Prognostic value of preoperative serum CEA level compared to clinical staging: II. Stomach cancer. *Br J Cancer* 1982, **45**, 718.
17. Wagener C, Müller-Wallraf R, Nisson S, Gröner J, Breuer H. Localization and concentration of carcinoembryonic antigen (CEA) in gastrointestinal tumor; correlation with CEA levels in plasma. *J Natl Cancer Inst* 1981, **67**, 539.
18. Shibata Y, Tamura K, Ishida N. *In vivo* analysis of the suppressive effects of immunosuppressive acidic protein, a type of α_1-acid glycoprotein, in connection with its high level in tumor-bearing mice. *Cancer Res* 1983, **43**, 2889.
19. Yip WCL, Tay JSH, Ho TE, Wong HB. Computers in paediatrics. 18. Medical decision making. Computer program to calculate sensitivity, specificity, false positive and negative rates, positive and negative predictive values and accuracy of a diagnostic test. *J Singapore Paediatr Soc* 1986, **28**, 74.
20. Colli A, Buccino G, Cocciolo M, Parravicini R, Mariani F, Scaltrini G. Diagnostic accuracy of sialic acid in the diagnosis of malignant ascites. *Cancer* 1989, **63**, 912.

Acknowledgement—We thank Miss A. Shimada for technical assistance.

Eur J Cancer, Vol. 27, No. 2, pp. 193–197, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Human Papillomavirus DNA in Cervical Intraepithelial Neoplasia Detected by *in situ* Hybridisation

Maria Rosaria Cardillo, Raffaele Marino and Vincenzo Pozzi

Human papillomavirus (HPV) infection was investigated by *in situ* hybridisation in histological sections from 38 women with abnormal Papanicolaou smears. 13 patients had condylomatous lesions without atypia, 15 cervical intraepithelial neoplasia (CIN) I, 4 CIN II, 3 CIN III and 2 carcinoma *in situ* (CIS). HPV DNA was detected in 29 cases (78%) (1 specimen was technically inadequate). HPV 16 and 18, and 31, 33 and 35 were both present (67%) in CIN III. HPV 6 and 11 were more frequent in CIN I (56%) and in condylomatous lesions (38%). 31% of the condylomatous lesions without atypia contained HPV 31, 33, and 35 and 31% of those with CIN I were infected with HPV 16 and 18. These data confirm the frequent association of HPV infection with cervical cancer and CIN, and indicate that *in situ* hybridisation can identify patients with specific types of HPV infection at risk for cervical cancer.
Eur J Cancer, Vol. 27, No. 2, pp. 193–197, 1991.

INTRODUCTION

SINCE 1976, when Meisel [1] first described cytohistological changes caused by human papillomavirus (HPV) in cervical epithelium, this infection has been diagnosed in 1–2% of women undergoing general screening [2] and in 5–10% of high-risk women being screened for cancer of the cervix [3]. In younger women, condylomatous changes generally occur without atypia; in women over 30, koilocytes are usually associated with various degrees of cervical intraepithelial neoplasia (CIN) and carcinoma *in situ* (CIS) [4].

Many low-grade lesions regress to normal or remain stable; others progress to dysplasia or invasive carcinoma. Although the mechanism underlying malignant conversion is unknown, current evidence implicates the type of HPV. Whether integration of HPV DNA by DNA in the host cell, other infective or viral agents, genetic predisposition, or the host's immunological defences influence the course of the infection is not known [5]. Because CIN develops faster in women infected with HPV [6], this high-risk group requires regular follow-up. With the use of DNA hybridisation, more than 60 HPV DNA types so far isolated have been detected in the uterine cervix. HPV 6 and 11 frequently coexist with cervical condylomata [7] and with low-grade intraepithelial neoplasia (CIN I) [8], but rarely with carcinoma. HPV 16, 18 and 31 have been found in all grades of CIN and also in invasive carcinomas of the cervix [9]. In worldwide reports, the virus most strongly associated with cervical intraepithelial neoplasia is HPV 16 [10], which is also associated with an increased risk of conversion from low-grade to high-grade CIN [11]. HPV was detected in 20% of CIN cases in the USA [12].

