HPV INFECTION AND CERVICAL CANCER: A REVIEW OF SCREENING AND PREVENTIVE STRATEGIES IN DEVELOPED COUNTRIES AND BRAZILIAN POLICIES

INFECÇÃO POR HPV E CÂNCER CERVICAL: UMA REVISÃO DE TRIAGEM E ESTRATÉGIAS PREVENTIVAS NOS PAÍSES DESENVOLVIDOS E POLÍTICAS BRASILEIRAS

Guilherme Lucas de Oliveira Bicca[1], Mariângela Freitas da Silveira[2], Kleber Roberto Siguel da Silva[3], Fernando Celso Lopes Fernandes de Barros[1,2]

ABSTRACT

Introduction: Cervical cancer is currently the third malignancy on number of female deaths in the world. Persistent HPV infection is the main agent involved in cervical cancer development, particularly of high risk (HR) HPV types 16 and 18, accountable for approximately 75% of cervical cancer cases. These aspect has increased demand for HPV detection molecular tests. **Objectives:** To summarise and update the current knowledge on HPV and cervical cancer screening techniques and, also, discuss HPV-related data and screening techniques in Brazil. **Methods:** We include articles published in the past 10 years, both in English and Portuguese. Scientific search engines as Scopus, Cochrane Library and Pubmed were used for the terms "cervical cancer", "HPV", "cervical carcinoma", "HPV vaccine". Only research articles and reviews were considered. **Results:** The most used techniques for HPV detection are PCR and Hybrid Capture 2 (HC2). However, techniques for detection of HPV E6/E7 mRNA and p16INK4a have been developed, which are still being validated. These tests may help distinguish transient from persistent HPV infections. **Conclusion:** To reduce the number of cervical cancer cases, screening strategies could be adjusted to contain the best combination of cytological and molecular tests. The ideal screening strategy require high sensitivity to minimize false negative results, and high specificity, to avoid false positives and over referral. Optimization may be achieved by using by co-testing, combining HPV genotyping and cytology triage with low-grade intraepithelial lesions (LSILs) or with atypical squamous cells of undetermined significance (ASC-US). Besides, strategies to prevent cervical cancer cases include HPV vaccination.

Keywords: papillomavirus infections; uterine cervical neoplasms; vaccines; Brazil.

RESUMO

Introdução: O câncer de colo uterino é a terceira causa de morte em mulheres no mundo. Infecção persistente pelo HPV é o principal fator no desenvolvimento do câncer de colo uterino, particularmente HPV de alto risco (AR), tipos 16 e 18, responsáveis por aproximadamente 75% dos casos de câncer de colo uterino. Este conhecimento incrementou a demanda de testes moleculares para detecção de HPV. **Objetivos:** Sumarizar e atualizar o conhecimento atual em técnicas diagnósticas para HPV e rastreamento de câncer cervical, e discutir dados a respeito do diagnóstico de HPV e métodos de rastreamento no Brasil. **Métodos:** Foram incluídos artigos originais ou revisões publicados nos últimos 10 anos, em inglês e português, utilizando as bases de dados Scopus, Cochrane Library and Pubmed, e os termos "*cervical cancer*", "*HPV*", "*cervical carcinoma*", "*HPV vaccine*". **Resultados:** As técnicas mais utilizadas para a detecção de HPV são a PCR e a captura híbrida. Contudo, técnicas para detecção de RNAm de HPV E6/E7 e p16NK4 já foram desenvolvidas estando em fase de validação. Estes testes poderão auxiliar na distinção de infecções transientes e persistentes. **Conclusão:** Para reduzir o número de casos de câncer cervical, estratégias de rastreamento podem ser ajustadas para a melhor combinação de testes citológicos e moleculares. Estratégias de rastreamento ideais requerem alta sensibilidade, minimizando resultados falso negativos e alta especificidade, evitando falsos positivos e excesso de encaminhamentos. A otimização pode ser obtida combinando testes de genotipagem de HPV e triagens citológicas. Além disso, estratégias para prevenção de casos de câncer cervical incluem a vacinação contra o HPV.

