

Universidade Católica de Pelotas

Guilherme Lucas de Oliveira Bicca

Infecção pelo HPV em mulheres da Zona Sul do Rio Grande do Sul e fatores associados ao desenvolvimento de lesões precursoras e câncer da cérvix uterina.

Pelotas 2014

Guilherme Lucas de Oliveira Bicca

Infecção pelo HPV em mulheres da Zona Sul do Rio Grande do Sul e fatores associados ao desenvolvimento de lesões precursoras e câncer da cérvix uterina.

Tese apresentada ao Programa de Pós Graduação em Saúde e Comportamento da Universidade Católica de Pelotas, como requisito parcial para obtenção do grau de Doutor em Saúde e Comportamento.

Orientador: Dr. Fernando Barros

Pelotas 2014

Infecção pelo HPV em mulheres da Zona Sul do Rio Grande do Sul e fatores associados ao desenvolvimento de lesões precursoras e câncer da cérvix uterina.

BANCA EXAMINADORA

Presidente e Orientador: Fernando Barros

1º Examinador: Dr. Jean Pierre Oses

2º Examinador: Dr. Maximiliano Sérgio Cenci

3º Examinador: Dra. Ludmila Gonçalves Entiauspe

Pelotas, 29 de janeiro de 2014
Sumário

PARTE I – Projeto

1	Identificação	6
1.1	Doutorando.....	6
1.2	Orientador	6
1.3	Programa de Pós Graduação	6
1.4	Instituição	6
1.5	Linha de Pesquisa	6
2	Delimitação do Problema.....	7
2.1	Introdução	7
2.2	Título	8
2.3	Justificativa.....	8
3	Objetivos.....	10
3.1	Objetivo geral	10
3.2	Objetivos específicos.....	10
4	Hipóteses.....	11
5	Materiais e Métodos	12
5.1	Delineamento do Estudo.....	12
5.2	Critérios de inclusão	12
5.3	Critérios de exclusão	12
5.4	Coleta de Dados.....	12
5.5	Aspectos Éticos	15
5.6	Análise estatística.....	15
6	Referências	17

Parte II – Artigos

18

Artigo 1 - HPV infection and cervical cancer: a review of screening and preventive strategies in developed countries and Brazilian policies.

Abstract	19
Resumo	20
Introdution	21
Scientific background on HPV	22
HPV and câncer	22
Development of cervical câncer	24
Cervical cancer screening	24
HPV detection	25
Screening strategies	27
Prevention	29
HPV in Brazil	31
Conclusion	32
References	33

Artigo 2 – Prevalence of Infection with High-Risk HPV in Women Using Hybrid Capture Conducting Prevention of Cervical Cancer in Southern Brazil.

Abstract	41
Resumo	42
Introdution	43
Methods	44
Results	46
Discusion	49
Conclusion	52
References	53

PARTE I
PROJETO

1 Identificação

1.1 Doutorando

Guilherme Lucas de Oliveira Bicca

1.2 Orientador

Prof. Dr. Fernando Barros

1.3 Programa de Pós Graduação

Saúde e Comportamento (PPGSC)

1.4 Instituição

Universidade Católica de Pelotas

1.5 Linha de Pesquisa

Câncer da cérvix uterina

2 Delimitação do Problema

2.1 Introdução

O câncer de colo uterino é o segundo tipo de câncer mais comum em mulheres no mundo. Aproximadamente 80% dos casos ocorrem em países em desenvolvimento. No mundo esta patologia acomete anualmente 370.000 mulheres, sendo responsável por aproximadamente 190.000 óbitos¹.

A evidente associação entre o Papilomavírus Humano (HPV), as lesões pré-invasoras e o câncer do colo uterino tem sido motivo de uma série de estudos científicos.

Entre os mais de 100 tipos de HPV identificados, nem todos são oncogênicos, podendo causar uma série de doenças tais quais verrugas, verrugas genitais, papilomatose de laringe, entre outras. No entanto, em 99,7% dos casos de carcinomas cervicais há presença de um ou mais dos seguintes sub-tipos de HPV de alto risco ou oncogênicos: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 e 68^{2,3,4}. Fatores como alta paridade, múltiplos parceiros, início precoce da vida sexual, uso prolongado de contraceptivos orais e tabagismo entre outros podem influenciar o desenvolvimento das lesões precursoras e a evolução destas ao câncer do colo uterino⁵.

Habitualmente, a infecção por HPV acomete jovens no início da atividade sexual, um fenômeno transitório em cerca de 80% dos casos. Entretanto, uma pequena fração de mulheres apresenta persistência da infecção, provavelmente por falha de mecanismos imunológicos, o que pode provocar alteração no epitélio cervical e transformação maligna⁶.

O método mais utilizado na prevenção do câncer cervical e de suas lesões precursoras é o Papanicolaou ou exame citopatológico da cérvix uterina, utilizado há várias décadas na maioria dos países, com diferentes estratégias de organização do programa de rastreamento, em especial relacionadas à periodicidade do exame e à faixa etária alvo, com variados níveis de sucesso na redução da morbimortalidade⁷.

Para a classificação dos resultados dos exames citológicos o sistema de Bethesda é atualmente o mais utilizado, classificando as anormalidades do epitélio escamoso cervical em lesão de baixo grau (LIBG), lesão de alto grau (LIAG), atipias celulares de significado indeterminado (ASCUS) e carcinoma invasor.

Quando da alteração do padrão normal do epitélio cervical no exame citopatológico geralmente encaminham-se as pacientes para avaliação colposcópica, que proporciona visualização da lesão e caso necessária direciona a realização de biópsia dirigida para a análise histológica, método padrão ouro para o diagnóstico e tratamento. A associação destes métodos mostra-se de grande importância para o diagnóstico e tratamento das lesões precursoras do câncer do colo uterino.

Recentemente testes específicos para a detecção da presença de HPV no colo uterino foram introduzidos ao screening do câncer do colo uterino no intuito de identificar as mulheres com risco de desenvolvimento desta patologia. Isto proporcionou um acréscimo à sensibilidade citológica além da capacidade de identificação dos subtipos virais e da quantidade de cópias virais presentes na amostra ou carga viral. Vários métodos moleculares de detecção do HPV existem no mercado sendo os mais utilizados a Hibridização Molecular ou Captura Híbrida II e o teste de PCR⁸.

Os testes de detecção do HPV associados à citologia podem ser úteis na identificação de mulheres de risco para lesões cervicais mais graves, auxiliar em achados citológicos e colposcópicos duvidosos como ASCUS ou atipia de significado indeterminado de células escamosas e AGUS ou atipia de significado indeterminado de células glandulares e também segundo alguns autores, no controle após tratamento das neoplasias intra-epiteliais cervicais⁹.

A Captura Híbrida II é um procedimento de hibridização molecular, de processamento rápido e leitura confiável para detectar 18 tipos de HPV divididos em grupos de baixo (6, 11, 42, 43 e 44), e de alto risco oncogênico (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 e 68).

2.2 Título

Infecção pelo HPV em mulheres da Zona Sul do Rio Grande do Sul e fatores associados ao desenvolvimento de lesões precursoras e câncer da cérvix uterina.

2.3 Justificativa

Os resultados do presente estudo poderão contribuir para aperfeiçoar a capacidade de detecção das lesões precursoras e do câncer do colo uterino

proporcionando redução da morbimortalidade pela doença e tratamentos menos agressivos às pacientes.

Possibilitar novas estratégias de prevenção e screening pelo maior entendimento dos fatores de risco associados a esta patologia e sua evolução com o passar dos anos.

3 Objetivos

3.1 Objetivo geral

Quantificar a Infecção pelo HPV de alto risco em mulheres da Zona Sul do Rio Grande do Sul em um Serviço especializado em Patologia do Trato Genital Inferior e Colposcopia e correlacionar os fatores associados ao desenvolvimento das lesões precursoras e câncer do colo uterino.

3.2 Objetivos específicos

Analisar a mudança nos padrões de sexualidade, paridade, tabagismo e presença de HPV de alto risco no decorrer dos 14 anos de análise.

Comparar e correlacionar os demais métodos de diagnóstico do câncer de colo uterino e suas lesões precursoras na população estudada: Exame citopatológico do colo uterino, colposcopia e exame histopatológico.

Avaliar os diferentes níveis de resultados citológicos e histológicos alterados e a presença de DNA de HPV AR.

Observar a variação da infecção pelo HPV nas diversas faixas etárias das pacientes.

4 Hipóteses

Haverá prevalência de HPV de alto risco na população estudada ao redor de 55%.

A infecção pelo HPV de alto risco será maior entre mulheres com início precoce da vida sexual, maior número de parceiros, maior número de gestações e fumantes.