We have analysed HPV DNA in specimens from 38 intraepithelial cervical lesions, by *in situ* DNA hybridisation. The study was designed to detect HPV, identify the types present (HPV 6 and 11, 16 and 18, and 31, 33 and 35) and correlate the results with the histological appearance of the lesions.

Correspondence to M.R. Cardillo.
M.R. Cardillo is at the Dipartimento di Biopatologia Umana, Sezione di Anatomia e Istologia Patologica and R. Marino and V. Pozzi are at the Istituto di Clinica Ostetrica e Ginecologica, Università di Roma "La Sapienza", viale Regina Elena 324, 00161 Rome, Italy.
Revised 22 Oct. 1990; accepted 5 Nov. 1990.

MATERIALS AND METHODS

Biopsy specimens were taken from 38 women aged 20–46 years, who were undergoing colposcopy because two consecutive Papanicolaou tests had disclosed atypia (CIN I–III) or CIS. The smears were taken by cervical scraping. Biopsy specimens taken

from the colposcopically abnormal areas were fixed in 10% formalin, embedded in paraffin wax and cut into 2 μm sections. Two sections were stained with haematoxylin and eosin for histological examination; five were left unstained for HPV DNA assay. The Papanicolaou smears were evaluated for abnormal cells according to the Bethesda system [13]: squamous intraepithelial lesions were classed as low-grade (CIN I) (including HPV-associated cell changes with mild dysplasia), high-grade (including moderate dysplasia (CIN II) or severe dysplasia (CIN III) or CIS. A histological diagnosis of condylomata was given for specimens that contained basal cell hyperplasia, koilocytosis of the superficial and intermediate layers, associated with crinkling nuclear degeneration and multinucleation, with dyskeratosis, parakeratosis or keratosis [14–17]. Condylomatous lesions with mitotic figures or atypical nuclei were classed as CIN and graded according to WHO criteria [18]. A histological diagnosis of CIN I, II or III was given for sections that contained anisocytosis, anaplasia and abnormal mitoses, respectively.

In situ hybridisation

Formalin-fixed paraffin-embedded sections for HPV-DNA assays were heated in an oven at 58°C for 30 min to enhance adherence of the cells to the slide. After being cooled to room temperature, the sections were dewaxed by two 10 min incubations in xylene, then immersed twice for 5 min in 100% ethanol and air-dried for 10 min [19, 20]. An HPV tissue hybridisation kit (ViraType *in situ* System, Life Technologies, Gaithersburg, Maryland) with three groups of *in situ* HPV DNA probes was used to detect the presence of HPV 6 and 11, 16 and 18, and 31, 33 and 35.

After incubation in digestion solution for 15 min at 35°C and washing in "Tris"-buffered saline, the sections were dehydrated through graded alcohols and air-dried. A biotinylated probe was then applied to the section.

Five sections from each biopsy specimen were investigated for the presence of HPV DNA: five test and two control probes were used. One probe detected HPV DNA 6 and 11, a second probe detected HPV 16 and 18 and a third detected HPV 31, 33 and 35. To ensure that tissue sections were properly fixed, digested and stained, each HPV type was also investigated with a positive (human genomic DNA) and a negative DNA control probe (pBR322). One drop of probe reagent was placed onto the appropriate tissue section and a coverslip applied. The slides were then put into a heating block that had been prewarmed to 100°C and were incubated for 5 min. Hybridisation was continued in a humidified incubation chamber at 37°C for 2 h. The slides were then rinsed in "Tris"-buffered saline to remove the coverslips, and washed twice in the buffer for 3 min at 37°C. Hybridisation was detected by incubating the sections at 37°C for 20 min in an alkaline-phosphatase reagent conjugate that binds specifically to the probe. Dephosphorylation was done by incubating the slides at 37°C for 60 min in alkaline phosphatase with 5 bromo-4-chloro-3-indolyphosphate (BCIP) as substrate. In the presence of nitroblue tetrazolium (NBI) a purplish-blue precipitate is deposited at the sites of probe hybridisation to HPV DNA. Finally, the sections were counterstained with nuclear fast red to allow for morphological examination and visualisation of target DNA. HPV DNA cell staining is seen as purplish-blue, mainly in the nuclei of infected cells containing target. Lesions were graded according to intensity of the colour and the number of positive cells per high-powered field. The intensity of the reaction was scored as +++ for strongly positive lesions (20–30 positive cells per field), ++ for moderately positive (10–20 cells), + for weakly positive (5–15 cells), and +/− borderline positivity (under 10 cells).