Palavras-chave: infecções por papillomavirus; neoplasias do colo do útero; vacinas; Brasil.

INTRODUCTION

Uterine cervical cancer is the third most common malignancy in women, and the seventh overall, with approximately 530,000 new cases in 2008[1] and 270,000 deaths annually[2]. Cervical cancer is responsible for more years of life lost in Latin America and the Caribbean than tuberculosis and AIDS[3]. It is estimated that viral infections are involved in 20% of human cancers worldwide, and just under 25% of cancer cases in developing countries[4]. Epidemiologic studies have shown that infection with high-risk (HR) types of Human Papillomaviruses (HPVs) is the main aetiological factor of cervical cancer[5]. Additionally, previous studies have shown that nearly all of cervical cancer cases test positive for HPV[6].

More precisely, persistent infection with HPV has been explicitly linked to the development of cervical cancer, with between 13 and 18 types of the virus characterized as conferring a high oncogenic risk[7]. Of these, the most carcinogenic, responsible for approximately 70% of all cervical cancers are types 16 and 18 HPV[8].

The better knowledge about the association between HPV and cervical cancer has increased the demand of tests for the presence of HPV for the diagnosis of abnormal cervical smears and screening for cervical cancer[9]. Also, it has led to the development of

[1]Postgraduate Program in Health and Behavior, Universidade Católica de Pelotas (UCPel) – Pelotas (RS), Brazil.
[2]Postgraduate Program in Epidemiology, Universidade Federal de Pelotas (UFPel) – Pelotas (RS), Brazil.
[3]School of Medicine, UFPel – Pelotas (RS), Brazil.

new screening techniques based on molecular biology testing. These strategies include PCR-based diagnosis and, more recently, Hybrid Capture 2 (HC2) assays.

METHODS

In this review we will summarise and update the current knowledge on HPV and cervical cancer screening techniques. Also, we will discuss HPV-related data and screening techniques in Brazil. This work comprises articles published in the past 10 years, both in English and Portuguese. Scientific search engines as Scopus, Cochrane Library and Pubmed were used for the terms "cervical cancer", "HPV", "cervical carcinoma", "HPV vaccine". Only research articles and reviews were considered.

DISCUSSION

Scientific background on HPV

Human Papillomavirus (HPV) are connected to epithelial proliferative diseases, both benign and malignant, with more than 100 types of the virus having been documented. Its genome encodes for only eight genes[10]. A new HPV type is determined by differences on three nucleotide sequences in its genome, namely in genes E6, E7 and L1, when differing more than 10% from those occurring in know HPV types. Two classes of HPV can be distinct, based on the location of the infection: cutaneous types that infect the epidermis, and mucosal types that infect the epithelia of the anogenital or the aerodigestive tract[11]. HPV types related to cervical cancer, termed high risk (HR), act by interfering with the cell cycle regulation. Its primary oncoproteins are E6 and R7, which mediate the degradation of proteins p53 and retinoblastoma tumour suppression protein (pRB)[10].

HPV and cancer

Of the HPV types related to cervical cancer, the 12 most common are included into two species: 7 (HPV 18, 39, 45, 59, 68) and 9 (HPV 16, 31, 33, 35, 52, 58, 67)[7], which convey greatly different risks. Of these, the most carcinogenic, responsible for approximately 60% of all cervical cancers is HPV type 16 (HPV16)[8], regardless of cytological appearance[12]. The second highest risk genotype is HPV18, accounting for 10 to 15% of cervical cancers[8].

Acute infections of these 12 types of HPV are common, particularly at younger ages[12]. The highest prevalence of HPV-positivity occurs in the late teens or early twenties[13]. There is a rapid decline on HPV infection after the age of 25, which continues until the around age 35–40, where they reach a plateau level[14]. However, adolescents have a high prospect of spontaneous clearance of cervical cell abnormalities, therefore a low risk of cervical cancer (HHS 2012). Although common, most HPV infections will be suppressed by the immune system within one or two years without causing cancer. They may, however, cause transient changes in cervical cells.

