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The use of urine in the follow-up of HPV vaccine trials

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Prevention and treatment of human papillomavirus related cervical cancer through vaccination is a relative new field with many scientific, technological and implementational challenges requiring numerous new clinical trials. The initial prophylactic HPV vaccine trials allowed to set new end-points based on persistent infection in order to determine vaccine efficacy for prevention of cervical cancer. Major progress has been made regarding detection of HPV DNA in urine and high correlations between urinary HPV DNA and cervical infections have been established. Urine sampling has a number of assets such as its non-invasive character, and allowing for self-collection at home creating options to simplify follow-up of HPV in women participating in HPV vaccine efficacy trials. The current reported variability in urinary HPV sampling and detection can be overcome through relative simple sampling and testing guidelines. Determining persistent infection or lack of therapy response by urinary HPV detection may be an interesting approach to assess a viral end-point in HPV prophylactic and therapeutic vaccine efficacy trials for women.

Keywords: human papillomavirus, HPV testing, urine, vaccine efficacy trials

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The Use of Urine in the Follow-up of HPV Vaccine Trials

Only three decades ago the link between infection with high risk human papillomavirus (HPV) types and cancer of the cervix was established.[1] As for viral hepatitis B this created an opportunity of preventing infectious disease-related cancer through vaccination. Two commercially available prophylactic vaccines, a bivalent and quadrivalent vaccine, have been tested on large cohorts and are licensed in the US, Europe and many other countries. Data from the clinical trials show that both vaccines have very high efficacy for prevention of vaccine type-related cervical precancers.[2-4] In December 2012 more than 40 countries had introduced HPV vaccine in their national immunization programmes.

Both licensed prophylactic HPV L1-based vaccines provide high antibody concentration and there are strong indications that these HPV vaccines induce an antibody mediated sterilizing protection, important in the prevention of infectious disease-related cancer. This assumption has been confirmed in animal studies where passive immunization of naïve recipients with immunoglobulin purified from immunized animals protected against high-dose viral challenge.[5] However, since only a small number of vaccine breakthroughs have been reported, which can be explained by undetected prevalent infections, no immune correlates of protection have been defined so far.[6] In addition to the currently available bi- and quadrivalent vaccines, a next generation HPV nonavalent vaccine has recently been developed, which has been shown effective in preventing persistent infection and precancerous lesions associated with HPV types 16/18/31/33/45/52/58 and genital warts caused by HPV types 6 and 11.[7]

Prophylactic HPV vaccines are relatively new and major progress at a scientific, technological and implementation level is ongoing, requiring additional clinical trials. A non-exhaustive list of pending vaccine-related questions would comprise the following topics: 1) one or 2 doses versus 3 doses; 2) infant or children vaccination vs. adolescent or adult vaccination; 3) bivalent and quadrivalent versus nonavalent or x-valent vaccines; 4) use of none-VLP vaccines based on viral capsomeres or

polypetides vs. VLP vaccines; 5) alternative administration routes, e.g. intradermal; 6) L2 vaccines containing cross-neutralization peptides; 7) use of HPV vaccines in already infected or treated patients; 8) documentation of long-term protection; 9) sustainability of the immunization programmes; etc.

The World Health Organization and International Agency for Research on Cancer (IARC) organized in 2013 an expert meeting to discuss primary end-points for prophylactic HPV vaccine trials.[8] As viral infection with a high risk HPV type has proven essential for the development of nearly 100% of cervical cancer cases, preventing viral infections should also prevent development of pre-malignant and malignant disease. The working group agreed that persistent infection of 6 months or longer is very likely to act with high fidelity as a surrogate for advanced disease/cancer. The value of persistent infection (6 months or 12 months) as viral end-point compared to CIN2+ as end-point was confirmed in the efficacy trials that have been performed.[9-14] For cervical cancer prevention, vaccine efficacy as determined by the viral end-point was similar to the CIN2+ end-point for vaccine types and similar or lower for non-vaccine types, the latter due to partial cross-protection of the vaccine. Furthermore, additional benefits for using type persistent infection instead of CIN2+ as end-point in trials were reported by the IARC working group: 1) more than 10-fold reduction of the sample size, 2) follow-up phase after final dose shortened by 2–3 years, and 3) substantially reduced complexity of study management.[8]