Table 1. *Detection of HPV DNA in cervical intraepithelial neoplasia by* in situ *hybridisation*

Case	Age (yr)	Histological diagnosis	HPV DNA 6/11	HPV DNA 16/18	HPV DNA 31/33/35
1	27	CIN II, condyloma lesion	−	+(*)	+++
2	23	Condyloma lesion	+	−	−
3	34	CIN I, condyloma lesion	+	−	−
4	35	Condyloma lesion	+	−	−
5	26	CIN I, condyloma lesion	−	+++	−
6	38	CIS	−	−	−
7	46	CIS	−	−	−
8	26	CIN I, condyloma lesion	+/−(*)	−	+++
9	30	CIN I, condyloma lesion	+	−	−
10	33	CIN II, condyloma lesion	+++	−	−
11	26	Condyloma lesion	+++	−	−
12	26	Condyloma lesion	+++	−	−
13	20	CIN I, condyloma lesion	+	−	−
14	32	Condyloma lesion	+	−	−
15	26	CIN I, condyloma lesion	−	−	+++
16	46	CIN III, condyloma lesion	−	+++(*)	+
17	40	CIN II, condyloma lesion	−	−	+++
18	22	Condyloma lesion	−	−	−
19	20	Condyloma lesion	−	−	+−
20	31	CIN I, condyloma lesion	−	++	−
21	34	CIN I, condyloma lesion	++	−	−
22	25	CIN I, condyloma lesion	++	−	−
23	23	CIN I, condyloma lesion	++	+/−(*)	−
24	21	Condyloma lesion	+(*)	−	+
25	28	Condyloma lesion	−	−	−
26	22	CIN I, condyloma lesion	−	−	−
27	28	CIN III, condyloma lesion	−	−	+
28	21	Condyloma lesion	−	−	−
29	25	CIN I, condyloma lesion	−	−	−
30	27	Condyloma lesion	−	+/−(*)	+++
31	28	Condyloma lesion	−	−	++
32	30	Condyloma lesion	−	−	−
33	31	CIN I, condyloma lesion	−	+++	−
34	32	CIN I, condyloma lesion	+++	−	−
35	34	CIN I, condyloma lesion	+++	+(*)	−
36	38	CIN II, condyloma lesion	−	++	−
37	39	CIN III, condyloma lesion	−	+++	−

*Cross-reactivity.

RESULTS

Table 1 shows HPV DNA detected in condylomas and CIN in the 37 patients from whom biopsy specimens were taken. 1 sample that contained only stroma was eliminated. In 13 patients the histological diagnosis was condyloma without atypia; 11 were flat condylomata, 1 was an acuminate wart (papillare) and 1 was an inverted condyloma. 15 condylomatous lesions were associated with CIN I, 4 with CIN II, 3 with CIN III, and 2 with CIS. In 3 cases, severe dysplasia had adjacent areas of mild or moderate dysplasia. In 2 other cases moderate dysplasia and slight dysplasia merged (condylomata sometimes overlie or merge with areas of CIN). The 5 patients who had condylomas that had recurred after cryotherapy were infected by the identical HPV type identified in the initial lesion. HPV genomes were detected in 29 of our 37 cases (78%).