HPV types are divided into high-risk (HR) and low-risk (LR), where HR HPVs are the ones associated with cervical cancer. Persistent infection with HR HPV genotypes is essential for the development of pre-cancer lesions, cervical intraepithelial neoplasia (CIN) grade 3 (CIN3) and, subsequently, cervical cancer[12]. Although the prevalence of HPV infection tends to decline with age, viral persistency tend to increase, leading to the increase of severe cervical dysplasia to rise on late twenties to early thirties and of cervical cancer in late thirties[13]. Studies have reported a prevalence of HR HPV about two times higher than of LR HPV types[15]. Data suggests that LR HPV infections tend to clear more rapidly than HR HPV infections, and the probability of an infection not clearing increases proportionally to its duration[16].

HPV persistence, from one to two years, particularly by HPV16, increases the prognostic for CIN3 or a more serious diagnosis (CIN3+) in the following years[6]. The risk of untreated CIN3 lesions becoming an invasive cancer goes up to 20% in 10 years and 30% in 30 years. However, when treated, only around 1% of the lesions will become invasive. In cases of women with both minimum disturbance of their lesion and persistent disease, the risk was of about 30% in 10 years, increasing to approximately 50% in 30 years[17]. Also, HPV16 and multiple-type infections have the lowest clearance rate, increasing the probability of cervical cancer[18].

Knowing the precise relation between HPV type specificity that may or not aggravate the risk of HPV infection is important to understand the dynamics of these infections, take actions toward prevention and determine the best course of treatment if they occur.

Development of cervical cancer

Cervical cancer begins with HPV acquisition, followed by viral persistence, proliferation of infected cells to pre-cancer and, finally, invasion[6], as shown in **Figure 1**. As previously seen, not all HPV infections will persist, and some will be cleared by the immune system. A less frequent outcome is the regression of pre-cancer cells to normality. Therefore, early onset of sexual activity and increased number of sexual partners may increase the risk of HPV infection and, possibly, that of cervical cancer[19].

However, there are independent risk factors associated with squamous cell carcinoma and adenocarcinomas[19]. Among them are smoking, number of pregnancies, other infectious agents[20] and early initiation on oral contraceptives[21]. A Finnish study found correlation of an increased risk of incident HPV-infection with the initiation of smoking beyond 13 years of age and for the initiation of oral contraceptives usage before the age of 20[21].

Cervical cancer screening

Cervical cancer screening comprises two types of tests: cytology-based and HPV testing. These tests are a way to detect HPV infections, abnormal cervical cells — including precancerous cervical lesions — and cervical cancers. High-quality screening using cytology has significantly reduced mortality from squamous cell cervical cancer, which constitutes up to 90% of cervical cancers[22].

Cytology-based screening traditionally involves 3 steps: finding cytological abnormalities in a Papanicolaou (Pap) smear; histological confirmation of a biopsy taken under colposcopic control and treatment of the lesion that otherwise could develop into invasive cancer[23]. When *in situ* lesions are confirmed, they are called cervical

