ELSEVIER

European Journal of Cancer 40 (2004) 1225–1232

European Journal of Cancer

www.ejconline.com

Detection of cervical precancer and cancer in a hospital population: benefits of testing for human papillomavirus

V. Dalstein[a], D. Riethmuller[b], J.L. Sautière[b], J.L. Prétet[a], B. Kantelip[c], J.P. Schaal[b], C. Mougin[a,*]

[a]*Laboratoire de Biologie Cellulaire et Moléculaire, CHU de Besançon, Hôpital Jean Minjoz, 25030 Besançon cedex, France*
[b]*Clinique Universitaire de Gynécologie, Obstétrique et de la Reproduction, Centre Hospitalier Universitaire, Besançon, France*
[c]*Laboratoire d'Anatomo-Pathologie, Centre Hospitalier Universitaire, Besançon, France*

Received 6 August 2003; received in revised form 15 January 2004; accepted 19 January 2004

Abstract

The aim was to determine the relevance of human papillomavirus (HPV) testing in identifying high-grade cervical intraepithelial neoplasia or worse (CIN2/3+) in a hospital population ($n=3574$) characterised by a high rate of cytological abnormalities and high-risk HPV infections. According to the results of the initial Papanicolaou and HPV test, women were directly referred for colposcopy/biopsy or recalled for a control visit. Sensitivity and specificity were corrected for verification bias. HPV-testing sensitivity was 94.3%, higher than that of cytological testing at any cut-off point (65.1%–86.8%), while specificity was greater for cytology than for HPV testing (99.3% or 91.8% versus 83.4%). The combination of both tests allowed 100% sensitivity and negative predictive value. We conclude that HPV testing is a relevant tool for the detection of cervical disease. The best way of combining cytology and HPV detection in screening programmes should be evaluated in large-scale studies.

Keywords: Human papillomavirus (HPV); Cervical cancer; Screening; Cervical intraepithelial neoplasia (CIN); Pap smear; Hybrid capture II (HCII)

1. Introduction

To date, the detection of precancerous cervical lesions by Papanicolaou (Pap) smear is widely recognised as the most effective method for preventing carcinoma of the cervix. The implementation of cytological screening has led to a major reduction in deaths from cervical cancer in certain countries. In France, the incidence of invasive carcinoma decreased by 3.5% per year during the period 1982–1992, while the numbers of carcinoma *in situ* rose sharply [1].

However, cervical screening based on conventional cytology is far from perfect. In addition to its low sensitivity, conventional cytology has poor reproducibility, even with the introduction of the Bethesda system [2,3]. In the early 1990s, liquid-based cytology was introduced, and large-scale studies demonstrated a slight improvement in the detection of high-grade lesions and carcinomas [4,5]. Moreover, it has advantage of preserving cells, which can be used later for human papillomavirus (HPV) DNA testing.

Indeed, it is now well established that high-risk (HR-) HPV are the main causal agent of intraepithelial and invasive cancer of the cervix [6,7]. However, only persistent infections may be of predictive value for present as well as future cervical intraepithelial neoplasia (CIN) [8,9]. The commercially available Hybrid Capture II (HCII) method for HPV detection, reproducible [10] and sensitive [11], provides objective results and could complement cytology in improving cervical screening. Numerous studies have evaluated HPV testing in primary screening [12–18], for the triage of atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesions (LGSIL) [19,20], and

* Corresponding author. EA3181, Laboratoire de Biologie Cellulaire et Moléculaire/Institut d'Etudes et de Transfert de Gènes, Centre Hospitalier Universitaire,
Besançon, France. Tel. +33-3-81-66-91-11; fax: +33-3-81-66-83-42.
E-mail address: christiane.mougin@ufc-chu.univ-fcomte.fr (C. Mougin).

0959-8049/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2004.01.021

as a predictive marker of residual or recurrent disease following surgical treatment [21]. Although many researchers and clinicians are deeply convinced of the utility of HPV testing [22,23], others are not [24,25]. These matters are also confusing because they involve important financial interests and deeply fixed habits. Currently, the point would be to find the best cost-efficient and clinically effective procedure for improving the prevention of cervical cancer.

In this study, we report the 5-year use of HPV testing in routine clinical practice in a French hospital, located in a county that is also a pilot area for organised cervical screening. Indeed, since 1993, Doubs County has been one of the pilot counties in France for such screening, targeting women between 20 and 65 years of age living in this administrative area [26,27]. Here we compare the results of different combinations of screening tests (standard cytology and HPV testing) in identifying precancerous cervical lesions.

