UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA

Gabriel Vitor da Silva Pinto

Determinantes do *clearance* da infecção pelo Papilomavírus Humano (HPV) em mulheres em idade reprodutiva: influência de fatores comportamentais, coinfecções sexualmente transmissíveis e resposta imune inata.

Tese apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, para obtenção do título de Doutor em Ciências, área – Patologia.

Orientadora: Profa. Dra. Márcia Guimarães da Silva
Coorientadora: Aline do Nascimento Bolpet

Botucatu
2019

Gabriel Vitor da Silva Pinto

Determinantes do *clearance* da infecção pelo Papilomavírus Humano (HPV) em mulheres em idade reprodutiva: influência de fatores comportamentais, coinfecções sexualmente transmissíveis e resposta imune inata.

Tese apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, para obtenção do título de Doutor em Ciências, área – Patologia.

Orientadora: Profa.Dra. Márcia Guimarães da Silva
Coorientadora: Aline do Nascimento Bolpet

Botucatu
2019

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP

BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Pinto, Gabriel Vitor da Silva.
Determinantes do *clearance* da infecção pelo Papilomavírus Humano (HPV) em mulheres em idade reprodutiva : influência de fatores comportamentais, coinfecções sexualmente transmissíveis e resposta imune inata / Gabriel Vitor da Silva Pinto. - Botucatu, 2019

Tese (doutorado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Medicina de Botucatu
Orientador: Márcia Guimarães da Silva
Coorientador: Aline do Nascimento Bolpet
Capes: 40101150

1. Papillomaviridae. 2. Mulheres. 3. Saúde reprodutiva. 4. Doenças sexualmente transmissíveis. 5. Coinfecção. 6. Resposta imune.

Palavras-chave: Achados ginecológicos; Características comportamentais; *Clearance*; Fatores sociodemográficos; Infecção por HPV.

Dedicatória

Dedico aos meus Pais, Silvana e Antônio, meu trabalho, meu empenho e minhas conquistas, pelo apoio incondicional e pela confiança em todas as minhas escolhas, por não medirem esforços para que eu chegasse até esta etapa da minha vida. Ao meu avô Tico que foi peça fundamental na formação do meu caráter e sei que onde estiver está muito orgulhoso de mim. A vocês, minha imensa gratidão e sempre amor.

Agradecimento Especial

À minha orientadora, Profa. Dra. Márcia Guimarães da Silva, meus mais sinceros agradecimentos pela confiança no meu trabalho desde o meu primeiro dia no laboratório. Muito obrigado por todos os ensinamentos, apoio, incentivo, dedicação e por ter me dado a oportunidade de trabalhar com aquilo que eu gosto.

Agradecimentos

À Deus que se faz presente em todos os momentos da minha vida, pelas conquistas até o momento, mas peço a Ele para me dar sabedoria para conquistar muito mais.

Foram muitas as pessoas que passaram pela minha vida ao longo desses quatro anos e que, de algum modo, tiveram participação nesse trabalho de Doutorado. Portanto, a missão de deixar aqui meus agradecimentos não é uma tarefa tão fácil.

À minha Coorientadora Profa. Dra. Aline do Nascimento Bolpet pela parceria, total apoio e dedicação no desenvolvimento deste trabalho.

Às queridas colegas de Laboratório de Imunopatologia da Relação Materno-Fetal: Amanda Della Coletta, Bruna Ramos, Camila Marconi, Carolina Pereira, Carolina Tafner, Jossimara Polettini, Larissa Marcolino, Laura Martin, Natália Moço e Nathália Noda pela total disponibilidade em fazer este trabalho se tornar possível. A dedicação de todas foi imprescindível. Agradeço pelo trabalho em grupo e a todos os momentos compartilhados.

De um modo especial as amigas conquistadas além laboratório Bruna Ramos, Laura Martin e Natália Moço, pela paciência e apoio incondicional em todos os meus momentos de maior necessidade. Foram fundamentais para a realização deste trabalho a amizade, o carinho e a prontidão de vocês em me ajudar sempre.

À secretária da Pós-Graduação em Patologia, Vânia Soler por sua competência e dedicação.

Ao Departamento de Patologia da Faculdade de Medicina de Botucatu e a UNIPEX onde este trabalho foi realizado, pelo apoio imprescindível para a realização e conclusão do meu Doutorado.

Ao Programa de Doutorado Sanduíche no Exterior (PDSE) da Capes, processo (88881.132503/2016-01), que proporcionou auxílio financeiro para a minha ida à McGill University no Canadá para realizar as análises estatísticas deste projeto.

Ao Department of Oncology and Epidemiology & Biostatistics, McGill University, Montreal, Canada e especialmente ao Dr. Eduardo Franco por me receber em seu grupo de pesquisa e total empenho para analisar os dados desse trabalho, além do treinamento em Epidemiologia que recebi durante meu estágio.

À secretaria de saúde do município de Botucatu, por possibilitar a realização deste trabalho.

Às enfermeiras das Unidades Básicas de Saúde do município de Botucatu que participaram da etapa crucial deste trabalho, por seu empenho em captar mulheres e seu cuidado com as pacientes visando sempre a saúde da mulher.

À CAPES pela bolsa de Doutorado e a FAPESP (Processo: 2012/01278-0) pelo auxílio pesquisa concedido.

Às mulheres que aceitaram participar do estudo, por sua disponibilidade e confiança no projeto.

A todos os colegas do Programa de Pós-Graduação em Patologia, com quem passei bons momentos no decorrer do curso.

Obrigado a todas as pessoas que contribuíram para a realização não só deste trabalho, mas para o meu crescimento como pessoa. Sou o resultado da confiança e da força de cada um de vocês.

Resumo

Resumo

Objetivo: O objetivo do presente estudo foi identificar determinantes do *clearance* da infecção pelo Papilomavírus Humano (HPV) em mulheres brasileiras em idade reprodutiva. **Métodos:** Trata-se de estudo de coorte denominado HPV-UNESP, no qual 1638 mulheres em idade reprodutiva foram recrutadas no período de setembro de 2012 e janeiro de 2013. Desse total, 544 mulheres positivas para a infecção pelo HPV participaram do seguimento longitudinal durante 30 meses, em mais 4 visitas. A infecção por HPV foi definida como detecção de qualquer um dos 36 genótipos testados pelo Linear Array Genotyping Test (Roche Molecular Systems, Inc.) e o desfecho de interesse foi o *clearance* da infecção, definido como a eliminação da infecção pelo HPV por, pelo menos, duas visitas consecutivas. Um questionário estruturado com 58 questões relativas à dados sociodemográficos, características comportamentais e ginecológicas foi aplicado em cada visita. Imediatamente após a entrevista, todas as mulheres realizaram exame ginecológico, no qual, após inserção de espéculo de Collins, não lubrificado, foi aferido o pH vaginal com fita (pH 4.0-7.0, Merck, Germany) no terço médio da parede vaginal. Para avaliação da microbiota vaginal, amostras foram coletadas com *swab* da parede vaginal e o padrão de microbiota foi classificado de acordo com os critérios de Nugent et al. (1991). O *whiff test* realizado por adição de solução de 10% de KOH ao conteúdo vaginal foi interpretado como positivo, negativo ou duvidoso. Amostras endocervicais foram coletadas com *cytobrush* para análises moleculares de infecção por HPV, *Chlamydia trachomatis* e *Neisseria gonorrhoeae*. Amostra adicional com representação de epitélio escamoso e glandular foi coletada para citologia oncótica. Finalmente, uma amostra do fundo de saco da vagina foi coletada com espátula de Ayre para pesquisa de *Trichomonas vaginalis* em meio líquido de Diamond. Em cada visita do seguimento longitudinal amostras vaginais e endocervicais foram recoletadas para realização dos mesmos exames, pelas mesmas técnicas descritas. Modelos de regressão logística foram usados para estimar proporções de riscos (HR), com intervalos de confiança de 95%, de *clearance* de HPV em função de covariáveis no momento da inclusão no estudo ajustadas por idade. **Resultados:** Os *status* civis solteira (HR = 0,67, IC 95% 0,49-0,92) e divorciada (HR = 0,53, IC95% 0.30-0,92) foram associados com atraso no tempo de *clearance* do HPV. O mesmo atraso no tempo de *clearance* foi observado em mulheres que declararam uso de anticoncepcional hormonal (HR = 0,72, IC95% 0,55-0,95) e em mulheres que declararam quatro parceiros sexuais ao longo da vida (HR = 0,55, IC 95% 0,34-0,90). Não houve associação entre concentração de Interleucinas (IL-) 1β, IL-6, IL-10, IL-12 e o Fator de Necrose Tumoral (TNF-α) e o tempo de *clearance* do HPV, somente a IL-4 mostrou correlação positiva (HR = 2,40, IC95% 1.11-5.21). Também não houve

associação entre coinfecções endocervicites por *Chlamydia trachomatis* e *Neisseria gonorrhoeae* e de infecção por T*richomonas vaginalis* com o *clearance* do HPV. **Conclusão:** Apesar da análise de fatores sociodemográficos, características comportamentais e achados ginecológicos, a maioria deles não foi significativamente associado com o *clearance* do HPV.

Palavras-chave: Infecção por HPV, *clearance*, fatores sociodemográficos, características comportamentais e achados ginecológicos.

Abstract

Abstract

Objective: The objective of the present study was to identify determinants of Human Papillomavirus (HPV) infection clearance in Brazilian women of reproductive age. **Methods:** This is a cohort study called HPV-UNESP, in which 1638 women of reproductive age were recruited from September 2012 to January 2013. Of this total, 544 women positive for HPV infection participated in longitudinal follow-up for 30 months, in 4 more visits. HPV infection was defined as detection of any of the 36 genotypes tested by the Linear Array Genotyping Test (Roche Molecular Systems, Inc.) and the outcome of interest was infection clearance, defined as the elimination of HPV infection by least two consecutive visits. A structured questionnaire with 58 questions regarding sociodemographic data, behavioral and gynecological characteristics was applied at each visit. Immediately after the interview, all women underwent a gynecological exam, in which, after insertion of the non-lubricated Collins speculum, the vaginal pH with tape (pH 4.0-7.0, Merck, Germany) was measured in the middle third of the vaginal wall. For vaginal microbiota evaluation, samples were collected with vaginal wall swab and the microbiota pattern was classified according to the criteria of Nugent et al. (1991). The whiff test performed by adding 10% KOH solution to the vaginal content was interpreted as positive, negative or doubtful. Endocervical samples were collected with cytobrush for molecular analysis of HPV, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection. Additional sample with representation of squamous and glandular epithelium was collected for cytology testing. Finally, a sample from the posterior fornix of the vagina was collected with Ayre's spatula for research of *Trichomonas vaginalis* in Diamond liquid medium**.** At each longitudinal follow-up visit, vaginal and endocervical samples were collected for the same exams, using the same techniques described. Logistic regression models were used to estimate hazard ratios (HR), with 95% confidence intervals, of HPV clearance as a function of covariates at the time of study inclusion adjusted for age. **Results:** Single (HR = 0.67, 95% CI 0.49-0.92) and divorced (HR = 0.53, 95% CI 0.30-0.92) marital status were associated with delayed HPV clearance time. The same delay in clearance time was observed in women who reported hormonal contraceptive use (HR = 0.72, 95% CI 0.55-0.95) and in women who reported four lifetime sexual partners (HR = 0 , 55, 95% CI 0.34-0.90). There was no association between Interleukin (IL-) 1β, IL-6, IL-10, IL-12 and TNF-α concentration and HPV clearance time, only IL-4 showed positive correlation (HR = 2.40, 95% CI 1.11-5.21). There was also no association between endocervical *Chlamydia trachomatis* and *Neisseria gonorrhoeae* co-infections and *Trichomonas vaginalis* infection with HPV clearance. **Conclusion:** Despite the

analysis of sociodemographic factors, behavioral characteristics and gynecological findings, most of them were not significantly associated with HPV clearance.

Keywords: HPV infection, clearance, sociodemographic factors, behavioral characteristics and gynecological findings.

Lista de Figuras e Tabelas

Capítulo I

Capítulo III

Lista de Abreviaturas, Siglas e Símbolos

ALT	*Arternative lengthening of telomeres* (Alongamento alternativo de telômeros)
ASC-US	*Atypical squamous cells of undetermined significance* (Células escamosas atípicas de significado indeterminado)
CDK	Quinase dependente de ciclina (Cyclin-dependent kinase)
CI	*Confidence interval* (Intervalo de confiança)
CO2	Dióxido de Carbono
CV	*Coefficients of variation* (Coeficiente de variância)
DNA	Ácido desoxirribonucleico
E1 e E2	Proteína do genoma viral de expressão precoce
E2F	Fator de Transcrição E2F
E6 e E7	Oncoproteínas do genoma viral
ELISA	*Enzyme-linked immunosorbent assay* (Ensaio de imunoabsorção enzimática)
FMB	Faculdade de Medicina de Botucatu
G1	Fase de interfase
HLA	*Human leukocyte antigen* (Antígeno leucocitário humano)
HPV	*Human Papillomavirus* (Papilomavírus Humano)
HR	*Hazards ratios*
HSIL	*High grade squamous intraepithelial lesion* (Lesão Intraepitelial escamosa de alto grau)
HSPG	*Heparan sulfate proteoglycans* (Proteoglicanos de sulfato de heparano)
IFNγ	Interferon γ
IL	Interleucina
INCA	Instituto Nacional do Câncer
IST	Infecção sexualmente transmissível
KOH	Hidróxido de potássio
L1 e L2	Proteínas do capsídeo viral de expressão tardia
LCR	*Long control region* (Região longa de controle)
LSIL	*Low-grade squamous intraepithelial lesion* (Lesão Intraepitelial escamosa de baixo grau)

mL	Mililitro
NILM	*Negative for intraepithelial lesion and malignancy* (Negativo para lesão intra-epitelial ou malignidade)
OR	*Odds ratio*
p21	Proteína de 21 kDa
p27	Proteína de 27 kDa
p53	Fosfoproteína com peso molecular de 53kDa
pb	Pares de base
PCR	*Polymerase chain reaction* (Reação em cadeia da polimerase)
pg	Picograma
pRb	Proteína Retinoblastoma
PSD95/Dlg/ZO-1 e PDZ	Domínio protéico PSD95/Dlg/ZO-1 e PDZ
PTPN13	Proteína tirosina fosfatase codificada pelo gene não receptor 13
S	Fase de síntese do ciclo celular
TERT	*Telomerase reverse transcriptase* (Trascripatase reversa da telomerase)
Th1	*T helper cells* (linfócitos T auxiliares)
TNFα	*Tumour necrosis factor α* (Fator de necrose tumoral α)
UNESP	Universidade Estadual Paulista
UV	Ultravioleta
VLP	*Vírus-like particles* (Partículas semelhantes aos vírus)

Sumário

Sumário

Capítulo I – Revisão de literatura

1. Introdução

1.1. Papilomavírus Humano

O Papilomavírus Humano (HPV) é responsável por uma das infecções sexualmente transmissíveis (ISTs) virais mais comuns no mundo e que se encontra presente em grande parcela da população, sendo a maior parte das mulheres infectadas logo após o início da atividade sexual[1,2]. Estabelecido como causa necessária para o câncer cervical, tendo a infecção persistente com os genótipos HPV16 e HPV18 responsáveis por causar 70% de lesões invasivas e 50% de lesões cervicais pré-invasivas em todo o mundo[3], estima-se também que contribua para 88% dos cânceres anais e mais de 50% dos cânceres da vulva, vagina e pênis[4]. Os HPVs também são causa importante para o desenvolvimento do câncer de orofaringe e das verrugas anogenitais[5,6].

Estima-se que a maioria das pessoas será infectada, por algum genótipo de HPV, em algum momento da sua vida[7]. No entanto, a maioria das pessoas elimina essas infecções espontaneamente, mesmo quando não são vacinadas, no período de seis meses a 1 ano[8,9]. Apenas uma minoria das pessoas permanece com a infecção de forma persistente e pode, eventualmente, resultar em lesões benignas ou malignas[10]. Enquanto a estimativa global de prevalência de HPV é reportada como sendo de 11,7%, as prevalências específicas variam de 1,6% a 41,9%[11]. Essa variação depende da população, região e país, sendo a prevalência de HPV maior em regiões em desenvolvimento (14,3%) do que em regiões desenvolvidas (10,3%). A distribuição da infecção HPV tipo-específica também varia nas regiões geográficas e alguns tipos específicos são mais prevalentes na região Ásia Pacífico do que em outras regiões do mundo[12].

O câncer cervical é a quarta causa mais comum de neoplasia do mundo, com uma incidência mundial estimada de 528.000 casos e 266.000 mortes em 2012[13]. Especificamente no Brasil, a doença, na região sudeste, é a quarta mais prevalente, com 16 casos para cada 100.000 mulheres e na região norte o câncer do colo do útero é a principal causa de câncer em mulheres[14].

1.1.1. Biologia do vírus

O HPV pertence à família *Papillomaviridae* e é um vírus de DNA circular de fita dupla, não envelopado, com aproximadamente 8000 pares de base que infecta células dos tecidos epitelial e mucoso[15, 16].

A organização genômica do vírus é bem conservada e o genoma viral está dividido em 3 regiões: a região regulatória (LCR, *long control region*), região precoce (*Early*) e região tardia (*Late*). A região LCR contém a origem de replicação do DNA, responsável pela regulação da expressão gênica. Os genes L1 e L2, localizados na região tardia, codificam as proteínas do capsídeo viral (Figura 1)[17]. Os genes da região precoce, E1 e E2, codificam proteínas envolvidas na replicação do DNA viral e no controle da transcrição. Os genes E6 e E7 codificam proteínas com atividade de transformação e imortalização celular[18].

