Cervical cancer screening following prophylactic human papillomavirus vaccination

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\textbf{Summary} The recognition that infection with certain human papillomavirus (HPV) types is a necessary cause of cervical cancer has opened new fronts for the prevention of this disease. Primary prevention is now possible via immunization with highly efficacious HPV vaccines and secondary prevention has gained impetus with the advent of sensitive HPV DNA testing to improve traditional Pap cytology screening programs. Although universal vaccination of teenagers and young women is a desirable policy cost remains a key obstacle. To achieve cost-effective reductions in the burden of cervical cancer prevention initiatives must consider screening and immunization as integrated and organized approaches that take advantage of HPV testing as primary screening test followed by triage with Pap cytology. This strategy has the added benefit of providing epidemiological surveillance of vaccinated populations.

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\textbf{Introduction}

The licensing of a first prophylactic vaccine (Gardasil\textsuperscript{TM}, Merck, Inc.) against the two most important oncogenic genotypes (16 and 18) of human papillomavirus (HPV) in 2006 has ushered a new era in cervical cancer prevention. A second vaccine (Cervarix\textsuperscript{TM}, GlaxoSmithKline, Inc.), which also targets these types, is expected to reach the market in 2007–2008 (already approved in Australia in May 2007 and received a favourable preliminary assessment in Europe). In clinical trials, these vaccines have been nearly 100\% efficacious in preventing incident persistent infection with the target types (Cervarix) and the precancerous high-grade lesions (both) that are caused by these viruses in women without prior exposure with the vaccine types\textsuperscript{[1–4]}. Mathematical models of the impact of these vaccines have projected a substantial public health benefit in most geographical areas\textsuperscript{[5–7]}.

Despite the enthusiasm with the initial results with HPV vaccination it is generally accepted that cervical cancer screening will have to continue after vaccination. Both vaccines are fully effective as pre-exposure prophylaxis for disease caused by HPV types 16 and 18, when used before the onset of infection; however, women currently infected with these viruses may not derive any benefit\textsuperscript{[8]}. Moreover, the target types included in the two vaccines are causally linked to about 70\% of all cervical cancers\textsuperscript{[9]}. Although some degree of cross-protection against infection with phylogenetically related HPVs (e.g., HPVs 45 and 31) could also exist\textsuperscript{[1]}, there is also a possibility of an increase in prevalence of other HPV types in vaccinated populations, as a result of the vacated ecologic niches following the progressive elimina-
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As already providing adequate protection against the onset of invasive cervical cancer and may be used as argument against adopting universal vaccination.

The benefits of vaccine protection are likely to be maximal in women before the age of sexual debut and as yet, little is known about the benefits of vaccination in women older than 26 years of age since efficacy RCTs have covered the age range of 15–26, and only bridging immunogenicity studies are available to document immune response in older women. Despite these uncertainties, policy decisions concerning HPV vaccination would benefit from considering the changes in future screening practices that are likely to ensue if vaccination were to be adopted. This could permit more realistic projections about the potential reductions in cervical cancer control costs due to a reformulation of screening recommendations.

Concerns about Pap cytology in cervical cancer screening

In spite of its track record, Pap cytology has important limitations. It is based on the subjective interpretation of morphologic alterations present in cervical samples that must be collected with proper attention to sampling cells of the transformation zone. Also, the highly repetitive nature of the work of screening many smears leads to fatigue, which invariably causes errors in interpretation. The average sensitivity of Pap cytology to detect high grade cervical intraepithelial neoplasia (CIN2+) or invasive cervical cancer has been reported as 53% and its average specificity as 97%. In addition there is large heterogeneity in sensitivity from about 30% to 75% [10]. Therefore, the Pap test’s high false negative rate is its most critical limitation. The advent of liquid-based cytology has helped to mitigate the problem of efficiency in processing cellular samples but because liquid-based cytology has not proven to be more sensitive than the conventional Pap smear the limitations of cytology remain the same [11]. This low sensitivity for an individual testing opportunity is compensated by the requirement in some countries (e.g., US and Canada) to have women entering screening age with an initially negative smear to repeat their tests at least twice over the next 2–3 years before they can be safely followed as part of an extended screening schedule. Examples of such safeguards can be found in guidelines by the Canadian screening programme [12] and the American Cancer Society [13].

