Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases

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Abstract

Geographical widespread data on human papillomavirus (HPV) type-distribution are essential for estimating the impact of HPV-16/18 vaccines on cervical cancer and cervical screening programmes. Epidemiological studies employing a variety of HPV typing protocols have been collated in meta-analyses. HPV-16/18 is estimated to account for 70% of all cervical cancers worldwide, although the estimated HPV-16/18 fraction is slightly higher in more developed (72–77%) than in less developed (65–72%) regions. About 41–67% of high-grade squamous intraepithelial lesion (HSIL), 16–32% of low-grade squamous intraepithelial lesion (LSIL) and 6–27% of atypical squamous cells of undetermined significance (ASCUS) are also estimated to be HPV-16/18-positive, thus highlighting the increasing relative frequency of HPV-16/18 with increasing lesion severity. After HPV-16/18, the six most common HPV types are the same in all world regions, namely 31, 33, 35, 45, 52 and 58; these account for an additional 20% of cervical cancers worldwide.

Keywords: HPV typing; HPV-16/18 fraction; Geographical heterogeneity

1. Methods used for the detection and typing of HPV-DNA in epidemiological studies

Presently, the two methodologies most widely used for HPV detection in epidemiological studies are the Polymerase Chain Reaction\textsuperscript{\textregistered} (PCR) using generic or consensus primers, and Hybrid Capture\textsuperscript{TM}-2 (HC2, Digene Co., Gaithersburg, MD, USA) [1]. Both assays are suitable for high-throughput testing, automated execution and reading. Furthermore, both assays have been optimised to detect the most clinically relevant HPV types so far, namely the high-risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Identification of specific HPV types in a biological specimen is preferentially done by PCR-based methods, since HC2 uses a cocktail of probes for 13 high-risk types and does not identify which HPV types are present.

The genotyping of HPV-positive samples is achieved by a variety of methods, which may be more or less comprehensive in their number of detectable HPV types, including Southern and Northern blot, dot blot or DNA sequencing, and rarely, HPV type-specific PCR may be done without a preceding generic or consensus PCR step.

The most widely used PCR protocols employ consensus primers that are directed towards a highly conserved region of the L1 gene [2]. Among these are the single pair of consensus primers GP5+/6+ and the MY09/11 degenerate primers, along with the modified version PGMY09/11. Another multiple set of consensus primers (SPF) is available that amplifies a smaller fragment (65 bp compared to 150 bp for the GP primers and 450 bp for MY09/11) of the L1 gene. These methods have been designed to perform in different formats and
can have very high analytical sensitivities [3]. They have been shown to be valuable to address the burden of HPV infections epidemiologically, although their clinical significance is not so evident [4].

In general, it seems that PCR systems using multiple primers such as PGM09/11 and SPF are more robust for detecting multiple infections than systems using single consensus primers such as GP5+/6+. In mixed infections, where one type is present in larger amounts than others, reverse line blot assays have been shown to be very useful [5].

The analytical sensitivities and specificities of HPV tests vary largely, depending on the assay characteristics, the type and quality of the biological specimen and the type and quality of the reagents employed, including the use of different DNA polymerases that affect test performance. Moreover, caution should be used when interpreting such comparisons because the assays differ in their ability to detect different HPV types either as single or multiple infections. In general, there are good to excellent agreement rates between tests performed with HC2 and generic PCR employing MY09/11 and GP5+/6+ systems [2,6]. Nevertheless, standardised methods and validated protocols, reagents and reference samples should be available to ensure the best test performance in different settings. A recent effort launched by the World Health Organisation, proposes the development of international standard reagents for calibration of HPV-DNA assays and kits to be used in different laboratories around the world [7] (see Chapter 23).

2. Methods for estimating HPV type-specific prevalence

Worldwide and regional estimates of HPV type-specific prevalence in women with and without cervical lesions have been estimated both from highly standardised multicentric studies [e.g., International Agency for Research on Cancer (IARC) cervical cancer series [8], IARC HPV prevalence surveys [19]] as well as from wider meta-analyses of all published data [9–12]. The strengths of the highly standardised studies include the inclusion of well-defined samples of women using a standardised protocol, standardised histological confirmation of the lesions under study, and further investigation of HPV-DNA-negative cases when considered appropriate (e.g., in invasive cervical cancer). In addition, the use of well-validated and standardised assays for HPV-DNA detection, and testing for a comprehensive range of HPV types, with the ability to separate single and multiple infections, have made these studies stand out. There are also limitations, however, with the main issues being the insufficient degree of geographical coverage and sample size. The strengths of the meta-analyses include the inclusion of a much larger sample size and a wider geographical coverage, whereas the main limitations are varied. For example, whilst many studies included in meta-analyses meet the above definition of highly standardised studies, others do not. In addition, the diversity in the techniques used for HPV detection and in the range of HPV types assessed in included studies is sizeable. This can lead to the underestimation of the prevalence of certain HPV types (although this may even be a problem for PCR primers used in highly controlled studies), and particularly of multiple infections. Finally, the use of diverse study populations with no common diagnostic protocol can introduce misclassification, particularly for lesser manifestations of HPV infection (i.e., ASCUS and LSIL), where a highly variable proportion of lesions are not HPV-related.