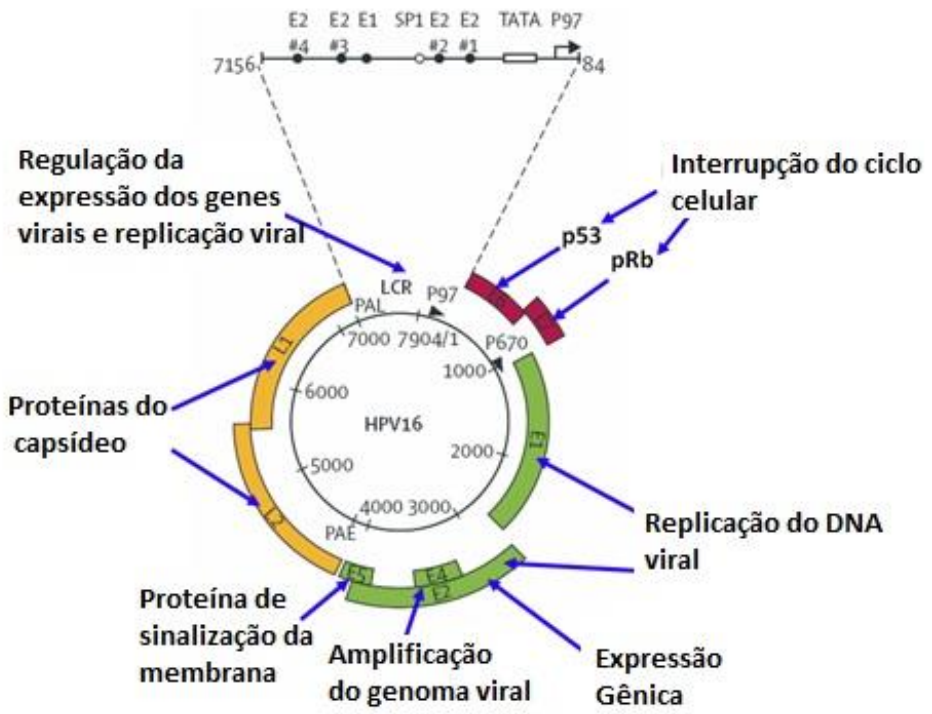

Figura 1: Representação esquemática do genoma do Papilomavírus Humano (HPV-16), evidenciando a região regulatória (LCR), as regiões precoces (E1, E2, E4, E5, E6 e E7) e as regiões tardias (L1 e L2). Adaptado de Schiffman et al. (2007).

Atualmente na literatura são descritos mais de 200 tipos de HPV, que são diferenciados em 5 grupos de acordo com seu tropismo epitelial e doenças associadas[19,20]. Os HPVs associados às infecções nas mucosas pertencem à família dos Alfa Papilomavírus, que apresentam tropismo por células do epitélio genital, e são classificados quanto ao seu

potencial oncogênico, baseado na espécie de origem e no grau de relação dos genomas virais mediante a comparação da sequência de nucleotídeos do seu genoma viral[21]. O gênero Alphapapilomavírus é o mais diverso com 14 espécies e neste gênero são encontrados os tipos que infectam preferencialmente a mucosa oral e anogenital em humanos e primatas (Figura 2)[21]. Os membros da família *Papilomaviridae* são comuns na natureza e já foram isolados e caracterizados de répteis[22], aves[23], marsupiais[24] e diversas espécies de mamíferos[25].

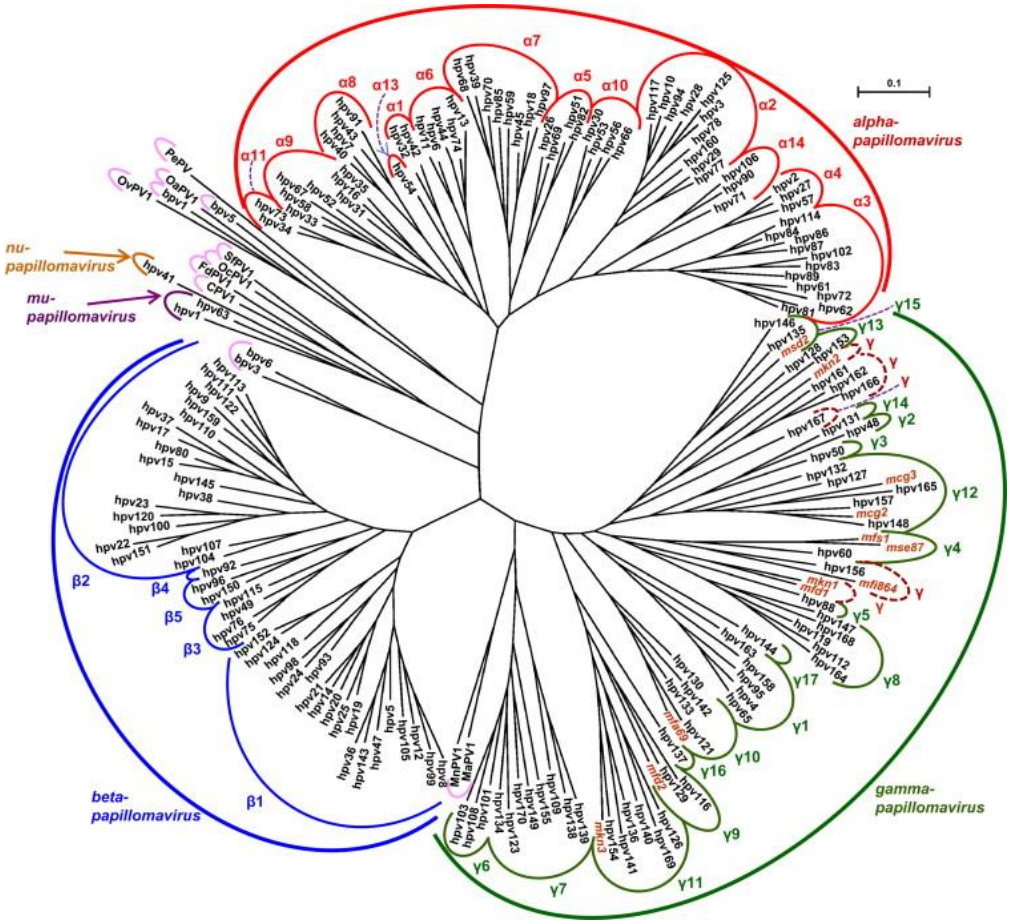

Figura 2. Árvore filogenética baseada na análise da sequência do gene L1 dos tipos de Papilomavírus. Os números nas pontas dos ramos identificam os tipos de HPV, "c" seguido de um número indica um novo candidato a tipo de HPV. Todas as outras abreviações se referem a tipos de Papilomavírus que infectam animais. Os semicírculos externos identificam os gêneros e os semicírculos internos, as espécies. Fonte: de Villiers (2013).

Os HPVs de baixo risco oncogênico estão associados ao desenvolvimento de verrugas genitais, enquanto os tipos de alto risco estão relacionados às lesões precursoras e ao câncer de colo do útero[26]. Os genótipos de HPVs de alto risco oncogênico mais comumente encontrados nas infecções anogenitais são: HPV-16, 18, 31, 33 e 45, sendo os HPVs 16 e 18

juntos são responsáveis por mais de 70% dos casos de carcinomas epidermóides e mais de 80% dos adenocarcinomas do colo do útero[21, 27]. Estima-se que em regiões menos desenvolvidas cerca de 17,8% das mulheres com citologia normal apresentem infecção latente pelo HPV[28].

O potencial oncogênico do HPV está relacionado principalmente à capacidade de expressar os oncogenes E6 e E7 que resultam na inativação das proteínas p53 e pRb, respectivamente, interferindo no ciclo celular e nos genes supressores de tumor[29]. A E6 medeia a degradação não somente de p53, mas também de proteínas que contém os domínios PSD95/Dlg/ZO-1 e PDZ que modulam as vias de sinalização celular que regulam a diferenciação celular, como por exemplo, o gene *PTPN13,* que pertence a uma família de moléculas sinalizadoras que regulam uma variedade de processos celulares incluindo crescimento celular, diferenciação do ciclo celular e transformação oncogênica [30-32]. A proteína E7 interage com membros da família Rb através de um *motif* conservado LXCXE que está presente na região amino-terminal dessas proteínas. A família Rb controla a transição da fase G1-S pela regulação da atividade de fatores de transcrição da família E2F[33]. Sítios de ligação para E2F são encontrados em muitos genes que estão envolvidos na regulação da progressão do ciclo celular, diferenciação, mitose e apoptose[34]. Em células normais, pRb reprime a transcrição de promotores dependentes de E2F por meio da ligação direta com o domínio de transativação de E2F[35]. A ligação de E7 de HPVs de alto risco à pRb perturba o complexo RB-E2F[36] resultando na expressão constitutiva dos genes que respondem a E2F, como ciclina A e ciclina E, e promove a entrada prematura na fase S e a síntese de DNA[37,38]. A proteína também leva as proteínas da família RB a degradação proteassomal por meio de vias dependente de ubiquitina[39, 40]. Em adição à desestabilização de RB, E7 contribui para a imortalização por meio da interação com proteínas chaves que controlam a progressão do ciclo celular. Os inibidores de CDK, p21 e p27, são importantes reguladores do controle do crescimento celular durante a diferenciação epitelial, e p21 é conhecido como um supressor tumoral na carcinogênese cervical[41]. A região carboxi-terminal da proteína E7 tem a capacidade de se ligar a p21 e p27 e inibir sua ação no controle do ciclo celular[42, 43].

Adicionalmente as proteínas E6 e E7 de HPVs de alto risco podem contribuir para a imortalização das células infectadas por meio da ativação da telomerase. E6 de HPVs de alto risco podem ativar a transcrição da trascripatase reversa da telomerase (TERT), que em conjunto com a inativação de RB pela E7 é um passo essencial para a imortalização celular[44, 45]. Alguns estudos sugerem que E7 atua promovendo o alongamento dos telômeros por meio da

via alternativa de alongamento de telômeros (*Arternative lengthening of telomeres* –ALT), que envolve recombinação homóloga entre os telômeros de cromátides irmãs[46, 47]. Acredita-se que a ativação de ALT pela E7 seja importante na manutenção do comprimento dos telômeros nos estágios iniciais do desenvolvimento do câncer[35].

1.2. Infecção Viral

O HPV apresenta tropismo por células epiteliais, causando infecções no tecido de revestimento, tais como a pele e mucosas. Os HPVs infectam células-tronco basais ou células transitoriamente amplificadoras do tecido epitelial da junção escamoso colunar do colo do útero[48, 49]. O vírus faz a sua entrada nas células epiteliais basais através micro abrasões ou lesões de continuidade epitelial. O seu ciclo de vida, que está diretamente relacionado ao processo de diferenciação da célula hospedeira, inicia-se com a infecção de células epiteliais basais que provavelmente foram expostas à microtraumas, lesões ou abrasões na epiderme (Figura 3)[19]. Nas células basais, o genoma viral é mantido em baixo número de cópias[50]. Somente após a diferenciação em células parabasais, ocorre a amplificação do genoma viral. A montagem do vírus e a eliminação das partículas virais para o meio extracelular ocorre nas camadas cornificadas ou mais superficiais do tecido epitelial[51].

O mecanismo molecular que facilita a transmissão do HPV é pouco estudado. No entanto, é claro que envolve os capsídeos sem envelope, que são construídos por L1 e L2, as principais e menores proteínas da cápside viral, respectivamente. A proteína tardia L1 se autoestrutura em um capsídeo com formato icosaédrico, que consiste em 72 pentâmeros, também chamados de capsômeros[52]. A proteína L2 menor do capsídeo auxilia na formação do capsídeo do genoma, que por sua vez é cromatinizada pelas histonas celulares[53]. A presença do DNA viral compacta e estabiliza ainda mais o capsídeo[54].

Os proteoglicanos de sulfato de heparano (HSPG) encontrados na superfície da célula hospedeira, voltados para a matriz extracelular, atuam como receptores iniciais para as *vírus-like particles* (VLPs) do vírus[55]. A Ligação inicial do HSPG a porções de L1 facilita as mudanças conformacionais em L2[56]. Subsequentemente, a proteína L2 é clivada por furina na superfície da célula em um local de clivagem de consenso que se conserva entre todos os papilomavírus[57]. A entrada de vírus em uma célula hospedeira é muito lento (até 12 h), possivelmente devido a mudanças conformacionais no capsídeo e nos receptores[58]. Após uma ligação bem-sucedida ao receptor, o vírus é internalizado na célula por endocitose[59]. O

genoma viral entra no núcleo através de quebra de envelope nuclear. Em seguida, ele localiza receptores nucleares[60]. Uma vez inserido no núcleo, o HPV replica um baixo número de cópias (10-200 número de cópias/célula) durante a amplificação inicial e estabelecimento de infecção[61].

O vírus se utiliza da alta capacidade das células epiteliais de se reproduzirem para a replicação de seu DNA viral, e, dependem da expressão dos genes precoces E1 e E2 que estimulam o ciclo celular que neste momento tem o aumento dos seus níveis[62]. Nas camadas mais diferenciadas do epitélio, proteínas do capsídeo são expressas para a produção de novos capsídeos, montagem das partículas virais e liberação da progênie[63]. O HPV é capaz de evadir-se do sistema imunológico do hospedeiro através de inúmeros mecanismos, dentre eles, está o de não indução de citólise e de necrose. Outro mecanismo de evasão consiste no fato de proteínas virais imunogênicas serem liberadas apenas por células epiteliais das camadas mais diferenciadas, portanto menos acessíveis pela vigilância imunológica[64].

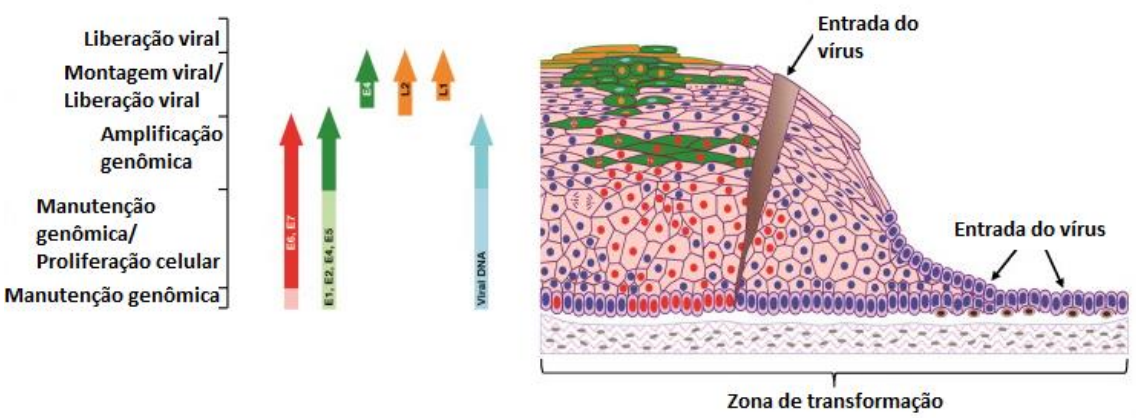

Figura 3: Estrutura das células epiteliais escamosas e os principais estágios do ciclo de vida do HPV após a infecção. Adaptado de Doorbar et al. (2012).

Embora a integração do vírus ao DNA da célula hospedeira não faça parte do ciclo de vida normal do HPV, os genótipos de alto risco oncogênico são frequentemente integrados no genoma humano em tecido de carcinoma cervical[65]. Foi proposto que a mudança da forma episssomal para a integração do HPV ao genoma da célula pode ser o evento inicial associado à progressão da lesão intra-epitelial de baixo grau para a lesão intra-epitelial de alto grau, portanto, espera-se que seja um biomarcador para a progressão do câncer[66].

1.2.1. Fatores associados à infecção por HPV

A transmissão do HPV se dá por contato direto, pele a pele, portanto a penetração sexual é a forma mais comum de transmissão do vírus[67]. Sendo assim, os comportamentos sexuais são determinantes para o aumento do risco da infecção. Os fatores associados à infecção pelo HPV podem ser divididos em três grupos: (1) Ambientais ou fatores exógenos, que incluem dietas deficientes, uso de contraceptivo hormonal, múltipla paridade, múltiplos parceiros sexuais, iniciação sexual precoce, consumo de álcool e tabaco, e coinfecção por outros agentes sexualmente transmissíveis; (2) Aspectos inerentes ao vírus, tais como, infecção por HPV de alto ou baixo risco oncogênico, infecções por múltiplos genótipos virais, infecções por variantes de HPV de alto risco que diferem quanto ao potencial oncogênico, carga viral e integração do DNA viral ao DNA da célula hospedeira; (3) Fatores inerentes ao hospedeiro, como os hormônios endógenos, fatores genéticos (polimorfismos de HLA) e fatores relacionados à resposta imunológica[68-70].

Com relação ao uso de preservativos como fator associado à infecção pelo HPV, existem ainda alguns paradoxos na literatura[71-75]. O uso de preservativos masculinos, mostrou efeito protetor considerável, quando usado corretamente e de forma consistente, em relação a aquisição de infecções sexualmente transmissíveis, incluindo: tricomoníase, cervicite clamidiana, gonorreia e HIV. Os dados sobre efeito protetor em relação às infecções de HPV e às lesões cervicais relacionadas têm sido menos consistentes. Em revisão sistemática, Manhart & Koutsky[76] identificaram 20 estudos relevantes e os resultados não são homogêneos. A inconsistência a respeito do efeito protetor sugere que os preservativos não constituem método altamente eficaz em prevenir as infecções por HPV. De forma geral, homens e mulheres optam por usar preservativos com parceiros que eles consideram ser de maior risco, como novos parceiros, parceiros casuais e profissionais do sexo. Por outro lado, não o utilizam com parceiros que consideram ser seguros, como os parceiros regulares e os cônjuges. As normas culturais desempenham papel importante na forma como as informações confidenciais são divulgadas para os pesquisadores. Como o uso de preservativos é socialmente desejável, pode ser relatado mais frequentemente do que representado pela realidade, o que torna os dados a cerca do uso de preservativos de difícil interpretação[77,78].

Com relação ao uso de contraceptivo oral, estudos relatam que a prevalência de HPV em mulheres que o utilizam é maior quando comparada com a prevalência em mulheres que nunca usaram contraceptivo[79, 80]. Segundo Lee et al.[81] os níveis hormonais afetam

sobremaneira a composição do microbioma vaginal, sugerindo que o uso de contraceptivo oral possa ser considerado um risco independente para infecção por HPV devido a sua habilidade de alterar o microbioma vaginal.