Possible short-term impact of HPV vaccination on screening practices

As the successive cohorts of vaccinated young women reach screening age, the reduction in cervical lesions will lead to a decrease in rates of colposcopic referral to about 40–60% or less of the existing case loads in most Western countries, judging from attributable proportion estimates [14] and preliminary findings from the vaccination trials [3]. A small proportion of currently referred cases are associated with low oncogenic risk HPVs, such as HPV 6. Merck’s Gardasil, which includes the latter type as immunogen, may thus lead to a more pronounced reduction in abnormalities than GSK’s bivalent vaccine, perhaps by an extra 5–10% in absolute terms [14]. Such reductions are likely to translate into initial savings to the health care system or to individuals but the vaccine-induced decrease in cervical lesions may
lead to a degradation of performance characteristics of Pap cytology (because of a decreased expectation of abnormalities on a day’s smear workload) with consequent concerns related to the need for heightened quality assurance. The positive predictive value (PPV) of Pap cytology will decline paralleling high vaccine uptake because clinically relevant lesions will become less common. This will lead to a decline in the performance of cytology because of a decrease in the signal (squamous abnormalities) to noise (inflammation and reactive atypias) ratio that characterizes the subjective and tedious work of reading and interpreting smears. In other words, a low lesion rate will lead to losses in sensitivity by causing a decrease in familiarity for recognizing abnormal cells as well as specificity, because fear of missing disease leads to more overcalls of benign abnormalities [15]. Fig. 1 illustrates the impact of combined changes in lesion prevalence and Pap performance on the positive predictive value of cytology screening. The lower PPV for cytology will require greater expertise to maintain good quality and this may be achieved by centralization of screening in larger laboratories. Use of liquid-based cytology may offset some of the problems but this technology is also likely to be affected. Likewise, use of automated cytology with optical recognition of abnormalities may reduce some of the problems related to rarity of relevant lesions but the altered signal-to-noise ratio expected post-vaccination may require recalibration of the computer-assisted recognition algorithms. Therefore, the negative impact on the PPV can be expected even with heightened quality control and improved cytology systems.

The above reductions in case loads will be a function primarily of two factors: (i) the overall uptake of HPV vaccination by the successive cohorts of adolescents and young women targeted by vaccination, and (ii) the time it will take for protected women to reach the age when they become eligible for screening [15]. In countries without a centrally managed health care system (e.g., the US) uptake of vaccination will require much effort in educating the public and health care providers. Vaccinated adolescents will reach the age of cervical cancer screening within 3 years after the onset of sexual activity. Therefore, the impact on screening and management case loads will be initially minimal for women vaccinated between the ages of 10 and 18 years. On the other hand, the benefits in risk reduction among young adult women receiving the vaccine will be realized almost immediately because of the short latency between the averted acquisition of HPV infection and the appearance of low grade or equivocal cervical abnormalities [15]. For countries where screening starts at age 30 or even 25 years (as happens in most of Europe), the effect on screening will be even more delayed.

**Possible long-term public health outcomes of HPV vaccination**

Even with high uptake, a statistically noticeable reduction of the burden of cervical cancer via HPV vaccination is unlikely to be observed for at least 10–15 years because of the dual facts that vaccination below age 20 will not affect high grade CIN rates appreciably for 5–10 years and another 5–15 years will be necessary for this to be translated into reductions in cancer incidence. A paradoxical situation may arise if high vaccine uptake occurs primarily among women who will eventually be adherent with screening recommendations. If adolescents and young women who are more likely to be vaccinated are the very ones destined to become screening-adherent the reduction in ASC-US and SIL abnormalities will be seen nearly exclusively among such women. There may be initial enthusiasm with the reduction in triage and management case loads consequent to the fewer abnormalities identified on screening. However, because of their high adherence with screening these women would not be the ones destined to develop cervical cancer. On the other hand, unprotected women may be less likely to be screened and their undetected precancerous lesions will progress until invasion occurs, when the attendant symptoms will then prompt the need for diagnosis [15]. This undesirable scenario of compounded inequity is unlikely to occur in countries that already enjoy the benefits of an organized screening program that reaches all women. Such countries are likely to adopt also an organized and universal vaccination program that benefits all segments of society.
Current evidence-based public health

