Prevaccination Distribution of Human Papillomavirus Types in Women Attending at Cervical Cancer Screening in Belgium

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Abstract

Introduction: **Before the introduction of vaccination against human papillomaviruses (HPV) as a new strategy of combating cervical cancer, it is required to describe the baseline prevalence of HPV infection as well as the distribution of the different HPV types in the population and among women with cervical lesions.**

Materials and Methods: **Approximately 10,000 liquid cervical cell samples from women, resident of Flanders (North Belgium) and participating in cervical cancer screening, were assessed cytologically and virologically with a multiplex real-time PCR using primers targeting the *E6/E7* genes of 16 HPV types. Correlations of HPV infection with age, geographic area, and occurrence of cytologic lesions were assessed.**

Results: **The prevalence of cytologic abnormalities was atypical squamous cells of undetermined significance (ASC-US), 1.6%; atypical glandular cells (AGC), 0.2%; low-grade squamous intraepithelial lesion (LSIL), 2.6%; atypical squamous cells, HSIL cannot be excluded (ASC-H), 0.3%; and high-grade squamous intraepithelial lesion (HSIL), 1.2%. The frequency of high-risk HPV infections was 11% in women without cytologic abnormalities, 77% in ASC-US, 32% in AGC, 85% in LSIL, and 93% in ASC-H and HSIL. The prevalence of high-risk HPV infection was highest in women of ages 20 to 24 years (29%) and decreased progressively with age. The percentage of women with HSIL in the entire study population attributable to infection with a particular type (AR_{pop} %) was highest for HPV16 (32%), followed by HPV31 (22%), HPV39 (11%), and HPV52 (11%). HPV18 was responsible for 7% of the HSIL lesions. Elimination of HPV16 and HPV18 is expected to reduce the prevalence of ASCUS with 24%, AGC with 19%, LSIL with 29%, ASC-H with 31% and HSIL with 37%.**

Discussion: **Compared to other West European studies, the prevalence of HPV infection was considerably higher in cytologically negative women but similar in women with cervical lesions. These differences could be due to the use of a PCR with high analytic sensitivity. These data are relevant for estimating the expected and theoretical levels of vaccine protection offered as vaccinated girls gradually age into the groups from which our observations stem. Further periodic laboratory-based surveys, including genotyping of cervical cell samples and linkage with vaccine registries, are an important resource to address pending questions of the effect of HPV vaccination. Research is warranted to disentangle the causal role of individual HPV types in case of multiple infections.** (Cancer Epidemiol Biomarkers Prev 2009;18(1):321–30)

Introduction

The recognition of the strong causal relation between persistent infection of the genital tract with oncogenic human papillomavirus (HPV) types and the occurrence of cervical cancer precursors and cervical cancer has resulted in the development of tests for HPV nucleic acid detection (1, 2). Such tests can be used in primary screening for cervical cancer, for triage of women with atypical or borderline cervical smears, or for surveillance after treatment of cervical intraepithelial neoplasia (3-5).

Prophylactic vaccination with the L1 capsid proteins elicits the production of virus neutralizing antibodies that protect against persistent infection and high-grade cervical, vaginal, and vulvar neoplasia caused by the HPV types included in the vaccine (6-9). In 2006, the use of a quadrivalent vaccine containing L1 virus-like particles of HPV types 6, 11, 16, and 18 was licensed by the U.S. Food and Drug Administration and authorized for marketing by the European Medicines Agency. A bivalent vaccine containing L1 from HPV types 16 and 18 was licensed more recently by the European Medicines Agency as well, but to date, not yet by the Food and Drug Administration.

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To assess the effect of current and future HPV vaccination programs, it is essential to assess the background prevalence of HPV infection, the distribution of HPV types according to age, and their association with cervical cancer precursors and cervical cancer.

Ideally, representative population-based surveys involving collection of cervical cellular material from women are needed to establish this baseline situation (10, 11). An alternative method consists in HPV testing and genotyping of liquid-based cervical samples collected as part of routine cytologic screening practices (which, due to its widespread nature, is then assumed to offer sufficient representation for general inferences). This last method is applied in our article, based on samples analyzed in a large laboratory localized in the province of Antwerp in Flanders, Belgium. This lab conducts analyses of ~100,000 cervical preparations per year from women living in Flanders (12).

Materials and Methods

Study Population and Used Tests. This is a laboratory-based, cross-sectional prevalence study. All cervical samples, received in October 2006 by the Laboratory for Clinical Pathology (labo RIATOL), Antwerp, Belgium, for cytologic examination, were tested for presence of HPV DNA. Cervical cells were collected with the Cervex-Brush Combi (Rovers Medical Devices B.V.). After collection, brush heads were transferred directly into a vial with BD-SurePath preservative fluid (13). From the fluid containing the cellular material, a liquid-based cytology sample was prepared with the robotic BD PrepStain Slide Processor, previously AutoCyte PREP System (BD Diagnostics-TriPath; refs. 14, 15). All slides are prescreened using BD-FocalPoint, a computerized scanning system for the primary screening of cervical smears (BD Diagnostics-TriPath), followed by targeted microscopic interpretation of selected suspicious fields using BD-FocalPoint guided screening review stations. Cytologic results were classified according to the Bethesda system 2001 (16, 17). The following classes were distinguished: negative for intraepithelial lesions and malignancies (NILM), which included atypical cells favoring reactive changes; atypical squamous cells of undetermined significance (ASC-US); atypical glandular cells (AGC); low-grade squamous intraepithelial lesions (LSIL); atypical squamous cells of undetermined significance, cannot exclude high-grade squamous intraepithelial lesions (ASC-H); and high-grade squamous intraepithelial lesions (HSIL).

DNA isolation from the liquid-based cytology leftover was done as previously described (12, 18). The presence of HPV genotypes was determined using a multiplex TaqMan-based real-time quantitative PCR targeting type-specific sequences of viral *E6* or *E7* genes: 6 *E6*, 16 *E7*, 18 *E7*, 31 *E6*, 33 *E6*, 35 *E6*, 39 *E7*, 45 *E7*, 51 *E6*, 52 *E7*, 53 *E6*, 56 *E7*, 58 *E6*, 59 *E7*, 66 *E6*, and 68 *E7* (18). The analytic sensitivity of the different PCR assays ranged from 1 to 100 copies and was calculated using standard curves for 16 type-specific PCRs constructed with plasmids containing the entire genome of the different HPV types (19). Real-time quantitative PCR for β-globin was used to verify the quality of DNA in the sample and to measure the amount of input DNA (18).

Viral load in each specimen was expressed as the number of HPV copies per genome equivalent or cell. The amount of β-globin DNA (in nanograms) present in each sample was divided by the weight of 1 genome equivalent (i.e., 6.6 pg/cell) and a factor of 2 (because there are 2 copies of β-globin DNA/genome equivalent) to obtain the number of genome equivalents in the sample (the number of cells in the sample).