A infecção pelo HPV de alto risco será maior nas pacientes mais jovens, reduzindo conforme o aumento da idade.

O teste de HC2 para HPV de alto risco tem ótima sensibilidade na triagem de pacientes com lesão intra-epitelial do colo uterino.

A positividade para HPV de alto risco é maior quando maior a gravidade citológica e histológica da lesão da cérvix uterina.

5 Materiais e Métodos

5.1 Delineamento do Estudo

Estudo transversal, realizado em pacientes encaminhadas para realização do teste de Hibridização Molecular (HM) para HPV no Serviço Especializado em Citologia Ginecológica (SECIG) no período de janeiro de 1998 e julho de 2012 (totalizando 14 anos), provenientes da cidade de Pelotas e Região Sul, encaminhadas por médicos ginecologistas e ou pelos serviços universitários do Ambulatório da Universidade Católica de Pelotas e da Faculdade de Medicina da Universidade Federal de Pelotas.

As principais indicações clínicas serão: o resultado de citopatológico alterado, exame físico alterado, história anterior de infecção pelo vírus HPV, entre outros. O SECIG é o único serviço especializado em Patologia do trato Genital Inferior e Colposcopia (PTGIC) que realiza integrados os exames de HM para HPV, Colposcopia Digital e Citopatologia na região Sul do Rio Grande do Sul.

O período de 14 anos do estudo será dividido em três grupos: 1998-2002; 2003-2008; 2009-2012, para possibilitar a avaliação da modificação do padrão de comportamento das pacientes estudadas, referente a: número de parceiros, sexarca, tabagismo e paridade bem como os resultados de prevalência de HPV de alto risco.

5.2 Critérios de inclusão

Mulheres com indicação para realização do Teste de HM para HPV, supra mencionadas.

5.3 Critérios de exclusão

1. Imunodepressão
2. Gestantes
3. Pacientes com histerectomia total (ausência do colo uterino)

5.4 Coleta de Dados

O presente estudo se propõe a realizar coleta de dados a partir dos prontuários das pacientes supracitadas que serão submetidas às técnicas a seguir. O período de

14 anos do estudo será dividido em três grupos (1998 - 2002; 2003 - 2008; 2009 – 2012) para possibilitar a avaliação da modificação do padrão de comportamento das pacientes referente a número de parceiros, sexarca, tabagismo e paridade, bem como os resultados de prevalência de HPV de alto risco.

Técnica Citológica

A coleta citológica será realizada utilizando a espátula de Ayre associada à escova endocervical. As lâminas serão fixadas em álcool 95% e a coloração a ser utilizada é a de Papanicolaou. O resultado da citologia das pacientes será analisado por um único citopatologista. Os resultados serão descritos conforme a Classificação Internacional de Bethesda em: (1) achados normais (2) atipias de células escamosas de origem indeterminada (ASCUS); (3) lesões escamosas de baixo grau (NIC 1 e HPV); (4) lesões escamosas de alto grau (NIC II e III); (5) atipias glandulares pré-invasoras, incluindo atipias glandulares de origem indeterminada (AGUS) e adenocarcinoma in situ; e finalmente (6) carcinoma invasor escamoso ou glandular. O esfregaço citológico será avaliado segundo critérios morfológicos: anofilia, halo perinuclear, disqueratose, critérios nucleares (binucleação, multinucleação), aumento da relação núcleo/citoplasma, anisocariose, hipercromasia, atipias nucleares, cariorrexe, além da realização da técnica de Papanicolaou, conhecida e difundida mundialmente.

Técnica Colposcópica

Todas as pacientes serão submetidas à Videocolposcopia. O aparelho a ser utilizado é o colposcopio DF Vasconcellos com 5 aumentos adaptado a vídeo-câmera digital para digitalização e armazenamento de imagens. Para a realização da colposcopia serão utilizados os seguintes reagentes: solução aquosa de ácido acético glacial a 5% e solução de lugol.

Os achados colposcópicos serão classificados de acordo com a classificação da International Federation of Cervical Pathology and Colposcopy (IFCCP) em: achados colposcópicos normais (epitélio escamoso original, epitélio colunar e zona de transformação normal) e anormais (epitélio aceto-branco, pontilhado, mosaico, leucoplasia, zona iodo-negativa e vasos atípicos), sendo esta última subdividida em alterações menores ou maiores dependendo da intensidade da alteração.

Serão realizadas imagens digitalizadas das colposcopias para a execução dos laudos colposcópicos.

Na presença de alterações colposcópicas maiores, as pacientes serão submetidas à biópsia dirigida utilizando pinça de Baby Tyschler MedGyn®. Algumas pacientes retornarão a seus médicos de origem para a realização das biópsias dirigidas. O tratamento das lesões será baseado nos resultados histopatológicos obtidos.

Técnica de Hibridização Molecular para HPV – Captura Híbrida

Para a detecção do DNA viral por captura híbrida, o material para a análise será coletado com o swab do kit coletor DIGENE® e irá passar por cinco procedimentos: desnaturação, hibridização, captura de híbridos, reação dos híbridos com o conjugado e detecção dos híbridos por quimiluminescência. Reagindo com sonda gênica específica, o material forma híbridos de RNA/DNA capturados por anticorpos que revestem as paredes dos tubos. A seguir, os híbridos imobilizados reagem com anticorpos específicos conjugados à fosfatase alcalina, formando um substrato estável, e são detectados por quimiluminescência ultra-sensível. Os testes de captura híbrida demonstram ao mesmo tempo resultados qualitativos e quantitativos.

A análise da HM será realizada para a sonda do Grupo B (alto risco; tipos 16, 18, 31,33, 35, 39, 45, 51, 52, 56, 58, 59 e 68) e os resultados serão classificados como positivos quando superiores a 1pg/ml o que é referente equivalente a 0,1 cópia de vírus por célula. Os resultados positivos serão posteriormente divididos em baixa (1pg/ml a 10pg/ml), moderada (10pg/ml a 100pg/ml), e alta carga viral (superior à 100pg/ml).

Algumas pacientes poderão apresentar mais de uma coleta durante o período do estudo, sendo possível observar as modificações na carga viral do HPV com o passar do tempo, em relação aos tratamentos realizados e seus resultados bem como mudança no padrão citológico e colposcópico de controle.

Demais Variáveis

A partir dos prontuários também foram coletados as seguintes variáveis para caracterização do perfil das pacientes:

- Idade: idade em anos completos no momento da coleta dos exames.
- Menarca: idade de início das menstruações, em anos completos.
- Sexarca: idade de início das relações sexuais, em anos completos.
- Número de parceiros: número de parceiros sexuais até o momento da coleta do exame.
- Fumo
- Número de gestações e filhos
- História de infecção por HPV: história anterior de infecção pelo vírus HPV.

5.5 Aspectos Éticos

O estudo foi submetido e aprovado pelo Comitê de Ética e de Pesquisa da Faculdade de Medicina da Universidade Federal de Pelotas, protocolo número 425607.

5.6 Análise estatística

Os dados serão armazenados sequencialmente de acordo com o número designado a paciente, em planilhas do programa Excel. Posteriormente serão transferidos para um banco de dados do programa STATA, sendo realizada a análise estatística através deste pacote estatístico. Os resultados serão sumarizados em gráficos e/ou tabelas descrevendo as variáveis estudadas em cada grupo, sendo em forma de percentuais se as variáveis forem categóricas; e média e desvio padrão se as variáveis forem numéricas contínuas.

A análise das variáveis categóricas será realizada pelo teste qui-quadrado de Pearson para heterogeneidade ou tendência linear, quando necessário. O nível de significância pré-fixado foi de 0,05.

A análise será processada considerando os grupos de acordo com o tratamento realizado.

O desfecho será considerado a presença de HPV de alto risco. Para fins de análise esta informação, obtida à partir da hibridização molecular, será categorizada da seguinte forma: positivo para HPV quando resultados superiores à 1pg/ml. Ausência de HPV quando resultado inferior à 1pg/ml.