2. Patients and methods

2.1. Study participants

Between August 1997 and July 2002, 9436 cervical specimens from 6691 women were tested for HPV DNA by the HCII system in the Cellular and Molecular Biology Laboratory of Besançon University Hospital. All the women who participated were carefully informed about HPV testing, the benefits of participation and the possibility of being recalled for additional visits. The present analysis included only women attending the Gynaecology Department of the University Hospital of Besançon, because of the well-defined follow up and the comprehensive availability of cytological/histological diagnoses and clinical histories. We excluded women attending physicians outside the hospital ($n=2573$), and those with unavailable cytological results ($n=411$), a previous history of conisation ($n=69$), hysterectomy ($n=5$) or cervical cancer ($n=6$), and infection with the human immunodeficiency virus ($n=19$). Also excluded were pregnant women ($n=22$), women aged <15 years ($n=6$) and women under suspicion for cervical cancer ($n=6$). We also excluded two cases of glandular abnormalities (AGUS). This left 3574 cases with full sets of data suitable for analysis.

At entry into the study, the women underwent a pelvic examination, and two samples of exfoliated cells were obtained from the cervix. The first sample was collected for conventional cytology with a Cytobrush Plus (Medscand Medical, Malmö, Sweden). A second sample was then collected with the Digene Cervical Sampler for HPV testing, placed in a vial containing 1 ml Digene specimen transport medium (STM), and stored at -20 °C until processed.

2.2. Cytology and histology procedures

Cytological and histological samples were analysed in the Cytology and Pathology Department of the University Hospital. Conventional cervical smears were classified according to the Bethesda system as normal (N); ASCUS; LGSIL; high- grade squamous intraepithelial lesion (HGSIL); suggestive of invasive cancer (IC); unsatisfactory smear (UNS). Cervical tissue samples were analysed using the CIN classification. Slides were reviewed in case of disagreement between cytology and histology. Cytopathologists were unaware of the HPV test results.

2.3. HPV DNA analysis

The HCII assay (Digene, Gaithersburg, MD, USA) was performed according to the manufacturer's instructions on the thawed STM with the two specific HPV RNA probe 'cocktails': one for low-risk HPV (LR-HPV) types and the other for HR-HPV types. Samples were considered positive when the ratio RLU/PC was equal or greater than 1.

2.4. Follow-up design

The follow-up algorithm is presented in Fig. 1. When the cytology was normal and the HPV test negative, women returned for standard biennial or triennial screening. Women with a smear showing ASCUS and a negative HPV test were invited for a control visit 6

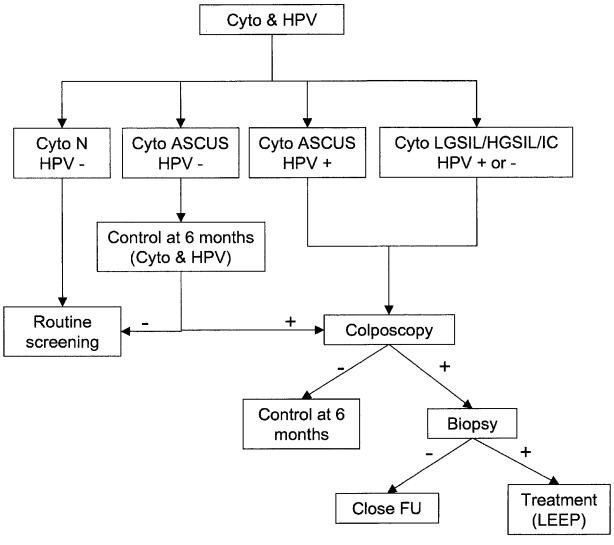

Fig. 1. Follow-up algorithm. ASCUS; atypical squamous cells of undetermined significance; Cyto, cytological testing; FU, follow up; HPV, human papillomavirus; LEEP, loop electro-excision procedure; LGSIL, low-grade squamous intraepithelial lesions; HGSIL, high-grade squamous intraepithelial lesions; N, normal.

months later, with a repeat Pap smear and HPV testing. Then, if the smear still showed abnormal cells, or if the HPV test had become positive, the women were referred for colposcopy and a biopsy was taken from any suspicious area. Women with an ASCUS smear and a positive HPV test were directly referred for colposcopy. Women with a smear suggestive of LGSIL, HGSIL or cancer at entry or at any further visit were immediately recalled for colposcopy, according to the French guidelines, whatever the result of the HPV test. In case of biopsy-confirmed moderate CIN, severe CIN or cancer (CIN2/3+), women were treated by the loop electroexcision procedure (LEEP). Women with discrepant results between cytology and histology were treated if recommended after a review of slides. Otherwise, they were closely followed up.

2.5. Data analysis

Statistical analysis was performed using the STATA Statistical Software (*Release 5.0*; StataCorp. 1997, College Station, TX, USA).

