HPV DNA detection: clinical applications

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Introduction

With the licensure and recent agreement by the Commonwealth Government to fund the prophylactic HPV vaccine [Gardasil®], preventative against cervical cancer and for females aged 12 to 26 from 2007, as well as the recent NHMRC guidelines on management of abnormal Papanicolaou (Pap) smears [1] recommending HPV DNA assays as a tool in the management post ablation for high-grade dysplasia, a good knowledge base by the medical profession, clinicians, scientists and the lay public will be critical to ensuring appropriate uptake of the vaccine and appropriate use of various diagnostic HPV DNA assays.

Disease association and natural history

Genital HPV infections are the most common viral sexually transmitted infection (STI), with the majority being transient and asymptomatic. Only in a small number of women does chronic infection of oncogenic or high-risk (HR) genotypes occur with resultant severe dysplasia (the precursor lesion of cervical cancer, otherwise known as high-grade squamous intraepithelial lesions [HSIL] in the new Australian modified Bethesda [AMBS], previously described as cervical intraepithelial neoplasia [CIN]) 2/3 in a proportion and if untreated, ultimately results in carcinogenesis via cofactors (eg cigarette smoking) and in complex pathways not totally understood.

HPV and cervical cytology

HPV is a non-enveloped DNA virus (see Figure 1), the replication of which is intimately linked to differentiation of squamous epithelium. Hence, HPV is unable to be cultured by conventional methods, although the cytopathic effect is that recognised on Pap smear cytology as low-grade sqaumous intraepithelial (LSIL). With this recognition it is now not recommended that LSIL be treated. In contrast HSIL, the true precursor lesion to cancer, must be treated to prevent neoplasia. This is the basis of the Pap screening programme: to detect HSIL, treat it and prevent cancer. The success of the programme is underpinned by lesions taking years to develop into cancer; so despite the low sensitivity of the test, regular screening eventually picks up the underlying lesions.

Impact of molecular biology and epidemiology: cancer risk

Detection of HPV has been revolutionised by molecular biology methods. Adaptation of molecular epidemiology has shown in large multi-national case-control studies that certain HR HPVs are the causative agent of cervical cancer, being found in almost 100% of cases [2]. Worldwide there is a consistent finding that HPV 16 and 18 are the two top ranking genotypes found in cervical cancers, collectively contributing to around 70%: this includes data from Australia [3,4]. Moreover, the strength of association of these carcinogenic HPVs is very strong with odds ratios of several hundred-fold, being the strongest ever observed for a human cancer and far greater than that of cigarette smoking to lung cancer. These HR HPVs also cause a proportion of LSIL (~25%) and around 70% of HSIL lesions. Therefore, the burden of disease from genital HPV is very large. In women worldwide, carcinoma of the cervix is a common cancer, second only to breast cancer, with 80% occurring in the developing world.

Disease association: low risk HPVs

In contrast to the HR HPVs there are low risk types, such as 6 and 11, which are the causative agents of the majority of anogenital warts, otherwise known as condylomata accuminata. These are

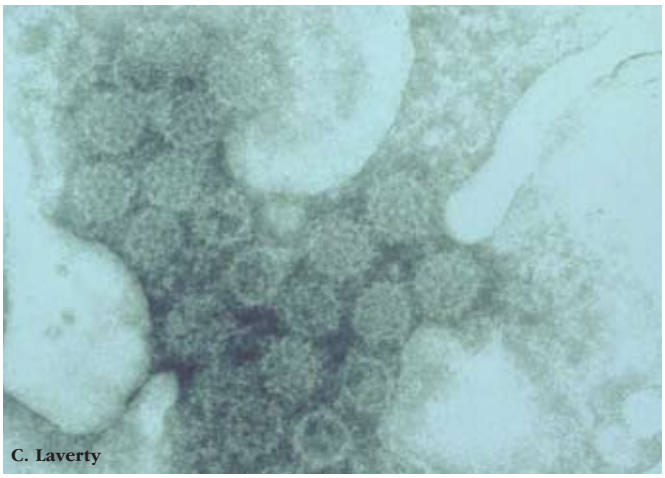

Figure 1. Electron micrograph of HPV from a condyloma accuminatum (with permission of Dr Colin Laverty).

a very common STI, having no specific antiviral treatment, largely being managed with ablative, cytotoxic or immunomodulatory treatment, all with relatively high recurrence rates.

