RENATA GUARISI

TABAGISMO, INFECÇÃO PELO PAPILOMAVÍRUS HUMANO E O DESENVOLVIMENTO DE NEOPLASIA INTRA-EPITELIAL CERVICAL

Tese de Doutorado

ORIENTADOR: Prof. Dr. LUIS OTÁVIO ZANATA SARIAN
CO-ORIENTADORA: Profª. Drª. SOPHIE FRANÇOISE M. DERCHAIN

Unicamp
2008

RENATA GUARISI

TABAGISMO, INFECÇÃO PELO PAPILOMAVÍRUS HUMANO E O DESENVOLVIMENTO DE NEOPLASIA INTRA-EPITELIAL CERVICAL

Tese de Doutorado apresentada à Pós-Graduação da Faculdade de Ciências Médicas da Universidade Estadual de Campinas para obtenção do Título de Doutor em Tocoginecologia, área de Tocoginecologia

ORIENTADOR: Prof. Dr. LUIS OTÁVIO ZANATA SARIAN
CO-ORIENTADORA: Profª. Drª. SOPHIE FRANÇOISE M. DERCHAIN

Unicamp
2008

FICHA CATALOGRÁFICA ELABORADA PELA
BIBLIOTECA DA FACULDADE DE CIÊNCIAS MÉDICAS
UNICAMP
Bibliotecário: Sandra Lúcia Pereira – CRB-8ª / 6044

G931t

Guarisi, Renata
Tabagismo, infecção pelo papilomavirus humano e o desenvolvimento de neoplasia intra-epitelial cervical / Renata Guarisi. Campinas, SP: [s.n.], 2008.

Orientadores: Luis Otávio Sarian, Sophie Françoise Mauricette Derchain
Tese (Doutorado) Universidade Estadual de Campinas. Faculdade de Ciências Médicas.

1. Neoplasias intra-epitelial cervical. 2. Papilomavirus humano. 3. Tabagismo. I. Sarian, Luis Otávio. II. Derchain, Sophie Françoise M. III. Universidade Estadual de Campinas. Faculdade de Ciências Médicas. IV. Título.

Título em inglês: Smoking, human Papillomavirus infection and the development of Cervical intraepithelial Neoplasia

Keywords:
- Cervical intraepithelial neoplasia
- Human Papillomavirus
- Smoking

Titulação: Doutor em Tocoginecologia
Área de concentração: Tocoginecologia

Banca examinadora:

Prof. Dr. Luis Otávio Sarian
Prof. Dr. Aarão Mendes Pinto Neto
Prof. Dr. Luiz Carlos Zeferino
Prof. Dr. Jesus de Paula Carvalho
Prof. Dr. Jurandyr Moreira de Andrade

Data da defesa: 31 - 07 – 2008

Diagramação e arte final: Assessoria Técnica do CAISM (ASTEC)

BANCA EXAMINADORA DA TESE DE DOUTORADO

Aluna: RENATA GUARISI

Orientador: Prof. Dr. LUIS OTÁVIO ZANATA SARIAN

Co-Orientadora: Profª. Drª. SOPHIE FRANÇOISE M. DERCHAIN

Membros:

1.
2.
3.
4.
5.

Curso de Pós-Graduação em Tocoginecologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas

Data: 31/07/2008

Dedico este trabalho...

...aos meus pais, Nelson e Maria Odete,
pelo amor incondicional, e exemplos de caráter e dedicação.

ao meu marido Claudio,
pela cumplicidade, amor e incentivo.

às minhas irmãs Telma e Cláudia,
e ao meu cunhado Luiz Henrique, pelo carinho e apoio constantes.

à minha avó Olympia,
tios, tias e primo, pelo exemplo de família.

às minhas sobrinhas, Ana Paula, Carolina e Renata,
fontes de amor e estímulo .

aos meus sogros, Nédis e José Pacheco,
e ao meu cunhado Cássio, pelo acolhimento generoso.

Agradecimentos

Ao Prof. Dr. Luíz Otávio Sarian, pelo apoio e incentivo, e cuja dedicação e competência foram fundamentais para a conclusão desta tese.

À Prof[a] Dr[a] Sophie Françoise Mauricette Derchain, grande exemplo de pesquisadora, pelo incentivo, confiança, e amizade sempre dedicados.

Ao Prof. Dr. Kari Syrjänen pela coordenação deste estudo multicêntrico, que propiciou o desenvolvimento desta tese.

Às amigas Roberta Nascimento e Joana Fróes Bragança Bastos que participaram coleta de dados, permitindo um trabalho em equipe.