For each woman, the following data were available: identity code, age, postal code of the residence, the cytologic result, the result of human β-globin DNA amplification, and the result of the HPV type-specific real-time-PCR.

Women with cytologic abnormalities were managed according to the Belgian follow-up expert guidelines (20, 21). Follow-up outcomes are currently collected and are to be reported later.

The following objectives were addressed: (*a*) to assess the prevalence of global type-specific HPV infection in the study population, stratified by cytologic finding(s) and by age; (*b*) to evaluate the cross-sectional association between HPV infection (all types, high-risk types, and individual HPV types; viral load; and occurrence of single or multiple infection) and cytologic abnormalities.

Statistical Analysis. The statistical package STATA version 7.0 and 9.2 (Stata Corp.) was used for data analysis. High-risk infection was defined as the presence of one or more of the following 14 HPV types: HPV16, HPV18, HPV31, HPV33 HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68 (2, 22, 23).

Geographic variation was assessed at the level of the province, district (termed ''arrondissement'' in Belgium) and municipality. Municipalities were grouped by level of urbanization: city agglomeration, suburbs, residential areas, and rural areas (24). Pearson's χ^2 test was used to study associations between two categorical variables. The change in the average viral load (at the $\log_{10}$ scale) by cytologic categories and the change in risk of cytologic abnormalities by increasing number of high-risk HPV (hrHPV) infections were assessed by ANOVA and by a χ^2 trend test, which generalizes the Wilcoxon test to several ordered groups (25). The rare categories, AGC and ASC-H, were excluded from this trend analysis. From the relative risk (RR, describing the association between a lesion and infection) and the prevalence (p) of type-specific HPV infection, the attributable risk percentage among the infected (AR_{exp} %) and the study population (AR_{pop} %) was calculated as follows:

$$AR_{exp}\% = \frac{RR - 1}{RR} * 100$$

$$AR_{pop}\% = \frac{p(RR - 1)}{p(RR - 1) + 1} * 100$$

The multivariate relation between occurrence of HSIL and the simultaneous presence of different HPV-genotypes and other factors was assessed by stepwise logistic regression, using probability levels of 0.01 and 0.05 for entry or removal of variables, respectively (26).

Results

The mean age of the study population was 42 years (range, 14-97; 1st quartile, 31; median, 41; 3rd quartile, 52 years). Eighty-three percent belonged to the target age group of 25 to 64 years, for whom screening is recommended (21). Ten percent was younger than 25 years and 7% was older than 64 years.

Only 0.1% of 9,297 processed samples were unsatisfactory for microscopic interpretation. The prevalence of cytologic abnormalities in the population with microscopically interpretable samples was 1.6% for ASC-US, 0.2% for AGC, 2.6% for LSIL, 0.3% for ASC-H, and 1.2% for HSIL.

In 11 (0.1%) samples, no human β-globin DNA could be amplified. However, one of these 11 samples contained high-risk HPV DNA. The 10 samples with no amplification of β-globin and viral DNA were excluded. The frequency of hrHPV infection was 15.2%. This frequency was 11% in women without cytologic abnormalities, 77% in ASC-US, 32% in AGC, 85% in LSIL, and 93% in ASC-H and HSIL (Table 1). The prevalence of hrHPV infection was highest in the age group 20 to 24 years (29%) and decreased progressively with increasing age up to 8% in the age group 55 to 59 years. After that age, the hrHPV positivity rate varied within the range of 7% to 10% (see Fig. 1). The geographic variation in hrHPV positivity rate was statistically insignificant by province ($P = 0.235$), but was significant by district ($P = 0.007$). Higher rates were observed in the city agglomerations (17%) compared with the other municipalities (14%), and this difference was statistically significant ($P = 0.001$). Seventy-three percent of hrHPV infections were single, whereas 27% were multiple infections (18% double, 6% triple, 2.3% quadruple, and 0.5% carrying five or more types).

The most common high-risk type in the general population was HPV16 (3.7%), followed by HPV31, HPV51, and HPV53, which all occurred in at least 2% of the tested population. HPV18 ranked at the 7th place (1.5%). HPV16 or HPV18 infection was found in 5.1% of all women.

Considering HPV6, HPV16, and HPV18, which are all included in the quadrivalent vaccine Gardasil (Merck & Co, Inc.), eight mutually exclusive groups of no, single, double, or triple infections can be considered. Their

Table 1. Prevalence of HPV types by cytologic category

Types		NILM	ASC-US	AGC	LSIL	ASC-H	HSIL+	Total
	Total	8,729	152	22	243	29	109	9,284
	%	94.0	1.6	0.2	2.6	0.3	1.2	100.0
hrHPV	N+	949	117	7	207	27	101	1,408
	% (n+/tot)	*10.9*	*77.0*	*31.8*	*85.2*	*93.1*	*92.7*	*15.2*
all types	N+	1,040	123	7	219	27	102	1,518
	% (n+/tot)	*11.9*	*80.9*	*31.8*	*90.1*	*93.1*	*93.6*	*16.4*
HPV06	N+	34	6	0	16	0	4	60
	% (n+/tot)	*0.4*	*3.9*	*0.0*	*6.6*	*0.0*	*3.7*	*0.6*
HPV16	N+	205	35	1	57	8	38	344
	% (n+/tot)	*2.3*	*23.0*	*4.5*	*23.5*	*27.6*	*34.9*	*3.7*
HPV18	N+	89	11	5	26	3	9	143
	% (n+/tot)	*1.0*	*7.2*	*22.7*	*10.7*	*10.3*	*8.3*	*1.5*
HPV31	N+	181	16	0	54	6	26	283
	% (n+/tot)	*2.1*	*10.5*	*0.0*	*22.2*	*20.7*	*23.9*	*3.0*
HPV33	N+	38	3	0	14	3	12	70
	% (n+/tot)	*0.4*	*2.0*	*0.0*	*5.8*	*10.3*	*11.0*	*0.8*
HPV35	N+	18	6	0	14	1	5	44
	% (n+/tot)	*0.2*	*3.9*	*0.0*	*5.8*	*3.4*	*4.6*	*0.5*
HPV39	N+	77	13	0	36	1	14	141
	% (n+/tot)	*0.9*	*8.6*	*0.0*	*14.8*	*3.4*	*12.8*	*1.5*
HPV45	N+	29	4	0	9	0	4	46
	% (n+/tot)	*0.3*	*2.6*	*0.0*	*3.7*	*0.0*	*3.7*	*0.5*
HPV51	N+	139	23	0	35	4	13	214
	% (n+/tot)	*1.6*	*15.1*	*0.0*	*14.4*	*13.8*	*11.9*	*2.3*
HPV52	N+	80	15	1	31	7	14	148
	% (n+/tot)	*0.9*	*9.9*	*4.5*	*12.8*	*24.1*	*12.8*	*1.6*
HPV53	N+	134	11	0	36	1	11	193
	% (n+/tot)	*1.5*	*7.2*	*0.0*	*14.8*	*3.4*	*10.1*	*2.1*
HPV56	N+	52	14	0	25	2	5	98
	% (n+/tot)	*0.6*	*9.2*	*0.0*	*10.3*	*6.9*	*4.6*	*1.1*
HPV58	N+	63	13	0	16	2	11	105
	% (n+/tot)	*0.7*	*8.6*	*0.0*	*6.6*	*6.9*	*10.1*	*1.1*
HPV59	N+	119	10	1	26	1	4	161
	% (n+/tot)	*1.4*	*6.6*	*4.5*	*10.7*	*3.4*	*3.7*	*1.7*
HPV66	N+	95	8	0	34	1	5	143
	% (n+/tot)	*1.1*	*5.3*	*0.0*	*14.0*	*3.4*	*4.6*	*1.5*
HPV68	N+	7	4	0	9	0	0	20
	% (n+/tot)	*0.1*	*2.6*	*0.0*	*3.7*	*0.0*	*0.0*	*0.2*