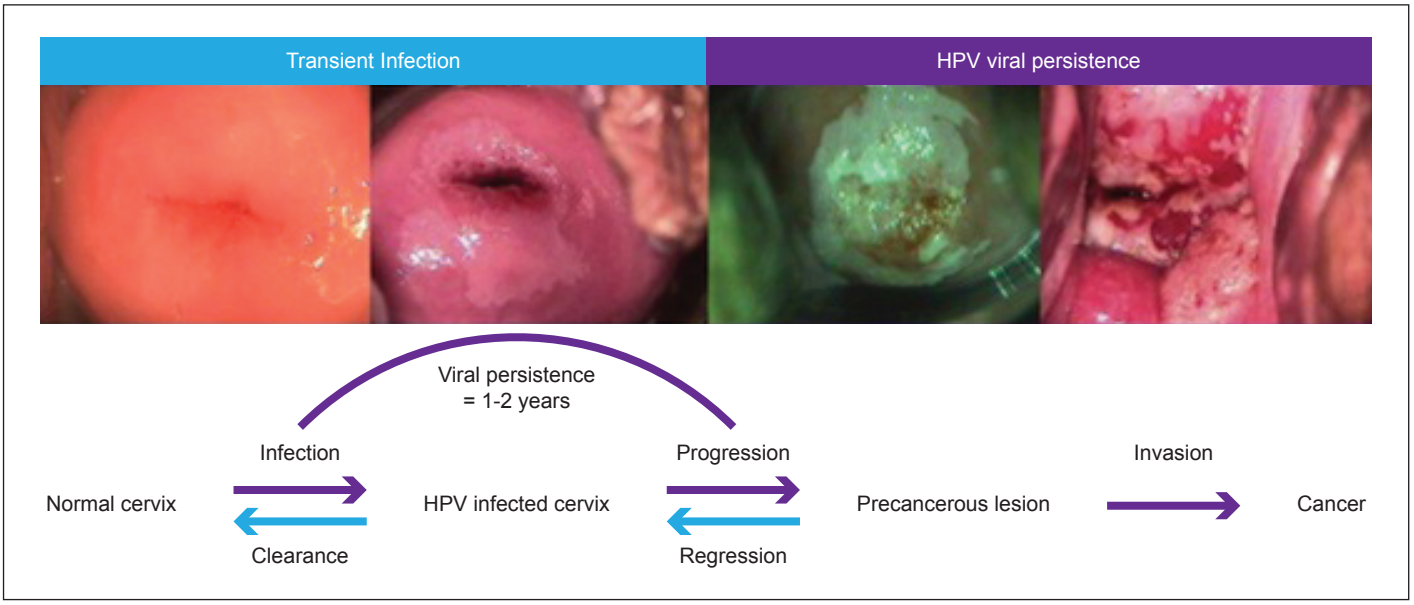

Figure 1 – Critical steps in the development of cervical cancer. Images refer to Colposcopy exams. Adapted from Muñoz et al.[8].

intraepithelial neoplasia (CIN). Depending on the severity of the lesion, it may be denominated CIN1, CIN2 and CIN3, indicating increasing levels of severity. Results from cytology-based tests are classified as LSIL+ for low-grade squamous intra-epithelial lesions or worse, or HSIL+ for high-grade intra-epithelial lesions or worse[23].

Liquid-based cytology (LBC) and Pap tests have similar accuracy as a test for detection of CIN2+. It is a simpler technique when compared to Pap test, its interpretation takes less time, and HPV testing can be performed on the same sample[23]. This could account for LBC replacing Pap tests as cytology exams.

Despite its great benefits toward cervical cancer prevention, cytology tests have weaknesses. In cytology, results are dependent on the collection of high quality sample during examination. Also, requiring identification of morphological changes within cells, interpretation of results is of a qualitative nature, which is subjective. Not only that, but a repetitive method can lead to larger number of interpretation errors[14]. In case of abnormal cytology, colposcopy is recommended as a diagnostic tool. However, shouldn't be considered for screening purposes.

HPV testing has the advantage of being objective (presence or absence of virus), removing the qualitative aspect present in cytology. Below we discuss current strategies of HPV detection in detail.

HPV detection

From the knowledge on the relation between HR-HPV types and cervical cancer came the need to develop new types of molecular detection systems, both for DNA and RNA recognition. Molecular tests offer increased sensitivity although they show lower specificity compared to cytology testing[6].

PCR has been used for over ten years in HPV detection. However, its high analytical sensitivity combined with the potential for contamination is a serious disadvantage for this method, once it may lead to false-positive results. The Hybrid Capture 2 (HC2) assay, a second-generation commercial HPV test, was introduced as a possible routine diagnostic test, including positive and negative controls. HPV DNA tests have been demonstrated to have higher sensitivity for CIN2+ lesions than the ones obtained by cytology in several studies[14].