À amiga Renata Gontijo, que trabalhou intensamente durante todo o processo de desenvolvimento do estudo LAMS, pela amizade e dedicação.

À Rozani Dufloth e Eliane Montemor, pela leitura das lâminas de colpocitologia oncológica.

Às biólogas Denise Pita Lima, Elizabete Campos e Lucia Carvalho, pela realização dos exames de captura de híbridos II.

Aos professores Aarão Mendes P. Neto e Maria Salete C. Gurgel, pela valiosa contribuição no processo de qualificação desta tese.

À Margarete, secretária da pós-graduação, pela amizade e carinho durante todo curso e grande contribuição nos aspectos burocráticos para realização desta dissertação.

À Sra. Maria do Rosário e à Sra. Cylene da ASTEC, pela dedicação e colaboração na estruturação e revisão desta tese.

Às Funcionárias do Centro de Saúde de Santa Bárbara, à coordenadora Suely C Correia Martins, pelo acolhimento e apoio.

À Dinalva Cassimiro e Roseméria Vilarino, pela ajuda nos questionários e na busca de mulheres para participarem do estudo.

À Adriana Müller, pela compreensão, apoio e incentivo, de grande importância na fase final desta dissertação.

Às secretárias Rosana e Bruna, pela dedicação, carinho e ajuda na organização das minhas atividades diárias.

Ao Professor Álvaro da Cunha Bastos, "in memoriam", exemplo de mestre e grande incentivador da minha vida acadêmica.

Às mulheres, que confiam em nós.

A Deus, que guia meus passos.

Este estudo foi parcialmente financiado:

Comitê Europeu de Pesquisa da Comunidade Econômica Européia (CEE)– INCO DEV 4-CT-2001-(10013) Coordenadores: Professor Kari Syrjänen e Professor Paulo Naud.

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) processo número 02/02091-9.

Os kits para Captura de Híbridos II foram doados pelo Professor Attila Lörincz.

O Estudo *LAMS* e a presente Tese

O estudo LAMS – Latin American Screening – é uma iniciativa multicêntrica financiada pela União Européia através do programa INCO-DEV. O objetivo principal do LAMS é avaliar a qualidade e comparar modalidades de programas de controle de câncer do colo do útero na América Latina, mais especificamente no Brasil e Argentina. Embora a coleta de dados tenha se restringido a estes dois países, participam do LAMS pesquisadores da Finlândia, Eslovênia e Itália, coordenados pelo Professor Kari Syrjänen. As mais de 12.000 mulheres participantes foram recrutadas entre janeiro de 2002 e novembro de 2003, em importantes centros médicos com experiência em patologia cervical, nas cidades de Campinas, São Paulo, Porto Alegre e Buenos Aires.

O LAMS permitiu: 1) avaliar oito diferentes testes diagnósticos como ferramenta de rastreamento e 2) testar a hipótese que diferentes taxas de incidência nestas regiões dependem do diferente curso clínico dos precursores do câncer ou da diferente exposição dessas mulheres a fatores de risco conhecidos.

O desenho do LAMS é uma combinação de estudo de base populacional, corte-transversal e de coorte prospectivo. As mulheres incluídas no estudo foram

entrevistadas com respeito a possíveis fatores de risco epidemiológicos e clínicos e em seguida submetidas à coleta de material para colpocitologia oncológica e captura de híbridos 2. Em seguida foi realizada a inspeção visual com ácido acético e/ou com solução de lugol iodado. Todas as mulheres com pelo menos um exame positivo, 5% daquelas com colpocitologia normal e 20% daquelas com Captura de Híbridos 2 (Digene do Brasil LTDA) normal foram recrutadas para seguimento semestral por pelo menos dois anos.

Espera-se que dos resultados do LAMS possam resultar conhecimento sobre as modalidades de rastreamento para o câncer do colo do útero e suas lesões precursoras, informações epidemiológicas sobre essas doenças nas populações estudadas e instrumentos para o planejamento de ações preventivas futuras, transportadas às populações brasileira e argentina e, possivelmente, extrapoláveis para outras populações de características clínicas e epidemiológicas semelhantes.

A presente tese de doutoramento é uma análise sobre as associações entre hábitos tabagísticos, infecção pelo HPV e desenvolvimento de lesões precursoras do câncer do colo. São usados, com autorização do Prof. Syrjänen, dados da biblioteca do LAMS associados a este fator epidemiológico. A autora participou das fases de coleta de dados em Campinas, da elaboração do banco de dados e das discussões sobre publicações do Estudo, mesmo aquelas não relacionadas ao tema desta tese.