6 Referências

1. Instituto Nacional de Câncer (Brasil). Câncer no Brasil: dados dos registros de base populacional, vol. IV. Rio de Janeiro: INCA; 2010. 487 p.
2. Vince A. *et al.* Clinical utility of molecular detection of human papillomavirus in cervical samples by hybrid capture technology. *J Clin Virol*, v. 25, p. S109-12, 2002.
3. Guimarães Eliane Machado, Brasileiro Filho Geraldo, Pena Sérgio Danilo Junho. Human papillomavirus detection in cervical dysplasias or neoplasias and in condylomata acuminata by in situ hybridization with biotinylated DNA probes. *Rev. Inst. Med. trop. S. Paulo* [serial on the Internet]. 1992 Aug [cited 2012 Oct 13]; 34(4): 309-314.
4. ZurHausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2002;2:342–50.
5. IARC Working Group. Evaluation of Carcinogenic Risks to Humans 2005 : Lyon F. Human Papillomaviruses. IARC Monographs. , editor. 2007.
6. Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F. Carcinogenicity of human papillomaviruses. *Lancet Oncol* 2005;6: 204.
7. Nieminen P, Kallio M, Hakama M. The effect of mass screening on incidence and mortality of squamous and adenocarcinoma of cervix uteri. *Obstet Gynecol* 1995;85:1017–21.
8. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. *Lancet* 2007; 370(9590):890–907.
9. Lorincz AT, Castle PE, Sherman ME, Scott DR, Glass AG, Wacholder S, Rush BB, Gravitt PE, Schussler JE, Schiffman M. Viral load of human papillomavirus and risk of CIN3 or cervical cancer. *Lancet* 2002;360:228–9.

PARTE II
ARTIGOS

ARTIGO 1

HPV infection and cervical cancer: a review of screening and preventive strategies in developed countries and Brazilian policies.

Abstract

Cervical cancer is currently the third malignancy on number of female deaths in the world. Studies have shown that 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. Knowledge on infection persistence and correlation with cervical cancer has increased the demand for HPV detection molecular tests. The most used techniques 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. Working on reducing the number of cervical cancer cases, screening strategies have been adjusted to contain the best combination of cytological and molecular tests. 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 overreferral. Optimization may be achieved by using by cotesting: 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). An important measure to reduce cervical cancer cases in prevention through the use of vaccination. Quadrivalent and bivalent vaccines have been approved and used in different countries. The Brazilian National Health System will, from 2014, offer the quadrivalent HPV vaccine free of cost for the vaccination of girls aged between 10 and 11 years old.

Key words: HPV, cervical cancer, vaccine, Brazil.

Infecção por HPV e o câncer de colo uterino: uma revisão da política brasileira e das estratégias de prevenção e screening em países em desenvolvimento

Resumo

Introdução: Atualmente o câncer de colo uterino é a terceira causa de morte em mulheres no mundo. Estudos têm demonstrado que infecção persistente pelo HPV é o principal agente envolvido no desenvolvimento do câncer de colo uterino. Especialmente HPV de alto risco (AR), tipos 16 e 18, são responsáveis por aproximadamente 75% dos casos de câncer de colo uterino. O conhecimento da persistência da infecção bem como de sua relação com o câncer de colo uterino promoveu um incremento na demanda de testes moleculares para detecção de HPV. As técnicas mais utilizadas são a PCR e a captura híbrida. Contudo, técnicas para detecção de RNAm de HPV E6/E7 e p16NK4 já foram desenvolvidas e estão em fase de validação. Estes testes poderão auxiliar na distinção de infecções transientes e persistentes. Buscando reduzir o número de casos de câncer de colo uterino, estratégias de seleção/diagnóstico combinando testes citológicos e moleculares tem sido ajustadas. As estratégias de screening ideais requerem alta sensibilidade para minimizar resultados falso negativos assim como alta especificidade a fim de evitar falsos positivos e excesso de encaminhamentos. A otimização pode ser obtida utilizando combinações testes de genotipagem de HPV com triagens citológicas. Uma medida importante para redução dos casos de câncer de colo de útero é a vacinação preventiva, vacinas quadrivalentes e bivalentes tem sido aprovados e utilizados em diferentes países. O Ministério da Saúde, a partir de 2014, oferecerá a vacina quadrivalente gratuitamente para imunização de meninas entre 10 e 11 anos de idade.

Palavras Chave: HPV, câncer de colo de útero, vacina, 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 HPV type 16 and 18 [8].

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

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.

Scientific background on HPV

Human Papillomavirus 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 known HPV types. Two classes of HPV can be distinct, based on location of 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 E7, 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]. In Figure 1 we can see the pattern of distribution of HPV infection based on HPV type.

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 tends 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 [16]. 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 [17].

HPV persistence, from one to two years, particularly by HPV16, increases the prognostic for CIN3 or more grave diagnosis (CIN3+) in the following years [6]. The risk of untreated CIN3 lesions becoming invasive cancer goes up to 20% by 10 years and 30% by 30 years. However, when treated, only around 1% of the lesions will become invasive. In cases of women both minimum disturbance of their lesion and persistent disease, the risk was of about 30% by 10 years, increasing to approximately 50% by 30 years [18]. Also, HPV16 and multiple-type infections have the lowest clearance rate, increasing the probability of cervical cancer [19].

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 2. 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 [20].

However, there are independent risk factors associated with squamous cell carcinoma and adenocarcinomas [20]. Among them are smoking, number of pregnancies, other infectious agents [21] and early initiation on oral contraceptives [22]. A Finnish study found correlation with an increased risk of incident HPV-infection for initiation of smoking beyond 13 years of age and for the initiation of oral contraceptives usage before the age of 20 [22].

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 [15]. High-quality screening using cytology has significantly reduced mortality from squamous cell cervical cancer, which constitutes up to 90% of cervical cancers [23].

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 [24]. When *in situ* lesions are confirmed, they are called

cervical 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 [24].

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 [24]. 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 it is a repetitive method and this 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 detection. Molecular tests offer increased sensitivity although it shows 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 that obtained by cytology in several studies [14].

There is debate to which of these two tests would be better. On a screening test using both techniques, Kulmala et al. [25] 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 [25]. 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 [25].

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. [26] 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 [26]. Nevertheless, the authors pronounce mRNA detection tool as a promising test as an adjunct to cytology.

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. [27] 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 [27].

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 could improve 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 [28].

The ideal cervical cancer screening strategy would require highest sensitivity to minimize false negative results, as well as highest specificity, in order to avoid false positives and over referral [29]. 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 [29].

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 sensitive for the detection of precancerous lesions in women with ambiguous cytology [13]. HPV testing is more sensitive 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 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 [29].

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 [30]. For this reason, development of HPV L1 virus-like-particle (VLP) vaccines is 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, 18 induced CIN2+ [31]. Figure 1 shows why research companies have focused in HPV types 16 and 18, given their priority on the relative burden of the disease.

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 [32].

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 [33]. 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) [31]. 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 [31].

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 [34]. 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) [34].

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 the 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 named that they did not include developing countries [14]. A program for screening for cervical cancer in Brazil now counts with 17 years of existence [35]. 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 [35]. It is estimated that Brazil has over 20,000 new cases of cervical cancer per year [16]. 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 & Silva (2010) 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% [16]. Also in Brazil HPV16 was the most prevalent irrespective of cytology results [16].

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, 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 [20]. 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 - [36]). Assuming the PPP rate used by Goldie et al. [2] was I\$ 0.8 (PPP rate of 2008 [36]), 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

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 [16]. Research and time will tell which screening strategies and programmes are best suited for different regions, adapting them to local resources and collective priorities.