A infecção concomitante por outros agentes sexualmente transmissíveis ou pelo *core* patológico da microbiota vaginal alterada, tem sido associadas com processos inflamatórios do colo do útero, situação que pode facilitar à aquisição de HPV[11, 82-84]. Lesões herpéticas ulcerativas podem agir como cofatores do HPV, facilitando o acesso do vírus para a camada de células basais. Respostas inflamatórias induzidas por infecções herpéticas podem interferir na capacidade do hospedeiro de montar resposta imune eficaz para a infecção por HPV, suprimindo linfócitos T auxiliares e alterando o estabelecimento de uma resposta imune celular mediada por linfócitos T[85].

A estimativa global da prevalência da infecção por *Neisseria gonorrhoeae* é de 0,8% (0,6–1,0%)[86]. Esta infecção sexualmente transmissível vem demostrado uma consistente evidência de associação com a infecção pelo HPV, achados de um aumentado risco para ≥ASC-US e principalmente HSIL em mulheres com coinfecção por HPV e *N. gonorrhoeae,* sugerindo uma possível ação sinérgica na progressão de lesões cervicais[87].

Outro agente sexualmente transmissível que tem sido associado à infecção por HPV é a infecção por *Chlamydia trachomatis*. O processo inflamatório, e consequente dano oxidativo decorrente da inflamação clamidiana, pode facilitar a infecção pelo HPV, e sua persistência pode estar associada ao desenvolvimento de lesão cervical[88]. Alguns estudos têm apontado a infecção por *C. trachomatis* como importante cofator associado ao desenvolvimento de neoplasias do colo uterino[89, 90], embora ainda existam controvérsias[91, 92]. Os estudos que descrevem associação entre presença de infecção por *C. trachomatis* e aumento do risco do desenvolvimento de doença induzida pelo HPV, sugerem que este patógeno é capaz de promover um cenário pró-inflamatório, levando a produção de grandes quantidades locais de citocinas pró-inflamatórias, produzindo um ambiente inflamatório crônico que pode contribuir possivelmente para a persistência do DNA do HPV no colo do útero[93]. O mecanismo biológico pelo qual a *C. trachomatis* atua a fim de facilitar a aquisição do HPV ainda é uma difícil questão a ser avaliada. Uma vez que ambos os agentes microbianos estão fortemente relacionados com a atividade sexual, dificultando a avaliação de um efeito independente da infecção por *C. trachomatis* sobre o risco de infecção por HPV[11].

1.2.2. Eliminação viral

Em cerca de 80% das mulheres infectadas pelo HPV, a eliminação viral ocorre antes da integração viral no genoma do hospedeiro, o estágio responsável pela carcinogênese[94]. Infecção por HPV persistente com risco elevado de câncer cervical ocorre em apenas uma pequena porcentagem de mulheres infectadas pelo vírus[95]. Embora a probabilidade e o tempo do *clearance* viral possam variar com base em fatores como idade das mulheres, tipo de HPV, comportamento sexual e *status* de tratamento no início, existem evidências de que a maioria das mulheres infectadas pelo HPV tende a eliminar o vírus dentro de 12 a 24 meses após a primeira detecção[96]. Estudos de coorte demostram que a depuração viral varia entre 55% e 64% aos 6 meses e entre 67% e 80% aos 12 meses[97-100].

A importância da eliminação/persistência do HPV foi reconhecida recentemente, e o número de estudos abordando essas questões aumentou substancialmente nos últimos anos. No entanto, os dados ainda são incompletos e, em parte, inconsistentes quanto aos cofatores que podem participar na regulação destes eventos[101,102].

O mecanismo envolvido na persistência viral é complexo e ainda não totalmente compreendido. Portanto, é fundamental identificar, entre uma coorte de mulheres infectadas pelo HPV, aquelas que não eliminam a infecção em um determinado momento. Além disso, a história natural de depuração de uma infecção por HPV cervicovaginal precisa ser melhor compreendida para prever seus possíveis resultados.

2. Referências bibliográficas

1. Giuliano AR, Nyitray AG, Kreimer AR, et al. EUROGIN 2014 roadmap: differences in human papillomavirus infection natural history, transmission and human papillomavirus-related cancer incidence by gender and anatomic site of infection. Int J Cancer. 2015;136(12):2752-2760.

2. Doorbar J, Egawa N, Griffin H, et al. Human papillomavirus molecular biology and disease association. Rev Med Virol. 2015;25 Suppl 1:20-23.

3. Hariri S, Bennett NM, Niccolai LM, et al. Reduction in HPV 16/18-associated high grade cervical lesions following HPV vaccine introduction in the United States - 2008-2012. Vaccine. 2015;33(13):1608-1613.

4. Plummer M, de Martel C, Vignat J, et al. Global burden of cancers attributable to infections in 2012: a synthetic analysis. Lancet Glob Health. 2016;4(9):e609-e616.

5. IARC monographs on the evaluation of carcinogenic risks to humans. Biological agents volume 100 B A review of human carcinogens. Lyon, France – 2012.

6. Lurie S, Mizrachi Y, Chodick G, et al. Impact of quadrivalent human papillomavirus vaccine on genital warts in an opportunistic vaccination structure. Gynecol Oncol. 2017;146(2):299-304.

7. Baudu A, Prétet JL, Riethmuller D, et al. Prevalence and risk factors of human papillomavirus infection types 16/18/45 in a cohort of French females aged 15-23years. J Epidemiol Glob Health 2014; 4:35-43.

8. Rositch AF, Koshiol J, Hudgens MG, et al. Patterns of persistent genital human papillomavirus infection among women worldwide: a literature review and meta-analysis. Int J Cancer 2013; 133(6): 1271–1285.

9. Cho HW, So KA, Lee JK, et al. Type-specific persistence or regression of human papillomavirus genotypes in women with cervical intraepithelial neoplasia 1: a prospective cohort study. Obstet Gynecol Sci 2015; 58(1): 40–45.

10. Goodman A. HPV testing as a screen for cervical cancer. BMJ. 2015 30;350:h2372.

11. Bruni L, Diaz M, Castellsagué X, et al. Cervical human papillomavirus prevalence in 5 continents: meta analysis of 1 million women with normal cytological findings. J Infection Dis 2010; 202:1789-1799.

12. Clifford GM, Gallus S, Herrero R, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet 2005; 366:991-998.

13. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer. 2015;136:E359–E386.

14. INCA. Instituto Nacional do Câncer [Internet]. Ministério da Saúde. [cited 2019 Jul 26]. Available at http://www.inca.gov.br 2019.

15. Sarchianaki E, Derdas SP, Ntaoukakis M, et al. Detection and genotype analysis of human papillomavirus in non-small cell lung cancer patients. Tumour Biol.2014;35:3203-3209.

16. Storey R, Joh J, Kwon A, et al. Detection of Immunoglobulin G against E7 of Human Papillomavirus in Non-Small-Cell Lung Cancer. J Oncol 2013; 240164.

17. Schiffman M, Castle PM, Jeronimo J, et al. Human papillomavirus and cervical cancer. Lancet 2007;370(9590):890-907.

18. Munger K, Howley PM. Human papillomavirus immortalization and transformation functions. Virus Res 2002; 89:213-228.

19. Doorbar J, Quint W, Banks L, et al. The biology and life-cycle of Human Papillomaviruses. Vaccine 2012; 30:F55–F70.

20. Sohrabi A, Hajia M, Jamali F, et al. Is incidence of multiple HPV genotypes rising in genital infections? J Infect Public Health. 2017;10(6):730-733.

21. de Villiers EM . Cross-roads in the classification of papillomaviruses. Virology. 2013;445(1-2):2-10.

22. Herbst LH, Lenz J, Van Doorslaer K, et al. Genomic characterization of two novel reptilian papillomaviruses, Chelonia mydas papillomavirus 1 and Caretta caretta papillomavirus 1. Virology. 2009;383(1):131-135.

23. Terai M, DeSalle R, Burk RD. Lack of canonical E6 and E7 open reading frames in bird papillomaviruses: Fringilla coelebs papillomavirus and Psittacus erithacus timneh papillomavirus. J Virol. 2002;76(19):10020-10023.

24. Bennett MD, Reiss A, Stevens H, et al. The first complete papillomavirus genome characterized from a marsupial host: a novel isolate from Bettongia penicillata. J Virol. 2010 ;84(10):5448-5453.

25. Bernard HU, Burk RD, Chen Z, et al. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology. 2010;401(1):70-79.

26. Burd EM, Dean CL. Human Papillomavirus. Microbiol Spectr. 2016;4(4).

27. de Oliveira CM, Fregnani JH, Carvalho JP, et al. Human papillomavirus genotypes distribution in 175 invasive cervical cancer cases from Brazil. BMC Cancer. 2013;13:357.

28. Alizon S, Murall CL, Bravo IG. Why Human Papillomavirus Acute Infections Matter. Viruses. 2017;9(10).

29. Liu S, Minaguchi T, Lachkar B, et al. Separate analysis of human papillomavirus E6 and E7 messenger RNAs to predict cervical neoplasia progression. PLoS One. 2018;13(2):e0193061.

30. Thatte J, Massimi P, Thomas M, et al. The Human Papillomavirus E6 PDZ Binding Motif Links DNA Damage Response Signaling to E6 Inhibition of p53 Transcriptional Activity. J Virol. 2018;92(16).

31. Mittal S, Banks L. Molecular mechanisms underlying human papillomavirus E6 and E7 oncoprotein-induced cell transformation. Mutat Res Rev Mutat Res. 2017;772:23-35.

32. Spanos WC, Hoover A, Harris, GF, et al. The PDZ binding motif of human papillomavirus type 16 E6 induces PTPN13 loss, which allows anchorage-independent growth and synergizes with ras for invasive growth. J Virol. 2008;82(5):2493-2500.

33. Thwaites MJ, Cecchini MJ, Passos DT, et al. Interchangeable Roles for E2F Transcriptional Repression by the Retinoblastoma Protein and p27KIP1-Cyclin-Dependent Kinase Regulation in Cell Cycle Control and Tumor Suppression. Mol Cell Biol. 2017;37(2).

34. Benevolenskaya EV, Frolov MV. Emerging links between E2F control and mitochondrial function. Cancer Res. 2015;75(4):619-623.

35. Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. Nat Rev Cancer. 2010;10(8):550-560.

36. Chellappan S, Kraus VB, Kroger B. et al. Adenovirus E1A, simian virus 40 tumor antigen, and human papillomavirus E7 protein share the capacity to disrupt the interaction between transcription factor E2F and the retinoblastoma gene product. Proc Natl Acad Sci U S A. 1992;89(10):4549-4553

37. Carrillo D, Muñoz JP, Huerta H, et al. Upregulation of PIR gene expression induced by human papillomavirus E6 and E7 in epithelial oral and cervical cells. Open Biol. 2017;7(11).

38. Zerfass K, Schulze A, Spitkovsky D. et al. Sequential activation of cyclin E and cyclin A gene expression by human papillomavirus type 16 E7 through sequences necessary for transformation. J Virol. 1995;69(10):6389-6399.

39. Boyer SN, Wazer DE, Band V. E7 protein of human papilloma virus-16 induces degradation of retinoblastoma protein through the ubiquitin-proteasome pathway. Cancer Res. 1996;56(20):4620-4624.

40. Jones DL, Thompson DA, MUNGER K. Destabilization of the RB tumor suppressor protein and stabilization of p53 contribute to HPV type 16 E7-induced apoptosis. Virology. 1997;239(1):97-107.

41. Shin MK, Balsits S, Brake T. et al. Human papillomavirus E7 oncoprotein overrides the tumor suppressor activity of p21Cip1 in cervical carcinogenesis. Cancer Res. 2009;69(14):5656-5663.

42. Zerfass-Thome K, Zwerschke W, Mannhardt B. et al. Inactivation of the cdk inhibitor p27KIP1 by the human papillomavirus type 16 E7 oncoprotein. Oncogene. 1996;13(11):2323-2330.

43. Jones DL, Alani RM, Munger K. The human papillomavirus E7 oncoprotein can uncouple cellular differentiation and proliferation in human keratinocytes by abrogating p21Cip1-mediated inhibition of cdk2. Genes Dev. 1997;11(16):2101-2111.

44. Hoppe-Seyler K, Bossler F, Braun JA, et al. The HPV E6/E7 Oncogenes: Key Factors for Viral Carcinogenesis and Therapeutic Targets. Trends Microbiol. 2018;26(2):158-168.

45. Vande Pol SB, Klingelhutz AJ. Papillomavirus E6 oncoproteins. Virology. 2013;445(1-2):115-137.

46. Pańczyszyn A, Boniewska-Bernacka E, Głąb G. Telomeres and Telomerase During Human Papillomavirus-Induced Carcinogenesis. Mol Diagn Ther. 2018;22(4):421-430.

47. Spardy N, Duensing A, Hoskins EE. et al. HPV-16 E7 reveals a link between DNA replication stress, fanconi anemia D2 protein, and alternative lengthening of telomereassociated promyelocytic leukemia bodies. Cancer Res. 2008;68(23):9954-9963.

48. Aksoy P, Gottschalk EY, Meneses PI. HPV entry into cells. Mutat Res Rev Mutat Res. 2017;772:13-22.

49. Schmitt A, Rochat A, Zeltner R, et al. The primary target cells of the high-risk cottontail rabbit papillomavirus colocalize with hair follicle stem cells. J Virol. 1996;70(3):1912-1922.

50. McBride AA. Mechanisms and strategies of papillomavirus replication. Biol Chem. 2017 Jul 26;398(8):919-927.

51. Longworth MS, Laimins LA. Pathogenesis of human papillomaviruses in differentiating epithelia. Microbiol Mol Biol Rev. Microbiol Mol Biol Rev. 2004;68(2):362-372.

52. Becker M, Greune L, Schmidt MA, et al. Extracellular Conformational Changes in the Capsid of Human Papillomaviruses Contribute to Asynchronous Uptake into Host Cells. J Virol. 2018;92(11). pii: e02106-e2117.

53. Buck CB, Pastrana DV, Lowy DR, et al. Efficient intracellular assembly of papillomaviral vectors. J Virol. 2004;78(2):751-757.

54. Fligge C, Schafer F, Selinka HC, et al. DNA-induced structural changes in the papillomavirus capsid. J Virol. 2001;75(16):7727-7731.

55. Richards KF, Bienkowska-Haba M, Dasgupta J, et al. Multiple heparan sulfate binding site engagements are required for the infectious entry of human papillomavirus type 16. J Virol. 2013;87(21):11426-11437.

56. Bienkowska-Haba M, Patel HD, Sapp M. Target cell cyclophilins facilitate human papillomavirus type 16 infection. PLoS Pathog. 2009;5(7):e1000524.

57. Richards RM, Lowy DR, Schiller JT, et al. Cleavage of the papillomavirus minor capsid protein, L2, at a furin consensus site is necessary for infection. Proc Natl Acad Sci U S A. 2006;103(5):1522-1527.

58. Havre PA, Yuan J, Hedrick L, et al. p53 inactivation by HPV16 E6 results in increased mutagenesis in human cells. Cancer Res. 1995;55(19):4420-4424.

59. Schelhaas M, Shah B, Holzer M, et al. Entry of human papillomavirus type 16 by actin-dependent, clathrin- and lipid raft-independent endocytosis. PLoS Pathog. 2012;8(4):e1002657.

60. Pyeon D, Pearce SM, Lank SM, et al. Establishment of human papillomavirus infection requires cell cycle progression. PLoS Pathog. 2009;5(2):e1000318.

61. De Geest K, Turyk ME, Hosken MI, et al. Growth and differentiation of human papillomavirus type 31b positive human cervical cell lines. Gynecol Oncol. 1993;49(3):303-310.

62. MCMurray HR, Nguyen D, Westbrook TF, et al. Biology of human papillomaviruses. Int J Exp Pathol. 2001;82(1):15-33.

63. Hebner CM, Laimins LA. Human papillomaviruses: basic mechanisms of pathogenesis and oncogenicity. Rev Med Virol. 2006;16(2):83-97.

64. Veldhuijzen NJ, Snijders PJF, Reiss P, et al. Factors affecting transmission of mucosal human papillomavirus. Lancet Infect Dis 2010;10: 862–874.

65. Duensing S, Münger K. Mechanisms of genomic instability in human cancer: insights from studies with human papillomavirus oncoproteins. Int J Cancer 2004;109:157-162.

66. Williams VM, Filippova M, Soto U, et al. HPV-DNA integration and carcinogenesis: putative roles for inflammation and oxidative stress. Future Virol. 2011; 6(1): 45–57.

67. Münger K, Howley PM. Human papillomavirus immortalization and transformation functions. Virus Res. 2002;89:213-228.

68. Lee H, Lee DH, Song YM, et al. Risk factors associated with human papillomavirus infection status in a Korean cohort. Epidemiol Infect. 2014;142: 1579-1589.

69. Figueiredo Alves RR, Turchi MD, Santos LE, et al. Prevalence, genotype profile and risk factors for multiple human papillomavirus cervical infection in unimmunized female adolescents in Goiânia, Brazil: a community-based study. BMC Public Health. 2013;13:1041.

70. Kotloff KL, Wasserman SS, Russ K, et al. Detection of genital human papillomavirus and associated cytological abnormalities among college women. Sex Transm Dis. 1998;25:243-250.

71. Thomas I, Wright G, Ward B. The effect of condom use on cervical intraepithelial neoplasia grade I (CIN I). Aust N Z J Obstet Gynaecol. 1990;30:236-239.

72. Ho GY, Bierman R, Beardsley L, et al. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med. 1998;338:423-428.

73. Moscicki AB, Hills N, Shiboski S, et al. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. JAMA. 2001;285:2995-3002.

74. Hogewoning CJ, Bleeker MC, van den Brule AJ, et al. Condom use promotes regression of cervical intraephitelial neoplasia and clearance of human papillomavirus: a randomized clinical trial. Int J Cancer. 2003; 107:811-816.

75. Winer RL, Hughes JP, Feng Q, et al. Condom use and the risk of genital human papillomavirus infection in young women. N Engl J Med. 2006; 354:2645-2654.

76. Manhart LE, Koutsky LA. Do condoms prevent genital HPV infection, external genital warts, or cervical neoplasia? A meta-analysis. Sex Transm Dis. 2002; 29:725-735.