The currently available prophylactic vaccines exhibit no therapeutic effects. Since the time between HPV infection and tumor development is 10–20 years, a large number of people worldwide already have a persistent HPV infection and, as a consequence, will not benefit from these prophylactic vaccines. Furthermore, current therapeutic interventions in women with high-grade cytological abnormalities are surgical and can cause unwanted side-effects.[15] Thus, non-invasive effective treatment strategies such as therapeutic vaccines are a desirable option. A comprehensive overview of completed and ongoing clinical trials in therapeutic studies is provided in the review of Khallouf et al.[16] Interestingly, virological end-points have not yet been introduced in the majority of these studies. Only for the PC10VAC01 study, the clinical response is reported as high viral clearance. The patient population of this trial included HPV 16 and/or 18 positive women with normal cervical cytology. Therefore, efficacy could not be based on cytological or histological response.[17] It is also important to notice that the current therapeutic vaccine trials are phase I or II studies with limited number of participants, whereas future larger studies may need less burdensome monitoring and would gain from very feasible virological end-points. It is obvious that alternative sampling, including urine sampling, then becomes attractive. Furthermore, urine samples have been successfully used in the post-treatment follow-up of cervical cancer.[18]

An essential part of any trial is the end-point related sampling. The sample should be of good quality, stable until processing, reproducible, feasible and user-friendly for the participants of the trial. From the cervical cancer screening field we learned that HPV DNA PCR testing on self-collected versus clinician collected samples showed similar sensitivity.[19] This denotes that a viral end-point on self-collected samples can also be considered in vaccine efficacy trials.

The potential advantages of using urine for HPV DNA testing as end-point in a vaccine trial have been reported earlier.[20,21] Urine sampling is non-invasive, and by consequence not interfering with the natural history of the infection. Conversely cytological, vaginal or cervical samples obtained by scraping the epithelium creates micro-lesions, and possibly induces an inflammatory reaction. In addition, urine samples can be obtained according to a self-sampling protocol, permitting at-home and if required more frequent sampling.[21,22] Urine and/or self-sampling is furthermore expected to increase participation in cervical cancer screening programs.[23] If used in clinical trials it may also increase participation, reduce operational burden and allow for repeated sampling required for defining persistent infection.

Urine HPV testing could also play a role to asses prevalent infection in potential vaccine trial participants prior to inclusion. A recent meta-analysis concluded that testing urine for HPV DNA seems to have good accuracy for the detection of cervical HPV.[24] However, the authors also launched a call for further investigation and standardisation. We showed already that urine testing for HPV DNA is feasible but a number of precautions need to be implemented: 1) HPV DNA may be rapidly broken down by DNA nucleases, therefore a preservative buffer is required; 2) the concentration of HPV DNA found in urine may be limited so performant DNA extraction and detection is essential; 3) finally, as confirmed by the meta-analysis of Pathak et al., we demonstrated that first void urine contains more HPV DNA than the subsequent fraction.[25]

The hypothesis for finding HPV DNA in urine of women with a cervical HPV infection is that, at the start of the void, urine gets contaminated by debris and impurities lining the urethra opening, including mucus and debris of exfoliated cells from the vagina, cervix and uterus. It hence follows that the initial flow of urine collects most of this debris, which explains why the first collected part of a urine void contains more HPV DNA than subsequent parts. This knowledge should avoid that women clean the genital area before taking a urine sample. A subsequent variable is the time of urine collection. Preliminary data show that the first void from the first urine of the day provides more HPV DNA copies. This finding strengthens the concept that more HPV DNA is present when the interval between 2 urinations increases, as more excreted mucus and debris have the time to accumulate.[26]

Convinced that standardisation of urine collection is a key step for obtaining high quality and reproducible results, a collection device was developed at the Antwerp University (dept. of product development) and further redesigned/refined at Novosanis, spin off company of the Antwerp University. Major advantage of using such a device is that the first 15 ml of the initial urine flow (i.e. first void) is guided to a collection tube were it is immediately mixed with a preservation

buffer. Without the need to interrupt the urine flow, the remaining void can exit the device directly in the toilet without diluting the first void sample. This device is currently being used in studies of early impact of national HPV vaccination programmes in Bhutan and Rwanda, co-ordinated by the IARC, and has been shown to have excellent acceptability in approximately 1,000 young women in each of the 2 settings (Gary Clifford, unpublished data).

An important aspect regarding the use of urine sampling for follow-up of vaccination is the fact that it is it much less performant in males. Indeed, the rationale for finding HPV DNA in urine of women is not transferable to males. The amount of HPV (if any) and human DNA in male first void urine is much lower compared to women.[22] In line with this observation, the use of urine in pre-menstruating young girls needs also further attention.

In summary, the use of HPV urine testing for monitoring the impact of HPV vaccination programmes or demonstrating the efficacy of prophylactic or therapeutic HPV vaccines in women looks promising. However, standard criteria for type of urine, volume, collection, storage, extraction, and testing are essential to maximise HPV DNA detection.

Disclosure of Potential Conflicts of Interest

AV and PVD are co-founders of Novosanis, a spin-off company of the University of Antwerp, responsible for valorisation of the urine collection device. SVK declares no conflicts of interest.

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