2.1. Methods for estimating HPV type-specific prevalence relevant to meta-analyses

The meta-analyses described in this chapter include only studies using PCR-based HPV detection assays that present prevalence of at least one type other than HPV-6, -11, -16 or -18. Whereas all studies have reported the prevalence of HPV-16 and -18, the prevalence of other types is inconsistently reported. Thus, the prevalence of each HPV type was estimated independently only among studies testing for the HPV genotype in question.

In this calculation, HPV type-specific prevalence includes that in single and in multiple infections. Because most of the studies included tested for only a subset of HPV types, or did not report the type-specific breakdown of multiple infections, it is unknown to what extent any given HPV infection exists in the presence of another HPV type. In particular, caution should be taken when interpreting the attributable fraction of rare or low-risk HPV types, which may largely represent benign infections in the presence of another high-risk type that is causally related to the given lesion.

HPV type-distribution is most often expressed as a proportion of all cases tested for the given HPV type. However, when HPV positivity varies considerably across studies (particularly for women without cervical abnormalities but also with ASCUS/LSIL), HPV type-distribution is also expressed as a proportion of HPV-DNA-positive women only.

3. HPV types in invasive cervical cancer

3.1. Pooled analysis of the IARC cervical cancer series

A pooled analysis of 12 studies conducted in 25 countries has estimated HPV type-specific prevalence in 3085 cervical cancer cases [8]. A standardised study protocol was applied and HPV-DNA testing with GP5+/6+ PCR primers was performed in a central laboratory. The overall HPV-DNA prevalence was 96% and the 15 most common types were, in descending order of frequency, HPV-16, -18, -45, -31, -33, -52, -58, -35, -59, -56, -39, -51, -73, -68 and -66 (see Fig. 1A).

HPV-16 and -18 account for 70% and the eight most common types (HPV-16, -18, -45, -31, -33, -52, -58 and -35) account for 89% of all cervical cancer cases worldwide.
A higher than average prevalence of HPV-16 was found in Northern Africa, Europe and North America, of type 45 in sub-Saharan Africa and of type 31 in Latin America [8].

Over 4% of cancers were classified as positive for "HPVX", but these most likely represent the failed detection of known types rather than infections of yet undiscovered types.

3.2. Meta-analyses of published literature

A comprehensive meta-analysis of 85 studies published up to February 2002 (including those in the aforementioned IARC series), comprised of 10,058 cervical cancer cases [9]. This meta-analysis was recently updated to include more than 14,500 cases from studies published up to January 2006 [11]. The most common HPV types identified were, in order of decreasing prevalence, HPV-16, -18, -33, -45, -31, -58, -52, -35, -59, -56, -51, -52, -35, -45, -39, -6, -68, -73, -66 and -70. The prevalence of high-risk HPV types obtained from the most recent meta-analysis is shown in Fig. 1A and B, respectively.

The most common HPV types in cervical cancer from the most recent meta-analysis are shown by world region in Fig. 2. The same eight HPV types (HPV-16, -18, -31, -33, -35, -45, -52, and -58) accounted for 90% of cases. Very consistent findings can therefore be seen when comparing the pooled analysis and meta-analysis approaches (Fig. 1A and B, respectively). The most common HPV types in cervical cancer from the most recent meta-analysis are shown by world region in Fig. 2. The same eight HPV types (HPV-16, -18, -31, -33, -35, -45, -52, and -58) accounted for 90% of cases. Very consistent findings can therefore be seen when comparing the pooled analysis and meta-analysis approaches (Fig. 1A and B, respectively).

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4. HPV types in high-grade lesions (HSIL)

A comprehensive meta-analysis of 53 studies published up to February 2002, included a total of 4338 HSIL cases [10]. This meta-analysis was recently updated to include, of more than 7000 cases from studies published up to January 2006 [11].