77. McCallum EB, Peterson ZD. Investigating the impact of inquiry mode on self-reported sexual behavior: theoretical considerations and review of the literature. J Sex Res 2012;49: 212-216.

78. Fenton KA, Johnson AM, McManus S, et al. Measuring sexual behaviour: methodological challenges in survey research. Sex Transm Infect. 2001;77: 84–92.

79. Moreno V, Bosch FX, Muñoz N. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. Lancet. 2002;359:1085-1092.

80. Castellsagué X, Bosch FX, Muñoz N. Environmental co-factors in HPV carcinogenesis. Virus Res. 2002; 89:191-199.

81. Lee JE, Lee S, Lee H, et al. Association of the vaginal microbiota with human papillomavirus infection in a korean twin cohort. PLoS One. 2013; 8: e63514.

82. Mendoza L, Mongelos P, Paez M, et al. Human papillomavirus and other genital infections in indigenous women from Paraguay: a cross-sectional analytical study. BMC Infect Dis. 2013;531.

83. McIver CJ, Rismanto N, Smith C, et al. Multiplex PCR testing detection of higher-than-expected rates of cervical mycoplasma, ureaplasma, and trichomonas and viral agent infections in sexually active australian women. J Clin Microbiol. 2009;47:1358-1363.

84. Moscicki AB, Hills N, Shiboski S, et al. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. JAMA. 2001; 285:2995-3002.

85. Smith JS, Herrero R, Bosetti C. Herpes simplex virus-2 as a human papillomavirus cofactor in the etiology of invasive cervical cancer. J Natl Cancer Inst. 2002;94(21):1604-1613.

86. Newman L, Rowley J, Vander Hoorn S, et al. Global Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2012 Based on Systematic Review and Global Reporting. PLoS One. 2015;10(12):e0143304.

87. de Abreu AL, Malaguti N, Souza RP, et al. Association of human papillomavirus, Neisseria gonorrhoeae and Chlamydia trachomatis co-infections on the risk of high-grade squamous intraepithelial cervical lesion. Am J Cancer Res. 2016;6(6):1371-1383.

88. Simonetti AC, Melo JH, de Souza PR, et al. Immunological's host profile for HPV and Chlamydia trachomatis, a cervical cancer cofactor. Microbes Infect. 2009; 11:435-442.

89. Koskela P, Anttila T, Bjørge T, et al. Chlamydia trachomatis infection as a risk factor for invasive cervical cancer. Int J Cancer. 2000; 85:35-39.

90. Wallin KL, Wiklund F, Luostarinen T, et al. A population-based prospective study of Chlamydia trachomatis infection and cervical carcinoma. Int J Cancer. 2002; 101:371-374.

91. Bhatla N, Puri K, Joseph E, et al. Association of Chlamydia trachomatis infection with human papillomavirus (HPV) & cervical intraepithelial neoplasia - a pilot study. Indian J Med Res. 2013; 137:533-539.

92. Castle PE, Escoffery C, Schachter J, et al. Chlamydia trachomatis, herpes simplex virus 2, and human T-cell lymphotrophic virus type 1 are not associated with grade of cervical neoplasia in Jamaican colposcopy patients. Sex Transm Dis. 2003; 30:575-580.

93. Silins I, Ryd W, Strand A, et al. Chlamydia trachomatis infection and persistence of human papillomavirus. Int J Cancer. 2005; 116:110-115.

94. Muñoz N, Castellsagué X, de González AB, et al. Chapter 1: HPV in the etiology of human cancer. Vaccine. 2006;24: 1–10.

95. Gravitt PE. The known unknowns of HPV natural history. J Clin Invest. 2011 ;121(12):4593-4599.

96. Akaaboune M, Kenfack B, Viviano M, et al. Clearance and persistence of the human papillomavirus infection among Cameroonian women. Womens Health (Lond). 2018;14:1745506518805642.

97. Rodriguez AC, Schiffman M, Herrero R, et al. Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. J Natl Cancer Inst. 2008; 100(7): 513–517.

98. Moscicki AB, Widdice L, Ma Y, et al. Comparison of natural histories of human papillomavirus detected by clinician- and self-sampling. Int J Cancer. 2010; 127(8): 1882–1892.

99. Plummer M, Schiffman M, Castle PE, et al. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. J Infect Dis. 2007; 195(11): 1582–1589.

100. Giuliano AR, Sedjo RL, Roe DJ, et al. Clearance of oncogenic human papillomavirus (HPV) infection: effect of smoking (United States). Cancer Causes Control. 2002;13(9):839-846.

101. Confortini M, Carozzi F, Zappa M. et al. Human papillomavirus infection and risk factors in a cohort of Tuscan women aged 18-24: results at recruitment. BMC Infect Dis. 2010;10:157.

102. Sammarco ML, Del Riccio I, Tamburro M, et al. Type-specific persistence and associated risk factors of human papillomavirus infections in women living in central Italy. Eur J Obstet Gynecol Reprod Biol. 2013;168(2):222-226.

Capítulo II – Objetivos

1. Objetivos

1.1. Objetivo geral

Identificar determinantes do *clearance* da infecção pelo Papilomavírus Humano (HPV) em mulheres brasileiras em idade reprodutiva.

1.2. Objetivos específicos:

Estimar a prevalência de infecções por HPV e determinar a diversidade genotípica em mulheres em idade reprodutiva, atendidas nas unidades de saúde do município de Botucatu-SP.

Estabelecer determinantes sociodemográficos, comportamentais e achados ginecológicos no *clearance* do HPV em mulheres com seguimento de 30 meses.

Capítulo III – Article

Determinants of human papillomavirus clearance among Brazilian women of reproductive age: the influence of behavior, sexually transmitted coinfection and innate immune response.

Pinto GVS[1,§], Bolpet AN[1,§], El-Zein M[2], Martin LF[1], Marconi C[3], Polettini J[4], Duarte MTC[1], Moço NP[1], Noda-Nicolau NM[1], Ramos BRA[1],Villa LL[5,6], Miot HA[7], Candeias JMG[8], Franco EL[2], Silva MG[1,*].

[1] UNESP - São Paulo State University, Department of Pathology, Botucatu Medical School, SP, Brazil; [2] Division of Cancer Epidemiology, McGill University, Montreal, Canada; [3] Department of Basic Pathology, Setor de Ciências Biológicas, UFPR – Univ Federal do Paraná, Curitiba, Paraná, Brazil; [4] Universidade Federal da Fronteira Sul, Campus Passo Fundo, Rio Grande do Sul; [5] Center for Translational Investigation in Oncology, Instituto do Câncer do Estado de São Paulo, São Paulo, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; [6] Department of Radiology and Oncology, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil; [7] UNESP - São Paulo State University, Department of Dermatology and Radiotherapy, Botucatu Medical School, SP, Brazil; [8] UNESP - São Paulo State University, Department of Microbiology and Immunology, Institute of Biosciences, Botucatu, SP, Brazil.

[§] These authors contributed equally to this work

***Corresponding author:**

Márcia Guimarães da Silva, MS, PhD

Email: marcia.guimaraes@unesp.br; Telephone: +55 14 38801580

Funding: This study was supported by: developmental funds granted by São Paulo Research Foundation − FAPESP (Grant 2012/01278-0) granted to Dr. Márcia Guimarães da Silva from the Department of Pathology, Botucatu Medical School, UNESP − Univ. Estadual Paulista, Botucatu, São Paulo, Brazil; and by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (88881.132503/2016-01) granted to Gabriel Vitor da Silva Pinto, PhD student in the Post-graduate Program in Pathology, Botucatu Medical School, UNESP – São Paulo State University, Botucatu, São Paulo, Brazil. Gabriel Vitor da Silva Pinto performed his training at the Division of Cancer Epidemiology, McGill University, Montreal, Canada with Dr. Franco financed by CAPES. This work was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

1. Abstract

Objective: The objective of the present study was to identify determinants of Human Papillomavirus (HPV) infection clearance in Brazilian women of reproductive age. **Methods:** This is a cohort study called HPV-UNESP, in which 1638 women of reproductive age were recruited from September 2012 to January 2013. Of this total, 544 women positive for HPV infection participated in longitudinal follow-up for 30 months, in 4 more visits. HPV infection was defined as detection of any of the 36 genotypes tested by the Linear Array Genotyping Test (Roche Molecular Systems, Inc.) and the outcome of interest was infection clearance, defined as the elimination of HPV infection by least two consecutive visits. A structured questionnaire with 58 questions regarding sociodemographic data, behavioral and gynecological characteristics was applied at each visit. Immediately after the interview, all women underwent a gynecological exam, in which, after insertion of the non-lubricated Collins speculum, the vaginal pH with tape (pH 4.0-7.0, Merck, Germany) was measured in the middle third of the vaginal wall. For vaginal microbiota evaluation, samples were collected with vaginal wall swab and the microbiota pattern was classified according to the criteria of Nugent et al. (1991). The whiff test performed by adding 10% KOH solution to the vaginal content was interpreted as positive, negative or doubtful. Endocervical samples were collected with cytobrush for molecular analysis of HPV, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection. Additional sample with representation of squamous and glandular epithelium was collected for cytology testing. Finally, a sample from the posterior fornix of the vagina was collected with Ayre's spatula for research of *Trichomonas vaginalis* in Diamond liquid medium**.** At each longitudinal follow-up visit, vaginal and endocervical samples were collected for the same exams, using the same techniques described. Logistic regression models were used to estimate hazard ratios (HR), with 95% confidence intervals, of HPV clearance as a function of covariates at the time of study inclusion adjusted for age. **Results:** Single (HR = 0.67, 95% CI 0.49-0.92) and divorced (HR = 0.53, 95% CI 0.30-0.92) marital status were associated with delayed clearance time. HPV. The same delay in clearance time was observed in women who reported hormonal contraceptive use (HR = 0.72, 95% CI 0.55-0.95) and in women who reported four lifetime sexual partners (HR = 0, 55, 95% CI 0.34-0.90). There was no association between Interleukin (IL-) 1β, IL-6, IL-10, IL-12 and TNF-α concentration and HPV clearance time, only IL-4 showed positive correlation (HR = 2.40, 95% CI 1.11-5.21). There was also no association between endocervical *Chlamydia trachomatis* and *Neisseria gonorrhoeae* co-infections and *Trichomonas vaginalis* infection with HPV clearance. **Conclusion:** Despite the

analysis of sociodemographic factors, behavioral characteristics and gynecological findings, most of them were not significantly associated with HPV clearance.

Keywords: HPV infection, Clearance, Sociodemographic factors, behavioral characteristics and gynecological findings.

2. Introduction

HPV is necessary for cervical cancer, and a frequent cause for other anogenital disease. It has a high economic impact, particularly in low-income countries[1, 2]. Most people get infected with HPV at some part of their life[3]. Risk factors associated with HPV infection can be divided into three groups: (1) Environmental or exogenous factors, which include deficient diets, hormonal contraceptive use, multiple parity, multiple sexual partners, early sexual initiation, alcohol and tobacco consumption, and coinfection with other sexually transmitted infections; (2) Inherent aspects of the virus, such as high and low oncogenic HPV infection, multiple viral genotype infections, high-risk HPV variants that differ in oncogenic potential, viral load, and viral DNA integration Host cell DNA; (3) Factors inherent to the host, such as endogenous hormones, genetic factors (HLA polymorphisms) and factors related to the immune response[4, 5, 6]. But most people clear the infection spontaneously, also when they are not vaccinated[7]. Only a minority of them has persistent HPV infections which can eventually result in HPV-related disease[8].

High-risk HPV infections are also associated with other anogenital diseases; they contribute to 85% of anal cancers and 50% of cancers of the vulva, vagina and penis[9]. The development of cervical lesions is the main complication of persistent HPV infection that may predict and progress to malignancy, recognized as the most important risk factor for cervical cancer development[10]. While the high-risk HPV types have been established as the main causative agent of both malignancies and the precursor lesions, other factors may also be involved, acting in conjunction with HPV, such as other sexually transmitted infections (STI)[11], smoking[12] and host immunological response[13]. Several behaviors, such as oral contraceptive use, sexual activities, tobacco and alcohol consumption, may influence the risk of initial viral infection and subsequent clearance of the virus[8, 14]. Specifically, at one year approximately 20%

of persistent infections with high risk HPV develop cervical intraepithelial neoplasia or cervical cancer within the subsequent 5 years[15]. The fact that only a small proportion of HPV-infected individuals will eventually develop cervical cancer and the long latency period between primary infections and cancer emergence suggest that additional factors are involved in the progression[16].

The immune response plays an important role in the natural history of HPV, including eliminating HPV infection as well as cervical cancer[16,17]. Cytokines are important regulators of HPV transcription[18]. It is well established that cytokines in immune responses to infection can be classified and divided into immunostimulating (tumour suppressing) Th1-type cytokines such as interleukin 2 (IL-2), and IL-12, interferon γ (IFNγ) and tumour necrosis factor α (TNFα), responsible for the induction of immune response mainly mediated by cells, and Th2-type cytokines (IL-4, IL-5, IL-6, IL-8, and IL-10), which modulate the inflammatory response to a predominantly humoral profile[16,19].

In the current study, we evaluated in detail determinants for clearance of HPV infections in a Brazilian cohort. Considering the economic importance of cervical cancer in developing countries, the need for prospective studies of HPV prevalence and its risk factors associates with clearance heightened in different populations. The aim of this study was to identify determinants of HPV clearance among Brazilian women of reproductive age.

3. Patients and Methods

3.1. Cohort description

The HPV-UNESP Cohort is a prospective study of HPV infection, clearance and risk factors among women at reproductive age. The focus of this cohort is to determine the influence of behavior, sexually transmitted coinfection and innate immune response on HPV infection natural history. The cross-sectional analysis performed using baseline demographic, behavioral and clinical history data has been described in previous publication[20]. From September 2012 to January 2013 the reproductive-age women were screened for eligibility and written informed consent was obtained from all women and this research project was approved by the research Ethics Committee Board of Botucatu Medical School, São Paulo State University, UNESP, under protocol 4121-2012. A total of 1638 healthy women in reproductive age attended the cervical screening program in 18 Primary Health Care Units Botucatu, an inner city of southeastern Brazil (Figure 1). Participants were eligible for the study if they were nonpregnant, non-menopausal, if they were no hysterectomy, no prior report of seroconversion for HIV, no antibiotic or vaginal cream used in the preceding 30 days and abstinence from sexual intercourse for 72 hours before the visit, for the follow-up visits a later appointment scheduled was made for each women and the inclusion criteria were maintained. Women under evaluation, treatment or follow up of a cervical cancer, presenting vaginal bleeding, urinary incontinence and women mentally unable to answer the questionnaire or provide informed consent were excluded of the study.

eligible
eligible

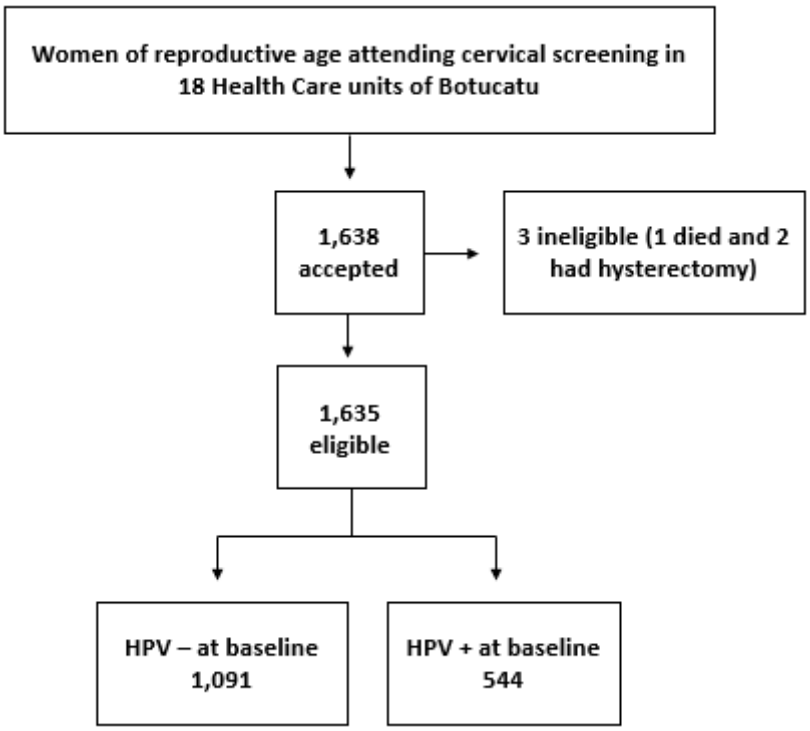

Figure 1. Flowchart of the eligible women for the study and patient's selection to compose the cohort.

Of the 1635 women in the cross-sectional study we identified a cohort with HPV positive women (n=544) who were eligible for long-term follow-up, examined every 4-6 months with 5 visits in this interval entering the study during the first two years (2013–2015), they were recruited and submitted to a follow-up questionnaire in addition to the molecular analyzes available from all of the 5 visits on study. The first 4 study visits were scheduled to occur every 6 months in the first and second year after enrollment on study. On average, the visits interval between each visit moment and the end of the follow-up as scheduled were at 0 ± 30.82 months, 4.08 ± 30.82 months, 10.92 ± 30.82, 15.89 ± 30.82 and 24.87 ± 30.82 months after enrollment.

3.2. Face-to-face interview and specimen collection

A standardized questionnaire containing 58 questions concerning sociodemographic characteristics, sexual behavior and clinical history was applied by a trained nurse from Botucatu Medical School of São Paulo State University (FMB-UNESP) at each visit. Following the interview, all women were submitted to a gynecological examination. After inserting a non-lubricated speculum, vaginal pH was assessed by pressing strips (4.0-7.0, Merck, Germany) against the vaginal wall. Whiff test was performed by adding 10%KOH solution to the samples with results interpreted by the practitioner as positive, doubtful or negative. For microscopic evaluation of the vaginal microbiota, samples were taken from the mid-lateral vaginal wall. Vaginal microbiota pattern was classified according to Nugent et al. (1991)[21]. Endocervical samples were collected with a cytobrush for molecular analysis of HPV, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection. Another remaining sample from endocervical and ectocervical were collected for Pap smears. Finally, a sample from the posterior fornix of the vagina using Ayre spatula was taken to assess the infection by *Trichomonas vaginalis*. Participant follow-up visits were scheduled every 6 months for a period of 24 months, for those women who presented HPV positivity for at least one genotype. At each visit, endocervical and mid-vaginal samples were recollected. Figure 1S shows the study design detailing the visit moments and all laboratory procedures performed.

