Self-sampling to detect cervical cancer

Professor Dr Chris JLM Meijer discusses his work to improve screening for cervical cancer. He describes the importance of self-sampling, a new detection method he has devised to increase health equity.

How did your interest in human papillomavirus (HPV) and cervical cancer develop?

I was initially trained as a pathologist, then as a medical immunologist and later I acquired molecular biology training. The main topics of my early research were in oncology, specifically lymphomas. When I moved to Amsterdam, researchers there were looking for immune reactions against tumours. I discovered it was very difficult to identify appropriate tumour antigens to which immune responses could be reliably measured. This turned my attention to viral oncology, and I became interested in Epstein-Barr virus, and later HPV and cervical cancer. Since 1986, I have led a group of scientists working on HPV and cervical cancer.

Could you expand on the experiments your group has worked on over the years?

We first developed a general primer-based polymerase chain reaction (PCR) to detect different HPV types in tissues and smears of cervical cancer and its precursor lesions. We showed that HPV was necessary for the progression of cervical intraepithelial neoplasia (CIN) lesions and that absence of HPV was associated with regression of the lesions. The team realised soon after that if we wanted to use HPV testing in clinical practice, we had to realise many HPV infections are transient. Simply referring all HPV-positive women to the gynaecologist would result in referring many more patients than if you only referred women based on abnormal cytology. We therefore launched a two-pronged approach. We first asked ‘Is it possible to define a test to detect HPV infections associated with disease, and not transient infections?’

With an international research team, we worked to formulate these criteria. They have been derived from large screening trials and clinical studies in which the efficacy of HPV testing had been proven. Secondly, we devised a triage algorithm for HPV positive women for use in population-based screening. HPV positive women with a positive triage test have a high risk for the most advanced precancer, cervical intraepithelial neoplasia 3 (CIN3), or cancer (CIN3+), which warrants immediate colposcopy referral. The CIN3+ risk of HPV-positive triage test negative women is perhaps too high to wait for the next 3-5 years. A threshold for developing CIN3+ in the next 1-2 years had to be defined for women who deserve further testing. In the Dutch screening setting we showed the three-year CIN3+ risk of HPV positive, cytologically negative women was 5 per cent – too high to send them to routine screening after five years. An additional cytology test after six to 12 months decreased the three-year CIN3+ risk below 1 per cent and seemed acceptable from a CIN3+ risk perspective.

To overcome the subjectivity of cytology, we then developed an assay to look for the tumour suppressor genes and to determine the level of methylation of the promotor regions. We were able to develop a threshold of methylation that corresponds with high grade CIN. Currently we are evaluating this assay in clinical settings.

Can you discuss your affirmations that self-sampled cervico/vaginal (c/v) specimens should be used for primary HPV testing for cervical cancer screening?

Women should have a choice; they should be able to choose to go to the doctor or take the sample themselves. HPV self-sampling and triage of HPV positive women with a methylation marker test on the same specimen opens the way to full molecular screening. You don’t need cytology, which is subjective. This enables incorporating HPV self-sampling into the screening of women in high-income and medium-income countries.

Your ultimate aim has been to develop full molecular cervical cancer screening on self-collected c/v material. Could you describe the hurdles you have overcome on your way to meeting this goal?

The Netherlands has one of the best cytology systems in the world, and it’s very well controlled. 70-80 per cent of smears are conducted inside the screening programme, whereas in many other countries, only 30-40 per cent take place within the programme. However, if you take smears outside the programme – what we call ‘opportunistic smears’ – it increases the cost associated with prevention. It was very difficult to make changes, as there are many people working in the cytology screening business. We faced a lot of criticism from gynaecologists, pathologists and cytotechnicians concerning the change. However, we finally succeeded in convincing physicians and health policy makers by showing that women with a negative HPV test had in the next screening round 70 per cent better protection against cancer and 50 per cent against CIN3+ compared to a negative cytology test.
Protecting against cervical cancer

Research taking place at VU University Medical Center in Amsterdam is revolutionising diagnosis and screening for cervical cancer. These new methods could increase early detection and, ultimately, survival rates.

PERSISTENT HUMAN PAPILLOMAVIRUS (HPV) infection causes 99.7 per cent of all cervical cancers – a much stronger link than that of smoking and lung cancer. It is also the second most common cancer in women worldwide. Although 80 per cent of people will be exposed to HPV in their lifetime, in most cases, it is cleared by the immune system and the individual is unaware of the infection. However, certain types of HPV infection can cause changes to the cells of the cervix in some women, leading to cancer. If detected early, cervical cancer is largely preventable and the survival rates are high. Unfortunately, in all too many cases, this prevention is not happening.

Professor Dr Chris JLM Meijer, Professor of pathology at VU University Medical Center in Amsterdam, is working to ensure cervical cancer is always detected before it is too late. Meijer leads a group of researchers integrating the basic and translational aspects of HPV in cervical cancer. The team is currently working to implement new preventive strategies in The Netherlands as well as developing countries. These strategies include using HPV testing as a primary tool in cervical screening, but also other biomarkers as secondary, so-called triage tests, for HPV positive women.

SELF-SAMPLING

Meijer’s interest in HPV began in 1986 when it became clear that the virus was associated with cervical cancer. His research today aims to develop comprehensive cervical cancer screening methods for vaginal brush- or lavage-based self-sampled cervico/vaginal (c/v) material. The anxiety associated with a smear test – which is the main preventive option available to women – and the associated reluctance to visit the doctor to receive one, is a major reason cervical cancer is often diagnosed too late. In fact, attendance rates in The Netherlands sit at 65 per cent. Meijer hopes to remove this obstacle by giving women the freedom to take the samples themselves.