Impact of the HPV vaccine

Therefore, with successful vaccination programmes, we look forward to not only a reduction in cervical cancer, but also in those vulvar cancers that are HPV-related and in the management of abnormal Pap smears, with a commensurate reduction in colposcopy, ablative therapy, and the adverse outcome of pre-term delivery. For the recently licensed quadrivalent vaccine, clinical trials show prevention of genital warts and vulval intraepithelial neoplasia [VIN] 2/3, the precursor lesion to HPV-related vulvar cancers of young women. Our group has been involved in several phase three clinical trials in young women, with the data soon to be published. We are also involved in similar trials in 'older' women (aged 26 years and above), the data to form the basis of whether these women will benefit from these prophylactic vaccines.

HPV detection

Initial molecular methods of HPV detection were direct probe hybridisation, such as dot blot and Southern blot. Besides being labour intensive and time consuming these methods had low sensitivity, and required large amounts of DNA in clinical sample. They have been largely superseded by amplification technology, which has allowed detection of low level virus copy numbers in clinical samples.

Two such methods currently used diagnostically include polymerase chain reaction (PCR) and the only FDA approved test to date, Hybrid Capture 2 [HC 2] (Digene Corporation, Gaithersburg, Maryland, USA). In their infancy are newer assays detecting HPV RNA that await large scale clinical trials to assess their clinical diagnostic value. PCR is a selective target amplification assay capable of exponential and reproducible increase in the HPV sequences present in biological specimens. The amplification process can theoretically produce one billion copies from a single double stranded DNA molecule after thirty cycles of amplification. However, when performing PCR, care must be taken to avoid false-positive results, which may be derived from cross contaminating specimens or reagents with the PCR products of previous rounds. Although this was a serious problem in laboratories when PCR was first utilised, most laboratories now implement procedures to overcome this.

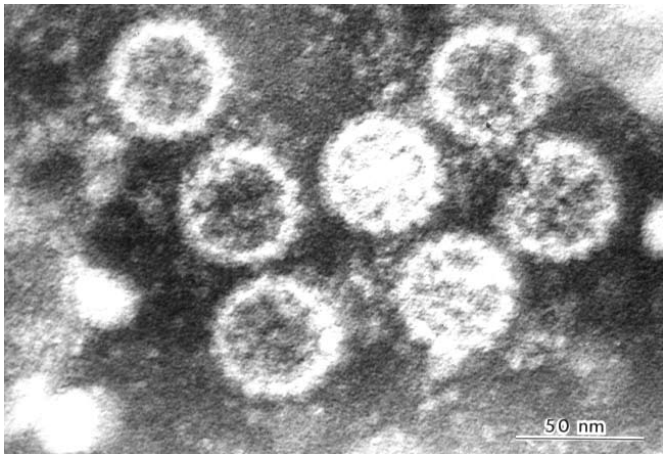

Figure 2. Electron micrograph of viral-like particle used in an HPV vaccine and showing similar morphology (as an empty shell with no DNA) to that of the complete virion as in figure 1. As an intramuscular vaccine these VLPs are sufficient to induce an immune response to levels far greater than that seen from natural infection.

It is to be noted that the sensitivity and specificity of PCR-based methods can vary depending on the DNA extraction procedures, site and type of clinical sample, sample transport and storage, primer sets, the size of the PCR product, reaction conditions and performance of the DNA polymerase used in the reaction, the spectrum of HPV DNA amplified and ability to detect multiple types. Generally a sensitivity of 1-10 copies per PCR reaction is achieved by most methods utilised.

Laboratories using molecular assays for detection of infectious organisms should use standardised tools when performing such assays. Although such standards are not available for HPV DNA assays yet, the World Health Organization (WHO) has initiated an international collaborative study enrolling several laboratories worldwide and these standards should soon be available [5].

The aim of developing such standards means that clinical diagnostic laboratories will be able to validate their own assays and determine their analytical sensitivity. Moreover, for epidemiological prevalence studies and surveillance studies, this will allow comparisons of HPV DNA detection and typing of results over time between different geographic locations, populations and anatomical sites. This is particularly important with the recent licensure of prophylactic HPV vaccine, as these standards will allow for accurate documentation and comparison of various methods in determination of the prevalence of HPV in trial pre- and post-vaccine population responses across various studies and geographic areas.

The WHINURS project (an acronym for 'Women, Human [papillomavirus genotype prevalence in] Indigenous and Non-Indigenous [Australian women, living in] Urban, Rural/Remote [areas] Study') is a study we have initiated with many partners around Australia. We are recruiting 3000 women presenting for Pap smears, largely from family planning clinics and Indigenous Australian health centres throughout Victoria, New South Wales, Queensland, Western Australia, the Northern Territory and

Tasmania. This study will determine the age stratified per geographical region HPV genotype prevalence that will form the baseline prior to HPV vaccination. We plan to subsequently set up a sentinel surveillance system in Australia to monitor the impact of the vaccine, particularly looking at disease burden of the vaccine related HPV genotypes.

