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Combined Pap and HPV testing in primary screening for cervical abnormalities: Should HPV detection be delayed until age 35?

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Abstract

In 2003, the United States Food and Drug Administration has approved the Hybrid Capture 2 assay for use with a Pap test to adjunctively screen women of 30 years and older for the presence of high-risk human papillomavirus (HR-HPV) infection. Although the predictive power of a negative test is strong, the number of false-positives may still be high. We investigated HPV prevalence in relation to age in a group of 2293 women, aged between 20 and 50, with normal cytology. Overall HR-HPV prevalence was 6.9% (95%CI = 5.9–8.0%). Regression analysis using 5-year intervals showed that the HR-HPV prevalence did not significantly decline up to age 34, whereas it declined significantly after age 35. This would suggest that postponing HPV detection in primary screening from age 30 to 35 would result in a decrease of almost 50% of the number of women with normal cytology and a transient HPV infection. However, larger scale studies are required to confirm this finding.

Keywords: Cervical cancer; Human papillomavirus; Primary screening

1. Introduction

The age-related prevalence of both high-risk and low-risk human papillomavirus (HPV) types in cytologically normal cervical smears has been shown by a number of studies [1–6]. Based upon these data and because of the frequency of asymptomatic HPV infections, as well as transient low-grade cervical lesions it has been suggested that HPV testing within primary cervical cancer screening would be unwise before the age of 30 years [7]. In 2003, the United States Food and Drug Administration (FDA) has approved Digene's Hybrid Capture 2 (HC2) assay for use with a Pap test to adjunctively screen women of 30 years and older for the presence of high-risk (HR) HPV infection, based on the theory that after the age of 30, the negative predictive value of both Pap and HC2 would improve the sensitivity of cervical cancer screening. Although the predictive power of a negative test is high, the number of HPV positive women may still be substantial. A fraction of these women will have a persistent infection and may go on to develop cervical lesions. Alternatively, a fraction of these women may have cervical lesions already, which were missed by cytology. However, a very large majority of HPV positive women, especially in younger age groups, will have

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a transient infection which will not cause progressive lesions, and may, therefore, be considered as false-positive. It has been shown previously that the median duration of HPV infection is between 8 and 12 months [8–10]. Furthermore, 10 months after incident infection, 50% of the women were no longer infected [10]. This number increased to 70% at 12 months, 91% at 24 months and 92% at 5 years [8,11].

In this study, we investigated HPV prevalence in relation to age in a group of 2293 women, aged between 20 and 50, with normal cytology.

2. Patients and methods

2.1. Study group

Between January 2001 and June 2003 cytology material from women attending the Department of Gynaecology at the University Hospital Antwerp for regular cervical cancer screening and women attending their general practitioner was included in this study. The study was performed anonymously; the only data available were age at sampling and result of cytology. Only women with normal cytology ($n = 2318$) were included in the analysis. The mean age of these women was 35.8 years. The study protocol was approved by the medical ethical board of Antwerp University.

2.2. Sample preparation and HPV detection

The residual material after preparation of thin layer cytology slides was used for HPV detection. The suspension was centrifuged for 5 min at 1207 g. Cells were resuspended in 0.5 ml TE (10 mM Tris–HCl pH 8.0, 1 mM EDTA) and frozen at −80 °C. After thawing, 100 μl of the suspension was taken, boiled for 10 min and centrifuged (5 min, 12,557 g). Isolation of DNA was checked by β-globin PCR [12]. Only from β-globin positive samples, 10 μl of the suspension was subjected to the GP5+/6+ HPV PCR [13]. Detection of PCR products was performed in an enzyme immunoassay format as described by Jacobs *et al.* [14]. Presence of HPV was detected with a high-risk (HR) HPV probe cocktail. The cut-off value for HPV positivity was calculated as the mean plus three times the standard deviation of six negative controls in each plate (DNA isolated from cell line A549, an HPV negative lung cancer cell line). Typing analysis on HPV positive samples was performed in an enzyme immunoassay with separate probes for HR HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

2.3. Statistical analysis

Logistic regression for HPV prevalence in different age groups was performed with age as a continuous variable and with 5- and 10-year intervals as categoric variables.