For cytological purposes, data were analysed for two thresholds of cytological positivity: (i) HGSIL or IC; (ii) ASCUS, LGSIL, HGSIL or IC. All histological diagnoses assessed within the 12 months following the screening procedure were taken into account. Histology was considered positive when CIN2/3+ was diagnosed. In cases of disagreement between the various histological diagnoses (biopsy, LEEP, hysterectomy), the worse diagnosis was taken into account.

Analytical characteristics of cytology and HPV testing for detecting CIN2/3+ were calculated. Sensitivity and specificity were estimated from contingency tables. We corrected for verification bias by adjusting the calculation of the screening indexes on the basis of the expected distribution of lesions among women without complete follow up, according to their initial smear and HPV test results. Only corrected values for specificity and sensitivity are presented. The Youden index ($Y = Se + Sp - 1$), which allows a global estimation of sensitivity (Se) and specificity (Sp), was also derived. Positive (PPV) and negative predictive values (NPV) were calculated from the Bayes' theorem, with an estimated CIN2/3+ prevalence of 7.39% for the global population studied (264/3574), and 7.78% for women $\geqslant 30$ years (196/2518). Different screening strategies were evaluated: (i) cytology alone, $\geqslant$HGSIL or $\geqslant$ASCUS threshold; (ii) HPV testing alone; (iii) the combination of cytology ($\geqslant$HGSIL or $\geqslant$ASCUS) and HPV testing. Finally, receiver operating-characteristic (ROC) curves were drawn, plotting sensitivity against (1- specificity) for different possible cut-off points of HPV testing. Three groups of women were considered: the whole population, women aged $\geqslant 30$ years and women aged < 30 years.

3. Results

The mean age of the 3574 women eligible at entry into the study was 37.2 ± 11.6 years (range 15.8–87.5 years).

Pap smears were as follows: 3019 normal (84.47%), 62 ASCUS (1.73%), 249 LGSIL (6.97%), 209 HGSIL (5.85%), 9 suggestive of invasive carcinoma (0.25%) and 26 unsatisfactory (0.73%). LGSIL smears were mostly observed at a young age (15.8% in the 20- to 24-year group), while HGSIL+ peaked between 35 and 39 years (8.8%).

3165 specimens out of 3574 (88.6%) were tested for both HR- and LR-HPV. Among these, 3.6% were positive for LR-HPV exclusively, 18.7% were positive for HR-HPV and 4.8% were positive for mixed low- and high-risk types. The highest rates of HR-HPV infections were found in women 20–24 years of age, while those with LR types as well as mixed infections were the most frequent before the age of 20 years. The rates then decreased, but with a slight increase in the oldest women (Fig. 2). 409 specimens were tested for HR types only. Overall, HR-HPV DNA was detected in 828 out of 3574 specimens (23.2%). The HR-HPV prevalence was significantly higher in women < 30 years old (32.3%) than in those $\geqslant 30$ years (19.3%) ($P < 0.0001$).

Table 1 shows the prevalence of HR- and LR-HPV DNA in relation to cytological results. The rate of HR-HPV infections increased with the severity of the cytological abnormalities, reaching 91.6% in HGSIL and 100% in IC smears. The detection rate for LR-HPV DNA (either alone or associated with HR types) reached 31.7% in LGSIL. None of the eight smears suggestive of an invasive carcinoma harboured LR-HPV.

In the forthcoming analyses, the data will be presented for HR-HPV only. Fig. 3 shows CIN2/3+ diagnosed according to the initial smear and HPV test.

At entry, 2625 women tested double negative (for cytology and HR-HPV). Subsequent routine control visits were registered for 26.7% in a mean period of 29 months (range 6–57 months). While 679 women showed

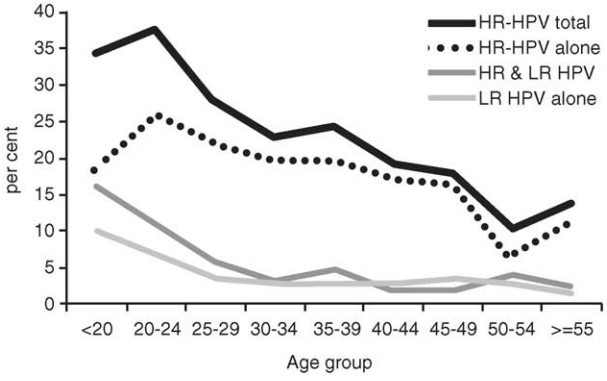

Fig. 2. Age-specific prevalence of high-risk (HR-) and low-risk (LR-) human papillomavirus (HPV) infections in the 3165 women tested with both 'cocktail' probes.