NOTE: Data are given as absolute numbers and percentages (in italics). *n*, absolute number.
Abbreviation: tot, total.

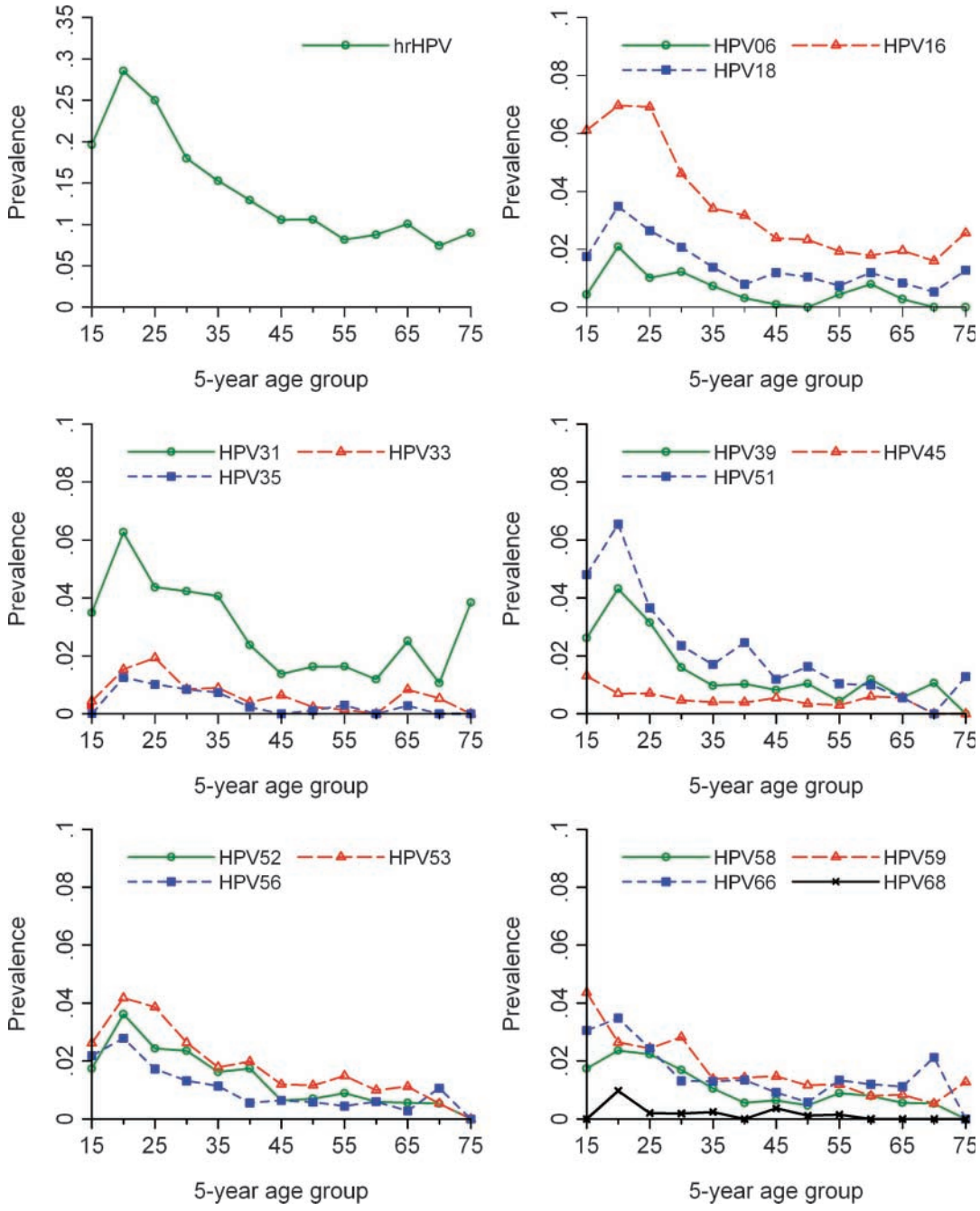

Figure 1. Prevalence of cervical infection with hrHPV infection (*top left*) and with individual HPV types (*other graphs*), by age.

frequencies in the study population according to age group are shown in Table 2. The very large majority (94.4%) carried none of the three types. Single infections were present in 0.5% (HPV6), 3.4% (HPV16), and 1.3% (HPV18), whereas double infections were found in 0.16% (HPV16/18), 0.12% (HPV6/16), and 0.06% (HPV6/18). Not a single woman was infected by all three types.

In general, prevalences were highest in the 20 to 24 year age group, decreased with aging, and showed some irregular increases in the oldest groups.

All tested HPV types, besides HPV59 and HPV68, were each associated with a significantly increased risk of HSIL+ lesions compared with women who did not carry that particular type (Table 3).