References

- [1] J. Ferlay, H.-R. Shin, F. Bray, D. Forman, C. Mathers, and D. M. Parkin, "Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008.," *International journal of cancer. Journal international du cancer*, vol. 127, no. 12, pp. 2893–917, Dec. 2010.
- [2] S. J. Goldie, J. J. Kim, K. Kobus, J. D. Goldhaber-Fiebert, J. Salomon, M. K. H. O'shea, F. Xavier Bosch, S. de Sanjosé, and E. L. Franco, "Cost-effectiveness of HPV 16, 18 vaccination in Brazil.," *Vaccine*, vol. 25, no. 33, pp. 6257–70, Aug. 2007.
- [3] B. H. Yang, F. I. Bray, D. M. Parkin, J. W. Sellors, and Z.-F. Zhang, "Cervical cancer as a priority for prevention in different world regions: an evaluation using years of life lost.," *International journal of cancer. Journal international du cancer*, vol. 109, no. 3, pp. 418–24, Apr. 2004.
- [4] F. X. Bosch, V. Tsu, A. Vorsters, P. Van Damme, and M. a Kane, "Reframing cervical cancer prevention. Expanding the field towards prevention of human papillomavirus infections and related diseases.," *Vaccine*, vol. 30 Suppl 5, pp. F1–11, Dec. 2012.
- [5] H. zur Hausen, "Papillomaviruses and cancer: from basic studies to clinical application.," *Nature reviews. Cancer*, vol. 2, no. 5, pp. 342–50, May 2002.
- [6] D. Saslow, D. Solomon, H. W. Lawson, M. Killackey, S. L. Kulasingam, J. Cain, F. a R. Garcia, A. T. Moriarty, A. G. Waxman, D. C. Wilbur, N. Wentzensen, L. S. Downs, M. Spitzer, A.-B. Moscicki, E. L. Franco, M. H. Stoler, M. Schiffman, P. E. Castle, and E. R. Myers, "American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer.," *American journal of clinical pathology*, vol. 137, no. 4, pp. 516–42, Apr. 2012.
- [7] K. a. Ault, "Human papillomavirus vaccines and the potential for cross-protection between related HPV types," *Gynecologic Oncology*, vol. 107, no. 2, pp. S31–S33, Nov. 2007.
- [8] N. Muñoz, F. X. Bosch, S. de Sanjosé, R. Herrero, X. Castellsagué, K. V Shah, P. J. F. Snijders, and C. J. L. M. Meijer, "Epidemiologic classification of human papillomavirus types associated with cervical cancer.," *The New England journal of medicine*, vol. 348, no. 6, pp. 518–27, Feb. 2003.
- [9] S. Kulmala and S. Syrjänen, "Human papillomavirus testing with the hybrid capture 2 assay and PCR as screening tools," *Journal of clinical microbiology*, vol. 42, no. 6, pp. 2470–2475, 2004.
- [10] M. Schiffman, P. Castle, J. Jeronimo, A. C. Rodriguez, and S. Wacholder, "Human papillomavirus and cervical cancer," *The Lancet*, vol. 370, pp. 890–907, 2007.
- [11] A. A. T. P. Brink, P. J. F. Snijders, and C. J. L. M. Meijer, "HPV detection methods," *Disease Markers*, vol. 23, no. 4, pp. 273–281, Jan. 2007.

- [12] M. Schiffman, "Integration of human papillomavirus vaccination, cytology, and human papillomavirus testing," *Cancer*, vol. 111, no. 3, pp. 145–153, May 2007.
- [13] M. Arbyn, P. Sasieni, C. J. L. M. Meijer, C. Clavel, G. Koliopoulos, and J. Dillner, "Chapter 9: Clinical applications of HPV testing: a summary of meta-analyses.," *Vaccine*, vol. 24 Suppl 3, pp. S3/78–89, Aug. 2006.
- [14] J. Cuzick, C. Clavel, K.-U. Petry, C. J. L. M. Meijer, H. Hoyer, S. Ratnam, A. Szarewski, P. Birembaut, S. Kulasingam, P. Sasieni, and T. Iftner, "Overview of the European and North American studies on HPV testing in primary cervical cancer screening.," *International journal of cancer. Journal international du cancer*, vol. 119, no. 5, pp. 1095–101, Sep. 2006.
- [15] HHS (U.S. Department of Health and Human Services), "Pap and HPV Testing." National Cancer Institute, pp. 1–5, 2012.
- [16] A. R. G. Ayres and G. A. E. Silva, "Cervical HPV infection in Brazil: systematic review.," *Revista de saúde pública*, vol. 44, no. 5, pp. 963–74, Oct. 2010.
- [17] H. Trottier, S. Mahmud, J. C. M. Prado, J. S. Sobrinho, M. C. Costa, T. E. Rohan, L. L. Villa, and E. L. Franco, "Type-Specific Duration of Human Papillomavirus Infection: Implications for Human Papillomavirus Screening and Vaccination," *Journal of Infectious Diseases*, vol. 197, no. 10, pp. 1436–1447, May 2008.
- [18] M. R. E. McCredie, K. J. Sharples, C. Paul, J. Baranyai, G. Medley, R. W. Jones, and D. C. G. Skegg, "Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study.," *The lancet oncology*, vol. 9, no. 5, pp. 425–34, May 2008.
- [19] K. Louvanto, K. J. Syrjänen, M. a M. Rintala, S. E. Grénman, and S. M. Syrjänen, "Genotype-specific clearance of genital human papillomavirus (HPV) infections among mothers in the Finnish family HPV study.," *Journal of Clinical Microbiology*, vol. 48, no. 8, pp. 2665–71, Aug. 2010.
- [20] C. M. Roteli-Martins, A. Longatto Filho, L. S. Hammes, S. F. M. Derchain, P. Naud, J. C. de Matos, D. Etlinger, L. Sarian, R. C. Gontijo, M. Y. S. Maeda, and K. J. Syrjänen, "Associação entre idade ao início da atividade sexual e subsequente infecção por papilomavírus humano: resultados de um programa de rastreamento," *Revista Brasileira de Ginecologia e Obstetrícia*, vol. 29, no. 11, pp. 580–7, 2007.
- [21] P. R. do Prado, R. J. Koifman, A. L. M. Santana, and I. F. da Silva, "Caracterização do Perfil das Mulheres com Resultado Citológico ASCUS/AGC, LSIL e HSIL segundo Fatores Sociodemográficos, Epidemiológicos e Reprodutivos em Rio Branco - AC, Brasil," *Revista Brasileira de ...*, vol. 58, no. 3, pp. 471–479, 2012.
- [22] K. Louvanto, M. a Rintala, K. J. Syrjänen, S. E. Grénman, and S. M. Syrjänen, "Incident cervical infections with high- and low-risk human papillomavirus (HPV) infections among mothers in the prospective Finnish Family HPV Study.," *BMC infectious diseases*, vol. 11, no. 1, p. 179, Jan. 2011.

- [23] D. M. Parkin and F. Bray, "Chapter 2: The burden of HPV-related cancers.," *Vaccine*, vol. 24 Suppl 3, pp. S3/11–25, Aug. 2006.
- [24] F. Hulstaert, M. Arbyn, M. Huybrechts, I. Vinck, M. Puddu, and D. Ramaekers, "Cervical Cancer Screening and Human Papillomavirus (HPV) Testing," Brussels, 2006.
- [25] S.-M. Kulmala, S. Syrjänen, I. Shabalova, N. Petrovichev, V. Kozachenko, J. Podistov, O. Ivanchenko, S. Zakharenko, R. Nerovjna, L. Kljukina, M. Branovskaja, V. Grunberga, A. Juschenko, P. Tosi, R. Santopietro, K. Syrjänen, and the N. I. S. C. S. Group, "Human Papillomavirus Testing with the Hybrid Capture 2 Assay and PCR as Screening Tools," *Journal of Clinical Microbiology*, vol. 42, no. 6, pp. 2470–2475, Jun. 2004.
- [26] T. Molden, I. Kraus, F. Karlsen, H. Skomedal, J. F. Nygård, and B. Hagmar, "Comparison of Human Papillomavirus Messenger RNA and DNA Detection: A Cross-sectional Study of 4,136 Women >30 Years of Age with a 2-Year Follow-up of High-Grade Squamous Intraepithelial Lesion," *Cancer Epidemiology Biomarkers & Prevention*, vol. 14, no. 2, pp. 367–372, Feb. 2005.
- [27] N. Wentzensen, M. Hampl, M. Herkert, A. Reichert, M. J. Trunk, C. Poremba, R. Ridder, and M. von Knebel Doeberitz, "Identification of high-grade cervical dysplasia by the detection of p16INK4a in cell lysates obtained from cervical samples," *Cancer*, vol. 107, no. 9, pp. 2307–2313, Nov. 2006.
- [28] P. Sasieni, A. Castanon, and J. Cuzick, "The impact of cervical screening on young women: a critical review of the literature 2002 – 2009," Sheffield, 2010.
- [29] J. T. Cox, P. E. Castle, C. M. Behrens, A. Sharma, T. C. Wright, and J. Cuzick, "Comparison of cervical cancer screening strategies incorporating different combinations of cytology, HPV testing, and genotyping for HPV 16/18: results from the ATHENA HPV study.," *American journal of obstetrics and gynecology*, vol. 208, no. 3, pp. 184.e1–184.e11, Mar. 2013.
- [30] R. L. Winer, J. P. Hughes, Q. Feng, S. O'Reilly, N. B. Kiviat, K. K. Holmes, and L. A. Koutsky, "Condom Use and the Risk of Genital Human Papillomavirus Infection in Young Women," *New England Journal of Medicine*, vol. 354, no. 25, pp. 2645–2654, Jun. 2006.
- [31] J. Horn, O. Damm, M. E. E. Kretzschmar, Y. Deleré, O. Wichmann, a M. Kaufmann, E. Garbe, a Krämer, W. Greiner, and R. T. Mikolajczyk, "Estimating the long-term effects of HPV vaccination in Germany.," *Vaccine*, vol. 31, no. 19, pp. 2372–80, May 2013.
- [32] CDC - Center for Disease Control and Prevention, "Human Papillomavirus (HPV) -Associated Cancers," 2013. [Online]. Available: http://www.cdc.gov/cancer/hpv/what_cdc_is_doing/. [Accessed: 14-Jul-2013].
- [33] Y. Deleré, C. Meyer, and S. Reiter, "Universal immunisation with human papillomavirus (HPV) vaccine among females aged 12-17 recommended in Germany.," *Euro Surveill.*, vol. 12, no. 14, p. pii=3169, 2007.
- [34] Ministério da Saúde, "Ministério da Saúde incorpora vacina contra HPV ao SUS," 2013. [Online]. Available:

<http://portalsaude.saude.gov.br/portalsaude/noticia/11662/893/ministerio-da-saude-incorpora-vacina-contrahpv-ao-sus.html>. [Accessed: 18-Jul-2013].