Table 1
Human papillomavirus (HPV) prevalence according to cytological diagnoses in the 3165 women tested with low-risk (LR) and high-risk (HR)-HPV 'cocktail' probes

Pap smear result	HR-HPV total (%)	HR-HPV associated with LR-HPV (%)	LR-HPV alone (%)
N/UNS ($n=2680$)	370 (13.8)	67 (2.5)	96 (3.6)
ASCUS ($n=56$)	30 (53.6)	3 (5.3)	2 (3.6)
LGSIL ($n=230$)	162 (70.4)	58 (25.2)	15 (6.5)
HGSIL ($n=190$)	174 (91.6)	25 (12.6)	1 (0.5)
IC ($n=8$)	8 (100%)	0	0
Total ($n=3165$)	744 (23.5)	153 (4.8)	114 (3.6)

N, normal; UNS, unsatisfactory smear; ASCUS, atypical squamous cells of undetermined significance; LGSIL; low-grade squamous intraepithelial lesion; HGSIL, high-grade squamous intraepithelial lesion; IC, suggestive of invasive cancer.

normal control Pap smears, 23 women (3.5%) were referred to colposcopy and biopsied because of abnormal cytology. No CIN2/3+ lesion was found in this subset of women.

Among the 420 women who tested normal on the Pap smear and were HR-HPV positive, 202 completed the follow up and 19 were diagnosed with a CIN2/3+.

Among the 102 women showing mild cytological abnormalities and a negative HR-HPV test, 87 completed the follow up and four were diagnosed with a CIN2/3+.

Among the 209 women who were diagnosed with an ASCUS or LGSIL and who harboured HR-HPV, 176 completed the follow up and 51 demonstrated cervical disease.

Among the 218 women with a cytological diagnosis of HGSIL or cancer, 199 were HR-HPV positive (91.3%). Most women (97.3%) completed the follow up and 89.6% were diagnosed with histologically proven CIN2/3+. Among these CIN2/3+, HR-HPV DNA was detected in 94.2%.

In all, 264 CIN2/3+ were detected among the 1379 women who completed the follow up (19.1%). Table 2 shows the prevalence of CIN2/3 and cancer according to age group. The highest rate of CIN2/3 was seen in the 35- to 39-year age group (22.3%) and the lowest after the age of 50 years. The prevalence of CIN2/3+ lesions was not statistically significantly different in women younger than 30 years (18.5%) than in older women (19.4%) ($P=0.726$). The highest prevalence of invasive cervical disease was observed in women above 55 years. However, one of the 15 women (range 26–77 years) with a cancer was younger than 30 years.

The sensitivity of HPV testing was higher than that of cytology with the $\geqslant$HGSIL threshold (94.6% versus 65.1%), while the specificity of cytology was better than that of HPV testing (99.3% versus 83.4%) (Table 3). The values obtained for the subset of women aged $\geqslant 30$ years showed slight improved specificity for HPV testing (83.4% up to 85.0%). All strategies demonstrated relatively high PPV, in particular for cytology, which is due to the high prevalence of CIN2/3+ diagnosed among this hospital population ($>7\%$), while NPV were all $>96\%$. When we considered the combination of cytology at an ASCUS+ threshold and HPV testing, sensitivity and NPV reached 100%, in the whole population as well as in women aged $\geqslant 30$ years.

ROC curves comparing the relative performance of the HCII assay in the global population and in women from two age groups ($<$or $\geqslant 30$ years) are shown in Fig. 4. In the whole cohort, the 2 pg/ml cut-off appeared the best, with the best estimated ratio between sensitivity (93.9%) and specificity (85.9%). The optimal capacity