Meijer has encountered several challenges on his way to successfully implementing HPV testing in cervical cancer screening. The first of these was the development of an effective, clinically validated test for HPV detection. Alongside collaborators Dr Jan Walboomers, Dr Peter Snijders and Dr Adriaan van den Brule, Meijer developed a novel polymerase chain (PCR) technique. PCR, a process that can be used to detect viral DNA, relies on primers – short pieces of DNA designed to match the section to be copied. The technique originally focused on general primers 5 and 6 to detect different HPV types in samples of cervical cancer, as well as precursor lesions. Meijer and Snijders built on their initial technology by elongating the primers and adding an easy readout. In the new and improved form, general primer mediated 5+/6+ (GP5+/GP6+) PCR can be used for high-throughput screening, enabling the rapid and automated analysis of samples.

Later, in collaboration with the International Agency for Research on Cancer (IARC), Meijer applied this pioneering detection method in controlled studies. It was shown that the majority of cervical cancers are associated with high risk HPV (hrHPV) types.

Building on this, Meijer was able to show that persistent hrHPV infection in women with abnormal smear results is associated with progressive cervical intraepithelial neoplasia (CIN), which describes the premalignant changes to the cervical cells. Moreover, analysis of samples from women who later developed cervical cancer showed that the HPV type found in the cancer was the same as detected in the earlier, normal smear, providing a strong causative link.

ELIMINATING SUBJECTIVITY

These findings provided fresh insight into the association of HPV and cervical cancer and facilitated rapid progress in the field. Furthermore, the team had successfully developed and validated an effective HPV detection test. The next step was to obtain robust, clinical evidence to prove this genetic method of HPV testing was superior to cytology – studying cells under a microscope and using morphological changes to diagnose disease.

Although cytology is the gold-standard for cervical cancer screening, its diagnosis sensitivity is, at most, 65 per cent. The technique also often yields false results, leading Meijer to hypothesise that HPV testing by his method could be an effective alternative.
INTENDING TO OFFERHPV TESTING ON SELF-SAMPLED CERVICO/VAGINAL SPECIMENS

OBJECTIVES

To improve the effectiveness of cervical cancer screening by increasing compliance and improving the primary screening test. Through biomarker testing combined with optimised self-collection methods for cervico/vaginal material, it is expected a full molecular self-screening for cervical cancer can be achieved.

KEY COLLABORATORS

Peter Snijders, Renske Steenbergen, Daniëlle Heideman; H Berkhof, VUMC Amsterdam • Pathologists Rence Rozendaal and Maaike Bleeker • Young scientists involved in clinical studies: Maaike Dijkstra; Bart Hesselink; Roos Luttmer. Nicole Polman; Viola Verhoef; Lisa de Strooper; Margot Uijterwaal; Marjolein van Zummeren

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Testing for HPV and methylation markers on self-collected vaginal material opens the way to full molecular screening

To examine the method in clinical practice, Meijer and his team, which includes Dr Peter Snijders, Dr Hans Berkhof, Dr Folkert van Kemenade, Dr Renske Steenbergen and Dr Daniëlle Heideman, among others, targeted women who did not attend cervical screening. They were sent a device called a Delphi Screener to collect their own c/v material for HPV testing. Analysis showed that the prevalence of CIN2, CIN3 and cervical cancer was higher in the non-attendees than in the regularly attending group, proving the importance of regular testing. Although only 30 per cent responded in this instance, the study was powerful evidence for HPV self-sampling as a feasible approach.

INCREASING SPECIFICITY WHILE PROTECTING SENSITIVITY

HPV detection identifies all women at risk of developing cervical cancer, but in doing so it also detects those with transient infections, which will be cleared from the body and will not lead to malignancy. Referring all HPV-positive women to the gynaecologist would lead to referral rates three times higher than screening by cytology. This would incur great healthcare costs and cause additional, sometimes unnecessary, stress to women. Meijer set about solving this problem in two ways. First, to prevent detection of transient infections, he defined a set of conditions that a HPV test must fulfil in order to be validated clinically. Second, based on data from the POBASCAM study, the group showed that cytology following an HPV test could be used to confirm those women who do require a colposcopy.

More recently, group members Dr Renske Steenbergen and Dr Saskia Wilting have identified several tumour suppressor genes involved in cervical carcinogenesis. CADM1, MAL and miR124-2 are increasingly methylated during the pathogenic processes following HPV infection. The methylation of these genes seemed a reliable biomarker for diagnosing and staging cervical abnormalities. The Meijer-Snijders team thus developed a test to determine the level of methylation, which could be used on physician-taken smears and self-collected specimens. Dr Viola Verhoef and Dr Bart Hesselink of the team showed in a trial of women who tested HPV positive on self-collected specimens that triaging for colposcopy using the methylation test was equally as sensitive as triage by cytology. This is a powerful finding, and it shows the HPV test can be made more exact by targeting specific methylation patterns, while remaining sensitive.

NOTHING IS MISSED

Meijer’s work could enhance protection for cervical cancer by as much as 70 per cent. The GP5+/GP6+ PCR assay is now a well-established HPV detection technique, and the triage method he has developed – ‘the molecular triage test’ – is much more directed. Together with self-sampling for HPV testing, it opens the way to full molecular screening. It gives patients the freedom to take the test without needing to visit a doctor. Meijer hopes the new molecular triage test will be available in six months to a year. “There may be a possibility that in the future, optimised methylation marker testing may be the primary screening test for cervical cancer,” he concludes.