3.3. HPV testing

DNA from cervical samples was extracted using an AmpliLute Liquid Media Extraction Kit (Roche Molecular Systems, Alameda, CA, USA), in accordance with the manufacturer´s instructions. HPV genotyping was performed using the Linear Array Genotyping Test (Roche Molecular Systems, Inc.), to detect 36 mucosal HPV genotypes (HPV 6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82,

83, 84, and 89), being the following genotypes of high oncogenic risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and (6, 11, 26, 34, 40, 42, 44, 53, 54, 61, 62, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, and 89) the low-risk oncogenic genotypes. The human β-Globin DNA was coamplified to assess DNA integrity as an internal control. Reactions were amplified in a PerkinElmer TC9600 Thermal Cycler. Positive and negative controls for HPV detection provided by Roche Molecular Systems were included in each assay.

3.4. Chlamydia trachomatis and *Neisseria gonorrhoeae* testing

For the evaluation of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection by PCR, DNA extraction was obtained with a previous digestion with proteinase K. The presence and integrity of DNA samples was confirmed by the amplification of β-globin housekeeping gene using primers GH20 (GAA GAG CCA AGG ACA GGT AC) and PCO4 (CAA CTT CAT CCA CGT TCA CC) which target a 268-base pair sequence[22]. To verify the presence of *Chlamydia trachomatis* infection in the sample a multiplex PCR were performed using two pairs of specific primers. The primer pairs used herein were CTP1 (TAG TAA CTG CCA CTT CAT CA); CTP2 (TTC CCC TTG TAA TTC GTT GC) product site: 201 bp sequence, and PL6.1 (5' AGA GTA CAT CGG TCA ACG A 3'); PL6.2 (5' TCA CAG CGG TTG CTC GAA GCA 3') product site: 155 bp sequence. Amplification parameters consisted of 40 cycles of 60 s at 95°C, 60 s at 55°C and 90 s at 72°C. In all reactions, negative and positive controls were run simultaneously, with respectively sterile water and *Chlamydia trachomatis* DNA extracted from infected McCoy cells[23]. The evaluation of *Neisseria gonorrhoeae* was performed with specific primers for PCR: HO1 (5' GCT ACG CAT ACC CGC GTT GC 3)' and H03 (5' CGA AGA CCT TCG AGC AGA CA 3'). PCR was performed using the Go Taq Green master mix (Promega Corporation, USA). Amplification parameters consisted of 40 cycles of 30 s at 94°C, 60 s at 55°C and 30 s at 74°C[24]. Amplicon

molecular weight was determined by comparing to a standard size marker, under UV transillumination.

3.5. Trichomonas vaginalis testing

Infection by *Trichomonas vaginalis* was examined by culture of vaginal posterior fornix samples in Modified Diamond's medium at 37°C in 5% CO2. Using a clean glass slide without cover and a sterile pipette, fresh wet-mount microscope slides were prepared with aliquots from the culture and examined microscopically under 10x and 40x objectives. Presence of the motile protozoan was checked daily, if it was not observed, the culture was incubated again with daily examination for up to 3 days in the same manner for the same trained observer. If *Trichomonas vaginalis* were not detected during the three days, the specimen was considered negative.

3.6. Vaginal microbiota pattern

Vaginal samples were spread onto glass microscope slides to classify vaginal microbiota following Gram-stain according to Nugent's scoring system: normal (scores 0-3), intermediate (4-6), and bacterial vaginosis (7-10), regardless the presence of symptoms or clinical findings[21]. Vaginal candidiasis was based on the presence of yeast blastospores or pseudohyphae on the smears with accompanying inflammatory cells in the presence of vulvovaginal pruritus[25]. All smears were evaluated by an experienced microscopist. All women with abnormal vaginal microbiota and/or positive for *Chlamydia trachomatis, Candida sp, and Trichomonas vaginalis* were treated in accordance with a protocol established by the Health Care Units from Botucatu city.

3.7. Cytology testing

Pap smears were fixed in absolute ethanol, stained and interpreted at the Cytology Unit of the Pathology Department, Botucatu Medical School FMB-UNESP by cytotechnologists and, when appropriate, cytopathologists through conventional means using locally prevailing practice standards and were classified according to the Bethesda system and the following cytological findings were reported: negative for intraepithelial lesion or malignancy (NILM), atypical squamous cells of undetermined significance(ASC-US), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL)[26].

3.8. Cervical cytokine analyses

IL-1-beta, IL-4, IL-6, IL-10, IL-12 and TNF-alfa levels were measured in the cervicovaginal fluid supernatants by enzyme linked immunosorbent assay (ELISA), Duo Set Kits (R&D Systems, Minneapolis, MN), according to manufacturer's instructions. All samples were tested in duplicate and those with values set above the standard curve range were diluted (1:5 and 1:10) and retested. The minimum detectable levels in the IL-1, IL-4, IL 6, IL-10, IL-12 and TNF-alpha assays were 0.092 pg/mL, 0.022 ng/mL, 0.007 pg/mL, 0.003 pg/mL, 0.125 pg/mL, respectively. To confirm acceptable assay performance, 10% of the samples were tested in duplicate and coefficients of variation (CV) intra assay calculated for each duplicate test, were 1%, 31%, 11%, 8%, 5% and 11% for IL-1, IL-4, IL-6, IL-10, IL-12 and TNF-α, respectively. The analyses were restricted to women which have normal vaginal microbiota according to Nugent´s scoring system independent of HPV genotypes.

3.9. Statistical Analyses

For the cohort study we analyzed only women with HPV infection. We used histograms for each cytokine analyzed and for all the follow-up moments due to estimate the cytokines

frequencies. The concentrations of each cytokine were categorized into quartiles. We assessed the relationship between HPV infection and sociodemographic characteristics, clinic history, lifestyle habits and cytokines by considering grouped HPV genotypes per their phylogenetic relationship within the genus Alpha-papillomavirus. Cox's proportional hazard ratio (HR) for HPV clearance, were analyzed in the perspective of HPV clearance of any HPV infection in at least one consecutive visit as a function of covariates.

Logistic regression

The odds ratio (OR), computed in logistic regression models with 95% confidence interval (CI), univariate associations between subject characteristics and one-year period prevalence of HPV infection as a function of covariates at baseline, this model restricted to women positive for any of the 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), persistence of prevalent infections detected at enrollment was assumed to occur at the midpoint between the last HPV-positive test result and a subsequent HPV-positive test result with a second visit at around 9-15 months (clinical follow-up perspective, time to allow for HPV persistence). We used the Kaplan-Meier technique to estimate the survival function of HPV infection.

3.9.1. Definitions

HPV exposure was defined as detection of any of the 36 HPV genotypes tested, where individual genotypes were considered. An additional analysis divided HPV genotypes by subgenus groups: Subgenus 1: HPVs 6, 11, 32, 40, 42, 44 and 54; Subgenus 2: HPVs 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73 and 82 Subgenus 3: HPVs 57, 61, 62, 71, 72, 81, 83, 84 and 89.

Clearance of infection was defined as one negative test for any HPV type following a positive test for any HPV DNA at baseline or during follow-up. Persistence was defined restricting to women positive for any of the 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59,

66 and 68), with a second visit at around 9-15 months (clinical follow-up perspective time to allow for HPV persistence). Cox proportional regression was used to estimate hazards ratios (HR) and corresponding 95% confidence intervals (CI), age-adjusted.

3.10. Role of the funding source

The funders of the study were not involved in study design; in the collection, analysis, and interpretation of data; neither in the writing of the report; and in the decision to submit the paper. The corresponding author had full access to all the data in the study and has final responsibility for the decision to submit for publication.

4. Results

4.1. Characteristics of the cohort

Table 1 presents characteristics of the cohort members. The median age was 32 years-old (14- 54), and the majority defined themselves as white (59%) and most of the women were married or living with a partner (47%). In relation to education status, 16.5% had completed high school. In this population, 92% were sexually active in the moment of enrollment. Among sexually active women, the median of lifetime sex partners was 4 (1-300). About their first sexual intercourse, 23% had initiated sexual intercourse before 15 years, 68% between 15 and 20 years old and 8% at 21 or over. Regarding previous pregnancies, 71% reported at least one previous gestation at enrollment. 462/544 (85%) of the women HPV positive selected from the cross-sectional study returned to the first follow-up moment of the study. 362/544 (66.5%) of women completed second follow-up, 242/544 (44%) completed eighteen-month follow-up and 105/544 (19%) the last visit moment. The clearance rate for HPV, considering any HPV genotype, with at least one negative consecutive visit within 30 months follow-up, was observed in 85% of patients.

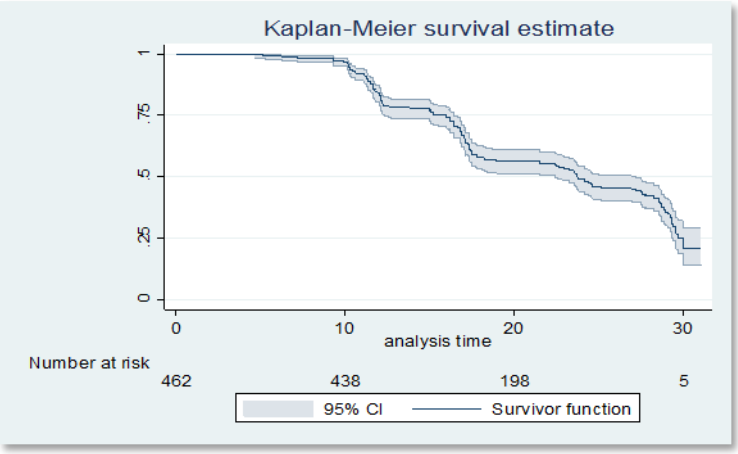

Figure 2. Kaplan-Meier survival estimates of time (in months) to clearance of Human Papillomavirus (HPV), age-adjusted hazards ratios (95% confidence intervals) of HPV liberal clearance (defined as clearance of an HPV infection in at least one consecutive visit)

The majority of women presented normal cytology result and most of the positive cases were observed at visits 1 (8.6%), 3 (8.5%) and 4 (10.1%). More than 89% of the women had normal cytologies at all visit moments, the prevalence of HPV-associated lesions was 8.6% (48/544) at the moment of inclusion. The highest prevalence of cytological abnormalities observed was 10.1% at visit moment 4, divided by 5.3% of ASC-US, 2.8% of LSIL and 2.0% of HSIL. ASC-US was the most observed cytological alteration with 17.8% considering all follow-up, followed by LSIL and HSIL, respectively, 11.4% and 6.1%, in this population were not observed cervical carcinoma.

More than 50% of cohort members had a normal vaginal microbiota pattern at all times, 34% of the women had bacterial vaginosis at the first visit, with progressive decline until the last visit with a slight elevation (Table 2). Of the 544 women originally sampled, 74 (13.6) women tested positive for *Chlamydia trachomatis* infection at visit 1 with progressive decline until the last visit. The prevalence of *Trichomonas vaginalis* infection was 1.1% in visit 1 and according to the protocol this infection was only diagnosed at the time of inclusion (Table 2).

Normal vaginal microbiota was observed in 52.2% (284/544) of the patients included in this study, the lactobacilli dominated flora was even higher in the subsequent moments observed, reaching 64.8% at visit moment 4. The prevalence of Bacterial vaginosis was 34.3% (187/544), decreasing its prevalence in the follow-up reaching 13.6% at visit moment 4, and a new high in prevalence observed at the last moment, 25.7%. Intermediate flora was detected in 5.1% (28/544) of women, with a small decline and variations in prevalence at the following moments, not reaching 5%. The prevalence of candidiasis was 4.0% (Table 2).

4.2. *HPV and Chlamydia trachomatis prevalence*

According to the HPV analysis, the overall prevalence of HPV infection was 33.3%. Within these HPV-positive women, 42.0% (229/544) of them had infections with multiple HPV types. Among women with multiple-type infection, 187 (82.74%) of them had one or more high-risk HPV genotype (data not shown). The most prevalent HPV type was HPV-16 (17.8%), followed by another high-risk HPV, HPV-52 (8.5%), 58 (8.3%) and 31 (7.2%). The most prevalent low-risk HPV was HPV-53 (9.9%), considering the subgenus, group 2 was the most prevalent (77.6%), followed by group 3 (36.2%) and group 1 (15.3%) (Table 1S). In the following visit moments where the samples were collected only from women who presented positivity for at least one HPV genotype, we observed a prevalence greater than 50 percent at all visit moments: visit 2 (69.9), visit 3 (58.3), visit 4 (60.3) and visit 5 (55.2). In relation to *Chlamydia trachomatis* infection, we detected 8.4% (138/1635) overall prevalence, and within HPV-positive women, 74 of them were also positive for *Chlamydia trachomatis* 13.6% (74/544). We observed a significant decline in the prevalence of *Chlamydia trachomatis* infection at moments 1 to 4 (13.6 - 1.2%), with a slight increase in prevalence at the last moment (3.8%), but the HPV coinfection rate in all the visit moments was high, 86.6% at visit moment 2, 88% at visit moment 3, 66.6% at visit moment 4 and 75% at the last visit moment.

Considering the age-adjusted Cox proportional regression, single women (HR 0.67, 95% CI, 0.49 – 0.92) or divorced (HR 0.53, 95% CI, 0.30 – 0.92), women using hormonal contraceptives (HR 0.72, 95% CI, 0.55 – 0.95) and have had 4 lifetime sexual partners (HR 0.55, 95% CI, 0.34 – 0.90) showed a delay in HPV clearance. The analyses considering HPV infection by HPV specific subgenus group showed in Subgenus group 2: Divorced women (HR 0.52, 95% CI, 0.29 – 0.98) women using hormonal contraceptives (HR 0.73, 95% CI, 0.56 – 0.97) and who did receptive oral sex (HR 0.75, 95% CI, 0.58 – 0.98) a delay in HPV clearance, the opposite situation was observed when women who had 11 years schooling or more (HR 2.36, 95% CI, 1.65 – 3.37) had a faster clearance rate compared to women with less than 11 years of schooling, when analyzed separately from the group with genotypes of HPV genotypes belonging to subgenus 2. Analyses performed with HPV clearance and sociodemographic characteristics or sexual behavior by group 1 and 3 subgenus showed no correlation (Table 3).

4.3. Cervical cytokine profile

The concentrations of each studied cytokine were categorized into quartiles, from the lowest concentrations to the highest (Table 2S). We assessed the relationship between HPV infection and cytokines concentration in all the visit moments by considering sociodemographic characteristics, clinic history, lifestyle habits and by considering grouped HPV genotypes per their phylogenetic relationship within the genus Alpha-papillomavirus. Considering any HPV infection, no associations between IL-1, 6, 10, 12 and TNF with HPV clearance were found, only IL-4 showed a positive clearance correlation (HR 2.40: 1.11-5.21 95% CI) in the quartile patients with the highest concentrations of cytokines. No association was observed in the models considering HPV subgenus 1, 2 and 3 separately or in the logistic regression model for 14 HR-HPV types with a second visit at around 9-15 months (Table 4).

4.4. Clinical follow-up perspective for HR-HPV persistence

Restricting to women positive for any of the 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), with a second visit at around 9-15 months (clinical follow-up perspective, time to allow for HPV persistence) associations between sexual behaviors characteristics at baseline and one-year period prevalence of HPV infection as a function of covariates, only hormonal contraceptive use women (OR 2.01, 95% CI 1.13 – 3.58) showed statistically significant viral persistence (Tables 5 and 6).

5. Discussion

In this study we demonstrated a high prevalence of HPV infection in our population and this prevalence rate is higher than that determined by other studies conducted in Brazil[27-30]. Even in subsequent visits where women positive for at least one HPV genotype at the time of inclusion had persistence rates above 50 percent of the women included at each visit. It is believed that HPV infections "clear" within 2 years in more than 90% of individuals[31-34]. Regarding this finding, we have to consider that the median age of our cohort was 29 years-old (15-50) among HPV-positive women, and several studies have demonstrated higher rates of HPV infection in younger women[35-37] suggesting that the prevalence of HPV infection decreases with the increasing age[8, 28, 34].

The overall prevalence of multiple HPV genotype infection was 42%. Among these women, 62% were infected with at least one high-risk HPV genotype (data not shown). The prevalence of multiple-type infection observed in our cohort was higher than previously reported by other epidemiological studies [38-40]. The most prevalent HPV genotype in multiple-type infection was HPV-16, present in 24.77% of the samples.

According to several epidemiological studies, there are certain well-known risk factors for HPV infection[41-44]. Most of these are related to sexual behaviors, which determine exposure to HPV. In our cohort, we did not observe an increased risk of HPV and *Chlamydia trachomatis* coinfection in women who had more than 1 partner in the year preceding evaluation as described in other studies[42, 43]. However, accurate information is difficult to obtain.

Therefore, we could access HPV prevalence and the determinants of HPV clearance in a time period of 24 months follow-up. We evaluated time to clearance of HPV infection in relation to covariates such as sociodemographic characteristics, clinic history, lifestyle habits, sexual behaviors and cytokines levels. Several studies have shown high HPV prevalence, especially in low developed countries, compared with the estimated global prevalence[45, 46].

The clearance of anogenital HPV infection has been attributed mainly to a successful adaptive immune response, suggesting that HPV clearance is mainly related to the host immune response or other intrinsic factors of the host and does not relate to behavioral factors[47].

Factors independently related to HPV clearance, considering the liberal perspective were, hormonal contraceptive use, four lifetime sexual partners, single and divorced women.