In the cervical tissue studied, all positive nuclei were localised

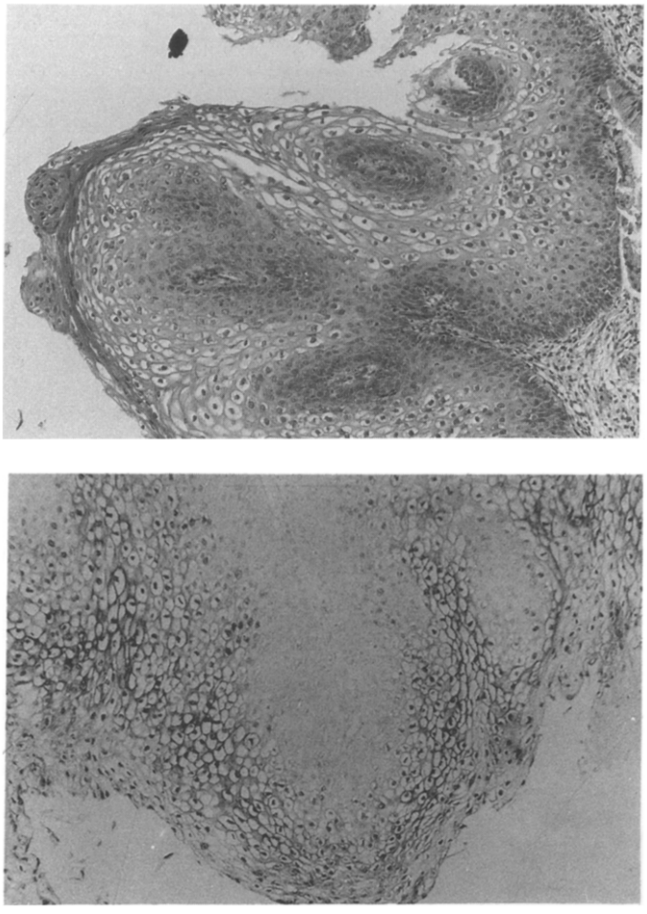

Fig. 1. Upper: acuminate condyloma of uterine cervix (case 24). Squamous epithelium shows koilocytosis, multinucleation, parakeratosis and dyskeratosis. Cellular architecture in the epithelial layers below human papillomavirus changes is not disturbed. Spikes are evident (haematoxylin/eosin, × 5). Lower: *in situ* hybridisation with HPV 6 and 11 DNA probe (nuclear fast red, × 13)

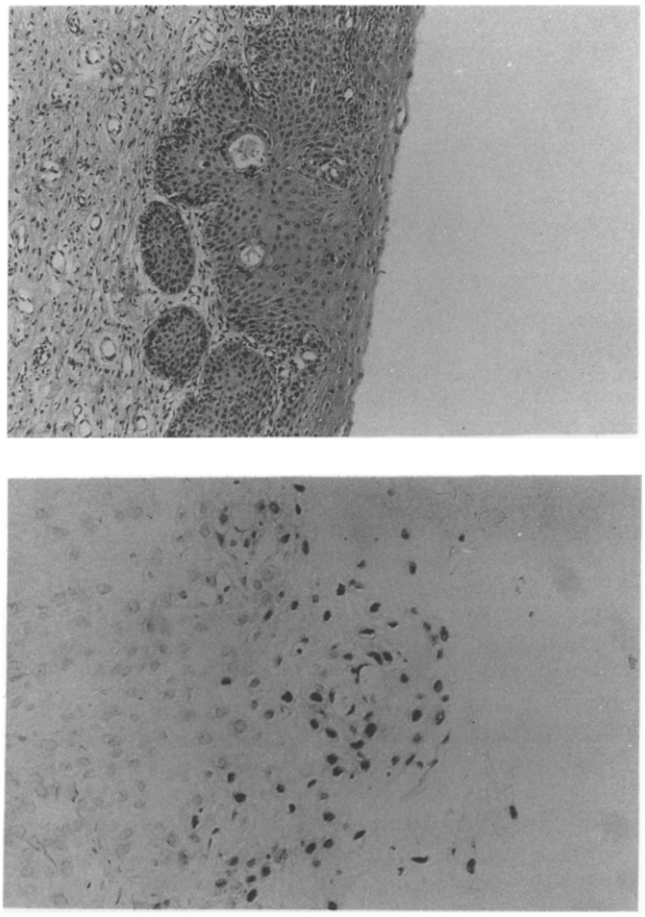

Fig. 2. Upper: flat recurrent condyloma of uterine cervix (case 21). Epithelium shows stromal papillae, koilocytotic changes in upper strata of epithelium (haematoxylin/eosin, × 13). Lower: *in situ* hybridisation with HPV 6 and 11 DNA probe. Distribution of viral sequence can be seen in the superficial squamous epithelium and koilocytic cells (nuclear fast red, × 13)

