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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™ (PCR) using generic or consensus primers, and Hybrid Capture™-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

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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 PGMY09/11 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.