All this sociodemographic characteristics had a lower time of clearance of HPV infections compared to women who did not use hormonal contraceptives, who had less than 4 lifetime sexual partners, and married women/ living with a partner. We did the same exercise considering factors independently related to; HPV Subgenus 2: HPVs 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73 and 82 HPV clearance and hormonal contraceptive use, receptive oral sex activity and divorced women had a lower time of clearance of HPV Subgenus 2 infections, on the other hand, women who had been in school for 11 years or more had a faster HPV Subgenus 2 time of clearance compared to women with lower schooling.

Cytokines are a diverse family of soluble small proteins, different cells and tissue can express types that act as immune mediators[48]. We found that while most cytokines were not associated with HPV time clearance, considering Age-adjusted hazards ratios (95% confidence intervals) of HPV liberal clearance (defined as clearance of HPV infection in at least one consecutive visits) as a function of the highest cervical IL-4 concentration quartile in our study population (230.09 pg/ml) was associated with increased HPV clearance. Secreted mainly by activated Th2 lymphocytes IL-4 exerts a diversity of effects on different cell types, including mast cells, monocytes, B-lymphocytes, and keratinocytes[49]. According to (Lembo et al., 2006)[50] IL-4 were added to the list of soluble factors with the potential to control HPV infections and the growth of HPV-associated carcinomas. Furthermore, expression of IL-4 demonstrated a downregulation of E6 and E7 levels also showing an+ active in suppressing HPV-16 LCR-driven transcriptional activity[51]. In addition, the role that IL-4 can play in the clearance of the virus from infected tissues and in the control of persistent HPV infections prior to the transformation of malignant cells merits further investigation.

There are two major limitations in this study that could be addressed in future research. First, the study had a loss in the number of patients during follow-up, and a second limitation is that we cannot accurately determine the initial moment of infection, this time between infection and detection by the study may probably influence clearance time. ON the other hand, the present study is a cohort, conducted in a population of low risk and low income, with a high prevalence of HPV. The study was conducted using high sensitivity and gold standard techniques for detection of infections and abnormal vaginal microbiota.

In conclusion, we described the prevalence of infections due to HPV, *Chlamydia trachomatis, Neisseria gonorrhoeae* besides vaginal flora patterns characterization in a

cohort in southeastern Brazil. According to our results, the overall STIs prevalence rates are high, suggesting that screening programs to detect these infections could be helpful in establishing prevention strategies in our population. Although many sociodemographic, behavioral and gynecology characteristics were tested, the vast majority of them were not significantly associated with HPV clearance.

6. References

1. Human papillomavirus vaccines. WHO position paper. Wkly Epidemiol Rec. 2009;84:118–131.

2. León-Maldonado L, Wentzell E, Brown B, at al. Perceptions and Experiences of Human Papillomavirus (HPV) Infection and Testing among Low-Income Mexican Women. PLoS One. 2016;11(5):e0153367.

3. Baudu A, Prétet JL, Riethmuller D, et al. Prevalence and risk factors of human papillomavirus infection types 16/18/45 in a cohort of French females aged 15-23years. J Epidemiol Glob Health 2014; 4:35-43.

4. Lee H, Lee DH, Song YM, et al. Risk factors associated with human papillomavirus infection status in a Korean cohort. Epidemiol Infect 2014;142: 1579-1589.

5. Figueiredo Alves RR, Turchi MD, Santos LE, et al. Prevalence, genotype profile and risk factors for multiple human papillomavirus cervical infection in unimmunized female adolescents in Goiânia, Brazil: a community-based study. BMC Public Health 2013; 13:1041.

6. Kotloff KL, Wasserman SS, Russ K, et al. Detection of genital human papillomavirus and associated cytological abnormalities among college women. Sex Transm Dis 1998;25:243-50.

7. Doorbar J, Egawa N, Griffin H, et al. Human papillomavirus molecular biology and disease association. Rev Med Virol. 2015;25 Suppl 1:20-23.

8. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006; 1:S1-15.

9. Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int J Cancer 2006; 118:3030-3044.

10. Burd E M. Human Papillomavirus and Cervical Cancer. Clin Microbiol Rev. 2003;16 (1): 1-17.

11. McCloskey JC, Kast WM, Flexman JP, et al. Syndemic synergy of HPV and other sexually transmitted pathogens in the development of high-grade anal squamous intraepithelial lesions. Papillomavirus Res. 2017;4:90-98.

12. Mzarico E, Gómez-Roig MD, Guirado L, et al. Relationship between smoking, HPV infection, and risk of Cervical cancer. Eur J Gynaecol Oncol. 2015;36(6):677-680.

13. Pontillo A, Bricher P, Leal VN, et al. Role of inflammasome genetics in susceptibility to HPV infection and cervical cancer development. J Med Virol. 2016;88(9):1646-1651.

14. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol 2005; 1:S16-24.

15. Tota JE, Ramanakumar AV, Villa LL, et al. Cervical Infection With Vaccine-Associated Human Papillomavirus (HPV) Genotypes as a Predictor of Acquisition and Clearance of Other HPV Infections. J Infect Dis. 2016; 214(5): 676–684.

16. Crosbie EJ, Einstein MH, Franceschi S, et al. Human papillomavirus and cervical cancer. Lancet. 2013;382(9895):889-899.

17. Welters MJ, de Jong A, van den Eeden SJ, et al. Frequent display of human papillomavirus type 16 E6- specific memory T-helper cells in the healthy population as witness of previous viral encounter. Cancer Res 2003;63:636-641.

18. Audirac-Chalifour A, Torres-Poveda K, Bahena-Román M, et al. Cervical Microbiome and Cytokine Profile at Various Stages of Cervical Cancer: A Pilot Study. PLoS One. 2016;11(4):e0153274.

19. Spellberg B, Edwards JE. Type1/type 2 immunity in infectious diseases. Clin Infect Dis 2001;32:76–102.

20. Bolpetti AN, Pinto GVS, Villa LL, El-Zein M, Franco EL, Silva MG. Association between Human Papillomavirus (HPV) and Chlamydia trachomatis in women attending cervical screening in the Southeast of Brazil. (manuscrito em preparação).

21. Nugent RP, Krohn MA, Hillier S L. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. J Clin Microbiol. 1991; 29:297-301.

22. Bauer HM, Ting Y, Greer CE, et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. JAMA. 1991;23-30;265(4):472-477

23. Griffais R, Thibon M. Detection of Chlamydia trachomatis by the polimerase chain reaction. Res Microbiol. 1989;140(2):139-141.

24. Ho BS, Feng WG, Wong B K, et al. Polymerase chain reaction for the detection of Neisseria gonorrhoeae in clinical samples. J Clin Pathol. 1992;45(5):439-442.

25. Sobel JD, Faro S, Force RW, et al. Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. Am J Obstet Gynecol. 1998;178(2):203-211.

26. Solomon D, Davey D, Kurman R, et al. Forum Group Members; Bethesda 2001 Workshop. The 2001 Bethesda System: terminology for reporting results of cervical cytology. Journal of American Medical Association 2002; 287: pp. 2114-2119.

27. Franco E L, Villa L L, Sobrinho J P, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis. 1999;180:1415-1423.

28. de Sanjosé S, Diaz M, Castellsagué X, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis. 2007;7:453-459.

29. Rosa MI, Fachel JM, Rosa DD, et al. Persistence and clearance of human papillomavirus infection: a prospective cohort study. Am J Obstet Gynecol. 2008;199:617.e1-7.

30. Miranda PM, Pitol BC, Moran MS, et al. Human papillomavirus infection in Brazilian women with normal cervical cytology. Genet Mol Res 2012; 11:1752-1761.

31. Gravitt P E. The known unknowns of HPV natural history. J Clin Invest. 2011;121(12):4593-4599.

32. Franco EL, Villa LL, Sobrinho JP, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis. 1999;180(5):1415-1423.

33. Woodman CBJ, Collins S, Winter H, et al. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet. 2001;357(9271):1831-1836.

34. Munoz N, Mendez F, Posso H, et al. Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results. J Infect Dis. 2004;190(12):2077-2087.

35. Howell-Jones R, de Silva N, Akpan M, et al. Prevalence of human papillomavirus (HPV) infections in sexually active adolescents and young women in England, prior to widespread HPV immunization. Vaccine 2012;30:3867-3875.

36. Mollers M, Hein JB, Henrike JV, et al. Prevalence, incidence and persistence of genital HPV infections in a large cohort of sexually active young women in the Netherlands. Vaccine 2013;31:394-401.

37. Smith MA, Tellier PP, Roger M, et al. Determinants of Human Papillomavirus coinfections among Montreal University Students: The Influence of Behavioral and Biologic Factors. Cancer Epidemiol Biomarkers. Prev 2014; 23:812-822.

38. Giuliano A R, Harris R, Sedjo R L, et al. Incidence, prevalence, and clearance of type-specific human papillomavirus infections: The Young Women's Health Study. J Infect Dis. 2002;186:462-469.

39. Molano M, Posso H, Weiderpass E, et al. HPV Study Group HPV Study. Prevalence and determinants of HPV infection among Colombian women with normal cytology. Br J Cancer. 2002;87:324-333.

40. Goodman MT, Shvetsov YB, McDuffie K, et al. Prevalence, acquisition, and clearance of cervical human papillomavirus infection among women with normal cytology: Hawaii Human Papillomavirus Cohort Study. Cancer Res. 2008; 68:8813-8824.

41. Herrero R, Castle PE, Schiffman M, et al. Epidemiologic profile of type-specific human papillomavirus infection and cervical neoplasia in Guanacaste, Costa Rica. J Infect Dis 2005;191:1796-1807.

42. Winer RL, Lee SK, Hughes JP, et al. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003;157:218-226.

43. Vinodhini K, Shanmughapriya S, Das BC, et al. Prevalence and risk factors of HPV infection among women from various provinces of the world. Arch Gynecol Obstet 2012;285:771-777.

44. Baudu A, Prétet J L, Riethmuller D, et al. Prevalence and risk factors of human papillomavirus infection types 16/18/45 in a cohort of French females aged 15-23 years. J Epidemiol Glob Health. 2014;4:35-43.

45. Bruni L, Diaz M, Castellsagué X, et al. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis. 2010;202(12):1789-1799.

46. Forman D, de Martel C, Lacey C J, et al. Global burden of human papillomavirus and related diseases. Vaccine. 2012;30 Suppl 5:F12-23.

47. Schmeink C E, Massuger L F, Lenselink C H, et al. Prospective follow-up of 2,065 young unscreened women to study human papillomavirus incidence and clearance. Int J Cancer. 2013 Jul;133(1):172-181.

48. Turner MD, Nedjai B, Hurst T, et al. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochim Biophys Acta. 2014 Nov;1843(11):2563-2582.

49. Nelms K, Keegan AD, Zamorano J, et al. The IL-4 receptor: signaling mechanisms and biologic functions. Annu Rev Immunol. 1999;17:701-738.

50. Lembo D, Donalisio M, De Andrea M, Cornaglia M, Scutera S, Musso T, et al. A cell-based high-throughput assay for screening inhibitors of human papillomavirus-16 long control region activity. FASEB J. 2006 Jan;20(1):148-150.

51. Donalisio M, Cornaglia M, Landolfo S, et al. TGF-beta1 and IL-4 downregulate human papillomavirus-16 oncogene expression but have differential effects on the malignant phenotype of cervical carcinoma cells. Virus Res. 2008;132(1-2):253-256.

Article tables:

Table 1. Sociodemographic, behavioral and gynecological history characteristics of the participants at enrollment (n=544).

Characteristic	Categories	n (%)
Age	≤32 years	354 (65.1)
	>32 years	189 (34.7)
	Missing	1 (0.2)
Marital status	Married	147 (27.0)
	Single	241 (44.3)
	Living with a partner	109 (20.0)
	Divorced	39 (7.2)
	Widow	5 (0.9)
	Missing	3 (0.5)
Ethnicity	White	322 (59.2)
	Black	50 (9.2)
	Indian	3 (0.5)
	Mixed	164 (30.1)
	Asian	5 (0.9)
Type of residence	Urban	509 (93.6)
	Rural	35 (6.5)
School level	None	4 (0.7)
	≤11 years	449 (82.5)
	>11	90 (16.5)
	Missing	1 (0.2)
Monthly family Income in US $ (quartiles)	Q1	151 (27.8)
	Q2	117 (21.5)
	Q3	137 (25.2)
	Q4	131 (24.1)
	Missing	8 (1.5)
Age at menarche	≤13 years	370 (68.0)
	>13 years	171 (31.4)
	Missing	3 (0.5)
Age at first intercourse	≤16 years	318 (58.5)
	>16 years	225 (41.4)
	Missing	1 (0.2)
Active sex life	No	41 (7.5)
	Yes	503 (92.5)
Hormonal contraceptive	No	229 (42.1)
	Yes	315 (57.9)
Condom use	No	377 (69.3)
	Yes	167 (30.7)
Number of lifetime sex partners	1	92 (16.9)
	2	89 (16.4)
	3	107 (19.7)
	4	67 (12.3)
	≥ 5	167 (30.7)

	Missing	22 (4.0)
Sexual orientation	Heterosexual	533 (98.0)
	Bisexual	9 (1.6)
	Missing	2 (0.4)
Previous pregnancy	No	158 (29.0)
	Yes	386 (71.00)
Anal sex	No	386 (71.00)
	Yes	150 (27.6)
	Missing	8 (1.5)
Douching	Yes, always	19 (3.5)
	Yes, frequently	11 (2.0)
	Never	514 (94.5)
Smoking	No	371 (68.2)
	Yes	172 (31.6)
	Missing	1 (0.2)
Alcohol	No	285 (52.4)
	Yes	258 (47.4)
	Missing	1 (0.2)
Drugs	No	512 (94.1)
	Yes	22 (4.0)
	Missing	10 (1.8)
Previously sexually transmitted disease	No	489 (89.9)
	Yes	55 (10.1)

Table 2. Clinical characteristics (n (%)) at baseline and follow-up visits .

	Visit 1 n= 544	**Visit 2 n=462**	**Visit 3 n= 362**	**Visit 4 n= 242**	**Visit 5 n= 105**
Cytology					
NILM	496 (91.1)	436 (94.4)	331 (91.4)	217 (89.6)	102 (97.1)
ASC-US	13 (2.3)	13 (2.8)	20 (5.5)	13 (5.3)	2 (1.9)
LSIL	26 (4.7)	9 (1.9)	4 (1.1)	7 (2.8)	1 (0.9)
HSIL	9 (1.6)	3 (0.6)	7 (1.9)	5 (2.0)	-
Missing	-	1 (0.2)	-	-	-
Chlamydia trachomatis					
Negative	469 (86.2)	409 (88.5)	330 (91.1)	236 (97.5)	99 (94.5)
Positive	74 (13.6)	45 (9.7)	25 (6.9)	3 (1.2)	4 (3.8)
Missing	1 (0.01)	8 (1.7)	7 (1.9)	3 (1.2)	2 (1.9)
Trichomonas vaginalis					
Negative	538 (98.9)	-	-	-	-
Positive	6 (1.1)	-	-	-	-
Vaginal microbiota					
Normal	284 (52.2)	258 (55.8)	219 (60.4)	157 (64.8)	67 (63.8)
Bacterial vaginosis	182 (34.3)	124 (26.8)	63 (17.4)	33 (13.6)	27 (25.7)
Intermediate flora	28 (5.1)	16 (3.4)	16 (4.4)	7 (2.8)	5 (4.7)
Aerobic vaginitis	-	1 (0.2)	-	-	-
BV and Candidiasis	5 (0.9)	10 (2.1)	3 (0.8)	-	-
Flora I and Candidiasis	22 (4.0)	18 (3.8)	11 (3.0)	1 (0.4)	2 (1.9)
Absent flora	10 (1.8)	-	3 (0.8)	1 (0.4)	2 (1.9)
Other	4 (0.7)	-	-	-	-
Not determined	-	18 (3.8)	3 (0.8)	2 (0.8)	2 (1.9)
Missing	9 (1.6)	17 (3.6)	44 (12.1)	41 (16.9)	-

NILM - *Negative for Intraepithelial Lesion and Malignancy*. **ASC-US -** *Atypical squamous cells of undetermined significance*. **HSIL -** *High grade squamous intraepithelial lesion*. **LSIL -** *Low-grade squamous intraepithelial lesion*.

Table 3. Age-adjusted hazards ratios (95% confidence intervals) of HPV liberal clearance (defined as clearance of an HPV infection in at least one consecutive visit) as a function of covariates at baseline, n=554.

