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International

HPV infection in 2014

Papillomaviruses (HPV) are non-cultivable viruses with circular DNA. They can establish productive infections in the skin (warts) and in mucous membranes (genitals, larynx, etc.). More than 100 genotypes of HPV are currently known, of which around fifteen are thought to have a high risk of oncogenic potential (highrisk HPV). They are very contagious with transmission usually taking place from first sexual intercourse, but infection is usually transient, and the viruses are eliminated within 6 to 18 months. Only persistent, chronic infections with high-risk HPV can lead to cell cycle abnormalities, which may - in the long term progress to cervical cancer.

HPV: Clinical aspects

HPV infections are very common and mundane, and such infections are often transient and without clinical consequences.

Low-risk HPV (primarily HPV 6 and 11) are mainly responsible for genital warts. High-risk HPV are implicated in cancer of the cervix (HPV 16 or 18 in 70% of cases), but also in cancers of the vagina, vulva, anus, oropharynx or penis.

High-risk HPV and cervical cancer

Cervical cancer is recognized as being a virus-induced cancer; HPV is required for the cancer to occur, although it is by itself not enough. Incidence of the disease is 500,000 new cases/year worldwide, causing 275,000 deaths. In France, it is the twelfth leading cause of cancer, resulting in about 1,000 deaths/year. This cancer is thought to be preventable through screening and use of the HPV vaccine. The current strategy is to vaccinate against HPV (6, 11), 16 and 18 before first sexual intercourse (at the age of 11-14 years), thus providing protection against 70% of cancers.

Natural history of HPV infection

LHPV infection is a common and banal infection: 70% of women will be infected with HPV during their lifetime. Infection usually takes place very early (peak prevalence 15-25 years), hence the importance of vaccination in adolescents and pre-adolescents.

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The infection is transmitted through sexual contact, and HPV are initially located in the mucous membranes. To survive, the virus must quickly reach the deepest cells and thus pass through all layers of the epithelium. There is only one area where the virus is able to penetrate, namely the junction between the vaginal portion of the cervix and the supravaginal portion of the cervix. This area is anatomically weak and vulnerable and is called the "transformation zone". It is here that HPV and its associated lesions develop. In the basal cells, HPV loses its capsid and its DNA persists in an episomal state. **The only way to detect the virus at this time is to perform a HPV test.**

In 90% of cases, the virus is eliminated naturally within 12 to 18 months. In 10% of cases, however, the HPV in its episomal state activates its replication genes after a period of several months or even years, leading to release of the virus and the development of so-called low-grade lesions. When detected, these lesions are not usually treated but they are instead monitored, as they resolve spontaneously in about 2/3 of cases. Only a small number of these lesions will progress, depending on the genotype of the virus causing the infection and on certain co-factors needed to enable them to develop into highgrade lesions. These co-factors are connected to the host (all causes of immunosuppression, in particular pregnancy, and other infections, especially infection with HIV) as well as the environment (smoking, oral contraceptives).

When the lesions develop from low-grade to high-grade, the viral DNA changes from an episomal state and becomes integrated into the genome of the host cell. The cleavage site of the loop opening is located at the E2 gene. This cleavage removes the inhibition of the E6 and E7 genes, which then code for their proteins E6 and E7, thus inactivating anti-oncogenes. These cells then develop an intraepithelial neoplasia: instead of making virus they form lesions which then progress to invasive cancer. The entire process takes place over 5 to 15 years. A high-grade lesion may simply persist unchanged or even regress, in about 30% of cases. Lesions such as these are the main detection targets of a cervical smear test.

Prevention of cervical cancer: the cervical smear test

Two smear methods can be used: the conventional smear (Pap smear) or liquid-based cytology.

In France, the recommendations of the ANAES [French national agency for accreditation and evaluation in health care] (published in 2002) are to perform cervical smears in all women between the ages of 25 and 65 years; after two normal smear test results performed one year apart, the smear test is to be repeated every 3 years.

In the past 50 years, this screening strategy has reduced the incidence of invasive cervical cancers by 75%. However, the cervical smear test has important limitations: the results are dependent on correct performance and interpretation of the test; the false negative rate is high (33% of cancers occur in patients with a negative smear); its sensitivity is highly dependent on age and is much better in women over 50 years (79.3%) than in younger (59.6%); likewise, its specificity in young women is low.