The most common HPV types identified were, in order of decreasing prevalence, HPV-16, -31, -58, -18, -33, -52, -35, -51, -56, -45, -39, -66 and -6 [11]. The eight most common types in HSIL were similar to those in cervical cancer, except for the under-representation of HPV-18 and the absence of the related HPV-45.

The prevalence of the eight most common HPV types in HSIL from the most recent meta-analysis, overall and by region, is shown in Fig. 3. HPV-16 is the most common HPV type in HSIL from all regions, but was ranged from 34% in Asia to 52% in Europe. HPV-18 prevalence varies from 13% in South/Central America to 22% in North America. The relative importance of HPV types 31, 33, 35, 52 and 58 appeared to differ somewhat by region, with HPV-58 prevalence being particularly high in Asia.

This meta-analysis also showed that HPV type-distribution varies significantly between squamous-cell carcinoma (SCC) and adenosquamous carcinoma (ADC), with HPV-16 being identified more often in SCC than in ADC and HPV-18 more in ADC than in SCC [9].
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5. HPV types in LSIL

A comprehensive meta-analysis of 53 studies published up to June 2004 included a total of 8308 LSIL cases [12]. Sixty-four percent of included cases were reported as cytologically diagnosed LSIL, and 36% as histologically confirmed CIN-1. The majority of cases came from studies in Europe (49%) and North America (29%), with African and Asian studies each representing only 3% of LSIL cases [12].

Only studies testing for HPV using one of four validated broad spectrum PCR primer sets, namely MY09/11, PGMY09/11, GP5+/6+ or SPF10, were included in the LSIL

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Fig. 2. Eight most common HPV types in more than 14,500 cervical cancer cases, by region (adapted from [11]).

Fig. 3. Eight most common HPV types in more than 7000 high-grade squamous intraepithelial lesions, overall and by region (adapted from [11]).
meta-analysis. Despite this additional restriction with respect to other meta-analyses [9–11], overall HPV positivity in LSIL varied from 29 to 100% across the included studies. For example, the three largest studies, each contributing over 1000 cases, detected HPV in 55% [13], 73% [14] and 92% [15] of LSIL, respectively. Variations in both cytological/histological assessment, sensitivity of PCR assays and regional definitions of LSIL are likely to account for these differences. To reduce heterogeneity, regional comparisons of HPV type-distribution were restricted to HPV-positive LSIL only.

Among 5910 HPV-positive LSIL, HPV-16 was the most common type (26%), followed by HPV-31 (12%), -51 (11%), -53 (10%), -56 (10%), -52 (9%), -18 (9%), -66 (9%) and 58 (8%). Many other HPV types were also detected in at least 5% of LSIL, thus highlighting the broad heterogeneity of HPV types in LSIL [12].

HPV-16 was the most prevalent HPV type in all regions, although the proportion of HPV-positive LSIL attributable to HPV-16 differed significantly from 16% in Africa to 29% in Europe. The proportion of HPV-positive LSIL attributable to HPV-18 differed significantly from 5% of HPV-positive LSIL in South/Central America to 12% in North America.

6. HPV types in women with ASCUS

No meta-analysis on HPV type-specific prevalence among women with ASCUS currently exists. As for LSIL, the diagnosis of ASCUS is not well standardised and HPV positivity varies greatly in published studies given the considerable intra- and inter-observer variation in its definition.

Furthermore, the HPV positivity of ASCUS varies with age. In the ASCUS/LSIL Triage Study (ALTS), 3363 ASCUS cases in the US were tested for 27 HPV types using PGMY09/11 primers; 2068 (61%) of these tested HPV-positive [16]. However, HPV positivity decreased from 78% in the 18–24 age group to 32% in women over 35 years of age (M. Schiffman, personal communication, April 2006).

The baseline prevalence of HPV-16 and -18 in the ALTS was 15% (24% in HPV-positive ASCUS) and 5% (8% in HPV-positive ASCUS), respectively [16]. HPV-16 and -18 were both present in 1%, and HPV-16 or -18 in 19%. However, the HPV-16/18 proportion decreases linearly with age, from 27% in 1455 women aged 18–24 years (35% in HPV-positive ASCUS) to 6% in 781 women over 35 (19% in HPV-positive ASCUS; M. Schiffman, personal communication, April 2006).

Women in Costa Rica have been tested for 27 HPV types using PGMY09/11 primers; 42% of 727 equivocal smears tested positive [17], and 15% with multiple types. HPV-16 and -18 prevalence was found to be 6.5% (15% in HPV-positive ASCUS) and 1.7% (4% in HPV-positive ASCUS), respectively [17].