Characteristics at baseline	N	Any HPV n=247	n	Subgenus 1 n=64	n	Subgenus 2 n=228	N	Subgenus 3 n=147
Age								
≤ 32 years	146	1.00	53	1.00	147	1.00	95	1.00
> 32 years	100	0.72 (0.45 – 1.15)	11	0.81 (0.28- 2.32)	81	0.63 (0.39 – 1.02)	51	1.40 (0.73 – 2.69)
Marital status								
Married	84	1.00	16	1.00	70	1.00	28	1.00
Single	96	0.67 (0.49 - 0.92)	34	0.99 (0.50 – 1.97)	96	0.76 (0.55 – 1.06)	75	0.81 (0.50 - 1.30)
Living with a partner	47	0.74 (0.51 – 1.07)	8	0.94 (0.39 – 2.31)	44	0.70 (0.48 – 1.03)	29	0.99 (0.58 – 1.68)
Divorced	15	0.53 (0.30 – 0.92)	6	1.09 (0.42 – 2.80)	14	0.52 (0.29 – 0.93)	11	0.83 (0.41 – 1.67)
Widow	3	1.34 (0.42 – 4.24)	0	1 (omitted)	3	1.54 (0.48 – 4.93)	3	1.30 (0.39 – 4.30)
Ethnicity								
White	144	1.00	30	1.00	135	1.00	82	1.00

Black	23	1.30 (0.82 – 2.02)	8	1.33 (0.57 – 3.12)	17	0.92 (0.37 – 1.60)	15	1.26 (0.71 – 2.22)
Indian	1	0.40 (0.06 – 2.87)	0	1 (omitted)	0	2.21 (0 – .)	2	0.99 (0.24 – 4.03)
Mixed	76	0.92 (0.70 – 1.22)	26	1.28 (0.75 – 2.20)	73	0.87 (0.65 – 1.15)	46	1.05 (0.73 – 1.51)
Asian	3	2.40 (0.76 – 7.55)	0	1 (omitted)	3	1.66 (0.53 – 5.22)	2	1.77 (0.43 – 7.25)
School level (years)								
None	1	1.00	0	1.00	0	1.00	2	1.00
≤ 11	201	2.83 (0.39 – 20.28)	56	1.28 (0.61 – 2.70)	192	2.36 (1.65 – 3.37)	118	0.74 (0.18 – 3.06)
>11	44	2.61 (0.36 – 19.04)	8	1 (omitted)	36	2.24 (. - .)	26	0.58 (0.14 – 2.51)
Monthly family Income								
R$ 0-930	74	1.00	23	1.00	66	1.00	44	1.00
R$ 931-1500	65	0.85 (0.61 – 1.19)	12	0.30 (0.14 – 0.62)	69	1.00 (0.72 – 1.42)	39	0.91 (0.59 – 1.40)
R$ 1501-2000	42	0.81 (0.55 – 1.18)	11	0.69 (0.33 – 1.42)	41	0.88 (0.59 – 1.29)	21	1.03 (0.61 – 1.74)
R$ 2001-20000	65	0.94 (0.67 – 1.31)	17	0.69 (0.36 – 1.31)	51	0.92 (0.64 – 1.33)	39	1.08 (0.70 – 1.67)

Monthly family Income US dollar (quartiles)								
Q1	74	1.00	23	1.00	66	1.00	44	1.00
Q2	46	0.78 (0.54 – 1.12)	11	0.30 (0.14 – 0.63)	50	0.97 (0.67 – 1.40)	29	0.77 (0.48 – 1.23)
Q3	64	0.87 (0.62 – 1.22)	13	0.65 (0.33 – 1.30)	63	0.94 (0.66 – 1.33)	36	1.20 (0.77 – 1.88)
Q4	62	0.96 (0.68 – 1.34)	16	0.66 (0.34 – 1.27)	48	0.92 (0.64 – 1.34)	34	1.07 (0.68 – 1.68)
Age at Menarche								
≤ 13 years	146	1.00	43	1.00	155	1.00	100	1.00
>13 years	80	1.13 (0.87 – 1.48)	21	1.23 (0.72 – 2.10)	72	1.06 (0.80 – 1.41)	45	1.01 (0.71 – 1.43)
Age at first sexual intercourse								
≤ 16 years	141	1.00	37	1.00	131	1.00	80	1.00
>16 years	105	0.94 (0.73 – 1.22)	27	1.33 (0.79 – 2.23)	97	0.96 (0.73 – 1.25)	66	0.97 (0.70 – 1.35)
Active sex life								
No	20	1.00	2	1.00	16	1.00	14	1.00
Yes	227	1.09 (0.68 – 1.75)	62	1.22 (0.29 – 5.10)	212	1.22 (0.72 – 2.06)	133	0.63 (0.36 – 1.11)

Hormonal contraceptive								
No	112	1.00	23	1.00	95	1.00	56	1.00
Yes	135	0.72 (0.55 – 0.95)	41	0.66 (0.38 – 1.14)	133	0.73 (0.56 – 0.97)	91	0.95 (0.67 – 1.35)
Hormonal contraceptive (sexlife ajusted)								
No	112	1.00	23	1.00	95	1.00	56	1.00
Yes	135	0.72 (0.55 – 0.94)	41	0.66 (0.38 – 1.14)	133	0.73 (0.55 – 0.96)	91	0.98 (0.96 – 1.40)
Condom use								
No	170	1.00	40	1.00	163	1.00	90	1.00
Yes	77	1.07 (0.82 – 1.40)	24	1.16 (0.68 – 1.97)	65	0.99 (0.74 – 1.31)	57	1.29 (0.92 – 1.80)
Number of lifetime sex partners								
1	45	1.00	12	1.00	39	1.00	14	1.00
2	37	0.77 (0.50 – 1.20)	12	0.73 (0.33 – 1.64)	35	0.82 (0.52 – 1.30)	27	1.37 (0.72 – 2.62)
3	49	0.68 (0.45 – 1.02)	18	1.20 (0.57 – 2.56)	51	0.71 (0.47 – 1.08)	30	1.37 (0.72 – 2.60)
4	26	0.55 (0.34 – 0.90)	4	0.74 (0.24 – 2.31)	27	0.66 (0.40 – 1.08)	22	1.28 (0.65 – 2.53)
≥5	81	0.82 (0.57 – 1.18)	15	0.61 (0.28 – 1.31)	66	0.77 (0.52 – 1.14)	49	1.45 (0.80 – 2.64)

Sexual orientation								
Just with men	241	1.00	63	1.00	233	1.00	142	1.00
With men and women	6	1.51 (0.67 – 3.42)	1	4.58 (0.60 – 35.00)	5	1.44 (0.59 – 3.51)	5	0.86 (0.35 – 2.12)
Previous pregnancy								
No	57	1.00	26	1.00	59	1.00	48	1.00
Yes	190	1.20 (0.87 – 1.66)	38	1.38 (0.75 – 2.54)	169	1.03 (0.75 – 1.41)	99	1.24 (0.83 – 1.86)
Receptive oral sex								
No	123	1.00	29	1.00	117	1.00	61	1.00
Yes	123	0.88 (0.69 – 1.14)	35	0.84 (0.51 – 1.39)	109	0.75 (0.58 – 0.98)	85	1.10 (0.79 – 1.55)
Active oral sex								
No	125	1.00	38	1.00	114	1.00	69	1.00
Yes	121	0.98 (0.76 – 1.27)	26	0.66 (0.40 – 1.10)	112	0.90 (0.69 – 1.17)	77	1.06 (0.76 – 1.48)
Anal sex								
No	169	1.00	46	1.00	156	1.00	98	1.00
Yes	75	1.01 (0.77 – 1.33)	18	1.22 (0.70 – 2.14)	69	0.93 (0.70 – 1.24)	46	1.26 (0.88 – 1.79)

Douching								
Never	215	1.00	58	1.00	200	1.00	126	1.00
Yes, sometimes	15	1.12 (0.66 – 1.90)	2	0.69 (0.14 – 3.40)	13	1.10 (0.63 – 1.94)	9	1.08 (0.54 – 2.15)
Yes, always	17	0.73 (0.44 – 1.20)	4	1.62 (0.58 – 4.53)	15	0.69 (0.40 – 1.17)	12	1.22 (0.67 – 2.22)
Smoking history								
No	164	1.00	50	1.00	150	1.00	109	1.00
Yes	82	1.19 (0.91 – 1.55)	14	0.98 (0.53 – 1.81)	78	1.25 (0.95 – 1.65)	37	1.18 (0.80 – 1.73)
Alcohol								
No	132	1.00	32	1.00	121	1.00	72	1.00
Yes	114	1.09 (0.85 – 1.40)	32	0.84 (0.51 – 1.39)	107	1.07 (0.82 – 1.39)	74	0.98 (0.70 – 1.36)
Drugs use								
No	231	1.00	60	1.00	216	1.00	139	1.00
Yes	9	1.21 (0.62 – 2.36)	1	0.82 (0.11 – 6.02)	9	1.20 (0.61 – 2.35)	5	1.75 (0.71 – 4.31)
Previous sexual disease								
No	225	1.00	53	1.00	205	1.00	131	1.00
Yes	22	0.71 (0.46 – 1.11)	11	0.68 (0.35 – 1.32)	23	0.79 (0.51 – 1.22)	16	0.86 (0.51 – 1.45)

Flora status								
Normal flora	130	1.00	28	1.00	116	1.00	81	1.00
Abnormal flora	115	1.13 (0.88 – 1.46)	36	1.43 (0.86 – 2.37)	110	1.05 (0.81 – 1.37)	64	0.86 (0.61 – 1.21)
Chlamydia trachomatis								
Negative	215	1.00	51	1.00	194	1.00	128	1.00
Positive	31	0.85 (0.58 – 1.24)	13	1.57 (0.84 – 2.93)	33	1.01 (0.70 – 1.47)	18	0.68 (0.41 – 1.11)
Trichomonas vaginalis								
Negative	245	1.00	63	1.00	224	1.00	147	1.00
Positive	2	0.63 (0.16 – 2.55)	1	1.31 (0.17 – 9.89)	4	0.99 (0.37 – 2.68)	0	4.84 (0 – .)
Residence								
Urban	225	1.00	60	1.00	205	1.00	137	1.00
Rural	22	1.19 (0.77 – 1.85)	4	1.38 (0.49 – 3.88)	23	1.27 (0.82 – 1.96)	10	0.80 (0.42 – 1.54)
Pap test								
No	64	1.00	8	1.00	12	1.00	10	1.00
Yes	182	0.80 (0.42 – 1.53)	56	0.55 (0.25 – 1.23)	216	0.79 (0.43 – 1.44)	137	0.90 (0.46 – 1.78)
Vaginal wash before the								

relation								
No	64	1.00	12	1.00	50	1.00	37	1.00
Yes	182	0.73 (0.55 – 0.97)	52	1.29 (0.69 – 2.43)	176	0.75 (0.55 – 1.03)	108	0.73 (0.50 – 1.07)
Vaginal wash after the relation								
No	13	1.00	0	1.00	12	1.00	8	1.00
Yes	233	0.96 (0.55 – 1.69)	64	4.34 (. – .)	214	0.88 (0.49 – 1.57)	138	0.99 (0.48 – 2.03)

Table 4. HPV liberal clearance (n=247) as a function of cytokines concentration-adjusted cox regression (N=554). Clearance of an HPV infection in at least one consecutive visit.

Cytokines	**n**	**Any HPV n=247**	**n**	**Subgenus 1 n=64**	**n**	**Subgenus 2 n=121**	**N**	**Subgenus 3 n=147**
IL-1β								
Q1	31	1.00	3	1.00	26	1.00	22	1.00
Q2	21	0.57 (0.32 – 0.99)	7	0.46 (0.11 – 1.91)	19	0.85 (0.47 – 1.54)	13	0.54 (0.27 – 1.08)
Q3	27	0.85 (0.50 – 1.43)	6	0.40 (0.09 – 1.81)	25	0.91 (0.52 – 1.60)	17	0.93 (0.49 – 1.76)
Q4	35	1.32 (0.81 – 2.17)	7	0.85 (0.20 – 3.55)	29	1.29 (0.75 – 2.21)	14	0.88 (0.45 – 1.73)
IL-4								
Q1	110	1.00	21	1.00	97	1.00	67	1.00
Q2	0	-	-	-	-	-	-	-
Q3	0	-	-	-	-	-	-	-
Q4	7	2.40 (1.11 – 5.21)	4	0.99 (0.34 – 2.94)	6	2.26 (0.98 – 5.22)	3	2.12 (0.66 – 6.81)
Q1	32	1.00	8	1.00	29	1.00	20	1.00
Q2	31	1.21 (0.74 – 1.98)	5	1.29 (0.40 – 4.10)	23	0.79 (0.46 – 1.37)	18	1.59 (0.82 – 3.06)
Q3	24	0.80 (0.47 – 1.37)	5	0.87 (0.27 – 2.80)	23	0.66 (0.38 – 1.15)	18	1.06 (0.55 – 2.03)

Q4	30	0.93 (0.56 – 1.54)	7	2.16 (0.78 – 6.02)	28	0.73 (0.43 – 1.24)	14	0.92 (0.46 – 1.82)
IL-10								
Q1	32	1.00	12	1.00	27	1.00	23	1.00
Q2	26	1.26 (0.75 – 2.11)	3	1.33 (0.37 – 4.84)	23	0.98 (0.56 – 1.71)	10	1.04 (0.49 – 2.21)
Q3	26	0.73 (0.43 – 1.24)	4	1.73 (0.53 – 5.65)	23	0.60 (0.34 – 1.05)	19	0.89 (0.48 – 1.63)
Q4	33	1.01 (0.62 – 1.65)	6	0.72 (0.26 – 1.98)	30	0.91 (0.54 – 1.55)	18	1.14 (0.61 – 2.13)
IL-12								
Q1	34	1.00	2	1.00	28	1.00	16	1.00
Q2	21	0.88 (0.51 – 1.53)	6	1.75 (0.35 – 8.75)	20	1.01 (0.57 – 1.80)	17	1.15 (0.58 – 2.28)
Q3	33	1.45 (0.89 – 2.37)	8	2.45 (0.48 – 12.59)	32	1.68 (1.00 – 2.83)	16	1.20 (0.60 – 2.41)
Q4	29	1.13 (0.68 – 1.87)	9	1.20 (0.25 – 5.74)	23	1.21 (0.69 – 2.12)	21	1.39 (0.72 – 2.69)
TNF-α								
Q1	98	1.00	21	1.00	88	1.00	58	1.00
Q2	0	-	-	-	-	-	-	-
Q3	0	-	-	-	-	-	-	-
Q4	19	0.79 (0.48 – 1.30)	4	0.66 (0.22 – 1.95)	15	0.64 (0.37 – 1.12)	12	1.11 (0.60 – 2.08)

Table 5. Univariate associations between subject characteristics and 9 - 15 months period prevalence of High-risk HPV infection as a function of covariates at baseline (n=239). Restricting to women positive for any of the 14 high-risk HPV types, with visits at around 9-15 months (clinical follow-up perspective, time to allow for HPV persistence)

Characteristics at baseline	n	HR-HPV n=152
Age		
≤ 32 years	106	1.00
> 32 years	46	1.91 (0.68 – 5.34)
Marital status		
Married	35	1.00
Single	72	1.35 (0.68 - 2.70)
Living with a partner	33	1.60 (0.71 – 3.64)
Divorced	11	1.14 (0.40 – 3.25)
Widow	1	0.29 (0.03 – 2.94)
Ethnicity		
White	79	1.00
Black	16	2.15 (0.73 – 6.32)
Indian	1	1 (empty)
Mixed	55	1.35 (0.76 – 2.42)
Asian	1	0.68 (0.04 – 11.34)
School level (years)		
≤ 11	123	1.00
>11	29	1.23 (0.61 – 2.49)
Monthly family Income US dollar (quartiles)		
Q1: 0-3,348	43	1.00
Q2: 3,471-5,208	32	1.15 (0.53 – 2.46)
Q3: 5,506-8,184	42	1.32 (0.64 – 2.72)
Q4: 8,556-55,800	34	1.09 (0.52 – 2.29)
Age at Menarche		
≤ 13 years	108	1.00
>13 years	44	0.74 (0.42 – 1.31)
Age at first sexual intercourse		
≤ 16 years	84	1.00
>16 years	68	1.34 (0.75 – 2.37)
Active sex life		
No	13	1.00
Yes	139	0.39 (0.12 – 1.29)
Hormonal contraceptive		
No	45	1.00
Yes	107	2.01 (1.13 – 3.58)
Hormonal contraceptive (sexlife ajusted)		
No	45	1.00
Yes	107	2.03 (1.13 – 3.63)
Condom use		
No	99	1.00
Yes	53	1.28 (0.71 – 2.30)

Number of lifetime sex partners		
1	30	1.00
2	23	0.66 (0.26 – 1.63)
3	31	0.78 (0.32 – 1.87)
4	21	1.40 (0.49 – 3.97)
≥5	45	0.82 (0.37 – 1.83)
Sexual orientation		
Just with men	149	1.00
With men and women	3	0.75 (0.12 – 4.86)
Previous pregnancy		
No	40	1.00
Yes	112	1.24 (0.65 – 2.37)
Receptive oral sex		
No	67	1.00
Yes	84	1.31 (0.77 – 2.25)
Active oral sex		
No	72	1.00
Yes	79	0.88 (0.51 – 1.52)
Anal sex		
No	116	1.00
Yes	35	0.91 (0.49 – 1.70)
Douching		
Never	136	1.00
Yes, sometimes	7	0.98 (0.28 – 3.52)
Yes, always	9	2.22 (0.57 – 8.72)
Smoking history		
No	108	1.00
Yes	44	1.01 (0.56 – 1.82)
Alcohol		
No	80	1.00
Yes	72	0.77 (0.44 – 1.31)
Drugs use		
No	142	1.00
Yes	8	4.05 (0.49 – 33.23)
Previous sexual disease		
No	137	1.00
Yes	15	1.45 (0.54 – 3.93)
Flora status		
Normal flora	80	1.00
Abnormal flora	67	0.67 (0.39 – 1.15)
Chlamydia trachomatis		
Negative	133	1.00
Positive	19	0.56 (0.27 – 1.17)
Trichomonas vaginalis		
Negative	149	1.00
Positive	3	2.17 (0.22 – 21.33)
Residence		
Urban	134	1.00
Rural	18	2.15 (0.76 – 6.07)

Pap test		
No	9	1.00
Yes	143	0.51 (0.10 – 2.46)
Vaginal wash before the relation		
No	24	1.00
Yes	126	1.55 (0.79 – 3.04)
Vaginal wash after the relation		
No	8	1.00
Yes	57143	0.23 (0.03 – 1.86)

Table 6. Univariate associations between cytokines levels and 9 - 15 months period prevalence of High-risk HPV infection as a function of covariates at baseline (n=239).

	IL-1β		IL-4		IL-6		IL-10		IL-12		TNF-α	
	n	**HR-HPV**	**N**	**HR-HPV**	**n**	**HR-HPV**	**n**	**HR-HPV**	**n**	**HR-HPV**	**n**	**HR-HPV**
Q1	17	1.00	71	1.00	18	1.00	21	1.00	19	1.00	62	1.00
Q2	15	1.10 (0.32 – 3.77)	-	-	16	1.26 (0.38 – 4.16)	17	1.17 (0.38 – 3.58)	20	2.85 (0.64 – 12.60)	-	-
Q3	22	2.85 (0.73 – 11.09)	-	-	18	1.74 (0.48 – 6.26)	24	2.53 (0.75 – 8.50)	20	0.67 (0.22 – 2.01)	-	-
Q4	16	0.63 (0.20 – 1.95)	3	0.34 (0.06 – 1.86)	22	0.97 (0.33 – 2.88)	12	0.75 (0.23 – 2.40)	15	0.68 (0.21 – 2.23)	12	1.06 (0.33 – 3.33)

Supplementary Tables and Figures:

	Screening (Pre-Enrolment:	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Inclusion/Exclusion: Eligibility Criteria	X	X	X	X	X	X
Informed Consent		X				
Enrolment Questionnaire		X				
Follow-up Questionnaire			X	X	X	X
Cervical Collection		X	X	X	X	X
HPV Typing and genotyping		X	X	X	X	X
***CT* Detection**		X	X	X	X	X
PAP test		X	X	X	X	X
***T. vaginalis* Detection**		X				
Vaginal Collection		X	X	X	X	X
Analysis of vaginal flora and treatment of the abnormal flora		X	X	X	X	X
Vaginal Wash Collection		X	X	X	X	X
Analysis of Cytokines		X	X	X	X	X
Medication Counselling of genital infection		X	X	X	X	X

Figure 1S. Study design detailing the visit moments and all laboratory procedures performed**.**

Table 1S. HPV prevalence**,** genotypic distribution of the 36 HPV genotypes found in our population and distribution according to HPV Alpha-papillomavirus subgenus groups at any visit moment.