- [35] J. de Andrade, "Limitações para o sucesso do rastreamento do câncer de colo no Brasil," *Revista Brasileira de Ginecologia e Obstetrícia*, vol. 34, no. 6, pp. 245–7, 2012.
- [36] World Bank, "PPP conversion factor (GDP) to market exchange rate ratio," 2013. [Online]. Available: <http://data.worldbank.org/indicator/PA.NUS.PPPC.RF>. [Accessed: 19-Jul-2013].

Legends for figures

Figure 1: Occurrence of HPV types in patients with cervical cancer. Group A9 encompasses HPV types 16, 31, 33, 35, 52, 58; group A7 types 18, 39, 45, 59, 68, 70; group A5 types 26, 51, 82 and group A6 types 53, 56, 66; all phylogenetically classified as HR HPV types. Although HPV16 belongs to group A9 and HPV18 to group A7, due to their high percentage of infection, they were plotted separately. “Others” include LR HPV types. HPV type X denotes specimens that were positive with the GP5+/6+ system but that did not hybridize with any of the probes used in the study. Adapted from Muñoz et al. [8].

Figure 2: Critical steps in the development of cervical cancer. Sexual transmission of HPV is common and tends to clear fast in younger women. Most HPV infections will show no cytological abnormality and will clear in less than two years. Of those that persist for two years, 10% will be linked to pre-cancer, which are usually detected in women with 25-30 years. CIN3 is a good predictor of eminent cancer risk, while CIN2 can be ambiguous. Images refer to colposcopy exams. Adapted from [10].

Figures

Figure 1

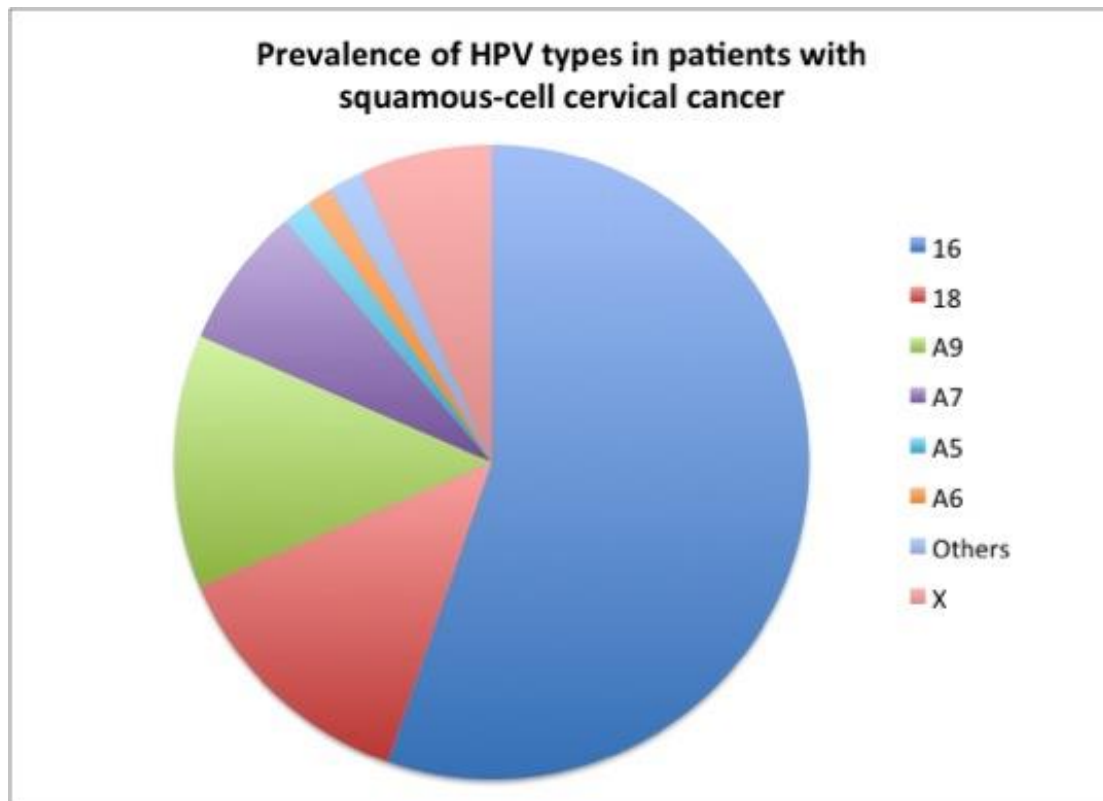
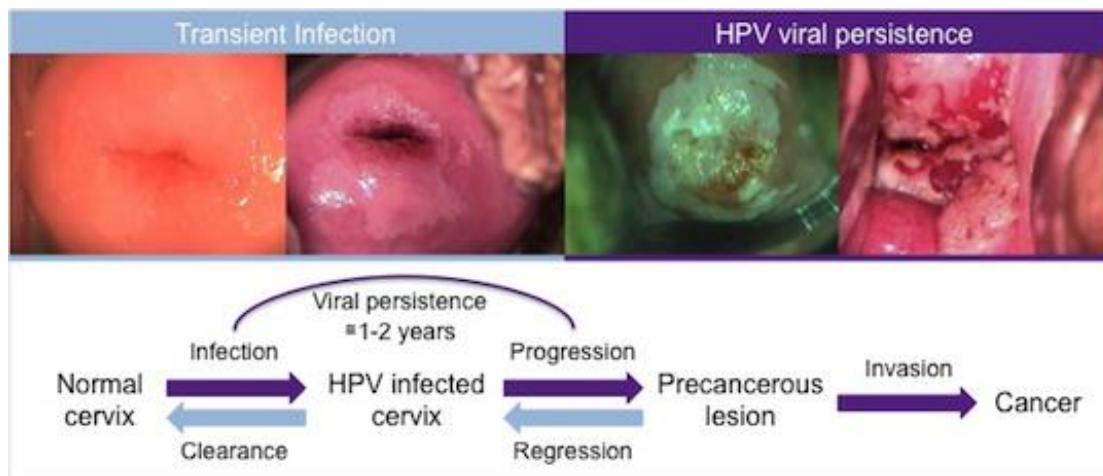


Figure 2



ARTIGO 2

Prevalence of Infection with High-Risk HPV in Women Using Hybrid Capture
Conducting Prevention of Cervical Cancer in Southern Brazil.

Abstract

Introduction: High risk (HR) HPV infection is known to be linked to cervical cancer, with molecular biology tests being an important tool in diagnosis. **Objective:** This study aimed to quantify the prevalence of HPV infection in women from the Southern part of the State of Rio Grande do Sul, Brazil, correlating factors associated with the development of precursor lesions and of cervical cancer. **Methods:** For that, 643 women were enrolled in the study, filling out a standardized questionnaire and undergoing cytology, colposcopy and HR HPV (Hybrid Capture 2) tests. **Results:** Most patients were aged between 20 and 39 years old (70.6%), decreased the percentage of smokers from 23% to 11% and the average age of sexual debut through the period studied was of 18 years old. HR HPV prevalence was correlated with younger ages, with fewer patients infected by HR HPV when they were older at first sexual activity. An almost 70% prevalence of infection was observed for women who had 4 or more sexual partners. Altered cytology and colposcopy results had significantly higher rates of HR HPV infection. 334 women were referred to biopsy. Of those, 321 had altered colposcopy results and cytopathologic of ASC-US/AGC-US, LSILs and HSILs, with 231 biopsies performed by the study. None of the results indicated cervical cancer. HC2 showed higher specificity than cytology, with high positive and negative prediction values (49.8% and 78.6%, respectively). **Conclusion:** The inclusion of HR HPV testing in screening programs in Brazil, according to international policies, will lead to fewer biopsies on women without infection and increased interval between screenings.

Keywords: High Risk HPV, cervical cancer, Hybrid Capture, Brazil.