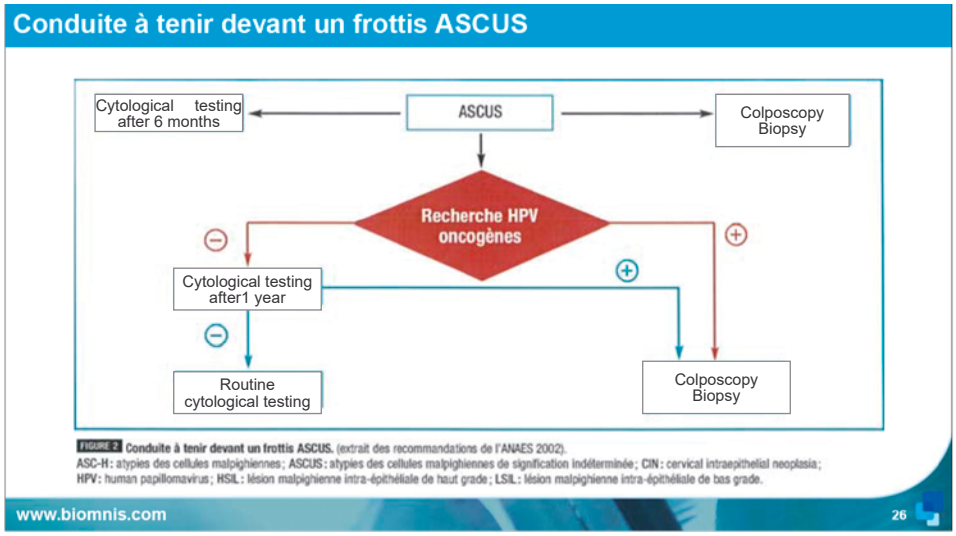
But performing a smear with liquid-based cytology results in fewer artifacts than the conventional smear and makes it possible to perform HPV testing (if the liquid medium used is validated for molecular biology technique used).

Value of HPV testing

Indications

Follow-up of cervical smears showing ASCUS (Atypical Squamous Cells of Undetermined Significance) This is the only indication currently eligible for reimbursement in France. In the case of a smear test showing ASCUS, a negative HPV test makes it possible to identify false positive cytology results, i.e. to classify

ANAES 2002 recommendations for the management of ASCUS smear results: 3 options to choose from.



"questionable" smears as normal smears. If the HPV test is positive, the woman may be referred for a colposcopy: in approximately 50% of cases a lesion will be detected.

Other indications

The test is also recommended for the monitoring of women who have had a precancerous CIN2 or 3 lesion that has been treated by cone biopsy. If the result is negative, a HPV test 6 months after the cone biopsy is adequate to conclude that the risk of recurrence of the lesion is remote, thus enabling monitoring to be performed at greater intervals. An application for reimbursement has been made for this indication.

Interpretation

A positive HPV test signals the presence of high-risk HPV DNA among the subtypes tested; it does not indicate the presence of lesions.

A positive HPV test is only really significant after the age of 3035 years. In younger women, the test is positive in at least 25-30% of cases (high prevalence of HPV infection). In those over 30 years of age, the test is positive in about 10% of women, i.e. those who have not eliminated the virus after several years and who are at risk of persistent infection and thus of developing cancer. In all cases, a positive HPV test should be interpreted alongside cytology results and/or clinical history.

The main advantage of this test is its very high negative predictive value (> 95%).

HPV testing: the techniques

As HPV is non-cultivable, it is only possible to detect the viral genome in the cells of the cervix by using molecular biology techniques on samples of mucosa. The methods available are direct molecular liquid-phase hybridization (Hybrid Capture 2 ®) or nucleic acid amplification tests (NAAT).

There are many tests on the market with various analytical and clinical features (ability to detect a precancerous CIN2 lesion) and varying detection thresholds (50 to 5,000 copies/ml). Certain kits allow the detection of a 'cocktail' of high-risk HPV, some enable partial genotyping and some enable full genotyping. Immunocytochemical approaches are currently being evaluated. These test are simply listed as "HPV testing" in the official list of laboratory tests (genotyping is not obligatory).

Tests available in 2014 (not an exhaustive list)

- **Pooled HPV testing:**Hybrid Capture® 2 HR (Qiagen), Amplicor®HPV (Roche Diagnostics), Cervista™ HPV-HR (Hologic)
- **Pooled HPV testing and partial genotyping for 16/18:**RealTime HR-HPV Assay (Abbott), COBAS® 4800 HPV test (Roche Diagnostics), Cervista HPV 16/18 (Hologic)
- **Full genotyping:** Linear Array HPV® genotyping test (Roche Diagnostics), INNO-LiPA HPV® Genotyping Extra (Innogenetics), Papillocheck® (Greiner Bio-One), CLART ®- HPV2 (Genomica), NucliSENS EasyQ® HPV (BioMérieux)
- **Pooled detection of E6/E7 mRNA:** APTIMA® HPV Assay (Gen-Probe)

The implementation of HPV testing is based on studies that have shown that the Hybrid Capture® 2 test has greater sensitivity and equal specificity to repetition of a cervical smear in the detection of precancerous lesions (CIN2 or higher) following a smear result showing ASCUS. With these tests, it is necessary to have internal control of cellularity and detection of inhibitors of the reaction. The difficulty is to differentiate between the presence of high-risk HPV associated with a lesion of CIN2-3 or above and a transient infection.