In Belgium, 59% of 549 ASCUS cases were found to be HPV-DNA positive and 5% had multiple HPV types (detected using MY09/11 PCR) [18]. HPV-16 prevalence was 13% (22% among HPV-positive ASCUS) and HPV-18 prevalence was 6% (10% among HPV-positive ASCUS) [18].

7. HPV types in women without cervical abnormalities

7.1. Pooled analysis of IARC HPV prevalence surveys

Women were randomly selected from the general population of 13 areas from 11 countries. A standardised protocol was used for cervical specimen collection and all HPV testing was by GP5+/6+ PCR-based enzyme immunoassay. A total of 15,613 women aged 15–74 years without cytological abnormalities were included in a pooled analysis of the proportion of HPV-positive women infected with different HPV types [19] (Fig. 4).

The most common types in women without cervical abnormalities were HPV-16, -42, -58, -31, -18, -56, -81, -35, -33, -45 and -52. HPV-positive women in sub-Saharan Africa were found to be significantly less likely to be infected with HPV-16 than their counterparts in Europe, although more likely to be infected with other high-risk and low-risk HPV types (Fig. 4). Women from South America had type distributions in between those from sub-Saharan Africa and Europe, and there was significant heterogeneity across Asian areas. HPV-35 was significantly more common in HPV-positive women in sub-Saharan Africa than in Europe, whereas the proportion of HPV-positive women infected with HPV-18 was similar across regions [19].

7.2. Meta-analysis of HPV types in women without cervical abnormalities

A comprehensive meta-analysis of all studies published up to January 2006 (including those in the aforementioned IARC series) should be completed in 2006. It will include more than 80 studies and more than 150,000 women without cervical abnormalities.
8. Shifts in HPV type-distribution across cervical lesions of increasing severity

A comparison of the HPV type-distribution from the IARC meta-analyses has shown that HPV types 16, 18 and 45 are significantly more common in SCC than HSIL, whereas the reverse is true for other HPV types [10]. A further comparison has shown that HPV-16 and -18 are also significantly more common in SCC than LSIL, whereas other types are much less frequent in SCC than LSIL, in some instances more than 10-fold so (e.g., HPV-39, -51 and -56), or even 30-fold (HPV-53 and -66) [20] (Fig. 5). These differences highlight the importance of HPV type in the risk of progression to cancer, even from HSIL.

Although inferences with progression based on simple cross-sectional data should be interpreted with caution and should not be used alone to determine the prognostic utility of any single HPV type, the findings of these large cross-sectional comparisons are, at present, the only approach available for comparing HPV types for their risk of progres-
sion to cancer, and are also in broad agreement with HPV type-specific risks for progression to ≥CIN-3 as estimated from prospective studies [21] (see Chapter 5).

9. Geographical variation in HPV type-distribution

When studies are combined, HPV type-distribution is broadly consistent across cervical cancer in all world regions, particularly with respect to HPV-16 and -18. Nevertheless, some inter- and intra-regional variations in the relative importance of the next most common types, namely HPV-31, -33, -45, -52, -58 and -35, have been reported. Whilst some of these apparent differences may arise simply by random fluctuation and/or a lack of representativeness of certain regional samples, some geographical differences are seen consistently across all pooled and meta-analyses, regardless of lesion severity. These differences are noteworthy for scientific reasons, even if they are not always relevant for cancer prevention.

The most consistently observed geographical variation concerns the prevalence of HPV-16 relative to non-HPV-16 types. In the IARC pooled analysis of women with normal cytology, a significantly larger proportion of HPV-positive women were found to be infected with HPV-16 in Europe than in sub-Saharan Africa [19]. Their counterparts in South America show intermediate proportions of HPV-16, and significant heterogeneity was seen across Asian populations. North America was not represented in the IARC pooled analysis, but appears to look most like Europe in terms of proportion of HPV-16 infection [22]. Similar regional differences between Europe, North America, South/Central America and Africa have consistently been observed among meta-analyses, estimating HPV-16 positivity, of LSIL (19, 19, 17 and 10%, respectively) and HSIL (51, 45, 41 and 41%, respectively). Concerning cervical cancer, the HPV-16 prevalence is still estimated to be highest among European cases, although the differences become less relevant (58, 54, 50 and 56%, respectively).