Resumo

Introdução: Sabe-se que infecções por HPV de alto risco (HPV AR) estão ligadas ao desenvolvimento de câncer cervical. **Objetivo:** Esse estudo teve como objetivo quantificar a prevalência de infecções por HPV em mulheres da metade sul do Estado do Rio Grande do Sul, Brasil, correlacionando fatores associados ao desenvolvimento de lesões precursoras e câncer cervical. **Métodos:** Para tanto, 643 mulheres foram incluídas no estudo, preenchendo um questionário padronizado e submetidas aos exames de citologia, colposcopia e HPV AR (Captura Híbrida 2). **Resultados:** A maioria das pacientes tinha idade entre 20 e 39 anos (70,6%), houve decréscimo na porcentagem de fumantes de 23% para 11% e a média de idade ao início da vida sexual era de 18 anos. A prevalência de HPV AR é correlacionada com idades mais jovens, com menos pacientes infectadas por HPV AR quando o início da atividade sexual foi mais tardio. Prevalência próxima a 70% foi observada em mulheres que tiveram 4 ou mais parceiros sexuais. Resultados citológicos e colposcológicos alterados tiveram taxas significativamente mais altas de infecção por HPV AR. 334 mulheres foram encaminhadas à biópsia. Destas, 321 tiveram resultados de colposcopia alterados e citopatologia ASC-US/AGC-US, LSILs e HSILs, com 231 biópsias realizadas neste estudo. Nenhum dos resultados indicou câncer. O teste de CH2 mostrou mais especificidade do que a citologia, com altos valores preditivos positivos e negativos (49,8% e 78,6%, respectivamente). **Conclusão:** A inclusão de testes para HPV AR nos programas de triagem no Brasil, de acordo com as políticas internacionais, levará à redução de biópsias em mulheres não infectadas e aumentará o intervalo entre exames.

Palavras-chave: HPV de alto risco, câncer cervical, Captura Híbrida, Brasil.

Introduction

Cervical cancer is the third most common malignancy in women, and the seventh in general, with approximately 270,000 deaths annually (1). Epidemiologic studies have shown that the main aetiological factor of cervical cancer is the infection with high-risk (HR) types of Human Papillomaviruses (HPVs)(2); in fact, nearly all of cervical cancer cases test positive for HPV (3).

Persistent HPV infection have been unequivocally linked to the development of cervical cancer, and at least 15 HPV subtypes are classified as high risk, including 16, 18, 31, 33 and 51(4). HPV types 16 and 18 are the most carcinogenic, responsible for approximately 70% of all cervical cancers(5).

The knowledge of the association between HPV and cervical cancer has led to the development of new screening techniques based on molecular biology testing. Some of these strategies include PCR-based diagnosis or Hybrid Capture 2 (HC2) assays, and between them, HC2 testing has better sensitivity for the detection of high-grade squamous intraepithelial lesions (HSIL) (6–9).

Among the pre-malignancy possible results of Pap smear, HSIL shows more severe cell abnormalities and are more likely to progress into cancer if left untreated(10). Hence the importance of identifying HR HPV in patients with a HSIL diagnosis.

Reviews on HPV infection, screening processes and treatment options indicate a lack of studies in developing countries(7). The current study aimed at quantifying HPV infection in women from the Southern part of the State of Rio Grande do Sul, and describes factors associated with the development of precursor lesions and cervical cancer.

Methods

A cross-sectional study was performed at the Gynecological Cytology Specialized Clinic (SECIG, Pelotas, RS, Brazil). All women included in the study were patients referred to be tested to HPV using uterine cervix samples and Hybrid Capture 2 (HC2) method, between January of 1998 and July 2012. This population comprises patients of gynecological practices from the entire Southern region of the State of Rio Grande do Sul (Brazil), private and insured. SECIG is the only service in the region, performing all the three following screening tests: cytopathology, videocolposcopy and HC2, being a reference also for the treatment of uterine cervix pathologies.

The study population was formed by 643 women aged between 15 and 81 years old. The hybrid capture test was requested by the patients physician mostly based on previous history of HPV infection or the presence of clinical findings. Pregnant women, patients with diagnosis or suspicion of immunosuppression and those who underwent total hysterectomy were excluded. Patients answered a standardized questionnaire including the following information: age, number of pregnancies, age of first sexual intercourse, number of sexual partners, smoking, previous history of HPV and birth control method of choice. Gynecological exam was then performed and screening test samples were obtained according to the following protocol.

For cervical cytology, an Ayre's spatula was used to collect cells from the transformation zone and a cytobrush for endocervical cells. Slides were fixed in 95% ethanol and Papanicolaou stained used. A single pathologist analyzed all cytological tests and results are described using Bethesda International Classification(11).

Colposcopy was performed using a *DFVasconcellos*[®] videocolposcope model CP-M1250, according to the literature(12). Colposcopy findings were classified according to the International Federation of Cervical Pathology and Colposcopy

(IFCCP) classification(13) and the images were digitalized. Results were catalogued as normal and abnormal according to the presence of: aceto-whitening at different levels, changed patterns of the blood vessels, and the stain pattern with iodine. Testing was unsatisfactory when the totality of the squamocolumnar junction was not visualized or intense atrophy and inflammatory process that prevented evaluation was observed.

Patients with altered colposcopies were submitted to biopsy, using *MedGyn*® Baby Tischler Cervical Biopsy Forceps, and subsequent histologic analysis with Hematoxylin/Eosin coloration. At colposcopy, 2 or more biopsy specimens should be taken. The used nomenclature was: low-grade cervical intraepithelial neoplasia (CIN1), high-grade cervical intraepithelial neoplasia (CIN2, CIN3) and invasive cervical carcinoma(14).

For the detection of HPV DNA by HC2, material was collected from the cervix using the swab and collection kit provided by *Digene*® HC2 HPV DNA Test (Qiagen N.V., Netherlands). The material was processed according to manufacturer's instructions. Samples were analyzed for HR HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The usual limit of 1 pg/ml of HPV DNA 16 was used as positive control, determining both quantitative and qualitative results (1 pg/ml equals 0.1 viral copy by cell). Positive results were then categorized in low (1pg/ml to 10pg/ml), moderate (10 pg/ml to 100 pg/ml) and high viral load (>100 pg/ml). Bivariate analyses were performed using chi-square and chi-square for linear trend. Statistical tests were considered to be statistically significant at a P value <0.05.

The study protocol was approved by the Ethics Committee of the Universidade Federal de Pelotas (Approval report number 425.607).

Results

The majority of the patients were aged between 20 and 39 years old (70.6%) (Table 1), and the age distribution did not change throughout the different periods of the study. The prevalence of smoking was 23% at the first period of years and showed a significant reduction through the years, reaching 11% at the last period. Regarding the age of sexual initiation, there was an increase on the number of women having their sexual debut before the age of 15 years in recent years, and a decrease in those who started at the age of 20 or more.

The number of sexual partners was evenly distributed between 1, 2 or 3, and 4 or more for the first third of the study. From 2003 onwards we can see a trend of increase in the number of sexual partners in this population. The number of pregnancies did not vary throughout the studied period. There was a significant trend of decrease in the number of women infected by HR types of HPV on recent years (Table 1).

Table 2 shows the positivity for HR HPV according to the sample characteristics. Regarding HR HPV prevalence, there was a linear correlation between a positive result for HPV and age, with a clear decrease in HPV infection with the increase in age ($p<0.001$). This study did not find a significant correlation between HPV infection and smoking ($p=0.4$). There was, however, a linear trend for reduction in HR HPV infection with the increase in the age of sexual initiation ($p<0.001$).

We found that an increase in the number of sexual partners was associated with an increase in HR HPV infection ($p<0.001$), reaching almost 70% of prevalence in those who had 4 or more partners. However, no correlation was found between the number of pregnancies and infection by HR HPV (Table 2). Although infection decreased in a linear pattern between those with no children and those with 1 or 2 pregnancies, the trend was not maintained. Those women with 3 or more pregnancies

showed similar values of HR HPV infection than women with only one pregnancy (Table 2).

Table 3 shows the relationship between positive results for HR HPV tests, colposcopy and cytopathology exams. Patients with altered colposcopy had significantly higher rates of HR HPV infection when compared with those patients whose colposcopy showed no alteration ($p<0.001$). There was also a significant difference according to the results of the cervical cytopathology tests. More severe results showed higher prevalence of HR HPV infection. Results varied from a prevalence of 37.2% of HR HPV infection among patients with normal cytopathology to a 98.4% prevalence among patients with HSILs.