Multiple hrHPV infections were associated with a higher risk of HSIL than a single hrHPV infection: 10.6% versus 7.9% [RR, 1.80; 95% confidence interval (95% CI), 1.23-2.63]. Table 4 shows the risk for cytologic lesions attributable to HPV16 or HPV18 infection. The percentages of ASC-US, AGC, LSIL, ASC-H, and HSIL

Table 2. Absolute and relative frequency of no, single, double, and triple HPV infections with type 6, 16, or 18, by age group

Age group	Total	HPV6 absent / HPV16 absent / HPV18 absent		HPV6 present / HPV16 absent / HPV18 absent		HPV6 absent / HPV16 present / HPV18 absent		HPV6 absent / HPV16 absent / HPV18 present		HPV6 present / HPV16 present / HPV18 absent		HPV6 present / HPV16 absent / HPV18 present		HPV6 absent / HPV16 present / HPV18 present		HPV6 present / HPV16 present / HPV18 present	
	n	n+	%	n+	%	n+	%	n+	%	n+	%	n+	%	n+	%	n+	%
10-19	230	213	92.60	0	0.00	12	5.22	3	1.30	1	0.43	0	0.00	1	0.43	0	0.00
20-24	718	636	88.58	10	1.39	44	6.13	20	2.79	3	0.42	2	0.28	3	0.42	0	0.00
25-29	984	886	90.04	6	0.61	63	6.40	23	2.34	3	0.30	1	0.10	2	0.20	0	0.00
30-34	1,062	985	92.75	10	0.94	43	4.05	17	1.60	2	0.19	1	0.09	4	0.38	0	0.00
35-39	1,231	1,167	94.42	7	0.57	39	3.17	14	1.14	1	0.08	1	0.08	2	0.16	0	0.00
40-49	2,350	2,259	96.13	4	0.17	63	2.68	21	0.89	1	0.04	0	0.00	2	0.09	0	0.00
50-59	1,532	1,484	96.87	2	0.13	32	2.09	12	0.78	0	0.00	1	0.07	1	0.07	0	0.00
60+	1,164	1,125	96.65	5	0.43	22	1.89	12	1.03	0	0.00	0	0.00	0	0.00	0	0.00
Total	9,271	8,755	94.43	44	0.47	318	3.43	122	1.32	11	0.12	6	0.06	15	0.16	0	0.00

lesions in the population attributable to one or both infections were 24.4%, 18.6%, 29.0%, 31.0%, and 37.2%, respectively.

The total high-risk viral load increased significantly with the number of high-risk infections (correlation coefficient $\rho = 0.36$). Moreover, the total high-risk viral load and the number of high-risk infections varied significantly by cytologic category ($P < 0.01$, ANOVA).

The trend of increasing load, considered over the range NILM→LSIL, was statistically significant for all HPV types, except for HPV68 (see Fig. 2). The average viral load did not increase further between LSIL and HSIL, except for HPV16 ($P = 0.043$). For certain types, the average load was even lower in HSIL than in LSIL, but this negative trend was only statistically significant for HPV56 ($P_{trend} < 0.01$).

Also for total hrHPV load, a significantly increasing trend was observed over the range NILM→LSIL ($P_{trend} < 0.01$), but between LSIL and HSIL, there was no statistically significant difference anymore ($P = 0.81$).

In a stepwise multivariate logistic regression, including all types and adjusting for age and geographic location, infections with HPV16, HPV18, HPV31, HPV33, HPV39, HPV45, HPV52, and HPV58 were significantly associated with presence of HSIL. Viral loads of HPV16, HPV18, HPV31, HPV33, HPV35, HPV51, HPV52, HPV53, and HPV58 were significantly associated with HSIL as well. Age group and geographic district were rejected from the logistic model.

Discussion

This study provides essential baseline information at the dawn of the introduction of HPV vaccination in Belgium. It documents the current prevalence of each HPV type in

Table 3. Percentage of HSIL attributable to infection with a given HPV type

Type(s)	RR*	RR (lcib)	RR (ucib)	Prevalence (%)	AR_{exp} %	AR_{exp} % (lcib)	AR_{exp} % (ucib)	AR_{pop} %
hrHPV	70.6	34.4	144.7	15.2	98.6	97.1	99.3	91.3
HPV all types	74.5	34.7	160.0	16.4	98.7	97.1	99.4	92.3
HPV06	5.8	2.2	15.4	0.65	82.9	55.2	93.5	3.0
HPV16	13.9	9.5	20.3	3.7	92.8	89.5	95.1	32.4
HPV18	5.8	3.0	11.2	1.5	82.6	66.3	91.0	6.8
HPV31	10.0	6.5	15.2	3.0	90.0	84.7	93.4	21.5
HPV33	16.3	9.4	28.3	0.8	93.9	89.3	96.5	10.3
HPV35	10.1	4.3	23.6	0.5	90.1	76.9	95.8	4.1
HPV39	9.6	5.6	16.3	1.5	89.5	82.1	93.9	11.5
HPV45	7.7	2.9	19.9	0.5	86.9	66.0	95.0	3.2
HPV51	5.7	3.3	10.1	2.3	82.6	69.4	90.1	9.8
HPV52	9.1	5.3	15.6	1.6	89.0	81.2	93.6	11.4
HPV53	5.3	2.9	9.7	2.1	81.1	65.4	89.7	8.2
HPV56	4.5	1.9	10.8	*1.1*	77.8	46.8	90.7	3.6
HPV58	9.8	5.4	17.8	*1.1*	89.8	81.6	94.4	9.1
HPV59	2.2	0.8	5.8	*1.7*	53.7	−24.2	82.7	2.0
HPV66	3.1	1.3	7.4	*1.5*	67.5	21.4	86.5	3.1
HPV68	0.0	—	—	*0.2*	100	—	—	0.2

NOTE: AR_{exp} %, percentage of HSIL attributable to infection in the exposed group (infected with HVP16 or HPV18). AR_{pop} %, percentage of HSIL attributable to infection in the population.

Abbreviations: lcib, lower confidence interval bound; ucib, upper confidence interval bound.

*The RR is computed considering as reference the women not infected with the particular type (mentioned in the 1st column), that is, women without any HPV or women with other HPV infections.

Table 4. Percentage of cytologic lesions attributable to HPV16 or HPV18 infections

Lesion	RR (95% CI)*	Prevalence HPV types (%)	AR_{exp} % (95% CI)	AR_{pop} %
ASC-US	7.4 (5.2-10.4)	5.2	86.4 (80.9-90.3)	24.4
AGC	5.5 (2.0-14.8)	5.2	81.8 (50.9-93.3)	18.6
LSIL	9.0 (7.0-11.6)	5.2	88.9 (85.9-91.4)	29.0
ASC-H	9.8 (4.6-21.0)	5.2	89.8 (78.2-95.2)	31.0
HSIL+	12.6 (8.7-18.3)	5.2	92.1 (88.5-94.5)	37.2

*The RR is computed considering as reference the women not infected with HPV16 or HPV18, this means women without any HPV or women with other hrHPV types.

the population and its association with cervical lesions. Moreover, it disentangles the contribution of each individual HPV type or combination of types as single or multiple infections in equivocal, low-grade, and high-grade cervical cytology.