HPV Genotype	**Visit 1 - Freq. (%) n= 1.635**	**Visit 2 - Freq. (%) n= 462**	**Visit 3 - Freq. (%) n= 362**	**Visit 4 - Freq. (%) n= 242**	**Visit 5 - Freq. (%) n=105**
Any HPV	544 (33.3)	323 (69.9)	211 (58.3)	146 (60.3)	58 (55.2)
HPV 6	18 (3.3)	11 (2.4)	7 (1.9)	2 (0.8)	3 (2.9)
HPV 11	7 (1.3)	4 (0.9)	2 (0.6)	3 (1.2)	0
HPV 16	97 (17.8)	63 (13.7)	37 (10.3)	29 (12.1)	6 (5.8)
HPV 18	31 (5.7)	21 (4.6)	14 (3.9)	9 (3.8)	4 (3.9)
HPV 26	9 (1.7)	10 (2.2)	3 (0.8)	4 (1.7)	1 (1.0)
HPV 31	39 (7.2)	28 (6.1)	21 (5.8)	13 (5.4)	2 (1.9)
HPV 33	8 (1.5)	5 (1.1)	2 (0.6)	3 (1.2)	1 (1.0)
HPV 35	22 (4.0)	17 (3.7)	12 (3.3)	9 (3.8)	5 (4.8)
HPV 39	17 (3.1)	22 (4.8)	11 (3.1)	4 (1.7)	2 (1.9)
HPV 40	6 (1.1)	2 (0.4)	1 (0.3)	0	0
HPV 42	13 (2.4)	6 (1.3)	3 (0.8)	4 (1.7)	0
HPV 45	43 (7.9)	17 (3.7)	11 (3.1)	3 (1.2)	4 (3.9)
HPV 51	34 (6.2)	18 (3.9)	12 (3.3)	6 (2.5)	2 (1.9)
HPV 52	46 (8.5)	26 (5.7)	17 (4.7)	13 (5.4)	5 (4.8)
HPV 53	54 (9.9)	39 (8.5)	16 (4.4)	10 (4.2)	4 (3.9)
HPV 54	34 (6.2)	24 (5.2)	13 (3.6)	11 (4.6)	2 (1.9)
HPV 55	13 (2.4)	5 (1.1)	5 (1.4)	3 (1.2)	2 (1.9)
HPV 56	26 (4.8)	16 (3.5)	9 (2.5)	7 (2.9)	2 (1.9)
HPV 58	45 (8.3)	27 (5.9)	17 (4.7)	12 (5.0)	2 (1.9)
HPV 59	29 (5.3)	21 (4.6)	7 (1.9)	3 (1.2)	3 (2.9)
HPV 61	52 (9.6)	31 (6.7)	19 (5.3)	16 (6.7)	8 (7.7)
HPV 62	50 (9.2)	39 (8.5)	24 (6.7)	14 (5.8)	4 (3.9)
HPV 64	1 (0.2)	0	0	0	0
HPV 66	52 (9.6)	25 (5.4)	10 (2.8)	5 (2.1)	2 (1.9)
HPV 67	9 (1.7)	4 (0.9)	2 (0.6)	3 (1.2)	1 (1.0)
HPV 68	21 (3.9)	12 (2.6)	6 (1.7)	3 (1.2)	0
HPV 69	4 (0.7)	2 (0.4)	0	0	0
HPV 70	22 (4.0)	11 (2.4)	7 (1.9)	7 (2.9)	3 (2.9)

HPV 71	9 (1.7)	3 (0.7)	3 (0.8)	3 (1.2)	0
HPV 72	10 (1.8)	8 (1.7)	3 (0.8)	4 (1.7)	1 (1.0)
HPV 73	26 (4.8)	16 (3.5)	11 (3.1)	4 (1.7)	1 (1.0)
HPV 81	28 (5.1)	19 (4.1)	10 (2.8)	4 (1.7)	1 (1.0)
HPV 82	16 (2.9)	13 (2.8)	3 (0.8)	4 (1.7)	4 (3.9)
HPV 83	20 (3.7)	9 (2.0)	7 (1.9)	3 (1.2)	3 (2.9)
HPV 84	30 (5.5)	17 (3.7)	8 (2.2)	3 (1.2)	1 (1.0)
HPV 89	30 (5.5)	17 (3.7)	11 (3.1)	8 (3.3)	2 (1.9)
HPV	83 (15.3)	44 (9.5)	30 (8.3)	21 (8.7)	7 (6.7)
Subgenus 1					
HPV	422 (77.6)	253 (54.8)	162 (44.8)	109 (45.0)	44 (41.9)
Subgenus 2					
HPV	197 (36.2)	120 (26.0)	71 (19.6)	48 (19.8)	16 (15.2)
Subgenus 3					

Table 2S. Concentrations of IL-1β, IL-4, IL-6, IL-10, IL-12 and TNF-α cytokines, median and range categorized into quartiles.

	IL-1β (pg/mL)	**IL-4 (pg/mL)**	**IL-6 (pg/mL)**	**IL-10 (pg/mL)**	**IL-12 (pg/mL)**	**TNF-α (pg/mL)**
Median (Range)	21.62 (0-459.4)	0 (0-230.09)	7.96 (0-530.27)	.371 (0-31.14)	21.27 (0-354.64)	0 0-1049.5
Quartile 1	60 (11.0)	241 (44.3)	63 (11.6)	75 (13.8)	63 (11.6)	207 (38.0)
Quartile 2	60 (11.0)	-	63 (11.6)	51 (9.4)	63 (11.6)	-
Quartile 3	60 (11.0)	-	63 (11.6)	63 (11.6)	63 (11.6)	-
Quartile 4	60 (11.0)	10 (1.8)	62 (11.4)	62 (11.4)	62 (11.4)	44 (8.1)
Not tested	304 (55.9)	293 (53.9)	293 (53.9)	293 (53.9)	293 (53.9)	293 (53.9)

Samples with abnormalities of flora have not been tested according to the Protocol, in this table they are shown as “Not tested”.

Anexos

QUESTIONÁRIO – Projeto HPV/*Chlamydia trachomatis*

Data da entrevista: dia_____ mês_____ ano_____ Horário:_____

Registro UBS:_____

Registro número no estudo:_____

Contatos telefônicos da entrevistada:_____

_____

Esta é a nossa primeira entrevista. As informações aqui obtidas são muito importantes para o nosso estudo. Gostaríamos que a senhora se sentisse a vontade para responder as nossas perguntas, não se preocupando com o que diz, porém sendo sincera. Devo informá-la que todas as respostas jamais serão reveladas a alguém. Agradecemos sua compreensão e colaboração com nossa pesquisa.

1. Qual o seu nome? _____
2. Qual a sua idade? _____ (Data de nascimento: dia____ mês____ ano_____)
3. Qual a cor da sua pele?

   [1] branca [2] negra [3] índia ou descendente

   [4] mulata ou parda [5] amarela
4. Qual seu estado civil?

   [1] casada [2] solteira [3] vive maritalmente

   [4] separada [5] viúva
5. Qual sua ocupação? _____
6. Quantos anos de aprovação escolar a senhora têm?_____
7. Na sua família, quantas pessoas contribuem para a renda mensal?_____
8. Qual a sua renda familiar? _____

   (Salário mínimo vigente – R$622,00 em Setembro de 2012)
9. Quantas pessoas vivem com esta renda? _____

10. Em que local a senhora mora?

[1] zona urbana/cidade [2] zona rural

11. Idade da primeira menstruação? _____

12. Quando foi a última menstruação? _____

13. Quais os métodos que utiliza para evitar gravidez?

[1] pílula anticoncepcional [2] D.I.U. [3] camisinha

[4] laqueadura [5] vasectomia [6] diafragma

[7] injeção hormonal [8] coito interrompido/ tabelinha [9] outro/não sabe

14. Se USA contraceptivo hormonal, há quanto tempo? _____

15. Se USOU contraceptivo hormonal, por quanto tempo? _____

16. História obstétrica: G____ P____ A____C_____

Quantas gestações a senhora teve? Quantos partos? Naturais ou cesáreas? Aborto?

17. Se teve algum aborto, quantos foram ESPONTANEOS_____ e quantos foram PROVOCADOS_____

18. No último ano, quantas vezes sentiu coceira na região genital?_____

[1] nenhuma [2] algumas vezes [3] muitas vezes

19. No último ano, quantas vezes sentiu dor/ardor na região genital? _____

[1] nenhuma [2] algumas vezes [3] muitas vezes

20. No último ano, quantas vezes teve corrimento vaginal? _____

[1] nenhuma [2] algumas vezes [3] muitas vezes

21. Na última semana, teve corrimento, coceira ou ardor na região genital?

[1] sim [2] não

22. A senhora fez uso de algum produto ginecológico na ultima semana?

[1] sim [2] não

23. Já fez exame de prevenção de câncer de colo do útero, o chamado Papanicolau?

[1] sim [2] não

24. Se sim, quantas vezes?_____

25. Se sim, quando foi a última vez? _____

[1] há menos de 1 ano [2] entre 1- 3 anos

[3] há mais de 3 anos [4] não se lembra

26. A senhora já teve algum exame de Papanicolau alterado?

[1] sim [2] não

27. Se sim, a senhora fez algum procedimento após este resultado?

[1] biópsia [2] cirurgia de alta frequência (CAF)

[3] conização [4] outro:_____

28. Já tomou a vacina contra HPV (vírus que causa o Câncer de Colo do Útero)?

[1] sim [2] não

29. Já teve alguma vez ferida na vagina ou vulva?

[1] sim [2] não

30. Alguma vez, soube por algum médico que tinha uma doença venérea ou sexualmente transmissível?

[1] sim [2] não

31. Se sim, qual?

[1] gonorréia [2] cancro [3] condiloma ou crista de galo

[4] sífilis [5] trichomoniase [6] herpes

[7] clamídia

32. Já teve alguma ferida no colo do útero que tenha sido diagnosticada por algum médico?

[1] sim [2] não

33. Se sim, recebeu tratamento para esta ferida?

[1] Não precisou [2] Não, ainda (está em seguimento)

[3] Não, recusou [4] Sim, cauterização

[5] Sim, não sabe o tratamento [6] Não sabe se foi tratada

Agora gostaria de lhe fazer algumas perguntas sobre sua vida intima, apesar de saber que é um assunto pessoal, gostaria de enfatizar que estas informações serão muito úteis para nossa pesquisa. A senhora deve saber que todas as respostas e informações aqui obtidas serão mantidas em total segredo, e nunca serão reveladas. Caso a senhora não queira responder qualquer pergunta, sinta-se a vontade para não responde-la.

34. Com que idade teve a primeira relação sexual? _____

35. A senhora tem vida sexual ativa?

[1] sim [2] não

36. Na sua vida, a senhora manteve/mantém relações sexuais com:

[1] somente com homens [2] com homens e mulheres [3] somente com mulheres

37. Durante toda a vida, com quantos parceiros manteve relações sexuais?_____

38. Durante toda sua vida, a senhora teve parceiros eventuais? Quantos?_____

39. Que a senhora saiba, quantos parceiros, dentre os fixos, NÃO foram fiéis, ou seja, quantos deles tiveram relações com outras mulheres? _____

40. No ultimo ano, com quantos parceiros manteve relações sexuais? _____

41. Com que freqüência tem relações sexuais?_____

42. O(s) seu(s) parceiro(s) tem o habito de praticar sexo oral em você?

[1] sim [2] não

43. A senhora pratica sexo oral no(s) seu(s) parceiro(s)?

[1] sim [2] não

44. Com quantos parceiros já praticou sexo oral? _____

45. A senhora pratica sexo ANAL?

[1] sim [2] não

46. Costuma lavar seus genitais ANTES de ter relações sexuais?

[1] sim [2] não

47. Costuma lavar seus genitais DEPOIS de ter relações sexuais?

[1] sim [2] não

48. Nos últimos meses se lembra de ter usado algum sistema que force água ou líquidos para o interior da vagina, tais como duchas ou bidês?

[1] sim, sempre [2] sim, com freqüência [4] sim, de vez em quando [5] nunca

49. A senhora usa preservativos durante a relação sexual?

[1] sim [2] não

50. Se sim, com que frequência?

[1] sempre, em todas as relações [2] somente com o(s) parceiro(s) eventual(s)

[3] somente com o(s) parceiro(s) fixo(s) [4] esporadicamente

51. A senhora fuma?

[1] sim [2] não

52. Se sim, quantos cigarros fuma em media por dia? _____

53. Se fumou, por quanto tempo? _____

54. A senhora consome bebida alcoólica?

[1] sim [2] não

55. Se sim, qual a freqüência que as consome?

[1] diariamente [2] semanalmente [3] mensalmente [4] raramente

56. Se sim, qual a quantidade? _____

57. A senhora utiliza alguma substancia?

[1] sim [2] não

58. Se sim, qual?

[1] maconha [2] cocaína [3] crack [4] cola [5] outra:_____

Nome da entrevistadora: _____

Unidade de Saúde: _____

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus Botucatu

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

Título da pesquisa: *"Avaliação da influencia da cervicite por Chlamydia trachomatis na persistência da infecção pelo Papilomavirus Humano (HPV) e no perfil de citocinas produzidas na secreção cervical de mulheres imunocompetentes"*

Nome da Instituição: Faculdade de Medicina de Botucatu – UNESP.

Nome dos Pesquisadores: Aline do Nascimento Bolpetti, Gabriel Vitor da Silva Pinto e Profa. Dra. Marcia Guimarães da Silva (Coordenadora)

A senhora esta sendo convidada a participar de um estudo no qual será pesquisada a influencia da infecção no trato genital causada pela bactéria *Chlamydia trachomatis* como co-fator para o risco de infecção persistente pelo Papilomavirus Humano (HPV). Assim como a infecção pelo vírus HPV, a infecção por *C. trachomatis* é uma das doenças transmitidas pelo ato sexual mais comum em todo o mundo. Trata-se de um estudo no qual as mulheres incluídas serão acompanhadas por um período de 24 meses (2 anos). Inicialmente, será realizado exame ginecológico e coleta de conteúdo vaginal para analise do padrão de bactérias encontradas no trato genital. A seguir a secreção cervical será coletada com uma escova similar a usada no exame de Papanicolaou, para a pesquisa do HPV e *C. trachomatis*. Uma vez identificada a presença do HPV, a sra será convidada a participar do estudo, e para isto será necessário que retorne a Unidade Básica de Saúde a cada 4 meses para nova coleta de secreção cervical. Desta forma, a coleta de material vaginal será feita 7 vezes durante o período de 2 anos do estudo, e nova coleta será feita somente no caso da amostra não se apresentar adequada. O presente estudo irá contribuir para um melhor entendimento do papel da *C. trachomatis* como co-fator para o risco de infecção persistente pelo HPV, sendo este vírus o principal fator de risco para o desenvolvimento do câncer de colo do útero.

A Sra terá acesso a todas as informações que julgar necessárias para melhor decidir sobre a sua participação ou não no estudo.

1. A participação é voluntária podendo se retirar do estudo a qualquer momento.
2. Para esta pesquisa serão cedidas regularmente amostras de secreção cervical, que serão obtidas conforme a necessidade para os experimentos propostos. Durante a coleta haverá um pequeno desconforto local igual ao de um exame ginecológico de rotina.
3. A sra terá garantido o sigilo de sua identidade e privacidade com relação aos resultados obtidos a partir de suas amostras biológicas.
4. Ao ser selecionada para participar do estudo e ter que retornar a Unidade Básica de Saúde a cada 4 meses, a sra receberá valor do transporte como bonificação.

As informações acima devem estar claras e o propósito do estudo entendido. Por esse motivo, a sra autoriza o pesquisador abaixo indicado e sua equipe de pesquisa a coletar e processar as amostras biológicas, bem como a levantar as informações que julgar relevantes.

Ao assinar esse termo, a sra estará fornecendo seu consentimento livre e esclarecido para participar do estudo. A sra receberá uma cópia deste documento assinado e datado. Ao assinar este termo, **a sra não estará**

desistindo de nenhum dos seus direitos previstos em lei. Por fim, a sra poderá a qualquer momento fazer as perguntas que desejar sobre o estudo, bastando entrar em contato com as pessoas indicadas.

Eu, _____ ao assinar este Termo de Consentimento Livre e Esclarecido, autorizo o pesquisador e sua equipe de pesquisa a coletar e processar minhas amostras biológicas, assim como a levantar as informações que julgar relevantes para o estudo.

Eu, abaixo assinado, expliquei todos os detalhes relevantes deste estudo para a voluntária identificada acima e forneci a ela uma cópia deste Termo de Consentimento Livre e Esclarecido datado e assinado.

_____
Aline Bolpetti
Nome legível do pesquisador, assinatura e data
E-mail: abolpetti@gmail.com
Tel.: (14) _97643998
Endereço: Departamento de Patologia
Faculdade de Medicina de Botucatu, UNESP.
Distrito de Rubião Jr s/n

Em caso de dúvidas, a qualquer momento contatar uma das pessoas abaixo:
Aline do Nascimento Bolpetti (abolpetti@gmail.com) – Tel.: (14) 97643998
Profa. Dra. Marcia Guimaraes da Silva (mgsilva@fmb.unesp.br) – Tel.: (14) 3811-6238, (14)97412496