HPV DNA testing as promising screening test

Of the molecular-based technologies for cervical cancer screening HPV DNA testing is the one eliciting the greatest interest. The Hybrid Capture™ (HC) assay (Digene, Inc., Gaithersburg, MD) is currently the most widely used in clinical and screening settings. It is a nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection in cervical specimens of HPV DNA of 13 high oncogenic risk genotypes, defined as those that are associated with cervical cancer: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Other HPV DNA testing formats based on polymerase chain reaction (PCR) with Luminex detection platforms are beginning to be commercially available and will permit identifying infection with individual oncogenic types, which will help in defining the prognosis of HPV infections. HPV testing has 25–35% higher sensitivity than cytology in absolute terms but somewhat lower specificity, 5–10% lower for detecting high grade lesions [10,16–19]. Screening of women older than 30 years tends to improve the performance of HPV testing because viral infections in this age group are less likely to be of a transient nature than those in younger women and are more directly related to high grade CIN [20]. It is noteworthy that the combination of Pap and HPV testing (called co-testing) attains very high sensitivity and negative predictive values (approaching 100%). This feature could potentially allow increasing screening intervals safely, e.g., from 1–3 years to 3–5 years, depending on the population. The drawback is an increased number of patients who would need additional evaluation including possibly colposcopy, many of which will turn out to be lesion-free. Resource-rich countries can absorb the extra costs related to the secondary triage of cases that will be referred via a dual-testing screening approach because this strategy may be cost-saving over time, because of the reduced patient flow in primary screening clinics afforded by the extension in the screening interval for women who are cytology and HPV negative [21]. Additional triage tests such as HPV typing, HPV E6/E7 mRNA and p16 testing may help to identify women most likely to harbour high grade disease [19]. A Pap-HPV co-testing approach has been recently recommended in professional guidelines in the US [22]. Fig. 2 shows the opportunities for screening intervention via Pap and HPV testing and their performance characteristics in identifying the succession of intermediate endpoints in the natural history of cervical neoplasia.

Emerging evidence in support of HPV testing in screening

In addition to the aforementioned strong body of evidence already published regarding non-randomized studies, a few large randomized controlled trials of HPV testing in primary cervical cancer screening are currently ongoing in the Netherlands, UK, Sweden, Finland, Italy, Canada, and India [23–29] and have already produced strong evidence in support of the adoption of HPV testing in primary screening [30–32]. These RCTs, embedded in on-going opportunistic or organized screening programs, will likely further add to the strength of evidence necessary for public health pol-

![Figure 2](image_url)

**Figure 2**  Pap cytology and HPV DNA testing in cervical cancer screening. Cytology relies on the recognition of morphological cellular abnormalities in a Pap test whereas HPV DNA testing detects DNA from oncogenic HPV types, which provides improved sensitivity compared with cytology. HPV testing is more "upstream" in the natural history of cervical neoplasia because it is based on detecting HPV DNA even before it becomes associated with morphological changes to the infected cervical epithelium. The downside of its greater sensitivity and in being more upstream than cytology is that it is less specific than the latter test. The extra referrals for colposcopy will lead to higher costs on initial screen that can be offset later on via extended screening intervals. Abbreviations: HR-HPV: infection with high oncogenic risk HPV types; HG: high grade.
icymakers to make informed decisions about the future of their cervical cancer screening programs. Evidently, all other features of organized screening programs, particularly coverage, quality assurance, and adequate case management also apply to any competing technology.