Sumário

Símbolos, Siglas e Abreviaturas

ALTS – ASCUS LSIL *Triage Study*

AGC – *Atypical glandular cells (*células glandulares atípicas)

ASC – *Atypical squamous cells* (células escamosas atípicas)

ASCCP – A*merican Society for Colposcopy and Cervical Pathology* (Sociedade Americana de Colposcopia e Patologia Cervical*)*

CAISM – Centro de Atenção Integral à Saúde da Mulher

CC – Câncer cervical *(Cervical cancer)*

CEP – Comitê de Ética em Pesquisa

CH2/HC2 – Captura de Híbridos 2 (*Hybrid Capture 2)*

CI/IC – Intervalo de confiança (*Confidence interval)*

CIN2+ – *High-grade cervical intraepithelial neoplasia*

CO – Citologia Oncológica

e.g. – Por exemplo

et al. – E outro(s); e outra(s)

FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo

FU – *Follow up* (seguimento)

HIV – *Human imunodeficience virus*

HPV – Papilomavírus humano (*Human papillomavirus)*

HR-HPV – HPV de alto risco *(Hight risk HPV)*

HR – *Hazard ratios*

HSIL – *High-grade intraephitelial lesion* (lesão intra-epitelial de alto grau)

IARC – *International Agency for Research on Cancer*

i.e. – Ou seja (*that is)*

IVA/ VIA – Inspeção visual com ácido acético *(Visual Inspection with acetic acid)*

LAMS – *Latin American Screening*

LSIL – *Low squamous intraepithelial lesion (*lesão intra-epitelial de baixo grau)

NC – *Not computable*

Neg. – *Negative/ normal* (Negativo/normal)

NIC/CIN – Neoplasia intra-epitelial cervical (*Cervical intraepithelial neoplasia)*

NIS – Novos Estados Independentes *(New Independent States)*

OR – *Odds ratio*

Pap – Papanicolaou

Pg/mcl – Picograma por microlitro; *Picogram per mililiter*

Ref – Referência *(Reference)*

RLU – Unidade relativa de luz; *Relative Light Unit*

RLU/CO – *Relative light unit; positive control*

RR – Risco relativo *(Relative risk)*

SPSS – *Statistical package for social sciences*

UNICAMP – Universidade Estadual de Campinas

VILI – *Visual Inspection with lugol iodine*

WMR – *Woman months at risk*

Resumo

Objetivo: Avaliar os efeitos do tabagismo na prevalência da infecção pelo papilomavírus humano de alto risco oncogênico (HR-HPV) e neoplasia intra-epitelial cervical (NIC). Avaliar prospectivamente os efeitos do tabagismo sobre a aquisição de HR-HPV e desenvolvimento de NIC em mulheres sem lesão histológica na primeira consulta. **Sujeitos e métodos**: Foram avaliadas 12.114 mulheres incluídas no estudo *Latin American Screening* (LAMS) entre janeiro de 2002 a novembro de 2003, em Campinas, São Paulo, Porto Alegre e Buenos Aires. Foram formados três grupos: 1) não tabagistas (n=7.499), tabagistas (n=2.706) e 3) ex-tabagistas (n=1.871). Para o seguimento prospectivo de 36 meses foram selecionadas mulheres com pelo menos um exame alterado na primeira consulta, mas que não tinham lesão histológica, e 10% daquelas com todos os exames normais, totalizando 1.011 mulheres. Um grupo formado por 150 mulheres com citologia compatível com células escamosas atípicas (ASC) ou lesão intra-epitelial de baixo grau (LSIL) e colposcopia normal foi estudado separadamente. A análise inicial avaliou a relação entre a história de tabagismo e outros fatores epidemiológicos com a prevalência de infecção por HR-HPV e NIC. As mulheres foram examinadas a cada seis meses com colposcopia, captura de híbridos 2 e colpocitologia oncológica.