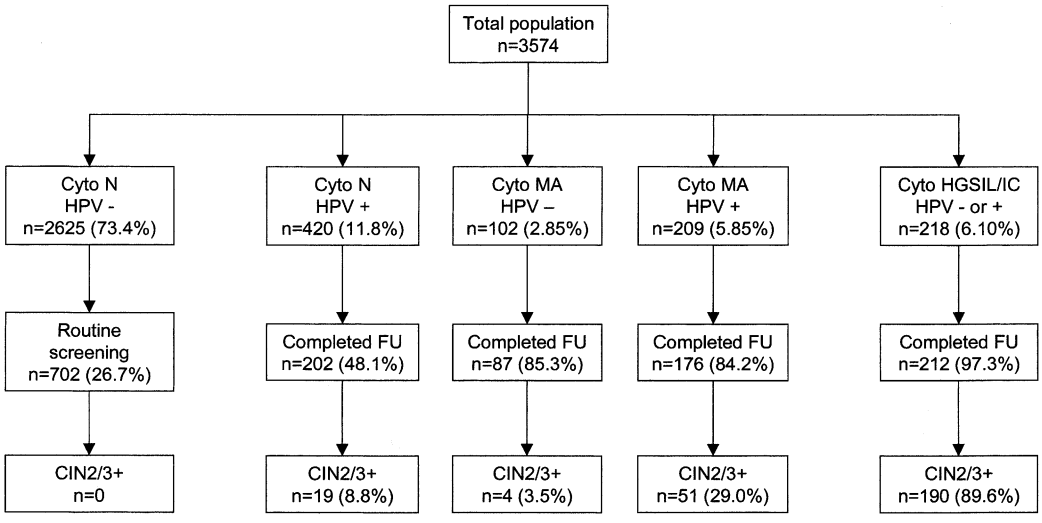

Fig. 3. Patients diagnosed with cervical intraepithelial neoplasia (CIN) 2/3+ within 12 months, according to the initial cytology (Cyto) and human papillomavirus (HPV) test results [MA, mild abnormalities (i.e. atypical squamous cells of undetermined significance (ASCUS) or high-grade squamous intraepithelial lesions (HGSIL)]; FU, follow up).

Table 2
Age-specific prevalence of histologically proven, moderate or severe cervical intraepithelial neoplasia (CIN) 2/3 and invasive cervical cancer (IC) among the 1379 women who completed the follow up

Age group (years)	CIN2/3 (%)	IC (%)
<20 ($n=38$)	5 (13.2)	0
20–24 ($n=143$)	30 (21.0)	0
25–29 ($n=186$)	32 (17.2)	1 (0.5)
30–34 ($n=256$)	48 (18.8)	3 (1.2)
35–39 ($n=264$)	59 (22.3)	3 (1.1)
40–44 ($n=189$	31 (16.4)	3 (1.6)
45–49 ($n=153$)	27 (17.7)	0
50–54 ($n=71$)	8 (11.3)	1 (1.4)
⩾55 ($n=79$)	9 (11.4)	4 (5.1)
Total ($n=1379$)	264 (19.1)	15 (1.09)

of this test to identify cases of confirmed CIN2/3+ in women aged ⩾30 years was also obtained at the threshold of 2 pg/ml, with a similar degree of sensitivity (91.6% instead of 93.2%) and an improved specificity (89.9% instead of 85%). For the subgroup of women aged <30 years, sensitivity remained high even with a cut-off of 10 pg/ml (⩾95.6%), while specificity was decreased.

4. Discussion

Here we report the 5-year routine use of HPV testing in addition to conventional cytology in women attend-

Table 3
Performance of cytology and human papillomavirus (HPV) testing for the detection of cervical intraepithelial neoplasia (CIN) 2/3+

Screening test	Sensitivity (%)	Specificity (%)	Youden index	PPV (%)	NPV (%)
Whole population					
Pap⩾HGSIL	65.1	99.3	0.644	88.1	97.3
Pap⩾ASCUS	86.8	91.8	0.786	45.8	98.9
HPV test	94.6	83.4	0.780	31.2	99.5
Pap⩾ HGSIL+HPV test	98.4	83.2	0.816	31.8	99.9
Pap⩾ASCUS+HPV test	100.0	80.2	0.802	28.7	100.0
Women ⩾30 years					
Pap⩾HGSIL	60.8	99.2	0.600	86.5	96.8
Pap⩾ASCUS	88.1	93.9	0.820	54.9	98.9
HPV test	93.2	85.0	0.782	34.4	99.3
Pap⩾HGSIL+HPV test	98.0	88.3	0.863	41.4	99.8
Pap⩾ASCUS+HPV test	100.0	85.0	0.850	36.0	100.0

PPV, positive predictive value; NPV, negative predictive value; other abbreviations in Table 1 and text.