High Analytic Sensitivity Yielding Elevated hrHPV Rates in Cytologically Normal Women. The HPV genotyping system we used, a type-specific real-time PCR targeting short sequences of *E6* and *E7* genes of 16 HPV types, can detect as low as 1 to 10 copies of each separate HPV type (19). In the current study, 11% of the NILM samples were positive for hrHPV types. By contrast, in two other Belgian surveys, prevalence of hrHPV in women with normal cervical specimen was 3% [area of Brussels, measured with HC2 (Qiagen); ref. 27] and 7% (area of Antwerp, measured with PCR using GP5+/6+ primers), followed by genotyping (28). In a meta-analysis, de Sanjosé found, for West Europe, a pooled prevalence of HPV infection of 8.4% (95% CI, 8.0-8.8%). These prevalence estimates are not comparable without age standardization. Nonetheless, it seems plausible that at least a part of the differences are attributable to the use of different HPV tests.

Inevitably, if the proportion of HPV positivity is too high in cytologically normal women, this will result in lower clinical specificity (29). This problem can be resolved by increasing the viral load threshold at type-specific level or by combination with a more specific triage test (30, 31). It was recently shown that, below certain critical virologic load thresholds, detection of visually detectable lesions becomes very rare (31). The definitive fine-tuning of the test should eventually be based on longitudinal outcomes based on histologically confirmed high-grade cervical intraepithelial neoplasia or worse disease (CIN2+; ref. 32).

hrHPV Positivity in Cytologically Abnormal Samples. In a survey on cytologic abnormal samples, conducted in the same region, using a very sensitive PCR with SPF10 primers followed by genotyping with InnoLiPa (Innogenetics), similar hrHPV rates were found in squamous intraepithelial lesions (79% in LSIL and 89% in HSIL; ref. 33). These prevalences were also similar to those derived from meta-analyses of European studies. The pooled hrHPV positivity rates were 71.9% (95% CI, 62.8-80.9%) in LSIL or CIN1 lesions (34) and 88.3% (95% CI, 85.8-90.8%) in cytologic HSIL or high-grade CIN2 (35).

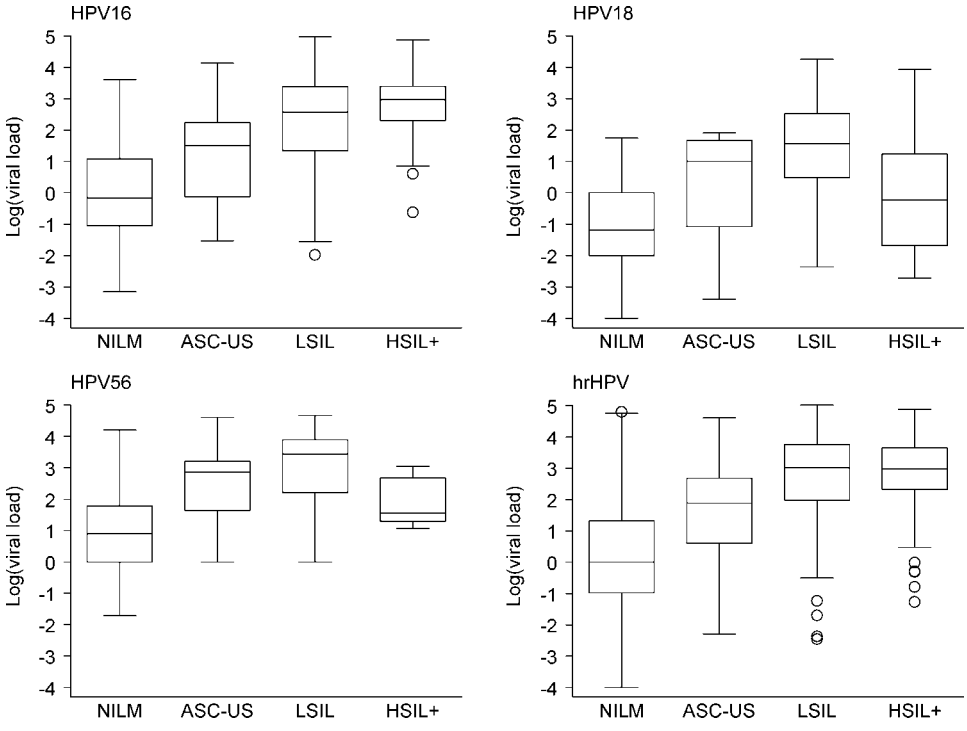

Figure 2. Box plots showing the distribution of viral load (logarithm of the number of HPV DNA copies per cell) by cytologic category: HPV16 (*top left*), HPV18 (*top right*), HPV56 (*bottom left*), and hrHPV (*bottom right*).