Recommendations

Primary screening via HPV testing followed by Pap cytology triage

Simply making cytology screening less frequent may not be a viable strategy to achieve a cost-effective combination of vaccination and screening in light of the aforementioned potential problems that may plague Pap cytology performance in conditions of low lesion prevalence (illustrated in Fig. 1). Although the "quantitative" effect shown in Fig. 1 will also negatively affect the PPV of HPV testing the latter is unlikely to be affected by the "qualitative" effects that further contribute to the decrease in PPV of cytology which are secondary to the degradation of sensitivity of specificity of the latter test due to the rarity of lesions (shown in the non-overlapping curves in Fig. 1). HPV testing has the screening performance characteristics that would make it an ideal primary cervical cancer screening test in such conditions. In addition, the interpretation of HPV testing results is objective and potentially automatable, which will make it less prone to the vagaries of subjective interpretation, particularly in conditions of low lesion prevalence. Pap cytology should be reserved for triage settings, i.e., in assisting management of HPV positive cases because it is more likely to perform with sufficient accuracy in conditions in which lesion prevalence is high, a situation that is artificially created when the workload includes only smears from women harbouring HPV infection (Fig. 1). The advantages of the approach of only using HPV testing as the primary screen and then triaging positive women with cytology have been described before [15,33–35] and are being evaluated in Finland [26], Northern Italy [27] and in British Columbia, Canada.

Integration of screening and follow-up of vaccinated populations

As a bonus, another key advantage of using HPV testing as the primary screening tool in prevention programs is the opportunity to create HPV infection registries with the provision to link test results from the same women over time, thus allowing an efficient and low-cost strategy to monitor long-term protection among vaccinated women. As HPV typing becomes incorporated in future HPV testing screening there will be an improved opportunity to manage HPV positive cases and to gain insights into the long-term effectiveness of vaccination [15].

Particularly for low resource regions in developing countries where screening is not very well established or effective, programmes which combine vaccination for adolescent girls with HPV-based screening of their mothers is very attractive. Given the difficulties with cytology, and the much higher sensitivity seen with HPV testing compared to cytology or visual inspection [36], an attractive strategy would be screening of the mothers by a rapid HPV test (2-h assay time) in the morning (along with vaccination of daughters), followed by any required treatment (comparable to a See-and-Treat approach) in the afternoon of the same day, using visual inspection with acetic acid in women who were HPV positive.

Economies of scale and market forces will lower costs of HPV testing in screening

At present, the main obstacle for the adoption of the above policy is the high cost of HPV testing. The fact that the market is dominated by a single manufacturer of a clinically approved HPV assay (Digene) is certainly a deterrent for achieving lower prices for HPV testing. Another problem comes from the current practice guidelines in most countries which at most approve HPV testing for the triage of ASC-US abnormalities, an admittedly restricted niche market that represents at most 5% of the total patient population that can benefit from this technology in screening. It is expected that once HPV testing is deployed in the high volume of primary screening there will be a reduction in the cost of individual tests because of the market expansion following an economy of scale. Governments and managed care organizations may be able to negotiate with the manufacturer(s) lower prices conditioned to high volume purchasing. Furthermore, a change in market potential from simple ASC-US triage to wide-scale primary screening will inevitably bring other biotechnology companies to compete in the field by bringing their own molecular HPV tests for validation and regulatory approval. This is already happening even before this change in market is realized. A few biotechnology companies are already in advanced stages of regulatory application for novel molecular HPV tests to compete in the Pap-HPV co-testing market in the U.S. Taken together, the combination of shifting trends in screening practices, economies of scale, and perception of new market opportunities for companies will further contribute to a reduction in the overall cost of the "HPV followed by Pap" screening approach.

Directions for further research

The above proposal for changes in screening practices among vaccinated women has at present strong theoretical underpinnings (likely loss of performance of Pap cytology in vaccinated women) and empirical support (proven value of HPV DNA testing). At present, however, we only have a limited understanding of the natural history of cervical lesions in vaccinated women from the initial published findings of HPV vaccine trials. RCTs of HPV testing followed by cytology triage compared with favoured local screening paradigms must be conducted in general and also in vaccinated populations in order to provide the evidence base that will inform future screening algorithms in vaccinated women. Historically, new screening technologies and combinations thereof, as well as new screening algorithms that combine old and new approaches are slow to be accepted in clinical practice. Professional guidelines take time to be updated as a reflection of the available evidence from controlled
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Frequency of screening in vaccinated women

Because of its enhanced sensitivity and improved negative predictive value a policy of HPV screening followed by Pap triage could be done at 3–5-year intervals even in today’s unvaccinated populations in North America. In Europe, even longer intervals could be possible because of the wide coverage of screening and proven effectiveness of policies with 5-year intervals when robust organized programs are in place. Pilot or demonstration projects and RCTs could be instrumental in demonstrating what could be acceptably safe intervals for both vaccinated and unvaccinated women as long as they remain negative. As these studies are implemented safety considerations dictate that extended screening intervals among vaccinated women should bear in mind the possibility of waning of immunity.