Resultados: A diferença mais importante entre os grupos foi a prevalência de HR-HPV entre tabagistas (21,7%) quando comparadas a não tabagistas (16,5%) e ex-tabagistas (13,5%). Teste HR-HPV positivo (OR=9,69; 95%CI=5-18,79), citologia com lesão intra-epitelial de alto grau (OR=40,52; 95%CI = 8,52-192,6) e história de não ter realizado Papanicolaou no passado (OR=2,65; 95%CI=1,21-5,79) estiveram associados ao maior risco de NIC 2 ou pior na consulta inicial. A incidência de anormalidades no Papanicolaou durante o seguimento foi mais freqüente nas tabagistas (5,8%) quando comparadas às ex-tabagistas (4,8%) e não tabagistas (1,7%). Durante o seguimento, HR-HPV+ na consulta inicial aumentou significativamente o risco de incidência de Papanicolaou anormal, independentemente da história de tabagismo. Tabagismo foi um significante preditor de incidência de HR-HPV durante o seguimento, e ter HR-HPV+ na consulta inicial, ser tabagista e ex-tabagista foram fatores de risco independentes para incidência de NIC2 ou pior. No grupo ASC/LSIL, apenas HR-HPV + inicial esteve associado ao risco de desenvolver NIC durante o seguimento de 36 meses (HR=3,42; 95CI%=1,11-9,43). Mulheres tabagistas tiveram maior risco de NIC de alto grau ou câncer durante o seguimento, quando comparadas às não tabagistas (p=0,04). **Conclusões:** O tabagismo, no passado ou presente, esteve associado a maior prevalência de infecção por HPV de alto risco oncogênico. O tabagismo também esteve associado ao aumento de risco de desenvolvimento de NIC 2 ou pior em mulheres com infecção por HPV de alto risco oncogênico. O tabagismo aumenta o risco de desenvolvimento de NIC2 ou pior em mulheres com citologia ASC/LSIL e colposcopia normal, em um período de 36 meses.

Summary

Objective: To assess whether smoking history interferes with the prevalence of hr-HPV infection and cervical intraepithelial neoplasia (CIN) and to prospectively examine the acquisition of hr-HPV infection and the development of CIN in baseline normal women. **Subjects and methods**: The study examines the baseline data on 12,114 women included in the Latin American Screening (LAMS) Study in São Paulo, Campinas, Porto Alegre and Buenos Aires, and the prospective data from 1,011 women, that included women with at least one positive test at baseline and no histological lesion and 10% of the women with normal cytology; and 150 women with ASC/LSIL and normal colposcopy at baseline, followed-up for a period of 36 months. Three groups were formed: 1) women that never smoked (n=7.499), 2) current (n=2.706) and 3) past smokers (n=1.871). The baseline assessment included the relation between the smoking history and several other epidemiological factors with the prevalence of hr-HPV infection and CIN. In the prospective analysis, women were controlled at 6-month intervals with colposcopy, HC2 and Pap to assess the cumulative risk of incident hr-HPV infection, smear abnormalities and CIN over a period of 36 months. **Results:** The most important significant differences were the higher prevalence (21.7%) of HR-HPV infections

among current smokers as compared to women who never smoked (16.5%) or those smoking in the past (13.5%). Testing HR-HPV positive at baseline (OR=9.69; 95%CI=5-18.79), high-grade squamous intraepithelial lesion in baseline Pap smear (OR=40.52; 95%CI = 8.52-192.6) and history of no previous Pap smear (OR=2.65; 95%CI=1.21-5.79) were significant predictors of baseline CIN2 or worse. Among women with baseline abnormal Pap, progression to CIN2or or worse was substantially more frequent in current (5.8%) and past smokers (4.8%) than in never smokers (1.7%). During follow-up, testing HR-HPV+ at baseline significantly increased the risk of incident abnormal Pap, irrespective of the smoking status. Being a current smoker was a significant predictor of incident HR-HPV during the follow-up (HR =1.44; 95%CI 1.03-2.04), and, baseline HR-HPV+ (HR=10.07; 95%CI 1.32-76.49), being a past smoker (HR=3.61; 95%CI 1.06-12.33) and current smoker (HR=3.51; 95%CI 1.21-10.14) for incident CIN2 or worse. Among women with ASC/LSIL at baseline, the HR-HPV+ (HR = 3.42; 95%CI 1.11 to 9.43) was the only factor related to an increased risk of developing CIN during the 36-month follow-up. While restricting the analysis to high-grade CIN, the probability of developing the disease was significantly higher for smokers (p= 0.04). **Conclusion:** The smoking history, past or current, was related to a higher prevalence of HR-HPV, and to a increased risk of developing CIN2 or worse in women with HR-HPV+. Being smoker was related to a higher risk of developing CIN2 or worse in women with baseline ASC/LSIL and normal colposcopy, during 36-months follow-up.