Table 4 presents results for colposcopy and cytopathology exams, since the criteria for referral to histological analysis by biopsy was the correlation between altered colposcopy and cytology results. The results on this table indicate that the patients that should be referred to biopsy are those with altered colposcopy results (atypical transformation zone) and cytopathologic results of ASC-US/AGC-US, LSILs and HSILs. This group corresponds to 321 women. Nevertheless, a further 6% of patients (13 women) were referred to biopsy despite having normal cytopathologic results. Their colposcopy findings included intense reaction to acetic acid, mosaic and punctation.

Therefore, 334 women were referred to biopsy, but only 231 biopsy exams were performed, since some patients returned to their physicians of choice to undergo the procedure. In the group of 231 women that had biopsy exams done by our team, results were as follows: 10% presented chronic cervicitis, 43% CIN I, 46% CIN II or III. None of the performed biopsies suggested cervical cancer.

From the total of patients with CIN II or III (107 women), 75% were referred to Loop Electrosurgical Excision Procedure (LEEP), 10% to cold knife conization and 3%

to electrocauterization of the cervix. The remaining patients were treated by their physician of choice. Histological surgical results, for both LEEP and cone, were 3% negative, 16% CIN I, 77% CIN II or III and 4% invasive carcinoma. Regarding the margins of LEEP/cone samples, 85% had noninvolved (negative) margins.

Figure 1 presents sensibility and specificity values of the methods when compared to the histological findings, while table 5 presents positive and negative prediction values according to the tests. Sensibility for altered cytopathology test was 98.1% and positive prediction of 48.4%. Specificity was 9.7% and negative prediction of 53.7%. Regarding HR HPV, sensitivity was 94.4% and positive prediction of 49.8%, while specificity was 17.7% and negative prediction was of 78.6%.

Discussion

There was an increase in the age of women seeking medical care with physicians for HPV-related exams throughout the studied period. From 2009 onwards it was observed a decrease in the proportion of patients aged less than 29 years and an increase in those aged over 30, relative to the two previous study periods. This agrees with current policy guidelines that advise the cotesting for HPV and cytopathology for women aged 30 or older(10). It was also observed a decline in smoking habits throughout the study, reaching less than half of the initial proportion of smokers. This trend has also been observed in other countries(15,16). In the United Kingdom there was a significant reduction in the percentage of female smokers, from 40% in the 1970's to 20% in 2007, an estimate that remains constant(15). The proportion of 16% for smoking found in our study is the same described in the United States(16).

A change in the age of sexual initiation is evident in this population, with a trend for earlier initiation. Other studies have shown an average decrease of four years in the sexual debut(17). Bajos et al.(17) also observed an increase in the number of sexual partners between the years of 1970 (1.8) and 2006 (4.4). The same was observed in the present study. As most middle-income countries, Brazil has made the transition to a low-fertility country, with women having less children, having near zero population growth rate (18). The data seen in this population reflects this tendency for smaller families. Nonetheless, we could not define a trend in HR HPV infections, with the prevalence fluctuating between different time periods.

Our study has shown that younger women were at greater risk of contracting HR HPV. This finding agrees with previous findings(19). Also, the early beginning of sexual activity may lead to an increased number of partners in life, and both variables increase the likelihood of HR HPV infection. In this study 21% of women had started sexual life before the age of 15, and over 73% of them tested positive for HR HPV

infection. Although there is limited information of sexual behavior data for Brazil (1), this report agrees with previous findings of another Brazilian group, where 20% of women reported having had their sexual debut at the age of 15 or younger (20).

With the average age of sexual initiation being 18 years, women who started sexual activity after the average age had lower risk of HR HPV infection than those whose onset was below average age. Regarding the number of partners, there was a significant increase (38%) in HR HPV infection with higher number of sexual partners.

Although there is an indication that smoking could be an independent factor associated with squamous cell carcinoma and adenocarcinomas (20,21), this study did not find correlation between smoking and infection by HR HPV. However, the actual role of independent factors still bears a level of uncertainty.

According to the literature, there is a relative increase of risk to develop cervical cancer with, not only an increasing number of sexual partners, but also with younger age at first intercourse, younger age at first full-term pregnancy, increasing duration of oral contraceptive use, and also with increasing parity (22). The findings of this study don't follow such trend. However, this may be explained by the distribution of the cohort. The number of women who had 3 or more pregnancies and 4 or more partners is only 5% of the number of women without children with 4 or more partners (data not shown). The absence of linearity for this variable may be due to the fact that, for this study, an increase in the number of pregnancies was proportional to a decrease in the number of sexual partners (data not shown).

The high sensitivity of HR HPV test is very important when colposcopy and cytology tests give false positive results or to reassure patients that had inconclusive results from the tests. Over 38% of patients diagnosed with ASC-US/AGC-US through cytopathology were HR HPV negative. Since the patient won't need to treat an abnormality that will probably disappear without treatment, the psychological tension

associated with a diagnosis that could lead to cancer is considerably lowered (10). Also, increasing the interval on follow-up visits benefits the patient emotionally, since the negative result on the HR HPV test would refrain the physician from referring the patient to colposcopy. In the Brazilian case, this wouldn't account for a significant financial difference, as colposcopy tests cost R\$ 3.38 on the Public Health System (SUS)(23). However, in countries where the test is only available through private medical care, the increase in costs might make a significant difference on both access to the test and its combination with other tests.

Although most research groups find that HPV testing is more sensitive, but less specific than cytology (7,19,24), we report different findings. As seen on figure 5, HR HPV testing showed opposite results, with lower sensitivity (although high percentage value) and higher specificity than cytological test. Since results showed higher positive and negative predictive values (PPV and NPV, respectively), the tendency is for higher costs of screening. As cytology is cheaper than HR HPV tests, when it has higher PPV values, this can reduce the referral of patients to colposcopy. A thorough screening strategy, as the one here presented, prevents the development of cervical cancer in apparently adequately screened women. Also, with such high NPV, the use of HR HPV test can lead to an extension on screening interval.

Conclusion

Therefore, for the 14 years of analysis in this study we see that women in the Southern part of Rio Grande do Sul are now smoking less, have first intercourse at younger age and increasing the number of partners through life, therefore exposing themselves to higher risks of HR HPV infection. However, there has been a decrease the number of children they have. Women and physicians are complying with international policies for HR HPV testing, since the percentage of women aged 30+ referred to testing increased. And with HR HPV tests showing high levels of specificity, screening intervals may be increased without compromise to the patient's health.

Acknowledgments

The authors thank Dr.Marilene Lucas de Oliveira Bicca, who performed all the analysis on the cytological exams throughout the years.

Conflict of interest

None declared.

References

1. Goldie SJ, Kim JJ, Kobus K, Goldhaber-Fiebert JD, Salomon J, O'shea MKH, et al. Cost-effectiveness of HPV 16, 18 vaccination in Brazil. *Vaccine* [Internet]. 2007 Aug 14 [cited 2013 Jul 1];25(33):6257–70. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17606315>
2. Zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat. Rev. Cancer* [Internet]. 2002 May [cited 2013 May 27];2(5):342–50. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12044010>
3. Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain J, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *Am. J. Clin. Pathol.* [Internet]. 2012 Apr [cited 2013 May 27];137(4):516–42. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22431528>
4. Ault K a. Human papillomavirus vaccines and the potential for cross-protection between related HPV types. *Gynecol. Oncol.* [Internet]. 2007 Nov [cited 2013 Jun 2];107(2):S31–S33. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0090825807006464>
5. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah K V, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N. Engl. J. Med.* [Internet]. 2003 Feb 6;348(6):518–27. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18248389>
6. Kulmala S-M, Syrjänen S, Shabalova I, Petrovichev N, Kozachenko V, Podistov J, et al. Human Papillomavirus Testing with the Hybrid Capture 2 Assay and PCR as Screening Tools. *J. Clin. Microbiol.* [Internet]. 2004 Jun 1;42 (6):2470–5. Available from: <http://jcm.asm.org/content/42/6/2470.abstract>
7. Cuzick J, Clavel C, Petry K-U, Meijer CJLM, Hoyer H, Ratnam S, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int. J. Cancer* [Internet]. 2006 Sep 1 [cited 2013 May 23];119(5):1095–101. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16586444>
8. Hulstaert F, Arbyn M, Huybrechts M, Vinck I, Puddu M, Ramaekers D. *Cervical Cancer Screening and Human Papillomavirus (HPV) Testing*. Brussels; 2006.
9. Park S, Kang Y, Kim DG, Kim E-C, Park SS, Seong M-W. Comparison of the analytical and clinical performances of Abbott RealTime High Risk HPV, Hybrid Capture 2, and DNA Chip assays in gynecology patients. *Diagn. Microbiol. Infect. Dis.* [Internet]. 2013 Jun [cited 2013 Jun 20];null(null). Available from: <http://dx.doi.org/10.1016/j.diagmicrobio.2013.04.012>
10. HHS (U.S. Department of Health and Human Services). *Pap and HPV Testing*. National Cancer Institute; 2012. p. 1–5.

11. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda system: Terminology for reporting results of cervical cytology. *JAMA* [Internet]. 2002 Apr 24;287(16):2114–9. Available from: <http://dx.doi.org/10.1001/jama.287.16.2114>
12. Sellors JW, Sankaranarayanan R. *Colposcopy and Treatment of Cervical Intraepithelial Neoplasia: A Beginners' Manual*. Lyon: International Agency for Research on Cancer; 2003. p. 132.
13. Bornstein J, Bentley J, Bösze P, Girardi F, Haefner H, Menton M, et al. 2011 Colposcopic Terminology of the International Federation for Cervical Pathology and Colposcopy. *Obstet. Gynecol.* [Internet]. 2012;120(1). Available from: http://journals.lww.com/greenjournal/Fulltext/2012/07000/2011_Colposcopic_Terminology_of_the_International.26.aspx
14. Richart RM. A Modified Terminology for Cervical Intraepithelial Neoplasia. *Obstet. Gynecol.* [Internet]. 1990;75(1). Available from: http://journals.lww.com/greenjournal/Fulltext/1990/01000/A_Modified_Terminology_for_Cervical.28.aspx
15. Cancer Research UK. Smoking [Internet]. 24 April 2012. 2012 [cited 2013 Aug 5]. Available from: <http://www.cancerresearchuk.org/cancer-info/cancerstats/types/lung/smoking/lung-cancer-and-smoking-statistics#page>
16. Centers for Disease Control and Prevention. Current Cigarette Smoking Among Adults—United States, 2011. *Morb. Mortal. Wkly. Rep.* 2012;61(44):889–94.
17. Bajos N, Bozon M, Beltzer N, Laborde C, Andro A, Ferrand M, et al. Changes in sexual behaviours: from secular trends to public health policies. *AIDS* [Internet]. 2010 May 15 [cited 2013 Aug 5];24(8):1185–91. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20299962>
18. Ezech AC, Bongaarts J, Mberu B. Global population trends and policy options. *Lancet* [Internet]. Elsevier Ltd; 2012 Jul 14 [cited 2013 Jul 31];380(9837):142–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22784532>
19. Arbyn M, Sasieni P, Meijer CJLM, Clavel C, Koliopoulos G, Dillner J. Chapter 9: Clinical applications of HPV testing: a summary of meta-analyses. *Vaccine* [Internet]. 2006 Aug 31 [cited 2013 May 24];24 Suppl 3:S3/78–89. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16950021>
20. Roteli-Martins CM, Longatto Filho A, Hammes LS, Derchain SFM, Naud P, Matos JC de, et al. Associação entre idade ao início da atividade sexual e subsequente infecção por papilomavírus humano: resultados de um programa de rastreamento. *Rev. Bras. Ginecol. e Obs.* [Internet]. 2007 [cited 2013 Jul 4];29(11):580–7. Available from: <http://www.scielo.br/pdf/rbgo/v29n11/a06v2911.pdf>
21. Prado PR do, Koifman RJ, Santana ALM, Silva IF da. Caracterização do Perfil das Mulheres com Resultado Citológico ASCUS/AGC, LSIL e HSIL segundo Fatores Sociodemográficos, Epidemiológicos e Reprodutivos em Rio Branco - AC, Brasil. *Rev. Bras. ...* [Internet]. 2012 [cited 2013 Jul 4];58(3):471–9. Available from: http://www.inca.gov.br/Rbc/n_58/v03/pdf/17_artigo_caracterizacao_perfil_mulhe

res_resultado_citologico_ascus_agc_lsil_hsil_segundo_fatores_sociodemograficos_epidemiologicos_reprodutivos_rio_branco_ac_brasil.pdf

22. The International Collaboration of Epidemiological Studies of Cervical Cancer. Comparison of risk factors for invasive squamous cell carcinoma and adenocarcinoma of the cervix: Collaborative reanalysis of individual data on 8,097 women with squamous cell carcinoma and 1,374 women with adenocarcinoma from 12 epidemiological studies. *Int. J. Cancer* [Internet]. Wiley Subscription Services, Inc., A Wiley Company; 2007 Feb 15;120(4):885–91. Available from: <http://dx.doi.org/10.1002/ijc.22357>

23. BRASIL. Ministério da Saúde. Tabela de procedimentos do SIA/SIH-SUS [Internet]. 2011. Available from: <http://sigtap.datasus.gov.br/tabela-unificada/app/sic/procedimento/publicados/consultar>

24. Schiffman M, Castle P, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. *Lancet* [Internet]. 2007 [cited 2013 Jul 13];370:890–907. Available from: <http://www.sciencedirect.com/science/article/pii/S0140673607614160>

TABLES

Table 1.Characterization of the cohort, on the three periods of the study, according to the variables from the standardized questionnaire.

Variables	1998 - 2002 n=199	2003 - 2008 n=227	2009 - 2012 n=217	Total n=643	p-value
Age					0.34*
Up to 19 years old	12.6	8.8	6.9	9.3	
20 to 29 years old	45.2	56.4	45.6	49.3	
30 to 39 years old	18.1	21.2	24.4	21.3	
40 to 49 years old	14.6	11.5	14.8	13.5	
50 years old or more	9.6	2.2	8.3	6.5	
Smoking					0.001*
No	76.9	85.5	88.9	83.9	
Yes	23.1	14.5	11.1	16.0	
First intercourse					0.005*
Before 15 years old	19.1	19.8	24.0	21.0	
16 to 19 years old	54.3	56.8	62.2	57.9	
20 years old or more	26.6	23.4	13.8	21.2	
Number of partners					<0.001*
1	36.2	25.6	17.1	26.0	
2 to 3	31.7	34.4	33.2	33.1	
4 or more	32.2	40.1	49.8	40.9	
Pregnancies					0.40*
0	64.8	72.3	64.5	67.4	
1	10.6	13.7	17.1	13.8	
2	17.1	7.9	13.4	12.6	
3 or more	7.5	6.2	5.1	6.2	
HR HPV					0.05*
Negative	38.2	28.2	47.0	37.6	
Positive	61.8	71.8	53.0	62.4	

*Chi-square for linear trend

Table 2. High Risk HPV prevalence, according to characteristics of the cohort.

Variables	High Risk HPV		p-value
	Negative n=242	Positive n=401	
Age			<0.001*
Up to 19 years old	25.0	75.0	
20 to 29 years old	30.3	69.7	
30 to 39 years old	36.5	63.5	
40 to 49 years old	57.5	42.5	
50 years old or more	73.8	26.2	
Smoking			0.40
No	38.3	61.7	
Yes	34.0	66.0	
First intercourse			<0.001*
Before 15 years old	26.7	73.3	
16 to 19 years old	37.1	62.9	
20 years old or more	50.0	50.0	
Number of partners			<0.001*
1	50.9	49.1	
2 to 3	33.8	66.2	
4 or more	32.3	67.7	
Pregnancies			<0.001*
0	31.4	68.6	
1	46.1	53.9	
2	55.6	44.4	
3 or more	50.0	50.0	

*Chi-square for linear trend

Table 3. High risk HPV prevalence, according to colposcopy and cytology results.

Variables	HR HPV viral load			p-value
	Negative	Positive	Total	
	n=242	n=401	n=643	
Colposcopy				<0.001
Normal	64.1	35.9	44.2	
Altered	16.7	83.3	55.8	
Cytopathologic				<0.001
Normal	62.8	37.2	41.8	
ASC-US/AGC-US	38.5	61.5	14.2	
CIN I	16.8	83.2	34.2	
CIN II e III	1.6	98.4	9.8	

Table 4. Relative results between colposcopy and cytology tests.

	Colposcopy				p-value
	Normal		Altered		
Cytopathologic	n	%	n	%	<0.001
Normal	231	85.9	38	14.1	
ASC-US/AGC-US	33	36.3	58	63.7	
CIN I	20	9.1	200	90.9	
CIN II e III	0	0	63	100.0	

Table 5. Positive and negative predictive values (PPV and NPV, respectively) for precursor lesion prediction on colposcopy, cytopathology and HR HPV tests.

	PPV	NPV
Colposcopy	46.3	-
Altered cytology	48.4	53.7
HR HPV	49.8	78.6

FIGURES

Figure 1. Sensibility and specificity results for colposcopy, cytopathology and HR HPV tests.