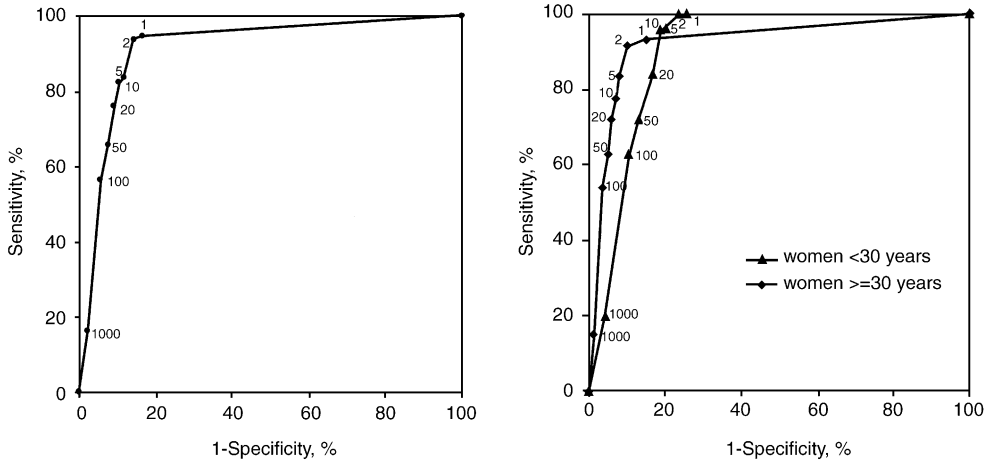

Fig. 4. Receiver operating-characteristic (ROC) curves for the Hybrid Capture II assay for the detection of cervical intraepithelial neoplasia (CIN) 2/3+, in the general population, and in women <30 years and ⩾30 years; sensitivity is plotted against (1-specificity) for each cut-off point of the test.

ing the University Hospital of the Doubs County, and evaluate the performance of both tests in detecting cervical precancer or cancer. Obstetrics/gynaecology specialists of this hospital are referent practitioners in the area for screening, management and treatment of cervical pathology. They are thus in charge of a large majority of the women participating in the Doubs' pilot screening programme and tested with borderline Pap smears.

Our hospital-based cohort therefore represents a population with a high prevalence of abnormal Pap smears. Indeed, the prevalence of cytological abnormalities was about 5-fold higher (15.5%) than in women attending the screening programme (3%) [26].

As a correlative, the overall prevalence of HR-HPV infections was also greater (23.2%) than that described in another French area in relation to primary screening (15.3%) [16]. As in the general population, HR-HPV positivity declined after the age of 25 years, as observed by others [12,13,28]. The rate of HR-HPV infections within each cytological diagnostic category (N, ASCUS, LGSIL, HGSIL+) was also in accord with published rates [16].

The proposed follow-up algorithm is in accord with the pilot screening programme and with French guidelines. The 12-month delay in assessing the histological diagnosis appeared sufficient to avoid the elimination of cases diagnosed belatedly.

The follow-up protocol was so that our physicians/colposcopists would refer, and if necessary biopsy, not only women with abnormal Pap smears, but also those with two successive positive HPV tests. Surprisingly, we noticed that women harbouring HR-HPV DNA and normal Pap were much less compliant with control visits (48.1%) than women with ASCUS or LGSIL smears, whatever their HPV status, negative (85.3%) or positive (84.2%). A similar phenomenon was reported by Kulasingam and colleagues [17]. Nevertheless, Pap-/HPV-positive patients should be followed up closely, given the high rate of CIN2/3+ diagnosed in this group. Among these 19 additional cases, seven were classified as CIN2 and 12 as CIN3. Women with HGSIL/IC smears demonstrated a higher rate of complete follow up (97.3%).

A major limitation of our study could be that the double-negative population was not controlled for disease by random colposcopy. However, no CIN2/3+ could be found in the 22 women who were colposcoped and biopsied because of an abnormal Pap at a subsequent screening test. In addition, we demonstrated in a former longitudinal study that no HPV-negative women, whatever the initial cytological diagnosis, developed CIN2/3+ during a 24-month follow up [9]. Furthermore, in a recent study that did provide such a control of double-negative women, no additional CIN2/3+ case was found in this population [11]. So it is unlikely that the number of putative missed diagnoses in the double-negative population would affect our results in a significant way.

As expected, the sensitivity of HPV testing was higher (94.6%) than that of cytology at any threshold (65.1% for HGSIL+), as already reported by others [12–18]. Indeed, as currently reported, the rate of false negatives for cytology was high: 74 of the 264 cases (28%) would have been missed at the HGSIL+ threshold. Nevertheless, the estimated sensitivity for cytology obtained with the ASCUS+ threshold was rather high (86.8%), which might be due to sampling by skilled gynaecologists and smear processing by experienced cytopathologists. In contrast, the specificity of the HPV test was lower (83.4%) than that of cytology (99.3%–91.8%), but might be acceptable in such a population with a high HPV prevalence. The combination of cytology (ASCUS+) and the HPV test revealed a 100% sensitivity and NPV, at the expense of specificity (80.2%). These estimated values are in accord with recently published data [14,15]. On the other hand, the estimated PPV were especially high compared with current results, the PPV depending greatly on the prevalence of the disease.

The improvement in specificity in women aged 30 years or above was low for the HPV test alone, and somewhat higher for the combined Pap/HPV strategies in our series. This can be explained by a high prevalence of HPV infections in our population, even after 30 years of age (19.3%).