1. Introdução

Embora o papilomavírus humano (HPV) seja considerado o agente etiológico primário para o desenvolvimento do câncer cervical, já que está presente em mais de 90% dos casos desta neoplasia (Walboomers et al., 1999), muitos estudos epidemiológicos têm sido realizados com o objetivo de identificar fatores de risco secundários, associados ao desenvolvimento de neoplasia intra-epitelial cervical (NIC) ou câncer, em mulheres infectadas pelo HPV de alto risco (HR-HPV). Entre os fatores de risco identificados estão: o uso de contraceptivo oral por longo período (Moreno et al., 2002; Castellsague e Munoz, 2003), multiparidade (Munoz et al., 2002), tabagismo (Castellsague e Munoz, 2003; Plummer et al., 2003), imunodepressão (Wang e Hildesheim, 2003), além de fatores relacionados ao hábito sexual, como início precoce da atividade sexual e grande número de parceiros sexuais (Herrero et al., 1989).

Dentre os potenciais co-fatores envolvidos no desenvolvimento de câncer cervical ou NIC, o tabagismo tem merecido especial atenção desde os anos 80 (Hellberg et al., 1983; Barton et al., 1988). Entretanto, estes estudos pioneiros,

por não conseguirem controlar outros fatores de risco - principalmente aqueles relacionados ao hábito sexual e à infecção pelo HPV - não foram conclusivos (Borck et al., 1989; Layde, 1989; Winkelstein, 1990). Uma recente análise, na qual foram avaliados oito estudos caso-controle da International Agency for Research on Cancer (IARC), mostrou que mulheres que fumam (ou fumaram no passado) têm risco aumentado de desenvolver câncer e NIC (Plummer et al., 2003).

Não existe, contudo, fundamentação biológica evidente para explicar este efeito. Detectou-se, por exemplo, que potenciais carcinógenos do tabaco costumam estar presentes em altas concentrações no muco cervical de mulheres tabagistas (Procopzyk et al., 1997; Melikian et al., 1999). Adicionalmente, esses carcinógenos poderiam contribuir ao dano no DNA das células epiteliais infectadas por linhagens do HPV de alto risco (HR-HPV), mais precisamente alterando o funcionamento dos genes supressores de tumor p53 e pRb (Trottier e Franco, 2006). O HPV penetra nas células da camada basal do epitélio cervical, e seus genes E6 e E7 se integram, respectivamente, com o produto dos genes supressores de tumor p53 e pRB. Essa integração promove alteração no ciclo celular e nos mecanismos de apoptose, sendo responsável pela indução e manutenção das alterações celulares (Snijders et al., 2006). Estima-se que cerca de 80% das mulheres sexualmente ativas entrarão em contato com o HPV em algum momento da vida. Entretanto, apenas 20% delas desenvolverão algum tipo de lesão cervical, o que sugere a participação de fatores adicionais na gênese da lesão, tornando a infecção pelo HPV necessária, mas não suficiente para o desenvolvimento do câncer cervical (Boyle e Smith, 1999; Munoz et al., 2003).

Ainda que sem confirmação por experimentação *in vivo* ou *in vitro*, existem indicações de que o cigarro facilitaria a aquisição e estabilização do HPV, desta forma favorecendo a persistência da infecção. É sabido que componentes do tabaco reduzem o número e afetam a função das células de Langerhans e linfócitos CD4 nos tecidos cervicais, desta forma também diminuindo a atividade das células *natural killer* (Ferson et al., 1979; Poppe et al., 1995; Poppe et al., 1996). Não está claro, entretanto, qual desses dois mecanismos tem um maior impacto no aumento do risco de desenvolvimento de NIC ou câncer entre mulheres tabagistas.

Estudos recentes mostraram maior prevalência de infecção por HR-HPV entre mulheres fumantes, e esta associação parece ser dose-dependente, isto é, depende do número de cigarros fumados por dia (Vaccarela et al., 2008). Similarmente, em um grande estudo multicêntrico realizado em três países do NIS (sigla em Inglês para "Novos Estados Independentes") da antiga União Soviética, mostrou que mulheres tabagistas apresentaram um risco maior de infecção pelo HR-HPV. Entretanto, neste estudo o tabagismo não foi um fator de risco independente para NIC 2 ou pior (Syrjänen et al., 2007).

Em contrapartida, em um estudo do grupo ALTS (ASCUS-LSIL Triage Study), mulheres que fumavam atualmente e mulheres que fumaram no passado tinham maior risco de diagnóstico de NIC 3 que mulheres não fumantes (McIntyre-Seltman et al., 2005).

