Early diagnosis of HPV16 cancers: a novel assay offers high specificity

The incidence of virus-related head and neck tumours is reaching epidemic proportions, particularly in the developed world. Here, Anna Huber provides an overview and discusses the need to improve patient outcomes while also offering cost-savings to the NHS.

A breakthrough in the detection of cancers caused by human papillomavirus (HPV) is on the horizon with the launch of the Abviris HPV16 subtype-specific serological assay. Unlike the more common molecular methods for the detection of HPV DNA in cells and tissues, the test relies on detecting an immune response that occurs when an HPV infection has led to tumour growth.

Experts have been warning of a 'virus-related cancer epidemic' and calling for improved methods of early detection, treatment control and post-treatment surveillance of HPV-induced cancers.[1] At the same time, they stress that any solution must be cost-effective and easy to integrate into clinical practice.

Healthcare agencies worldwide view HPV vaccination as the silver bullet against HPV cancers. However, vaccinated populations will not reach cancer-relevant age for many decades and there are still over seven billion individuals currently unprotected.[2] As a result, incidence of HPV-induced cancer will continue to rise, alongside the need to expand detection strategies beyond cervical cancer.

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Biomarker blood test offers diagnostic potential in HPV-related cancers.

Upward trend in head and neck cancers

Human papillomavirus belongs to a family of DNA viruses with over 200 different subtypes, which are transmitted through skin-to-skin contact and infect the basal cells of the skin or mucous membranes. It is estimated that at least 80% of the population contract HPV at least once in their lives, and most infections are cleared within two years without symptoms occurring. In rare cases, persistent infections with HPV16 and other high-risk HPV types can lead to the development of cancer of the infected epithelia, in particularly in the mouth, throat, cervix and anogenital region.

Although cervical cancer still accounts for the largest proportion of cases worldwide, epidemiologists are concerned about the continuing upward trend in HPV-associated head and neck cancers, seen predominantly in developed countries.[3]

In the UK, the number of head and neck cancer cases has already overtaken those of cervical cancer, with those for oropharyngeal cancer tripling since 1995.[4,5] Over half of oropharyngeal cancer cases are caused by high-risk HPV types, in particular HPV16, which is responsible for 90% of all HPV-induced cases.

While cervical cancer screening is an undoubted success, it is mainly due to the cancer cells being easy to locate and access. This is not the case if the cancer is in the back of the mouth and throat, where most HPV-induced cancers are located. For this reason, around 70% of oropharyngeal cancers are usually only detected as T3 or T4 late-stage tumours, reducing survival. According to Cancer Research UK, 44–88% of head and neck cancer cases could be prevented; however, methods for early detection are lacking. As a consequence, twice as many

people die from head and neck cancers compared to deaths from, for example, skin cancer, which is diagnosed more frequently but detected earlier.

Immune response indicates cancer cell growth

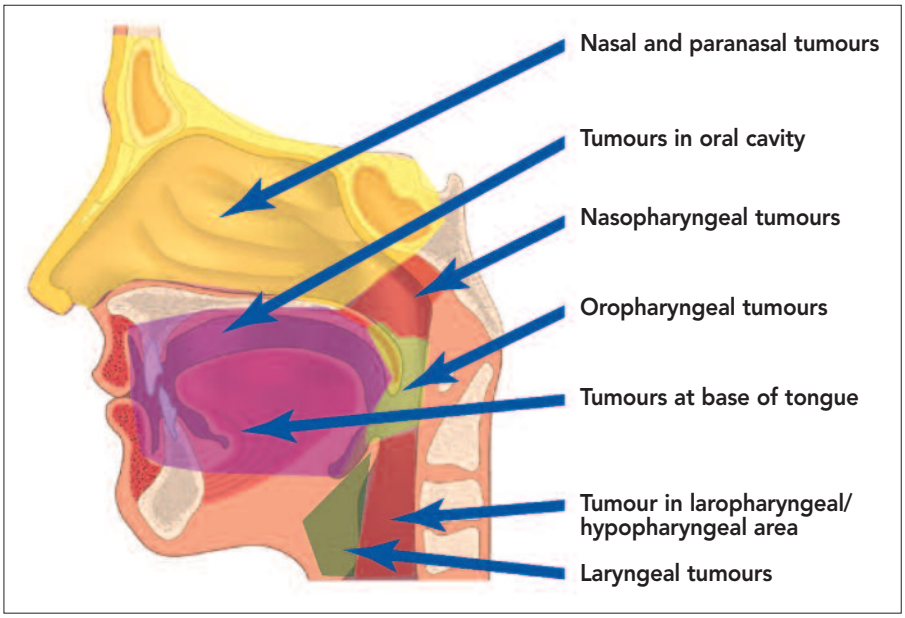
German biotech company Abviris specialises in developing innovative serological assays to detect HPV-induced cancers. Unlike traditional HPV genotyping tests that detect subclinical, latent HPV infections, the test developed by Abviris detects an immune response that occurs in the event of HPV-induced cell growth. The blood-based assay is easy to use and can be run as a lateral-flow rapid test, with a hands-on time of less than five minutes and results delivered within 20 minutes, all at the point of care.