There is a debate about which of these two tests would be better. On a screening test using both techniques, Kulmala et al.[24] found that the results of PCR and HC2 were consistent for 85% of the samples. However, the sensitivity of HC2 for the detection of high-grade squamous intraepithelial lesions (HSILs) was slightly better[24]. The authors also highlight that the HC2 assay is technically well designed, being easily controlled and performed by lab personal, while PCR needs to have many of its steps optimized, making it more difficult to have rigid standards[24].

Other promising screening techniques being developed detect carcinogenic HPV E6/E7 mRNA and $p16^{INK4a}$, which may help distinguish transient from persistent HPV infections. Molden et al.[25] compared the detection of HPV mRNA from carcinogenic HPV types with the detection of HPV DNA. E6/E7 mRNA expression was detected by the PreTect HPV-Proofer assay, whereas the presence of HPV DNA was detected by Gp5+/6+ consensus PCR followed by type-specific PCR. PreTect HPV-Proofer had lower detection rate of HPV for cases of abnormal cytologic diagnosis; cytologic normal, atypical squamous cell of uncertain significance (ASC-US); and low-grade SIL (LSILs) diagnosis. No significant difference was observed for the detection of high-grade squamous intraepithelial lesion (HSIL) when comparing the tests[25]. Nevertheless, the authors pronounce mRNA detection tool as a promising test as an adjunct to cytology.

The $p16^{INK4a}$ is a cell-cycle regulator that is overexpressed in cervical pre-cancer and cancer cells induced by the deregulated expression of HPV oncogenes. Wentzensen et al.[26] tested $p16^{INK4a}$ levels in lysates of cervical cells that were obtained from a disease-enriched population by using a $p16^{INK4a}$-specific sandwich ELISA. Nonetheless, the overall content of this protein may be higher in specimens derived from patients with high-grade cervical intra-epithelial neoplasias (HGCIN) compared with specimens derived

from patients with low-grade dysplasia or patients without cervical intraepithelial lesions. Still, the authors suggest that ELISA-based quantification of solubilized $p16^{INK4a}$ protein may have high sensitivity for detecting cervical pre-cancer[26].

As there is evidence suggesting that only persistent infections are associated with precancerous lesions, detecting the persistence of HPV — specially types 16 and 18 — would give even more specific markers of clinically significant infections. However, this will require robust assays and feasible clinical protocols[10].

After the treatment of cervical lesions, HPV testing detects residual infection quicker and with higher sensitivity and comparable specificity compared to follow-up cytology[13]. The absence of HPV infection will most likely shorten the follow-up period, yet more data is needed to confirm this hypothesis.

Screening strategies

Cervical cancer prevention programmes vary extensively by country, but most of them could be improved immensely by new techniques. The suitable programme depends on affordability, different social demands for protection against cancer and willingness to prevent complications even at low risk conditions. These will have an effect on when screening begins, the appropriate interval between tests and age to stop assessments. However, studies suggest that screening women within 5 to 10 years of sexual initiation wouldn't be cost effective, as the risk of benign HPV infections is high but the risk of cancer is still low[10]. Overall, evidence suggests that, if screening under the age of 25 is at all valuable, the benefit would be modest at best. It should also be taken into account that women treated for cervical lesions prior to childbearing have preterm delivery chances increased[27].

The ideal cervical cancer screening strategy would require the highest sensitivity to minimize false negative results, as well as the highest specificity, in order to avoid false positives and over referral[28]. Unfortunately, strategies that favour one of these points will inevitably lack in quality for the other. Namely, when maximizing sensitivity, tests have usually presented relatively poor specificity[28].

Incorporating molecular tests into cervical cancer screening strategies may lead to an increase in disease detection and in length of screening intervals. Increase in detections will improve benefits of treatment and longer time between screenings may reduce distresses as the psychological impact of screening positive and proceed with treatment of lesions that might have cleared by themselves[6]. Also, there is evidence that testing for HR HPV is cost effective and sensible for the detection of precancerous lesions in women with ambiguous cytology[13]. HPV testing is more sensible but less specific than Pap tests, can be useful on the follow-up of women post-colposcopy when pre-cancer is not found and can guide an evaluation of cure post-treatment[10]. Testing negative for HR HPV types provides more reassurance against the development of pre-cancer and cancer than cytology-based testing[10].