Contudo, em muitos estudos epidemiológicos, a associação entre tabagismo e câncer cervical está sujeita a viés e fatores de confusão (Castellsague e Muñoz,

2003; Campo, 2006; Trottier e Franco, 2006). Além dos efeitos biológicos dos carcinógenos do cigarro e do potencial efeito sobre a imunidade da cérvice uterina, o aumento no risco de neoplasia cervical entre tabagistas pode ser atribuído a fatores de risco epidemiológico relacionados à atividade sexual (Herrero et al., 1989). Pesquisas recentes estabeleceram forte associação entre tabagismo e alguns fatores de risco, como início precoce de atividade sexual e grande número de parceiros sexuais. Estes fatores estão relacionados com aumento no risco de adquirir infecção pelo HR-HPV (Vaccarella et al., 2006).

Existem, portanto, muitas frentes a explorar com relação à contribuição epidemiológica do tabagismo para o desenvolvimento do CC e de suas lesões precursoras. Devem-se destacar os questionamentos ainda pendentes sobre

1) O efeito do tabagismo sobre a probabilidade de aquisição de infecção pelo HPV,
2) O efeito do tabagismo sobre a persistência do HPV,
3) O efeito dos carcinógenos do tabaco em mulheres já infectadas pelo HPV,
4) O efeito do tabagismo, mediado pela redução da imunidade, na promoção da proliferação de clones neoplásicos,
5) O efeito do tabagismo sobre o tempo de desenvolvimento de lesões precursoras e sua degeneração para lesões invasoras,
6) Se os efeitos do tabagismo, mencionados acima, não são simplesmente produto da associação estatística entre o hábito de fumar e outros elementos comportamentais, nutricionais e sociais associados ao risco de câncer do colo.

Todos os questionamentos acima já foram, individualmente ou em combinação, avaliados por estudos anteriores. Contudo, como exposto anteriormente, as conclusões são heterogêneas e metodologicamente questionáveis.

Outro fator regionalmente importante é o fato de a infecção pelo HPV ser a doença sexualmente transmissível mais freqüente no mundo, a mais comum entre mulheres jovens sexualmente ativas. Por existirem atualmente mais de 120 subtipos descritos de HPV, 50 deles infectantes do trato genital, a variabilidade epidemiológica regional na distribuição destes tipos é enorme. Desses, aproximadamente 15 são conhecidos por seu alto potencial oncogênico como, por exemplo, os HPV 16, 18, 31, 45 e 58 que, juntos são responsáveis pela maioria dos casos de câncer cervical e suas lesões precursoras (Hart et al., 2001).

Mesmo assim, o comportamento biológico, com suas resultantes clínicas, de cada tipo de HPV é sabidamente ímpar. Acrescentando ainda mais fatores complicadores, a distribuição regional destes tipos virais, em termos epidemiológicos, é extremamente heterogênea. É enorme a variedade de distribuição dos tipos de HPV ao redor do mundo, e ainda dentro de países de grande território, como, por exemplo, o Brasil (Reeves et al, 1989; Cavalcanti et al., 2000). Há uma clara indicação que diferentes tipos de HPV podem ter propriedades biológicas e epidemiológicas distintas, produzindo resultados inconsistentes e algumas vezes conflitantes.

Outra consideração sobre estudos de base populacional sobre o HPV é considerar o fato que diferentes populações apresentam dessemelhança de fatores epidemiológicos, econômicos e geográficos, evitando desta forma, a generalização

de resultados de uma região para outra (Bosch e de Sanjosé, 2007). Da mesma forma, as estratégias médicas derivadas de estudos conduzidos em uma região do globo podem ser invalidadas em outra.

Oportunamente, o estudo LAMS (Latin American Screening) surgiu com o objetivo de estudar as características e hábitos de uma população específica, e melhorar a qualidade dos programas de controle de câncer cervical, comparando a colpocitologia oncológica, inspeção visual com ácido acético (IVA), CHII, cervicografia e colposcopia. Este estudo foi desenvolvido entre 2002 e 2005 no Brasil (Campinas, São Paulo e Porto Alegre) e Argentina (Buenos Aires), formando uma coorte predominantemente composta por populações urbanas, e que foi seguida por 24 meses. Resultados preliminares do LAMS mostraram que os exames estudados tiveram um desempenho diferente nesta população quando comparados a populações geograficamente distintas (Gontijo et al., 2002; Sarian et al, 2005; Syrjänen et al., 2005).