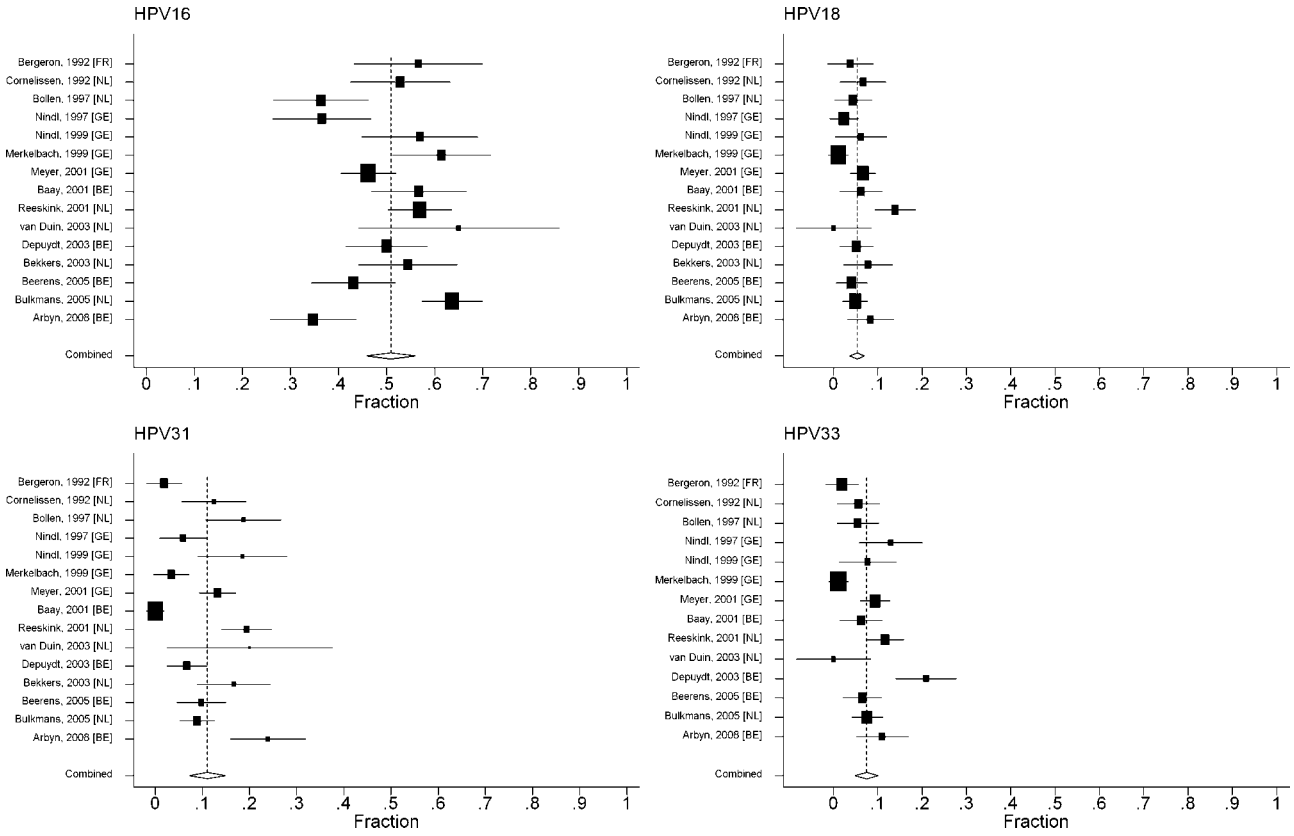

Figure 3. Prevalence of HPV types 16, 18, 31, and 33 in high-grade cervical lesions (HSIL, CIN2, or CIN3) observed in West European studies included in the meta-analysis of Smith et al. (35) and in the current study. (*BE,* Belgium; *FR,* France; *GE,* Germany; *NL,* the Netherlands).

Our study confirms the high hrHPV positivity rates in women with LSIL (85%), observed in the ASCUS/LSIL Triage study (36) and in recent meta-analyses (3, 37). These data show that the triaging capacity of a hrHPV cocktail test is quite low in case of low-grade cytologic abnormalities.

Type-Specific HPV Infections. The five most prevalent types in the Belgian study population without cytologic abnormalities were HPV16 (2.3%), HPV31 (2.1%), HPV51 (1.6%), HPV53 (1.5%), and HPV59 (1.4%), whereas in HSIL, the most prevalent types were HPV16 (34.9%), HPV31 (23.9%), HPV39/HPV52 (both 12.8%), and HPV51 (11.9%). HPV18 was present in 1.0% of women without lesions, in 8.3% with HSIL, but it was the most prevalent type in cases with AGC (23%).

HPV6, although associated with a significantly increased risk of HSIL lesions in this study, is not considered to be involved in the development of cervical cancer (2).

The West European pooled rates of the most common HPV types, reported in the aforementioned meta-analysis, were 1.6% for HPV16 and 0.7% for HPV18 and HPV31 (38).

In the forest plots in Fig. 3, we compare the type-specific prevalence of four common types in high-grade intraepithelial lesions to those reported in neighboring countries. The prevalence in our Belgian study was among the lowest for HPV16, among the highest for HPV31, and similar to other West European countries for HPV18 and HPV33. However, one should be aware of the visibly large heterogeneity in the forest plots, which may be due to variable background risks, age composition of study populations, nonstandardized conditions of sample handling, and the use of different tests.

Potential Effect of HPV Vaccination on Burden of Cytologic Lesions. Because cytology and virology results were available for all samples, we were able to assess the percentage of cervical lesions attributable to each individual HPV type or combination of types. The AR_{exp} % (see Table 3) can be seen as the potential effect of vaccination representing the reduction in prevalence of lesions among women infected with a certain HPV type if infection with that type is eliminated by a 100% effective prophylactic vaccine (assuming absence of cross-protection or type replacement). In addition, the AR_{pop} % corresponds with the reduction in prevalence of lesions in the whole population when infection with one or more types is eliminated. We found that elimination of HPV16 and HPV18 is expected to result in a reduction of the prevalence of ASC-US by 24%, AGC by 19%, LSIL by 29%, ASC-H by 31%, and HSIL by 37%. The reduction in the burden of high-grade cervical lesions, estimated from our study as the expected result of vaccination against HPV16 and HPV18 (37%), was similar to other

estimates also derived from population-attributable risk percentages evaluated in two HPV screening trials, 39% (39) and 45% (40), respectively, and also was not so far from observed outcomes of intention-to-treat analyses of phase III vaccination trials (41). However, considerably higher estimates of reduction in high-grade lesions were derived from meta-analyses where possible effect of vaccination was simply equalized to the prevalence of type-specific HPV infection in cervical lesions (39). These latter estimates are often used in cost-effectiveness analyses.

There is no established method to confirm the causal role of a particular hrHPV infection in the development of neoplastic lesions in case of multiple infections. A plausible assumption proposed by Sargent et al. (40) is that vaccination against HPV16 and HPV18 would have brought women with a single HPV16, a single HPV18, or a double infection with HPV16 and HPV18 to the profile of cervical abnormalities observed in women without a hrHPV infection. If infected with HPV16 and/or HPV18 together with other hrHPV infections, the vaccine would move the profile to those with other hrHPV without concomitant HPV16/18 infection (40). Applying this assumption on our Belgian study would yield a population-attributable risk percentage of 46% for HSIL instead of 37% (see Table 4). Other methods can be considered as well, such as the estimation of the adjusted RR (or odds ratio) by multivariate regression (Poisson or logistic model) controlling for infection with other hrHPV types, which would yield, in our Belgian study, a RR associated with HPV16 infection of 7.5 instead of 13.9 and an AR_{pop} % of 20% instead of 32% (see Table 3).