These differences in the relative prevalence of HPV-16 might be related to complex geographical and biological interplay between virus and host immunogenetic factors. HPV-16 seems to be less influenced by immune impairment than other HPV types, as can be seen in women infected with HIV [23] (as discussed in Chapter 16). Defects in cellular immunity (e.g., through chronic cervical inflammation, parasitic infection, malnutrition and, more recently, HIV), might contribute to a higher penetrance of HPV types other than HPV-16 in certain populations. The effect of this would be an apparent decrease in the relative importance of HPV-16 in these populations.

Regardless of the underlying mechanism, trying to identify which HPV types account for geographical variation in the non-HPV-16 fraction of cervical lesions is more difficult because of their lower frequency and the variability in the sensitivity of different HPV assays to detect them. Among meta-analyses of LSIL [12], HSIL [10] and cancer [9] HPV-58 appears to have greater importance in certain parts of Asia. Six percent of cervical cancers from Asia were found to be HPV-58-positive, compared to 3% of cases from South America and 0–2% from other regions [9]. A consistent excess of HPV-58 was also seen in Asia among LSIL [12] and HSIL [10]. High HPV-58 prevalence appears to be particularly concentrated in studies from China, Taiwan and Korea.

10. Preventive potential of HPV-16/18 vaccines

The proportional impact of an HPV-16/18 vaccine on cervical abnormalities is estimated in Fig. 6 using crude HPV-16/18-positive fractions, both overall and separately, for five world regions. The HPV-16/18-positive fractions are drawn from meta-analyses on cervical cancer [11], HSIL [11] and LSIL [12], and from large individual studies for ASCUS [16–18], where they exist.

The estimated HPV-16/18-positive fraction in cervical cancer is approximately 70%, but is estimated to be largest in North America and Oceania (76 and 77%, respectively), slightly lower in Africa and Europe (72 and 74%, respectively), and lowest in South/Central America and Asia (65 and 69%, respectively; Fig. 6).

Certain caveats should be considered when interpreting these crude fractions:

(1) HPV-16/18-positive fractions were estimated by simple addition of the type-specific prevalence for HPV-16 to that for HPV-18, meaning that HPV-16/18 multiple infections were unavoidably counted twice. This may lead to a small over-estimation of the proportional impact of an HPV-16/18 vaccine.

(2) The HPV-16/18 fraction also assumes that HPV-16 and -18 are causally related to the lesion in which they are found, even when in the presence of another HPV type. This assumption may lead to an over-estimation of the proportional impact of an HPV-16/18 vaccine, particularly on LSIL/ASCUS, where many multiple infections with a broad range of HPV types are involved.

(3) Although HPV is found in over 99% of all cervical cancers, HPV was detected in only 87% of cervical cancers in the meta-analysis from which the data on cancer were drawn. Many of the remaining 13% of HPV-negative cases came from studies that did not use the broad spectrum of HPV detection assays available, and so are likely to be positive for high-risk types other than HPV-16/18. However, if all HPV-negative cases were re-investigated using gold-standard investigation techniques [24], it is likely that the HPV-16/18-positive fraction would increase further.

(4) If cross-protection against other HPV types, as recently reported for one of the two HPV-16/18 vaccine candidates [25], proves not only relevant for prevent on of HPV infection but also for preventing cervical lesions...
and cancer, then the fraction of preventable lesions may be slightly higher (see also Chapter 13).

Reassuringly, among the 3607 cancer cases included in the highly standardised, IARC multicentric series [8], the overall estimated attributable fraction for HPV-16 and -18, taking into account 1.8% of HPV-16/18 multiple infections, and using gold-standard re-investigation of HPV-DNA negative cases, was identical to that reported from meta-analysis, namely 71% of the cervical cancer burden worldwide [9]. This equates to 350,000 potentially preventable cervical cancer cases annually by current estimations [26].

11. Priority of HPV types to be included in next-generation vaccine products

Given the very low propensity of some, even high-risk, HPV types to progress from lesser manifestations (i.e., normal cytology, ASCUS, LSIL, HSIL) to cervical cancer, an order of priority for HPV types to be included in the next generation of vaccine products should be established using only the HPV type-distribution among cervical cancer cases.

Fortunately, after HPV-16 and -18, the six most common HPV types are almost exactly the same in all world regions,
namely 31, 33, 35, 45, 52 and 58. The extension of existing HPV-16/18 vaccines to include these six additional types would cover an estimated 90% of the worldwide cervical cancer burden, which equates to 444,000 preventable cases annually by current estimations [26]. If it is not possible to include all six additional types in next-generation vaccines, then there are some regional differences in the priorities for HPV types [8,9,11].

Disclosed potential conflicts of interest

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