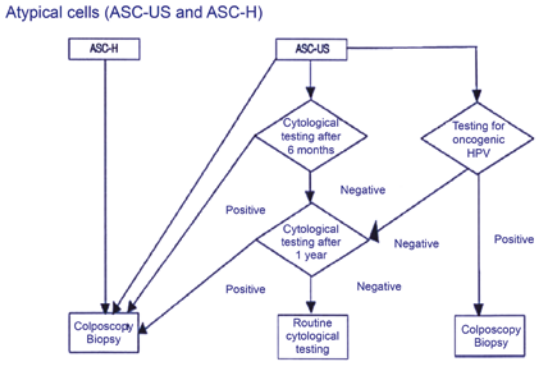
The choice of kit is based on a balance between clinical sensitivity (it must not miss lesions of type CIN2 or higher in women over 30 years old) and specificity (presence of high-risk HPV predictive of lesions of type CIN2 or above, to avoid repeated, unnecessary and redundant examinations). The test must of course have an excellent NPV (if negative for high-risk HPV: risk of lesions is ruled out).

Samples for HPV testing

In France, the CNQ 2011 [quality control guidelines] identified 19 different possible transport mediums In 2013, the French National Health Authority (HAS) stipulated pre-analytical conditions for HPV genome testing for oncogenic human papillomavirus using cervical smear samples. In practice, it is best to use the transport medium recommended or validated by the manufacturer of the HPV test (otherwise, provide validation details for the transport method used) and comply with the transport and storage conditions recommended by that same manufacturer.

Best practice is to perform HPV testing after a smear test showing ASCUS on the sample that was used to perform the liquid-based cytology (if the fluid medium used is validated). For example, HPV testing using Cobas 4800 Roche can be performed 6 months after collection of the sample if the liquid medium used is Cobas collect® Rochie or Preservcyt® stored at between 2 and 30°C. Testing is also possible after 6 months if the medium Surepath® is used and if storage is between 2 and 15°C (or only 14 days if storage is at between 15 and 30°C).

Cervical cancer: screening strategies (ANAES 2002 recommendations)



While in France screening for cervical cancer is based primarily on the cervical smear test in the first instance, other countries have adopted a strategy of combined screening: HPV testing + cervical smear (sensitivity 35% > than smear test only). This strategy increases the sensitivity of the test and the NPV of screening and could make it possible to increase the interval between screening tests (5 years if HPV testing and cervical smear are normal). If the cervical smear is negative and HPV testing is positive, genotyping becomes of great interest, as women could be referred for a colposcopy in the event of detection of HPV16 or 18 (the most common, most persistent, most high-risk), or simply receive follow-up at 12 months if any other type is detected.

Finally, due to its high sensitivity, some authors have also considered the use of HPV testing as primary screening to replace the cervical smear. However, before the age of 30, this test holds little value (high prevalence of HPV). After the age of 30 years, it seems that this approach is valid, but it remains under discussion.

Prevention of cervical cancer: vaccination

Prevention of cervical cancer is difficult because HPV transmission takes place through direct contact and can even occur after genital contact without sexual intercourse; therefore the use of condoms does not provide total protection against infection.
Currently, screening through the use of cervical smears is performed at a stage when a (potentially premalignant) lesion may already be present; it is therefore secondary screening. The benefit of vaccination is early intervention, before exposure to the risk, i.e. prior to HPV infection: it then becomes primary prevention.
HPV 16 and 18 are the two virus types most frequently responsible for causing cancer and which have the greatest oncogenic potential, hence the interest in offering vaccine protection against these two viruses. In terms of low-risk HPV, types 6 and 11 are responsible for almost all genital warts. Two vaccines are currently available: Gardasil® (MSD) (anti-HPV 6, 11, 16, 18), 3 injections at 0, 1 and 6 months, and Cervarix® (GSK) (anti-HPV 16, 18). The vaccination protocol is vaccination at between 11 and 14 years of age, according to a regimen of 2 doses at an interval of 6 months, and a possible booster at the age of 15 to 19 years (in 3 doses). Vaccine coverage in France is about 35%.

Key points for proper clinical use of HPV testing

- Understand the natural history of HPV infection in order to be able to inform patients clearly before performing the test.
- HPV testing is not a diagnostic test. It only measures a risk: do not initiate treatment solely on the basis of the presence of high-risk HPV.
- Reassure patients who are HPV positive and for whom it is not possible to precisely date the exposure.
- Only test for high-risk HPV; specify that the presence of high-risk HPV does not mean a lesion or cancer.
- Do not use HPV testing as primary screening before the age of 30 years or after a pathological smear (high-grade dysplasia or cancer).

Carole Emile, based on a report by Anne Ebel, Biomnis Ivry-sur-Seine, October 2014.