Targets for Future HPV Vaccination. Three- to five-year follow-up outcomes from randomized clinical trials in 15- to 26-year-old women showed excellent protection rates (95-100%) for HPV16- or HPV18-related CIN2+ or adenocarcinoma *in situ* in women that were not infected with these types just before or during the administration of the vaccine (8, 42-45). Women infected with an HPV type included in the vaccine are slightly or not protected against disease related to that type (46, 47) but are protected against pathology related to the other vaccine types (48). Preliminary data from a phase III trial enrolling women 25 to 45 years of age also indicate good protection (~90%) against HPV6/11/16/18–related CIN or extragenital lesions if HPV naïve for these types (49). The phase III vaccination trials have also shown that DNA negativity for the vaccine types conditions vaccine efficacy (50). Given this evidence, the data shown in Table 2 look interesting because they could allow defining the age strata in the population that would fully or partially be protected by a bivalent or quadrivalent vaccine. However, we should be careful and avoid wishful interpretation about the effects of vaccinating older women, which are not supported by observed data. Wide consensus exists among vaccinologists and immunologists that HPV DNA negativity in sexually inexperienced girls or young women corresponds with a high probability of protection offered by HPV vaccination. Seropositive but HPV DNA–negative women seem to be protected as well, but observations are based on too small sample sizes to be conclusive. No data are available on the risk of HPV-induced disease in women that have cleared a prior infection. It would be interesting to assess differences in risk and protective effect of vaccination in HPV DNA–negative women who were previously HPV DNA positive and those who were previously HPV DNA negative. This could be done by HPV DNA testing on archived smears from cytology biobanks. For instance, one could look for previous smears stored in laboratories taken from women who were DNA negative and seronegative for the vaccine types at enrollment in the trials. Then, one could distinguish HPV16/18 experienced (archived smear positive for HPV16/18) from inexperienced women (archived smear negative for HPV16/18). If this could not be done for women enrolled in a phase III trial, a new prospective study could be set up. If such a study would reveal no difference in risk and protection, an argument for vaccinating older women would be provided. In the absence of this, we cannot reject the hypothesis that HPV DNA–negative women having cleared the virus have sufficient cellular immunity and do not need vaccination anymore.

Limitations of the Study and Future Research. The current study is cross-sectional and, therefore, observed correlations between infection and lesions should be interpreted with caution before accepting them as causal. Extension of the cohort and follow-up of HPV-positive, cytologically negative women will provide more relevant data about the cumulative incidence of CIN2, CIN3, adenocarcinoma *in situ*, or cancer associated with type-specific HPV infections. Unfortunately, HPV11 was not included in the panel of the multiplex PCR reactions, although included in the quadrivalent vaccine. Specific primers for this type have been added more recently. Preliminary data indicate very low prevalence (~0.1% in a similar population screened in July 2009).[5]

Another unresolved issue is the attribution of a lesion to a specific HPV type in case of multiple infections. Persistence of a type over time, sensitive type-specific *in situ* hybridization, or use of mRNA based tests could clarify this question (51, 52). Storage of all residual cellular material, extraction and freezing of DNA, and several feasibility studies involving testing of archived material further enhance the possibility to disentangle the natural history of HPV infections at the type-specific level (53-55).

Conclusion. With the current study, an important first step is made for a comprehensive series of surveillance activities of effects of HPV vaccination (56, 57).

Removing the most oncogenic HPV types through vaccination will likely result in less cervical abnormalities. Nevertheless, this decrease will be less than usually expected because a multitude of HPV types cause cervical intraepithelial neoplasia. This statement should not be considered as vaccine failure. On the contrary, HPV16 and, to a lesser degree, HPV18 are particularly carcinogenic, and elimination of them would therefore preferentially prevent the progressive lesions.

More research, virologic and epidemiologic, is warranted to disentangle the causal role of multiple infections in carcinogenesis.

Currently, systematic screening, organized according to evidence-based guidelines, should be the first priority

[5] C. Depuydt, personal communication.

for women who are older than 25 years (58). Periodic surveys, nested in the screening program with HPV genotyping on cervical cell samples, linked to vaccination registries, will be an important tool to address pending questions such as type replacement, incidence of breakthrough infections, breakthrough lesions, and duration of protection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F. Carcinogenicity of human papillomaviruses. Lancet Oncol 2005; 6:204.
2. zur Hausen H, Arbyn M, Villa L, et al.; IARC Monograph Working Group. IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 90. Human papillomaviruses. Lyon: IARC Press; 2007.
3. Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Chapter 9: Clinical applications of HPV testing: a summary of meta-analyses. Vaccine 2006;24 S3:78–89.
4. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007;370: 890–907.
5. Arbyn M, Buntinx F, Van Ranst M, Paraskevaidis E, Martin-Hirsch P, Dillner J. Virologic versus cytologic triage of women with equivocal Pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. J Natl Cancer Inst 2004;96:280–93.
6. Koutsky LA, Harper DM. Chapter 13: Current findings from prophylactic HPV vaccine trials. Vaccine 2006;24:114–21.
7. Stanley M, Lowy DR, Frazer I. Chapter 12: Prophylactic HPV vaccines: underlying mechanisms. Vaccine 2006;24:106–13.
8. Ault KA. Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3, and adenocarcinoma *in situ*: a combined analysis of four randomised clinical trials. Lancet 2007;369:1861–8.
9. Joura EA, Leodolter S, Hernandez-Avila M, et al. Efficacy of a quadrivalent prophylactic human papillomavirus (types 6, 11, 16, and 18) L1 virus-like-particle vaccine against high-grade vulval and vaginal lesions: a combined analysis of three randomised clinical trials. Lancet 2007;369:1693–702.
10. Franceschi S, Herrero R, Clifford GM, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. Int J Cancer 2006;119:2677–84.
11. Clifford GM, Gallus S, Herrero R, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet 2005;366:991–8.
12. Depuydt C, Vereecken AJ, Salembier GM, et al. Thin-layer liquid-based cervical cytology and PCR for detecting and typing human papillomavirus DNA in Flemish women. Br J Cancer 2003;88:560–6.
13. Arbyn M, Herbert A, Schenck U, et al. European guidelines for quality assurance in cervical cancer screening: recommendations for collecting samples for conventional and liquid-based cytology. Cytopathology 2007;18:133–9.
14. Vassilakos P, Saurel J, Rondez R. Direct-to-vial use of the AutoCyte PREP liquid-based preparation for cervical-vaginal specimens in three European laboratories. Acta Cytol 1999;43:65–8.
15. Vassilakos P, Griffin S, Megevand E, Campana A. CytoRich liquid-based cervical cytologic test. Screening results in a routine cytopathology service. Acta Cytol 1998;42:198–202.
16. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002; 287:2114–9.
17. Herbert A, Bergeron C, Wiener H, Schenck U, Klinkhamer PJ, Arbyn M. European guidelines for quality assurance in cervical cancer screening: recommendations for cervical cytology terminology. Cytopathology 2007;18:213–9.
18. Depuydt C, Benoy IH, Bailleul EJ, Vandepitte J, Vereecken AJ, Bogers JJ. Improved endocervical sampling and HPV viral load detection by Cervex-Brush Combi. Cytopathology 2006;17:374–81.
19. Depuydt CE, Boulet GA, Horvath CA, Benoy IH, Vereecken AJ, Bogers JJ. Comparison of MY09/11 consensus PCR and type-specific PCRs in the detection of oncogenic HPV types. J Cell Mol Med 2007; 11:881–91.
20. Weyers S, Crijns P, De Sutter P, et al. Opvolging na afwijkende cervixcytologie. Tijdschr voor Geneeskunde 2005;61:129–34.
21. Arbyn M, Van Oyen H. Cervical cancer screening in Belgium. Eur J Cancer 2000;36:2191–7.
22. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518–27.
23. Munoz N, Castellsague X, de Gonzalez AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. Vaccine 2006;24:1–10.
24. Merenne B, Van Der Haegen H, Van Hecke E. La Belgique: diversité territoriale. Bulletin du Crédit Communal 1997;202:1–144.
25. Sasieni P, Stepniewska K, Altman D. Test for trend across ordered groups revisited. Stata Tech Bull 1996;6:193–6.
26. Hosmer DW, Lemeshow S. Applied logistic regression. New York: John Wiley & Sons; 1989.
27. Arbyn M, Bourgain C, De Sutter P, et al. Triage HPV detection versus primary HPV screening in combination with liquid-based cytology: a randomised trial. Brussels: Scientific Institute of Public Health; 2001. Report No.: 6.
28. Baay MF, Tjalma WA, Weyler J, et al. Human papillomavirus infection in the female population of Antwerp, Belgium: prevalence in healthy women, women with premalignant lesions and cervical cancer. Eur J Gynaecol Oncol 2001;22:204–8.
29. Meijer CJ, Snijders PJ, Castle PE. Clinical utility of HPV genotyping. Gynecol Oncol 2006;103:12–7.
30. Arbyn M, Dillner J, Schenck U, et al. Chapter 3: Methods for screening and diagnosis. In: Arbyn M, Anttila A, Jordan J, et al., editors. European guidelines for quality assurance in cervical cancer screening. 2nd ed. Luxembourg: Office for Official Publications of the European Communities; 2008. p. 69–152.
31. Depuydt CE, Arbyn M, Benoy IH, Vandepitte J, Vereecken A, Bogers JJ. Quality control for normal liquid-based cytology: rescreening, high risk HPV targeted reviewing and/or high risk HPV detection? J Cell Mol Med. 2008 Jun 9 (Epub ahead of print).
32. Arbyn M, Depuydt C. Distribution of HPV types in a population of 10 thousand Belgian women, screened for cervical cancer. Brussels: Scientific Institute of Public Health, RIATOL; 2007. Report No.: 20.
33. Beerens E, Van Renterghem L, Praet M, et al. Human papillomavirus DNA detection in women with primary abnormal cytology of the cervix: prevalence and distribution of HPV genotypes. Cytopathology 2005;16:199–205.
34. Clifford GM, Rana RK, Franceschi S, Smith JS, Gough G, Pimenta JM. Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. Cancer Epidemiol Biomarkers Prev 2005;14:1157–64.
35. Smith JS, Lindsay L, Hoots B, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007;121:621–32.
36. Sherman ME, Schiffman MA, Cox JT. Effects of age and human papilloma viral load on colposcopy triage: data from the randomised atypical squamous cells of undetermined significance/low-grade intraepithelial lesion triage study (ALTS). J Natl Cancer Inst 2002;94: 102–7.
37. Arbyn M, Paraskevaidis E, Martin-Hirsch P, Prendiville W, Dillner J. Clinical utility of HPV DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN. An update of pooled evidence. Gynecol Oncol 2005;99:7–11.
38. de Sanjose S, Diaz M, Castellsague X, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 2007;7:453–9.
39. Naucler P, Ryd W, Tornberg S, et al. HPV type-specific risks of high-grade CIN during 4 years of follow-up: a population-based prospective study. Br J Cancer 2007;97:129–32.
40. Sargent A, Bailey A, Almonte M, et al. Prevalence of type-specific HPV infection by age and grade of cervical cytology: data from the ARTISTIC trial. Br J Cancer 2008;98(10):1704–9.
41. Arbyn M. Effects of quadrivalent human papillomavirus vaccination. Lancet 2007;370:1031–2.
42. Harper DM, Franco EL, Wheeler CM, et al. Sustained efficacy up