Precaution should be taken on the use of molecular testing of LSIL lesions. LSIL is usually the manifestation of a current HPV infection with low potential for neoplastic transformation. Consequently, molecular testing of these lesions will frequently produce a positive result, limiting its capacity to discriminate between cases that may lead to severe lesions[13].

Two strategies have been described as being able to optimize the balance between specificity and sensitivity. One consists of cotesting HPV genotyping and cytology triage with low-grade intraepithelial lesions (LSILs), and the other is HPV genotyping and cytology triage with atypical squamous cells of undetermined significance (ASC-US). The authors state that the latter strategy can lead to 50% reduction in the number of required screenings, also being more sensitive and requiring less colposcopies to detect CIN3 or more severe cases[28].

Prevention

Reduction of HPV infection rates can be achieved, to some level, by health education programs and conscientious condom use, decreasing the risk of cervical cancer at the population level. Nevertheless, condom use does not entirely protect against HPV transmission, as the male anogenital skin is not completely covered[29]. For this reason, development of HPV L1 virus-like-particle (VLP) vaccines is a considered a major advance in prevention of cervical cancer. These vaccines are based on the self-assembly of recombinant L1 protein into non-infectious capsids that contain no genetic material[10].

Two types of vaccine against HPV were recently approved: the quadrivalent Gardasil (against HPV types 6, 11, 16, 18) (Merck and Co, Bluebell, PA, USA), and the bivalent Cervarix (against HPV types 16, 18) (GlaxoSmithKline, Rixensart, Belgium). Both vaccines are almost completely effective against HPV 16, and 18 induced CIN2+[30].

In the United States, the federal Vaccines for Children (VFC) program includes HPV vaccination. This program covers vaccine costs for children and teens who don't have insurance and for some children and teens who are underinsured. Vaccination is recommended for girls and boys aged 11 or 12 years. Depending on the jurisdiction, HPV vaccines are also recommended for teen boys and girls who did not get the vaccine when they were younger, teen girls and young women through age 26, as well as teen boys and young men through age 21[31].

Germany has a vaccination program against the most oncogenic types of HPV (namely 16 and 18) since 2007. The Standing Committee on Vaccination (STIKO) recommends vaccination for girls between the ages of 12 and 17 years old[32]. A recent study predicts that, over the next 100 years, HPV vaccination will have prevented approximately 37% of cervical cancer cases even if vaccination coverage is only 50% (as currently observed in Germany)[30]. According to the same study, cross-protection could result in a further reduction of approximately 7% of all cervical cancer cases for the bivalent and about 5% for the quadrivalent vaccine[30].

The Brazilian Department of Health has recently announced that, from 2014, the HPV vaccine will be available free of cost through the National Health System, where girls aged between 10 and 11 years old will be immunized[33]. The aim is to vaccinate 80% of the cohort, approximately 3,3 million people. Federal investments of over R$ 360 million have been announced for the acquisition of 12 million doses of the vaccine. The vaccine, quadrivalent, will be produced in partnership between the Butantan Institute (affiliated to

the São Paulo State Secretary of Health) and Merck (Merck Sharp & Dohme; Merck, Co., Inc. Brazilian subsidiary)[33].

To ensure that a program is cost-effective and vaccination will protect young women through the age of greatest risk of HPV exposure, vaccination durability should be of 10–15 years or greater or that boosting would be safe and effective[10]. Also, HPV vaccines available today would give best public-health benefits when applied to girls who haven't started sexual activity. The determination of the appropriate age to proceed with vaccination will require research on the age of first sexual activity for each region, developing programs that are suitable for the population in question.

