar B. High incidence of HPV in 146 cervical carcinomas. A study using three different pairs of consensus primers, also detecting genomes with putative deletions. *Eur J Cancer* 1995, **31A**, 1511–1516.
23. Van Bommel PFJ, Van Den Brule AJC, Helmerhorst TJM, *et al.* HPV DNA presence and HPV genotypes as prognostic factors in low-stage squamous cell cervical cancer. *Gynecol Oncol* 1993, **48**, 333–337.
24. Van den Brule AJC, Walboomers JMM, Du Maine M, Keenemans P, Meijer CJLM. Difference in prevalence of human papillomavirus genotypes in cytomorphologically normal cervical smears is associated with a history of cervical intraepithelial neoplasia. *Int J Cancer* 1991, **48**, 404–408.
25. Rutledge FN, Mitchell MF, Munsell M, Bass S, McGuffee V, Atkinson EN. Youth as a prognostic factor in carcinoma of the cervix: a matched analysis. *Gynecol Oncol* 1992, **44**, 123–130.
26. Bjørge T, Thoresen SØ, Skare GB. Incidence, survival and mortality in cervical cancer in Norway, 1956–1990. *Eur J Cancer* 1993, **29A**, 2291–2297.
27. Barnes W, Delgado G, Kurman RJ, *et al.* Possible prognostic significance of human papillomavirus type in cervical cancer. *Gynecol Oncol* 1988, **29**, 267–273.
28. Chen TM, Chen Ca, Wu CC, Huang SC, Chang CF, Hsieh CY. The genotypes and prognostic significance of human papillomaviruses in cervical cancer. *Int J Cancer* 1994, **57**, 181–184.
29. Hagmar B, Christensen J-JP, Johansson B, *et al.* Implications of human papillomavirus type for survival in cervical squamous cell carcinoma. *Int J Gynecol Cancer* 1995, **5**, 341–345.

Acknowledgement—This study was supported by a grant from The Norwegian Cancer Society.