Age at initiation of screening

In women vaccinated as part of a school-based program screening will not have to start until 8–12 years later. Which approach should be used in this regard, traditional cytologic screening or the combined HPV-Pap algorithm described above? Should separate algorithms be envisaged for vaccinated and unvaccinated women? Should the screening interval be different between these two groups, e.g., 3–5 years for unvaccinated and 5–7 years for vaccinated women? With a high coverage of vaccination among young women it is likely that there will be a shift of the peak age of precancerous lesions to older ages. Continued surveillance via the HPV with Pap triage approach will demonstrate whether this phenomenon will occur and the extent of the age shift in different populations.

Also germane to this discussion is the fact that HPV testing has been proven useful in women 30 years of age or older. Studies are ongoing that could perhaps permit cost-effective screening via HPV testing at age 25 years and older, particularly with cytologic triage, as proposed above.

Follow-up algorithm for HPV positive/Pap negative women

What should be the frequency of testing for a woman harbouring an oncogenic HPV infection but with no signs of cytological abnormalities? How soon after her last HPV test becomes negative should she be returned to the regular frequency of screening for average risk women? Much research is needed to determine safe and cost-effective intervals for following up women who are found to harbour HPV or cytological abnormalities. Should different policies be evaluated for vaccinated and unvaccinated women? What is the value of adjunctive tests such as HPV E6/E7 mRNA, HPV typing, p16, and other biomarkers to triage those patients into those needing immediate referral, enhanced surveillance, or only routine screening? As of today, these tests have had only limited clinical testing for risk stratification and are yet to be validated as screening or triage tools. In particular, HPV typing may contribute to risk prediction in HPV positive/Pap negative women.

Clinical perspective

In conclusion, much has been achieved during the last 10 years from research on screening and prevention of cervical cancer. Progress in this area has been grounded on the recognition that HPV infection is the central, necessary cause of this important neoplastic disease. However, it is imperative that screening and primary preventive strategies be adapted to and meshed with one another in well-designed and managed organized programs to permit cost-effective reductions in the burden of cervical cancer. With the advent of HPV vaccination there may come a day when screening algorithms, such as described here, may be applied differently to vaccinated and unvaccinated women. There is always much hesitation to use complex risk-based approaches to decide on how to screen because they may cause confusion and fail to be properly applied in clinical practice. Common sense dictates that screening must be simple but policies should take into account prior history of vaccination to be able to be cost-effective. Breast and colorectal cancer screening practices are two examples in which risk-based differences in policies are already in place and widely promulgated by professional guidelines. Cervical cancer screening may eventually be added to the list. As research on the subject continues to provide additional evidence for public health action the next 5–10 years will bring many changes in practice standards and guidelines.

We realize that many of the predictions made in this article are based purely on theoretical grounds and epidemiologic principles of the performance of screening tests in conditions of varying prevalence. Much of our rationale is also based on current understanding of the value and robustness of HPV testing in screening. By definition, the subject matter of our article is one that requires writing about theoretical concerns and the indirect evidence that supports potential changes in practice. Many of our views, despite their theoretical underpinnings and some empirical support, may not be widely acceptable. Colleagues who are convinced that no changes are necessary to Pap cytology as a technology per se may view HPV testing as potentially causing many more colposcopy referrals than would be acceptable. Our arguments to the effect that this issue is offset by the extra safety margin of HPV testing (which would permit extended screening intervals and thus be cost-saving in the long run) still require empirical support as suggested above. However, our proposal is made from a strong foundation of theory and practice which has emerged in recent years and underscores the importance of reaching
cost-effectiveness via a careful integration of primary (vaccination) and secondary (screening) prevention strategies. Therefore, the essential assumptions in our proposal are: (i) that HPV vaccination will have its intended effects as predicted above, (ii) that HPV testing will maintain its performance levels upon deployment as a primary screening test, and (iii) that Pap cytology would falter in the conditions of low lesion prevalence consequent to high vaccine uptake. Our statements should not be viewed as firm recommendations for practice guidelines but a roadmap for the merging of technologies in cervical cancer control that are likely to earn evidence-based status in the future.

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