in the parakeratocytes and koilocytes in the superficial and squamous epithelium (Figs 1 and 2) and in cells that displayed greater squamous differentiation. In cases 16, 18 and 38 (Table 1), in which CIN III adjoined areas of CIN I and II, positive staining was seen in the superficial cells of foci of mild and moderate dysplasia, but not in those of severe dysplasia (Fig. 3). Areas of positive staining alternated with areas of negative reaction.

DISCUSSION

Attempts to localise HPV DNA in intraepithelial lesions (CIN and CIS) and in invasive carcinoma of the cervix have been equivocal. Whereas HPV was reported in 95% of cases of CIN and 89% of invasive carcinomas of the cervix [7, 9, 21], more recent studies with the commonest HPV genotype probes have detected HPV DNA in 73–74% of precursor lesions and in 80% of invasive carcinomas [22]. In worldwide reports HPV 6 is strongly associated with condylomas and low-grade intraepithelial lesions [22, 23]. HPV 16, 18 and 31 are prevalent in high-grade intraepithelial lesions and invasive carcinoma [22–27].

Nevertheless, some low-grade lesions contain HPV 16, 18 and 31 and some high-grade lesions contain HPV 6 and 11. Colgan [27] demonstrated HPV 16 and 31 in normal epithelium adjacent to areas of condylomata and dysplastic cervical intraepithelium.

In contrast to these studies, we found HPV 31, 33 and 35 in 4 of the 13 condylomata, a finding that we are unable to explain. Not all the lesions in our study population were positive for HPV infection. The 8 lesions that did not hybridise may have contained other as yet untyped HPVs [12]. Under the highly stringent conditions of hybridisation in the ViraType technique, HPV DNA might also have escaped detection [25]. Areas of positive staining alternated with areas of negative reaction. This suggests that an overproduction of HPV nucleic acid is correlated with production of viral capsid protein or concentration of the mature virus. Alternatively if production levels of the virus are correlated with the histological evolution of the condyloma, then biopsy specimens would merely reflect one particular moment in the course of the lesion [28]. In 4 cases (8, 16, 23 and 31), although the sample reacted strongly for a specific HPV type, for others it was borderline. The most likely explanation is cross-hybridisation: in these sections, one virus happened to be more strongly expressed than the others. The cases (1, 16, 24, 35) of double hybridisation are presumably due to cross-reactivity or dual infection.

Evidence of HPV infection in condylomatous lesions which have recurred after cryotherapy implies that after local ablation of the wart and the epithelial lesions, HPV genomes may persist in the epithelium. These patients would therefore be likely to develop a neoplasm within the squamous lesion or elsewhere in the lower genital tract [29]. Similarly, the frequency of HPV