O desenho do estudo LAMS permite avaliar, seja para esta população específica, seja com fins de generalização para outras populações de características semelhantes, inúmeros aspectos relacionados aos efeitos do tabagismo sobre o desenvolvimento de câncer cervical e suas lesões precursoras. Entre as potencialidades analíticas do LAMS, estão: a) avaliar concomitantemente, em uma população de aproximadamente 12.000 mulheres, as associações entre o tabagismo e a1) infecção pelo HPV, a2) prevalência de anormalidades citológicas, NIC e câncer cervical; b) avaliar, prospectivamente, em uma coorte de mais de 1.000 mulheres, a probabilidade de aquisição da infecção pelo HPV e posterior

desenvolvimento de NIC em função do b1) histórico de tabagismo; b2) tempo e b3) intensidade do tabagismo. Não obstante, pela dimensão do tamanho amostral, a casuística do estudo LAMS também permite avaliar subpopulações, como por exemplo, as mulheres portadoras de citologia ASC/LSIL, que merecem atenção específica em função da já conhecida associação com HR-HPV (Solomon et al., 2001; Castle et al., 2005), e também do risco aumentado de desenvolver NIC e câncer cervical (Kinney et al., 1998; Schiffman e Solomon, 2003).

Esta tese de doutoramento tem por objetivo explorar, através da extensa biblioteca de dados do estudo LAMS, os efeitos do tabagismo sobre a epidemiologia e o comportamento clínico da infecção pelo HPV e a prevalência e incidência de neoplasias precursoras do câncer cervical em uma amostra significativa de mulheres latino-americanas vivendo em condições urbanas.

2. Objetivos

2.1. Objetivo Geral

Determinar os efeitos do tabagismo sobre a probabilidade de aquisição de infecção por HPV de alto risco oncogênico e sobre o desenvolvimento de neoplasia intra-epitelial cervical.

2.2. Objetivos Específicos

– **Artigo1:** Avaliar o papel do tabagismo como um preditor de aquisição da infecção pelo HPV de alto risco oncogênico, e também no desenvolvimento de NIC2 ou pior durante o seguimento de uma coorte de mulheres do LAMS study.

– **Artigo 2:** Comparar o risco de desenvolvimento de NIC em tabagistas e não tabagistas portadoras de colpocitologia oncológica compatível com células escamosas atípicas ou lesão intra-epitelial de baixo grau e colposcopia normal ao início do seguimento.

3. Publicações

Artigo 1 - **Increased risk of oncogenic Human papillomavirus (HR-HPV) infections and incident high-grade cervical intraepithelial neoplasia (CIN) among smokers: Experience from the Latin American Screening (LAMS) Study.**

Artigo enviado para a revista *Sexually Transmitted Diseases*

Artigo 2 - **Smoking contributes additional risk for cervical intraepithelial neoplasia in women with normal colposcopy and atypical squamous cells or low-grade squamous intraepithelial lesion.**

Artigo enviado para a revista *Diagnostic Cytopathology*

3.1. Artigo 1

---------- Cabeçalho original ----------

De: em.std.0.bdd47.fe011119@editorialmanager.com
Para: sarian1@terra.com.br
Cópia:
Data: 24 Jun 2008 08:33:00 -0400
Assunto: A manuscript number has been assigned to Increased risk of oncogenic Human papillomavirus (HR-HPV) infections and incident high-grade cervical intraepithelial neoplasia (CIN) among smokers: Experience from the Latin American Screening (LAMS) Study.

Dear Prof. Sarian,

Your submission entitled "Increased risk of oncogenic Human papillomavirus (HR-HPV) infections and incident high-grade cervical intraepithelial neoplasia (CIN) among smokers: Experience from the Latin American Screening (LAMS) Study." has been assigned the following number: STD08-180.

You will be able to check on the progress of your submission by logging on to Editorial Manager as an author.
The URL is http://std.edmgr.com/.

Thank you for submitting your work to Sexually Transmitted Diseases.

Kind regards,

Jeanne Moncada, MT
Managing Editor
Sexually Transmitted Diseases

Increased risk of oncogenic Human papillomavirus (HR-HPV) infections and incident high-grade cervical intraepithelial neoplasia (CIN) among smokers: Experience from the Latin American Screening (LAMS) Study.

Authors:

Luis Otavio Sarian[1], M.D, PhD.

Luciano Serpa Hammes[2] , M.D., PhD.

Adhemar Longatto-Filho[3,4], M.Sc, PhD, PMIAC.

Renata Guarisi[1],M.D.

Sophie FM Derchain[1], M.D., PhD.

Cecília Roteli-Martins[5], M.D., PhD.

Paulo Naud[2], M.D., PhD.

Mojca Eržen[6], M.D., PhD, MIAC.

Margherita Branca[7], M.D., PhD., MIAC.

Sílvio Tatti[8], M.D., PhD.

Jean Carlos de Matos[2], M.D.