Two recently published, peer-reviewed studies evaluated the ability of Prevo-Check, the Abviris CE-marked HPV16-specific rapid test, to detect HPV16-induced oral, oropharyngeal and anal cancer. In the Blatt study,[6] Prevo-Check was able to identify HPV16-induced cases among 107 oral cancer patients with an accuracy of 100%.

In a second study involving over 1000 blood samples representative of the general population, Weiland *et al*.[7] established the assay's specificity to be over 99%. It also examined patients diagnosed with either HPV16-induced oropharyngeal or anal squamous cell carcinoma, demonstrating sensitivities for these cancers of 95% and 90%, respectively. In the anal cancer population, blood samples were available in the year (up to nine months) prior to cancer diagnosis. Nine out of 10 of these retrospective samples were found to be serologically positive, suggesting that these patients would have received an earlier cancer diagnosis if they had been offered this rapid test.

In the patient population with oropharyngeal cancer, antibody concentrations were monitored for a period of 24 months after treatment onset. The study team observed a decrease in antibody levels during successful treatment, while a rise during post-treatment follow-up was associated with cancer recurrence.

These studies provide the first evidence that an easy-to-use, blood-based test is able to track HPV-tumour development closely. The low rate of positive results among the general population, combined with high sensitivity observed in target patient groups, supports the view that the assay would be able to detect HPV-induced malignancies, without identifying transient, subclinical HPV infections.



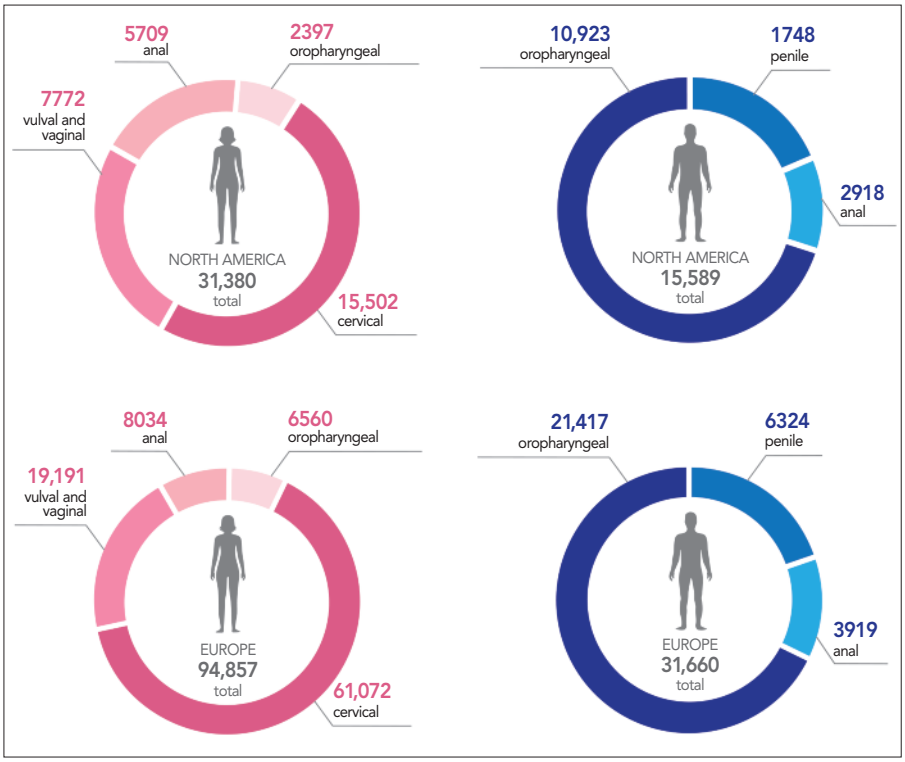
Head and neck cancer sites. Most HPV-driven head and neck tumours occur in the tonsils and at the base of the tongue.