Which HPV assay one uses is determined by the indication: for epidemiology one requires a sensitive assay; by contrast, in clinical settings the particular assay needs to be able to detect disease rather than the low copy number of inconsequential infection. Therefore, it is pertinent that any new diagnostic assay is validated so that clinical sensitivity, as well as analytical sensitivity, is determined.

Moreover, from a public health perspective it is not recommended to screen sexually active women prior to HPV vaccination, either for HPV DNA or for type specific antibodies.

Clinical indications

Worldwide, there are three areas in clinical care where HPV DNA testing has been endorsed or considered, whereas to date only indication 3 is endorsed in Australia.

1. In primary screening in conjunction with the Pap, or as a stand alone test for women aged over 30 years

Well organised, high quality Pap cytology screening programmes that adequately reach a high proportion of those at high risk, have markedly reduced cervical cancer incidence and mortality rates in Australia. However, sensitivity of Paps ranges from 30% to 87%, with a mean of 51% [6]. Longitudinal studies show HPV DNA testing has a higher sensitivity for predicting prevalent HSIL than cytology. Based on this, it has been suggested that the more sensitive assay be used for screening, followed by Pap for triage of those found to be HPV DNA positive. This is currently being evaluated. Combining HPV DNA and Pap cytology could result in increasing the screening interval for those with a normal Pap and negative HPV DNA, making the combination cost-effective. Moreover, with the introduction of the prophylactic vaccine, screening intervals will need to be revised. In the meantime, vaccinated women will require Paps to pick up the 30% of types not covered by the vaccine.

Most published studies have been conducted utilising HC2, with studies based on PCR detection only just coming to light. In the ALTS study the authors concluded that ASCUS and LSIL patients who are HPV 16 positive are at significantly greater risk for detection of HSIL during a two-year follow-up phase when compared to those who test positive for another HR HPV or are HPV-negative [7]. Therefore, in the near future genotyping may well have a greater role in clinical management. However, to date there is not an FDA or TGA approved genotyping assay: nor did the recent NIH Bethesda system review meeting (September 2006) endorse genotyping in clinical algorithms of care.

Mathematical modelling to evaluate clinical and economic outcomes for those aged more than 30 years, predict that HPV DNA plus cytology were more effective in reducing cancer incidence and were more cost effective than conventional cytology [8]. Thus, less frequent screening with more sensitive tests is likely to provide a reasonable balance between benefits and costs.

Furthermore, HPV DNA testing, in particular PCR, can be performed on self-collected samples – these have similar sensitivity to clinician-collected samples, and could provide an option in low-resource settings many of which have no Pap programmes.

2. Triage of women with minimally abnormal Paps (ie In AMBS 2004 nomenclature "possible LSIL, possible HSIL"[1]) to discriminate those lesions truly HPV related and requiring colposcopic follow-up.

In the USA, HPV DNA testing has an accepted role in the management of women with minor cytological abnormalities [9], the rationale being that a high proportion of this group, on consensus cytological review, are normal when HPV DNA is negative (around 50%) and so at extremely low risk for HSIL. Clinical trials also show that HPV DNA testing predicted abnormalities sooner and with greater sensitivity, and resulted in around 50% requiring referral to colposcopy, compared to two-thirds by repeat Pap [10].

3. As a test of cure after ablation for HSIL, whereby persistence of HPV DNA after treatment could be an accurate predictor of residual disease or relapse.

Following ablative treatment for HSIL, up to 10% may have residual disease. The standard of care has been close cytological and colposcopic follow-up at 6, 12 and 24 months post-procedure. In a meta-analysis of eleven studies, the NPV for residual disease by HPV DNA testing was 98%, resection margins 91%, cytology 93%, with combined HPV and Pap 99% [11]. Therefore, combining HR HPV DNA testing with cytology allows women who are double negative for HR HPV and Pap (70% of this population), less intensive follow-up is the basis for the current NHMRC recommendation [1].

Communication

With all these new changes there is a necessity to appropriately educate the health profession and the general public, particularly women and their partners, about HPV infection [12]. Many women would not be aware that Pap cytological abnormalities relate to a viral infection, nor for that matter that the virus is transmitted sexually. There is a need to convey clear and consistent information about HPV to the general community and to destigmatise and demystify the whole area of HPV. This will be particularly relevant to ensure appropriate use of HPV DNA assays in clinical care, as well as in the introduction of the HPV prophylactic vaccine in the very near future.

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