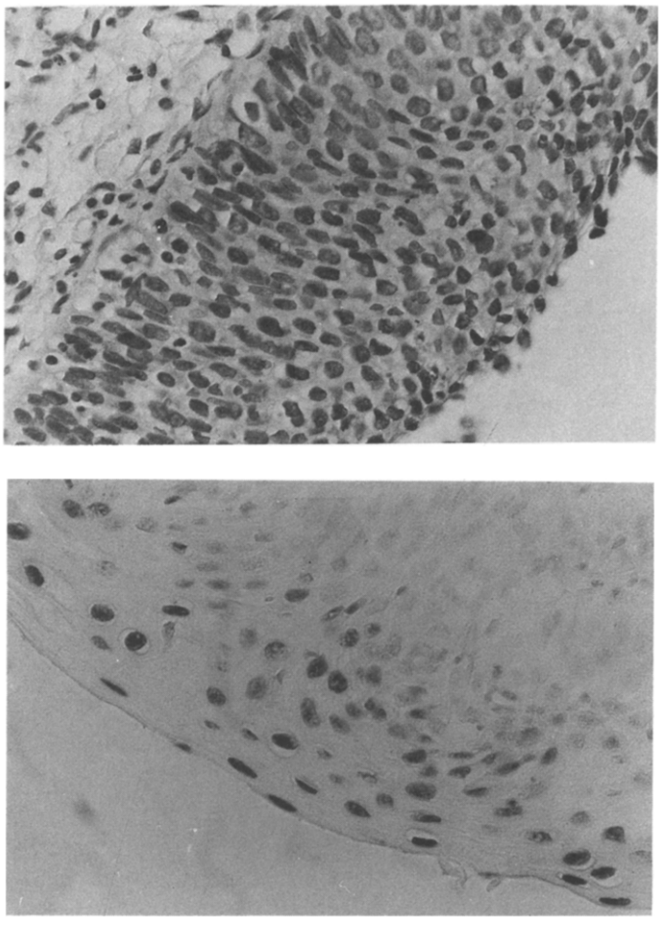

Fig. 3. Upper: CIN grade II (case 16), haematoxylin/eosin, × 13). Lower: *in situ* hybridisation with HPV 16 and 18 DNA probe (nuclear fast red, × 13)

31, 33 and 35 that we observed in condylomatous lesions without atypia (31%), and that of HPV 16 and 18 in CIN I (31%), suggest that condylomas and intraepithelial lesions only arise because of immunosuppression or when a combination of cofactors intervenes [30, 31]. A study of these patients before and after immunomodulator therapy will be reported.

Patients with condylomas therefore need more frequent follow-up. Even if the infective agents are HPV 6 and 11, the likelihood of the infection persisting or evolving is considerable. Despite the limits of *in situ* hybridisation in comparison with Southern blotting [32, 33], we have shown that the *in situ* technique is useful in selecting women at high risk of developing an invasive carcinoma.