Abviris is already planning several further clinical studies to confirm the efficacy of the biomarker, some of which may also demonstrate its usefulness as a screening tool for high-risk patients such as those who are HIV-positive. The protocol for the Weiland findings will form the basis of a separate and much larger study across at least 15 hospital sites, which is due to start later this year. It will look at diagnosis, characterisation and its ability to monitor the success of treatment, seeking to confirm the biomarker's ability to identify early recurrence.

Improving tumour characterisation

The ability to classify correctly whether a head and neck tumour is HPV-induced or non-HPV-induced has consequences for staging, treatment and prognosis. This is reflected in the most recent edition of the TNM staging guidelines issued by the American Joint Committee on Cancer and the Union for International Cancer Control, which recommends stratification of oropharyngeal carcinoma by HPV status.[8]

Some studies indicate that patients with oropharyngeal cancer caused by HPV



Numbers of HPV-related cancers in Europe and North America.

may respond better to treatment, with clinical trials already underway to investigate if de-intensification of treatment could be an option.[9] However, despite the clinical imperative for accurate tumour classification, there are currently no methods specifically approved for HPV detection in head and neck cancers, and there is ongoing debate about which diagnostic tools are the most appropriate.

Current guidelines recommend staining of tumour tissue for p16, a cellular protein which can be upregulated as a result of HPV activity.[10] However, the limitations of relying on p16 immunohistochemistry alone as a surrogate marker for indicating HPV status is well documented, as p16 upregulation can be caused by factors other than HPV. The Royal College of Pathologists in the UK has therefore recently recommended use of a second-line test to confirm that HPV is indeed present in tumours previously tested positive for p16.[11]

A call for single-step diagnostics

A 2019 *British Journal of Cancer* paper looking at methods for determining HPV status in patients with oropharyngeal cancers under TNM8 guidelines reports on the lack of standardisation of current practices and the negative impact this is having on patient management.[12] At the same time, National Institute for Health and Care Excellence (NICE) guidances stress the need to simplify current two-step practices, pointing to the prognostic value and significant cost-savings for the NHS if a single-step test could be proved to be effective.[13]

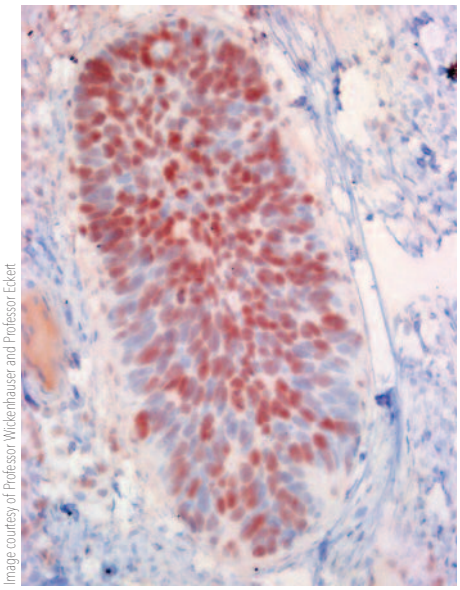


Image courtesy of Professor Wickenhauser and Professor Eckert

HPV16 L1 in cell nuclei of a squamous carcinoma (immunohistochemisty staining).