3. Results

Cervical smear material was obtained from 2318 women with normal cytology. Material from 25 samples (1.1%) was negative on β-globin PCR, and these were excluded from the analyses. Overall HR-HPV prevalence in this study was 6.9% (158 of 2293 women, 95%CI = 5.9–8.0%). The age-related HR-HPV prevalence was analysed in 10-year intervals (Table 1, Fig. 1), in 5-year intervals (Table 1, Fig. 2) and in intervals of 1 year (data not shown). Using 5-year intervals the HPV prevalence remained stable at approximately 9% in the age groups 20–24, 25–29 and 30–34, and decreased significantly to approximately 5% in the age

Table 1
Age-specific HPV prevalence in women with normal cytology

Age group	*N*	HPV+	% HPV+	95%CI	*P*-value[a]
20–29	710	64	9.0	7.0–11.4	Ref.
30–39	772	55	7.1	5.4–9.2	0.182
40–50	811	39	4.8	3.4–6.5	0.001
20–24	314	28	8.9	6.0–12.1	Ref.
25–29	396	36	9.1	6.5–12.4	0.936
30–34	411	38	9.2	6.6–12.5	0.879
35–39	361	17	4.7	2.8–7.4	0.031
40–44	343	16	4.7	2.7–7.5	0.032
45–50	468	23	4.9	3.1–7.3	0.028
Total	2293	158	6.9	5.9–8.0	

[a] Logistic regression analysis with age groups as categorical variable.

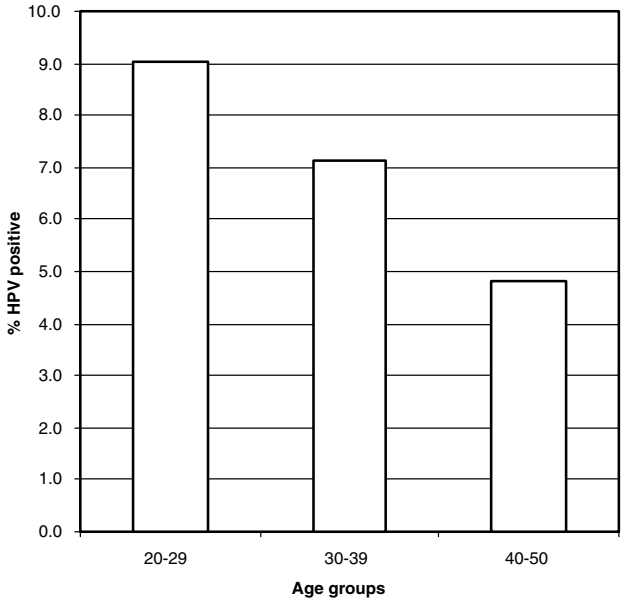

Fig. 1. Age-specific prevalence of high-risk HPV in women with normal cytology, 10-year intervals.

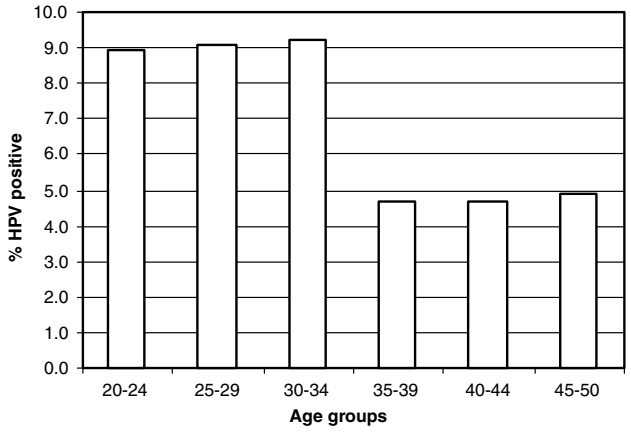

Fig. 2. Age-specific prevalence of high-risk HPV in women with normal cytology, 5-year intervals.

groups 35–39, 40–44 and 45–50. Using 1-year intervals, peaks were found at the ages 22, 23 (11.6% and 12.5%, respectively) and the ages 31, 32 (12.4% and 11.6%, respectively).

All HR-HPV genotypes that could be detected in this study were found at least once. HPV type 16 ($n = 49$, 31.0% of the infections) was most prevalent, followed by HPV types 35 ($n = 28$, 17.7%), 31 ($n = 23$, 14.6%), 18 ($n = 19$, 12.0%), 56 ($n = 18$, 11.4%), 51 ($n = 17$, 10.8%) and 45 ($n = 16$, 10.1%). All other genotypes accounted for less than 10% each. In one case, the HPV type could not be determined due to lack of material. There was no significant difference in genotype distribution with age.

The sum of infections with individual HPV types was higher than the total number of infections due to the presence of multiple infections. Of the 157 infections that could be typed, 103 were single infections, 42 were double infections, 8 were triple infections and 4 were quadruple infections. No infections with more than four HPV types were found.