1. Meisels A, Fortier R. Condylomatous lesions of the cervix and vagina 1. Cytologic pattern. *Acta Cytol* 1976, **20**, 505–509.
2. Ludwig ME, Lowell DM, LiVolsi VA. Cervical condylomatous atypia and its relationship to cervical neoplasia. *Am J Clin Pathol* 1981, **76**, 255.
3. Jenson AB, Kurman RJ, Lancaster WD. Human papillomaviruses. In: Belsche R, ed. *Textbook of Human Virology*. Littleton, Maryland, PSG Publishing, 1984, pp. 951–968.
4. Fenoglio CM. Viruses in the pathogenesis of cervical neoplasia: an update. *Hum Pathol* 1982, **13**, 785–787.
5. Syrjanen KJ. Current concepts of human papillomavirus infections in the genital tract and their relationship to intraepithelial neoplasia and squamous cell carcinoma. *Obstet Gynecol Surv* 1984, **39**, 252–265.
6. Syrjanen KJ. Female genital tract infections by human papillomavirus (HPV) and their association with intraepithelial neoplasia and squamous cell carcinoma. *Cervix Lower Female Genital Tract* 1984, **2**, 103–126.
7. Gissmann L, Wolnik L, Ikenberg H, *et al.* Human papillomaviruses type 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. *Proc Natl Acad Sci USA* 1983, **80**, 560.
8. McCance DJ, Campion MJ, Clarkson PK, *et al.* Prevalence of human papillomavirus type 16 DNA sequences in cervical intraepithelial neoplasia and invasive carcinoma of the cervix. *Br J Obstet Gynecol* 1985, **92**, 1101–1105.
9. Durst M, Gissmann L, Ikenberg H *et al.* A papillomavirus from a cervical carcinoma and its prevalence in cervical biopsy samples from different geographic regions. *Proc Natl Acad Sci USA* 1983, **80**, 3812–3815.
10. McCance DJ. Human papillomavirus and cancer. *Biochim Biophys Acta* 1986, **ii**, 237–240.
11. Campion MJ, McCance DJ, Cuzick J, Singer A. The progressive potential of mild cervical atypia: a prospective cytological, colposcopic and virological study. *Lancet* 1986, **ii**, 237–240.
12. Lorincz AT, Lancaster WD, Temple GF. Cloning and characterisation of the DNA of a new human papillomavirus from a woman with dysplasia of the uterine cervix. *J Virol* 1986, **58**, 225–228.
13. The 1988 Bethesda system for reporting cervical/vaginal cytologic diagnoses: developed and approved at the National Cancer Institute Workshop, Bethesda, USA, 12–13 December. *Acta Cytol* 1989, **33**, 567–574.
14. Koss LG, Durfee GR. Unusual patterns of squamous epithelium of the uterine cervix: cytologic and pathologic study of koilocytic atypia. *Ann NY Acad Sci* 1956, **63**, 1245.
15. Reid R, Crum CP, Herschman BR, *et al.* Genital warts and cervical cancer. III. Subclinical papillomaviral infection and cervical neoplasia are linked by a spectrum of continuous morphologic and biologic changes. *Cancer* 1984, **53**, 943–953.
16. Meisels A, Morin C, Casas-Cordero M. Human papillomavirus infection of the uterine cervix. *Int J Gynecol Pathol* 1982, **1**, 75–94.
17. Crum CP, Ikenberg H, Richart RM, *et al.* Human papillomavirus 16 and early cervical neoplasia. *N Engl J Med* 1984, **310**, 880–883.
18. *Histologic Typing of Female Genital Tract Tumours*. Geneva, World Health Organization, 1975.
19. Brigati DJ, Myerson D, Learly JJ, *et al.* Detection of viral genomes in cultured cells and paraffin-embedded tissue sections using biotin-labeled hybridization probes. *Virology* 1983, **126**, 32–50.
20. Lewis FA, Griffiths S, Dunnicliff R, *et al.* Sensitive *in situ* hybridization technique using biotin-streptavidin phosphatase complex. *J Clin Pathol* 1987, **40**, 163–166.
21. Boshart M, Gissmann L, Ikenberg H, *et al.* A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. *EMBO J* 1984, **3**, 1151–1157.
22. Collins JE, Jenkins D, McCance DJ. Detection of human papillomavirus DNA sequences by *in situ* DNA–DNA hybridisation in cervical intraepithelial neoplasia and invasive carcinoma: a retrospective study. *J Clin Pathol* 1988, **41**, 289–295.
23. Fukushima M, Okagaki T, Twiggs LB, *et al.* Histological types of carcinoma of the uterine cervix. *Cancer Res* 1985, **45**, 3252–3255.
24. Kadish AS, Burk RD, Kress Y, *et al.* Human papillomaviruses of different types in precancerous lesions of the uterine cervix. Histologic, immunocytochemical and ultrastructural studies. *Human Pathol* 1986, **17**, 384–392.
25. Ostrow RS, Dawn A, Manias BA, *et al.* Detection of human papillomavirus DNA in invasive carcinomas of the cervix by *in situ* hybridization. *Cancer Res* 1987, **47**, 649–653.
26. Wilczynski SP, Bergen S, Walker J, *et al.* Human papillomaviruses and cervical cancer. *Hum Pathol* 1988, **19**, 697–704.
27. Colgan TJ, Percy ME, Suri M, *et al.* Human papillomavirus infection of morphologically normal cervical epithelium adjacent to squamous dysplasia and invasive carcinoma. *Hum Pathol* 1989, **20**, 316–319.
28. Stoler MH, Wilbur DC, Broker TR, *et al.* *In situ* hybridization for HPV in squamous carcinoma and its precursors. *Med Pathol* 1988, **1**, 88A.
29. Wickenden C, Steele A, Malcolm ADB, *et al.* Screening for wart virus infection in normal and abnormal cervices by DNA hybridization of cervical scrapes. *Lancet* 1985, **i**, 65–67.
30. Carson LF, Twiggs LB, Fukushima M, *et al.* Human genital

papilloma infections: An evolution of immunologic competence in the genital neoplasia papilloma syndrome. *Am J Obstet Gynecol* 1986, **155**, 784–789.
31. Blaustein A, Sedlis A. Diseases of the vagina. In: Blaustein A, ed. *Pathology of the Female Genital Tract*, 2nd ed. New York, Springer, 1984, p. 81.
32. Syrjanen SM, vonKrogh G, Syrjanen KJ. Detection of papillomavirus DNA in anogenital condylomata in men using *in situ* DNA hybridisation applied to paraffin sections. *Genitourinary Med* 1987, **63**, 32–39.
33. Beckamann AM, Myerson D, Daling JR, *et al.* Detection and localization of human papillomavirus DNA in human genital condylomas by in situ hybridization with biotinylated probes. *Med Virol* 1985, **16**, 256–273.