On the basis of our results obtained in women tested for both HR- and LR-HPV genotypes, we confirm that testing for HR-HPV types only is an adequate strategy. Indeed, none of the IC harboured LR-HPV alone, and only two CIN2 harboured LR types alone (0.75%).

Nevertheless, there were 15 CIN2/3+ diagnosed in women in whom HR-HPV was not detected (5.7%). All of these were older women (mean age 45 years; range 37–63 years). We hypothesised that these lesions might be regressive [29]. Additionally, we noticed that for two of them (13.3%), the positive histological diagnosis was made on the biopsy specimen, but no residual dysplastic lesion could be found on the LEEP specimen.

Another possible limitation of our study concerns the 'gold standard' diagnosis of cervical disease. Ideally, the routine histological diagnosis should have been confirmed by experts because of the substantial variability in histological interpretation [3]. Here, slides were only reviewed in case of disagreement between cytology and histology.

The analysis of ROC curves allowed exploration of age-restriction effects, and confirmed the slight improvement in specificity in women aged 30 years or above. In addition, it suggested that the 2 pg/ml cut-off might be more accurate than the usual 1 pg/ml in such a population with a high HR-HPV prevalence.

In conclusion, even in a population with a high HPV infection rate, HPV testing appears to be a relevant

tool, by itself or as an adjunct to cytology. HPV-negative testing allows the selection of a population with a very low risk of developing severe dysplasia whatever the result of cytology [9,29,30], with an acceptable specificity, especially in a wealthy country setting. For these women, the screening interval might be safely lengthened [22]. In contrast, the combination of cytology and HPV-positive testing is able to identify with 100% sensitivity women who currently have high-grade disease, and also those at greatest risk of developing disease. Therefore, it appears relevant to use the HPV DNA test as an adjunct in women reaching hospital. In this setting, reliable tests, as sensitive and specific as possible, could avoid the misdiagnosis of precancer and cancer. Although our study was not designed for this purpose, we can expect that the use of HPV testing might lead to a decrease in mortality from cervical cancer, or at least in morbidity, which is also a huge issue in the female population concerned.

In order to exploit the excellent sensitivity of HPV testing and the excellent specificity of cytology, and also to consider cost-effectiveness, a new approach combining these two screening methods is emerging [16,31]: HPV testing as the primary screening and cytology in HPV-infected women only. Women with cytological abnormalities would then be directly referred for colposcopy, while those with a normal Pap smear would be recalled for a control visit several months later to ensure that they had cleared their HPV infection. Since the prevalence of HPV infection in the general population is about 10–12% and the large majority of these HPV-positive women will clear their infection in the following months, and since the screening interval could be safely lengthened for HPV-negative women, this new strategy might not only be the most effective, but also cost saving [31].

Acknowledgements

We are indebted to Sylviane Coumes-Marquet for excellent technical assistance with HPV testing. We thank all the women who participated in the study. The grant sponsors were Ligue contre le Cancer (comité du Doubs). Mrs V. Dalstein is the recipient of a predoctoral scholarship from the Fondation de France.