4. Discussion

In this group of 2293 women with normal cytology, the overall HR-HPV prevalence was 6.9%. This is consistent with data from the literature which suggest that the prevalence of HR-HPV in women with normal cytology does not exceed 10% in the North-Western part of Europe. In a study from the Netherlands on 3305 cytologically normal smears from the general female population, 3.3% of the women were found to have a HR-HPV infection [6]. In Germany, 8466 women attending routine cervical screening were tested for HR-HPV by HC2. Of the 7832 women with a cytologically normal smear, 460 (5.9%) were HPV positive [15]. In a Swedish study on women aged 32–38, 417 women out of 6123 were HR-HPV positive (6.8%) [16]. Finally, HR-HPV was found in 257 out of 3089 (8.3%) women with normal cytology from a routine cervical screening population in Scotland [17]. However, higher HR-HPV prevalences (20%) have also been reported in Belgium [18]. This may be due to a different detection method or to age differences in the screened populations.

The decision of the FDA to approve the HC2 assay for use with a Pap test to adjunctively screen women of 30 years and older for the presence of HR-HPV infection prompted us to look carefully at the age-specific prevalence in our cohort, with logistic regression analysis. Using age as a continuous variable, a highly significant gradual decline of HR-HPV with age was obtained ($P < 0.001$), as shown previously [1–6]. This model, however, does not clarify where the decline is most obvious. Regression analysis using 5-year intervals as a categoric variable showed that the HR-HPV prevalence did not significantly decline in the age groups 25–29 and 30–34, whereas it declined significantly after age 35, to remain fairly stable in the age groups 35–39, 40–44 and 45–50. Other studies, summarised in Table 2, have shown similar results.

Postponing HPV detection in primary screening from age 30 to age 35 would result in a decrease in the number of HPV positive women with normal cytology of almost 50%. The large majority of these women (>80%) will have transient infections and are at low risk of developing cervical lesions. These results suggest that thousands of women in North-Western European countries alone could be saved from being diagnosed with a wide-spread sexually transmitted infection. Investigation of the emotional and psychosocial impact in people with genital warts and asymptomatic HPV infections taking part in an HPV support group showed that a positive diagnosis of HPV resulted in emotional distress (anger, anxiety, depression, fear of rejection, shame and guilt), sexual problems and a negative self-image [19]. Similarly, female students imagining a positive HPV diagnosis expected to feel fear, anxiety, regret, anger and confusion [20]. Furthermore, increasing awareness of the link between HR-HPV and cervical cancer might cause women with an abnormal Pap smear and/or a positive HPV diagnosis to resent or distrust their partner and to feel unable to disclose their test result for fear of stigma, associated with the diagnosis of a sexually transmitted infection [21]. Bearing this burden in mind, HR-HPV testing should be introduced with caution. We have shown previously, that HPV detection might be useful in determining a safe age to stop cervical smear screening. Because of the low HPV prevalence in elderly women, nearly 94% of the women of 50 years and older could potentially be withdrawn from screening [22]. The results of the present study suggest that HPV detection might be more appropriate from the age of 35. However, larger scale studies are needed to confirm this finding.

Table 2
HPV prevalence in women with normal cytology in North-Western Europe

Reference	Area	Method used	Age groups													
			20–24 *n*/*N*	(%)	25–29 *n*/*N*	(%)	30–34 *n*/*N*	(%)	35–39 *n*/*N*	(%)	40–44 *n*/*N*	(%)	45–50 *n*/*N*	(%)	Total *n*/*N*	(%)
Jacobs *et al.* [6]	The Netherlands	GP5+/6+ PCR-EIA	4/23	17.4	7/46	15.2	47/389	12.1	8/316	2.5	22/679	3.2	27/708	3.8	115/2161	5.3
Petry *et al.* [15]	Germany, North	HC2					110/1000	11.0	89/1144	7.8	47/941	5.0	49/764	6.4	295/3849	7.7
Petry *et al.* [15]	Germany, South	HC2					53/882	6.0	50/949	5.3	33/762	4.3	17/519	3.3	153/3112	4.9
Forslund *et al.* [16]	Sweden	GP5+/6+ PCR-EIA					259/3149	8.2	174/3069	5.7					433/6218	7.0
Cuschieri *et al.* [17]	Scotland	GP5+/6+ RT-PCR	91/488	18.6	53/420	12.6	24/419	5.7	24/475	5.1	14/364	3.8	12/291	4.1	218/2457	8.9
This study	Belgium	GP5+/6+ PCR-EIA	28/314	8.9	36/396	9.1	38/411	9.2	17/361	4.7	16/343	4.7	23/468	4.9	158/2293	6.9
	Total		123/825	14.9	96/862	11.1	531/6250	8.5	362/6314	5.7	132/3089	4.3	128/2750	4.7	1372/20,090	6.8

Conflict of interest statement

None declared.

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