Eur J Cancer, Vol. 27, No. 2, pp. 197–200, 1991.
Printed in Great Britain

0277-5379/91 $3.00 + 0.00

Diploid Predominance and Prognostic Significance of S-phase Cells in Malignant Mesothelioma

S. Pyrhönen, A. Laasonen, L. Tammilehto, J. Rautonen, S. Anttila, K. Mattson and L.R. Holsti

70 histologically verified, malignant mesotheliomas were analysed by flow cytometry for DNA content and S-phase fraction (SPF) of tumour cells. 60% (42/70) were DNA diploid. 18 of the 28 aneuploid tumours were near-diploid with DNA indices of 1.3 or less. SPF could be calculated in 51 cases. SPF was significantly higher in aneuploid (median 16.0%) than in diploid tumours (median 5.6%). DNA ploidy was not a prognostic determinant; survival was the same for both aneuploid and diploid tumours. SPF, however, was significantly correlated ($P = 0.039$) with prognosis. Patients who had tumours with a low SPF survived almost twice as long as those with a high SPF. Thus malignant mesothelioma has a peculiar DNA ploidy pattern compared with many other solid tumours, with a predominance of diploid or near-diploid type cells. As in many other tumours, SPF may be used as a clinically relevant prognostic indicator.
Eur J Cancer, Vol. 27, No. 2, pp. 197–200, 1991.

INTRODUCTION

DNA FLOW CYTOMETRY (FCM) analysis of malignant tumours provides information on DNA ploidy and the proliferative activity of tumour cells, which may be correlated with clinical characteristics to identify prognostic factors and to increase knowledge of tumour biology. Malignant mesothelioma is increasing in frequency in most countries, with the use of asbestos between 1940–1970 [1–5]. However, little is known of the biology of mesothelioma, and only limited information is available from DNA FCM [6, 7].

Our group has examined systematically various biological characteristics of malignant mesotheliomas such as chromosomes [8], *in vitro* growth ability [9] and asbestos fibre content [10], as well as clinical aspects of this disease [11]. We use thoracotomy for routine staging in the management of mesothelioma. This provides samples for histological verification as well as for other methods of tissue examination, such as FCM.

For this report we have analysed DNA ploidy profiles and the proliferative activity of 70 histologically verified malignant mesotheliomas by measuring the S-phase fraction (SPF) of tumour cells. We have also correlated FCM parameters with prognosis, with the specific aim of testing the clinical application of FCM in the examination panel for malignant mesothelioma.

PATIENTS AND METHODS

Patients

Tumour samples were obtained from 70 patients with malignant mesothelioma all diagnosed and treated at the Helsinki University Central Hospital between 1978 and 1989. Histological diagnosis and subtyping was done by the panel of the Lung Cancer Cooperative Group of the European Organization for Research and Treatment of Cancer. The patients participated in clinical trials of multimodality therapy [11] consisting of debulking surgery, chemotherapy and hemithorax irradiation (Table 1).

Correspondence to: S. Pyrhönen.
S. Pyrhönen, A. Laasonen and L.R. Holsti are at the Department of Radiotherapy and Oncology, L. Tammilehto and K. Mattson are at the Department of Pulmonary Medicine; and J. Rautonen is at the Department of Pediatrics, Helsinki University Central Hospital, Haartmanikatu 4, S.F. 00290 Helsinki; and S. Anttila is at the Institute of Occupational Health, Helsinki, Finland.
Revised 6 Aug. 1990; accepted 23 Nov. 1990.