HPV in Brazil

There is a need for further documentation of HPV infection, screening processes and treatment options in developing countries. When assessing studies on HPV testing and screening, authors have markedly stated that they did not include developing countries[14]. A program for screening of cervical cancer in Brazil now counts with 17 years of existence[34]. Through its data we see that the number of deaths due to cervical cancer in Brazil are similar to those in developing countries, being far from rates observed in countries where cervical cancer screening is well structured and established[34]. It is estimated that Brazil has over 20,000 new cases of cervical cancer per year[15]. The expected number of cases will increase from 19,603 (estimate for the year 2002) to approximately 36,800 in 2030[1].

In a review regarding HPV infection in Brazil, between the years 1989 and 2008, Ayres and Silva[15] only found 14 articles that met their inclusion criteria. From the data collected in these papers, they could infer that the overall prevalence of HPV cervical infection varied widely from 13 to 54%. When analysing the HPV infection in women with normal cytology results, rates varied between 10 and 24%[15]. Also in Brazil HPV16 was the most prevalent irrespective of cytology results[15].

As stated in previous study, provided that the cost per vaccinated woman is I$ 25 (International Dollars) or below, it appears that vaccination alone would be cost-effective in Brazil. However, there is uncertainty in the price of vaccines and for the programmatic costs related with adolescent vaccination[2]. But if we assume coverage of 70%, HPV16, and 18 vaccination of adolescent girls (before age 12) could reduce the lifetime risk of cervical cancer by 43%. Combining vaccination and three screenings after the age of 30, both at 70% coverage, may lead to a reduction of 53 to 70% in the risk of cancer[2].

Regarding the age indicated for vaccination, a Brazilian report, part of the Latin American Screening (LAMS) study, indicates the ideal age as being 15 years old[19]. This result is based on the average age of the first sexual intercourse of the women interviewed for the study. This differs from the age 12 determined on an international study, where ages 9 to 12 are determined as prior to sexual debut and ideal for vaccination[2].

All this taken into account, the Brazilian Government has decided to drop the age of vaccination from what was recommended in previous Brazilian study, agreeing with the findings of Goldie et al.[2]. The approximate cost of the vaccine in Brazil will be of I$ 28 (accepting the PPP conversion factor (GDP) to market exchange rate ratio in Brazil as I$ 1.07, according to the 2012 World Bank Report[35]). Assuming the PPP rate used by Goldie et al.[2] was I$ 0.8 (PPP rate of 2008[35]), the value of the vaccine in Brazilian Reais, estimated by the group at the time, would be of R$ 31.25. Therefore, with a current cost of R$ 30.05, the program reveals itself to be cost-effective.

CONCLUSION

The ideal screening strategy would require high sensitivity to minimize false negative results, as well as high specificity, in order to avoid false positives and over referral. Optimization may be achieved by using by co-testing: a combination of HPV genotyping and either cytology triage with low-grade intraepithelial lesions (LSILs) or with atypical squamous cells of undetermined significance (ASC-US). It is believed that programmes worldwide are moving from a morphologic prevention model (based on cytology, colposcopy and/or histology) to a model based on HPV virology and its molecular interaction with the human host[12]. Knowing how HPV infections are distributed in the population is key for the development of new tests and for the evaluation of the impact of vaccines in different scenarios[15]. Research and time will tell which screening strategies and programmes are best suited for different regions, adapting them to local resources and collective priorities.

An important measure to reduce cervical cancer cases is prevention of HPV infection through the use of vaccination. Quadrivalent and bivalent vaccines has been approved and are being used in different countries. The Brazilian National Health System has make a huge step in this direction offering the quadrivalent HPV vaccine free of cost for the vaccination of girls aged between 10 and 11 years old.

Conflict of interests

The authors report no conflict of interests.

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Address for correspondence:
GUILHERME LUCAS DE OLIVEIRA BICCA
Rua Padre Anchieta 2396 – Centro
Pelotas (RS), Brazil
CEP: 96015420
Tel: +55 (53) 3222-7398
Fax:+55 (53) 3025-7398
Email: gbicca@yahoo.com

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