Following NICE research recommendation, Blatt *et al.* set out to compare the performance of the new serological assay with the current two-step approach for tumour classification in 107 patients with oral cancer.[6] In line with previous data, Blatt found positive p16 staining in some tumours that were in fact HPV-negative. Without a second-line HPV DNA test, these cases would have been misclassified as HPV-induced, with potentially detrimental consequences to patient management.

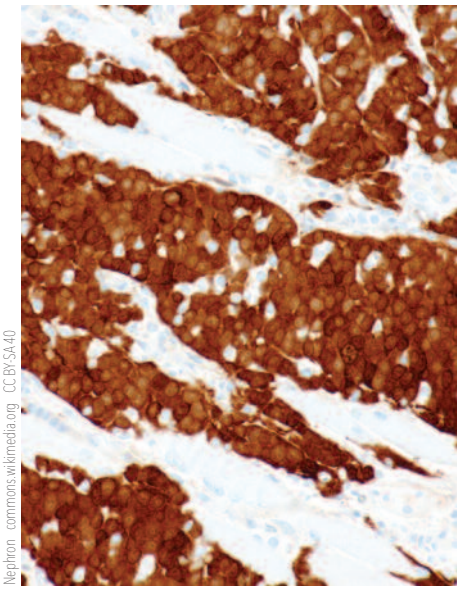
In contrast, the Abviris test identified only cases that were positive for both p16 and HPV16 DNA, therefore exhibiting an accuracy of 100% in identifying HPV16-induced tumours. Although further studies are needed to confirm these findings in a larger population, it is tempting to speculate that it may soon be possible to replace the cost- and time-consuming two-step approach to determine the HPV status of head and neck tumours with a simple blood-based single-step test.

HPV16 L1 – rediscovering an overlooked target

The excellent performance characteristics of the new serological assay are explained by the highly specific detection of a patient's immune response to HPV16 L1. The HPV genome contains eight genes (*E1–E7*, as well as *L1* and *L2*), and most studies have focused on oncogenes *E6* and *E7*.[14]

Previously, it was believed that there was insufficient evidence that tumour cells produced L1 or that serum antibodies against L1 could discriminate between cancer and subclinical HPV infection. However, several historic studies[15–17] indicated a potential role for this target, but they had not been pursued until now. For example, one earlier study clearly showed that immune cells programmed to kill L1-positive cells were also able to destroy tumour cells.[15]

This was taken up in the current study by Weiland *et al.*, which described an immunostaining protocol with which it is possible to visualise L1 expression in tumour tissue. This provided strong new evidence that L1 is indeed expressed in HPV-induced oropharyngeal squamous cell carcinomas.



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Oropharyngeal squamous carcinoma showing p16-positivity (immunohistochemical staining).

Impact on budgets and patient outcomes

The most immediate application for a blood-based biomarker test lies in the post-treatment monitoring of patients treated for HPV-induced cancers. At present, treatment success and cancer recurrence can only be monitored using high-cost imaging methods including positron-emission tomography (PET-CT) or invasive endoscopies performed under general anaesthetics.

Using the test to identify rising antibody levels, and therefore potentially tumour recurrence, could lead to earlier intervention. This would also reduce the need for invasive procedures and imaging, so that higher-cost resources could be targeted more appropriately.

Another possible application lies in offering early detection of HPV cancer or precancer in high-risk but asymptomatic people, such as those with recurring genital warts, multiple sexually transmitted infections (STIs) and/or HIV, alongside men engaging in anal sex. Testing could be offered at sexual health clinics, and those with a positive result fast-tracked for further investigation.

There is significant and wide-ranging diagnostic potential for a biomarker capable of tracking HPV tumour development. It would enable NHS clinical and pathology services to target resources more effectively. Furthermore, by offering patients improved and early detection of HPV-induced cancers, it would bring closer the NHS Long Term Plan, that by 2028 "three in four cancers (75%) will be diagnosed at an early stage".[18]

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Further information may be found on the Abviris website (https://abviris.com).