References

1. Weidmann C, Schaffer P, Hédelin GH, *et al*. L'incidence du cancer du col de l'utérus régresse régulièrement en France. *Bull Epidémiol Hebdomadaire* 1998, **5**, 17–19.
2. Sasieni P. Cervical sampling devices. *Br Med J* 1996, **313**, 1275–1276.
3. Stoler MH, Schiffman M. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. *JAMA* 2001, **285**, 1500–1505.
4. Papillo JL, Zarka MA, St John TL. Evaluation of the ThinPrep Pap test in clinical practice. A seven-month, 16,314-case experience in northern Vermont. *Acta Cytol* 1998, **42**, 203–208.
5. Bernstein SJ, Sanchez-Ramos L, Ndubisi B. Liquid-based cervical cytologic smear study and conventional Papanicolaou smears: a metaanalysis of prospective studies comparing cytologic diagnosis and sample adequacy. *Am J Obstet Gynecol* 2001, **185**, 308–317.
6. Bosch FX, Manos MM, Muñoz N, *et al*. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *J Natl Cancer Inst* 1995, **87**, 796–802.
7. Walboomers JM, Jacobs MV, Manos MM, *et al*. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999, **189**, 12–19.
8. Wallin KL, Wiklund F, Angstrom T, *et al*. Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. *N Engl J Med* 1999, **341**, 1633–1638.
9. Dalstein V, Riethmuller D, Prétet JL, *et al*. Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: A longitudinal French cohort study. *Int J Cancer* 2003, **106**, 396–403.
10. Riethmuller D, Gay C, Bertrand X, *et al*. Genital human papillomavirus infection among women recruited for routine cervical cancer screening or for colposcopy determined by Hybrid Capture II and polymerase chain reaction. *Diagn Mol Pathol* 1999, **8**, 157–164.
11. Bory JP, Cucherousset J, Lorenzato M, *et al*. Recurrent human papillomavirus infection detected with the hybrid capture II assay selects women with normal cervical smears at risk for developing high grade cervical lesions: a longitudinal study of 3,091 women. *Int J Cancer* 2002, **102**, 519–525.
12. Cuzick J, Beverley E, Ho L, *et al*. HPV testing in primary screening of older women. *Br J Cancer* 1999, **81**, 554–558.
13. Schneider A, Hoyer H, Lotz B, *et al*. Screening for high-grade cervical intra-epithelial neoplasia and cancer by testing for high-risk HPV, routine cytology or colposcopy. *Int J Cancer* 2000, **89**, 529–534.
14. Schiffman M, Herrero R, Hildesheim A, *et al*. HPV DNA testing in cervical cancer screening: results from women in a high-risk province of Costa Rica. *JAMA* 2000, **283**, 87–93.
15. Ratnam S, Franco EL, Ferenczy A. Human papillomavirus testing for primary screening of cervical cancer precursors. *Cancer Epidemiol Biomarkers Prev* 2000, **9**, 945–951.
16. Clavel C, Masure M, Bory JP, *et al*. Human papillomavirus testing in primary screening for the detection of high-grade cervical lesions: a study of 7932 women. *Br J Cancer* 2001, **84**, 1616–1623.
17. Kulasingam SL, Hughes JP, Kiviat NB, *et al*. Evaluation of human papillomavirus testing in primary screening for cervical abnormalities: comparison of sensitivity, specificity, and frequency of referral. *JAMA* 2002, **288**, 1749–1757.
18. Petry KU, Bohmer G, Iftner T, *et al*. Factors associated with an increased risk of prevalent and incident grade III cervical intraepithelial neoplasia and invasive cervical cancer among women with Papanicolaou tests classified as grades I or II cervical intraepithelial neoplasia. *Am J Obstet Gynecol* 2002, **186**, 28–34.
19. Manos MM, Kinney WK, Hurley LB, *et al*. Identifying women with cervical neoplasia: using human papillomavirus DNA testing for equivocal Papanicolaou results. *JAMA* 1999, **281**, 1605–1610.
20. Solomon D, Schiffman M, Tarone R. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. *J Natl Cancer Inst* 2001, **93**, 293–299.
21. Nobbenhuis MA, Meijer CJ, van den Brule AJ, *et al*. Addition of high-risk HPV testing improves the current guidelines on follow-up after treatment for cervical intraepithelial neoplasia. *Br J Cancer* 2001, **84**, 796–801.
22. Meijer CJ, Helmerhorst TJ, Rozendaal L, van der Linden JC, Voorhorst FJ, Walboomers JM. HPV typing and testing in gynaecological pathology: has the time come? *Histopathology* 1998, **33**, 83–86.

23. Cuzick J. Time to consider HPV testing in cervical screening. *Ann Oncol* 2001, **12**, 1511–1514.
24. Cain JM, Howett MK. Preventing cervical cancer. *Science* 2000, **288**, 1753–1755.
25. Herbst AL, Pickett KE, Follen M, Noller KL. The management of ASCUS cervical cytologic abnormalities and HPV testing: a cautionary note. *Obstet Gynecol* 2001, **98**, 849–851.
26. Monnet E, Carbillet JP, Meslans Y, Petitjean A, Gautier CP. [Participation of women in cervical cancer screening. Results of the first 5 years of a pilot program in the province of Doubs]. *Presse Med* 1999, **28**, 2093–2097.
27. Schaffer P, Sancho-Garnier H, Fender M, *et al*. Cervical cancer screening in France. *Eur J Cancer* 2000, **36**, 2215–2220.
28. Sigurdsson K. Effect of organized screening on the risk of cervical cancer. Evaluation of screening activity in Iceland, 1964–1991. *Int J Cancer* 1993, **54**, 563–570.
29. Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, *et al*. Cytological regression and clearance of high-risk human papillomavirus in women with an abnormal cervical smear. *Lancet* 2001, **358**, 1782–1783.
30. Rozendaal L, Walboomers JM, van der Linden JC, *et al*. PCR-based high-risk HPV test in cervical cancer screening gives objective risk assessment of women with cytomorphologically normal cervical smears. *Int J Cancer* 1996, **68**, 766–769.
31. Cuzick J, Szarewski A, Cubie G, *et al*. Management of women who test positive for high-risk types of human papillomavirus: the HART study. *Lancet* 2003, **362**, 1871–1876.