to 4-5 years of bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet 2006;367:1247–55.
43. Villa LL, Costa RLR, Andrade RP, et al. Efficacy of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine through up to 5 years of follow-up. European Research Organization on Genital Infection and Neoplasia; 2006 Apr 26; Paris. 2006. p. 1.
44. FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med 2007;356:1915–27.
45. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med 2007;356:1928–43.
46. FUTURE II Study Group. Supplementary appendix. N Engl J Med 2007;356:1915–27.
47. Hildesheim A, Herrero R, Wacholder S, et al. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine among young women with preexisting infection: a randomized trial. JAMA 2007; 298:743–53.
48. Ferris D. Efficacy of a quadrivalent HPV (types 6/11/16/18) L1 virus-like particle (VLP) vaccine in women with virologic evidence of HPV infection: a combined analysis [Abstract S11-2]. European Research Organization on Genital Infection and Neoplasia; 2006 Apr 26; Paris. 2006.
49. Luna J. The safety, efficacy and immunogenicity of quadrivalent HPV (types 6/11/16/18) L1 virus-like-particle (VLP) vaccine in women aged 24 to 45; oral presentation of abstract, plenary session (PA1-04). 24th International Papillomavirus Conference and Clinical Workshop; 2007 November 3-10; Beijing 2007.
50. Harper DM, Paavonen J. Age for HPV vaccination. Vaccine 2008;26: A7–11.
51. Paavonen J, Jenkins D, Bosch FX, et al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. Lancet 2007;369:2161–70.
52. Gravitt PE, van Doorn LJ, Quint W, et al. Human papillomavirus (HPV) genotyping using paired exfoliated cervicovaginal cells and paraffin-embedded tissues to highlight difficulties in attributing HPV types to specific lesions. J Clin Microbiol 2007;45:3245–50.
53. Arbyn M, Andersson K, Bergeron C, Bogers JP, von Knebel-Doeberitz M, Dillner J. Chapter 16: cervical cytology biobanks as a resource for molecular epidemiology. Methods in biobanking. Tutowa (NJ): The Humana Press, Inc.; 2008.
54. Boulet GA, Horvath CA, Berghmans S, et al. Cervical cytology biobanking: quality of DNA from archival cervical Pap-smears. J Clin Pathol 2008;61:637–41.
55. Horvath CA, Boulet G, Sahebali S, et al. Effects of fixation on RNA integrity in a liquid-based cytology setting. J Clin Pathol 2007;10:1–13.
56. Dillner J, Arbyn M, Dillner L. Translational mini-review series on vaccines: monitoring of human papillomavirus vaccination. Clin Exp Immunol 2007;148:199–207.
57. Arbyn M, Dillner J. Review of current knowledge on HPV vaccination: an appendix to the European guidelines for quality assurance in cervical cancer screening. J Clin Virol 2007;38: 189–97.
58. European Commission. European guidelines for quality assurance in cervical cancer screening. 2nd ed. Luxembourg: Office for Official Publications of the European Communities; 2008.