Unilabs

HPV and cervical cancer

Human papillomavirus (HPV) are a group of more than 120 related DNA viruses that infect skin cells. A subgroup of some thirty of these virus types can cause genital infections and are sexually transmitted. Some, particularly, types 6 and 11, cause genital warts (condyloma). Other types are capable of causing genital cancers, particularly cervical cancer if infection persists for a long time. The most important cancer-associated types in the western world are HPV types 16, 18, 31, 33 and 45. Papillomaviruses are extremely difficult to cultivate and infections give only a weak antibody response. For this reason all HPV tests detect virus DNA or RNA.

The current Swedish cervical screening program is based on the detection of microscopically observable changes in cell samples from the cervix. Women between 23 and 49 are called in for a cervical smear every three years, while women between 50 and 60 are called every five years. The vast majority of samples are normal, but every year around 30 000 smears have some form for abnormality which requires follow-up. These changes are classified as mild, moderate or high-grade dysplasia, for which the terms CIN1, CIN2 and CIN3 are often applied. CIN is an abbreviation of 'cervical intraepithelial neoplasia', strictly speaking a histological term defined by examination of a biopsy. The majority of such abnormalities disappear spontaneously.

In a minority of cases where the abnormality persists or the cellular changes are more severe, a closer gynecological examination is required. For this purpose a special instrument called a colposcope to examine a magnified image of the cervix and identify abnormal areas. A biopsy of such structures is taken for microscopic analysis, and if pre-cancerous changes are found the patient is treated by removing all of the affected part of the cervix, a process known as conisation. If lower grade lesions are found these can be treated by less aggressive methods such as cryotherapy or laser vaporisation.

Need for a complementary test

The Swedish cytological screening program has been in operation since the 1960s and both incidence of cervical cancer and cervical cancer deaths have been halved. However this decline has stopped in the last ten years. Some 450 women still die of cervical cancer in Sweden each year. There is therefore a need for supplementary test, such as testing for HPV, and there is good reason to believe that the authorities will recommend use of HPV testing in secondary screening as has already been introduced in neighbouring countries.

Vaccination

A further exciting development in the field of cancer prevention is the introduction of an HPV vaccine. Two vaccines have been approved for use in Sweden. Both are contain 'virus-like particles' which are essentially empty virus particles. These particles stimulate an immune response against HPV, but are unable to cause infection. Both the vaccines give good protection against HPV16 and HVP18 which causes som 70% of cervical cancer. One of the vaccines also protects against HPV6 and HVP11 which cause genital warts.

At a consensus meeting organised by the National Institute of Public Healt and the Swedish organisation of obstetrics and gynecology in May 2006 the long term aim of elimination of HPV types 16 and 18 from the Swedish population was agreed upon. The plan is to introduce a universal vaccination program encompassing girls at primary school. The vaccine is now included in the list of subsidised medicines for girls between 13 and 17 and gi-

ves good protection against infection with HPV16 and 18 and the cellular abnomalities that they cause.However, as cervical cancer rarely occurs in women under 30 years of age it will take many decades before the benefits of such a vaccination can be assessed.

Diagnosis of HPV infection

There are a variety of methods, all based on the detection of viral nucleic acids (DNA or RNA), available for detection of HPV infections. Most of these use the same kind of cell sample as is used for liquid-based cytology. Some methods can also use parafin-embedded tissues.

In situ hybridisation allows direct visualisation of HPV in cells by microscopy. Although the method has limited sensitivity it has the advantage that HPV infection is seen in the context of histopathological findings in the sample. The preferred sample for in-situ hybridisation is a parafin embedded biopsy.

In most other tests a suspension of cervical cells collected with a brush or swab is the preferred sample, and this is a less invasive sampling method. Many of these methods are based on PCR, although the Digene High Risk HPV test (Digene/Qiagen) uses a different signal amplification technique that does not involve nucleic acid amplification. The methods differ in their sensitivity and specificity. The most sensitive methods are PCR-based, but it should be noted that clinical sensitivity and specificity are not necessarily the same as analytical performance. In all the methods the first step is extraction of nucleic acid from the sample. In PCR short sequences of complementary DNA (primers) bind to viral DNA and cause it to be repeatedly copied. Once the viral DNA has been copied (amplified) there are a wide range of methods available for detection and/or typing of the viral DNA, including hybridisation to DNA or RNA probes, melting point analysis and DNA sequencing of the copied DNA.

Examples of commercially available HPV test follow. The AMPLICOR Human Papilloma Virus (HPV) test (Roche) is based on PCR followed by hybridisation in microtiter plates; typing can be achieved by the linear array test, which uses a different PCR reaction followed by hybridisation to a membrane strip. Abbott's Real-Time High Risk HPV test is based on real-time PCR. Microarrays may be used for HPV typing, as in in the CLART HPV2 low density array (Genomica) or PapilloCheck (Greiner Bio-one). An alternative is the use of addressable microparticles (Luminex technique) which is used in the Qiagen and Multimetrix HPV genotyping tests. The sensitivity of PCR tests may be so high that only a handful of HPV copies is necessary for a positive result. On the other hand the Digene Hybrid Capture (HCII) test, which does not use amplification has a sensitivity which is deliberately adjusted to 5000 viral copies in order to achieve better specificity. All of the above tests detect most or all of the clinically relevant HPV types. Distinction between types may be at the genotype level, or the types may be grouped, as in the Low-Risk/High Risk type destinction used in the Digene/Qiagen hybrid capture test.

The critical viral genes for cancer development er the E6/E7 oncoprotein genes and some tests focus on these genes as cancer development markers, rather than detecting the virus per se as DNA tests do. These test detect viral mRNA from the E6/E7 genes. There are two such tests on the market. The Aptima HPV test (Gen-probe) which is based on realtime transcription mediated amplification (TMA) and detects 14 high-risk HPV types and the HPV Proofer (Norchip/Biomerieux) based on the NASBA technique and which focuses on the dominant high-risk types.

Unilabs offer a variety of HPV tests

Unilabs in Norway and England offer the well-established Hybrid Capture (HCII) test. In this test the samples are first treated with a basic solution in order to break up the cells and release and denature the DNA. The target HPV DNA is then hybridised to an RNA probe and the resulting DNA-RNA hybrid is captured by antibodies bound to the walls of a microtiter plate. New antibodies, labelled with alkaline phosphatase are bound to this complex and the enzyme is detected by a chemiluminescent reaction. The signal strength is proportional to viral load. Samples with less than 5000 copies of virus are classed as negative; this improves the specificity for high-grade lesions without appreciably reducing the sensitivity. Thirteen high-risk types are detected.

Unilabs Skövde in Sweden use HPV Proofer, a realtime NASBA technique for detection of mRNA from the E6/E7 oncogenes. This test is appropriate for identifying women with a high risk of developing cervical cancer and is considered more speficic than DNA-based methods. Total RNA is extracted from the cells either manually or robotically and viral mRNA is then detected using a kit which specifically amplifies E6/E7 mRNA using the NASBA amplification technique. mRNA is copied to DNA and the DNA is copied to anisense RNA. In contrast to PCR, NASBA runs at a constant temperature (isothermal) using a mixture of three enzymes. The RNA copies thus produced are detected using a special kind of fluorescent probe called molecular beacons. The test specifically detects expression of E6/E7 oncogenes from HPV types 16, 18, 31, 33, and 45 in cytological samples.

We are currently working on an in-house HPV detection and typing method based on multiplex real-time PCR. Development and validation is in progress at our research laboratory in Norway.

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