Renata Gontijo[1], M.D.

Marina YS Maeda[4], M.Sc.

Temístocles Lima[5], M.D.

Silvano Costa[9], M.D., PhD.

Stina Syrjänen[10], D.D.S., PhD.

Kari Syrjänen[11], M.D., PhD, FIAC.

[1]Campinas, Brazil

[2]Hospital de Clinicas de Porto Alegre, Brazil

[3]Life and Health Sciences Research Institute, School of Health Sciences, University of Minho, Braga, Portugal

[4]Instituto Adolfo Lutz , São Paulo, Brazil

[5] Hospital Leonor Mendes de Barros (HLMB), São Paulo, Brazil

[6]SIZE Diagnostic Center, Ljubljana, Slovenia

[7]Unit of Cytopathology, National Centre of Epidemiology, Surveillance and Promotion of Health, National Institute of Health (ISS), Rome, Italy
[8]First Chair Gynecology Hospital de Clinicas, BA, Argentina
[9]Department of Gynecological, Obstetric and Pediatric Science, S.Orsola-Malpighi Hospital, Bologna, Italy
[10]Department of Oral Pathology, University of Turku, Finland
[11]Department of Oncology & Radiotherapy, Turku University Hospital, Finland

Address for correspondence:

Luis Otávio Sarian
Department of Obstetrics and Gynecology Faculty of Medical Sciences, PO Box 61 University of Campinas – UNICAMP, Zip Code 13083-970, Campinas, SP, Brazil.

Acknowledgements
This report is a part of the LAMS (Latin American Screening) study, entitled: IMPROVING HEALTH SYSTEMS TOWARDS EQUALITY-BASED CONTROL OF CERVICAL CANCER IN LATIN AMERICA, and supported by the INCO-DEV Program of the European Commission (Project# ICA4-CT-2001-10013). The generous contribution of DIGENE Inc. (USA) providing the HC2 tests at our disposal is gratefully acknowledged.

Manuscript figures: abstract word count: 249; text word count: 4624; 6 tables; 1 figure

Short summary:
In a report on over 12,000 Latin American women, tobacco smoking was related to a higher prevalence of high-risk HPV infection and increased incidence of CIN in HPV+ women.

Abstract

Background: The purpose of this study was to assess the effect of smoking on the prevalence and incidence of hr-HPV infection and cervical intraepithelial neoplasia (CIN) in a large sample of Latin American women. **Methods:** The study examines baseline data on over 12,000 women included in the Latin American Screening (LAMS) Study (Brazil and Argentina), and over 1,000 women followed-up for a period of 36 months. Three groups were formed: never smokers; current; past smokers. The prevalence of hr-HPV infection and CIN were compared between the study groups. In the prospective analysis, women were controlled at 6-month intervals to assess the cumulative risk of incident hr-HPV infection, smear abnormalities and CIN. **Results:** A higher prevalence (21.7%) of HR-HPV infection was found among current smokers as compared to never smokers (16.5%) or past smokers (13.5%). Being current smoker was significantly (p=0.002) associated with HR-HPV detection (OR=1.36; 95%CI=1.12 to 1.66). Being a current smoker was a significant predictor of incident HR-HPV during the follow-up (HR=1.44; 95%CI 1.03-2.04). For incident CIN2+, being a past smoker (HR=3.61; 95%CI 1.06-12.33) or current smoker (HR=3.51; 95%CI 1.21-10.14) were the significant independent predictors. Current and past smokers had a significantly increased risk of incident CIN2+ (p=0.00105). **Conclusions:** smoking increases the risk of contracting HR-HPV infection, making current smokers with persistent HR-HPV+ true high-risk patients for disease progression. Similar incidence and predictive power of incident CIN2+ among past and current smokers suggest that this increased risk associated with cigarette smoking persists several years after cessation of smoking.

Key Words: Cervical cancer, screening, smoking, HPV, CIN

Introduction

Recent epidemiological evidence lists cigarette smoking among the risk factors of cervical cancer (CC) and its precursor (CIN) lesions. In a pooled analysis of IARC multi-centre, case-control studies, women who ever smoked were at a significantly increased risk of developing CC and CIN [1]. It seems likely that the effects of smoking on cervical disease could be ascribed to potent tobacco carcinogens, present in high concentrations in the cervical mucus of women smokers [2,3]. These carcinogens may contribute additional DNA damage to epithelial cells infected by oncogenic (high-risk, HR) Human papillomavirus (HPV) types, most notably the disruption of p53 and pRb gene pathways by over-expression of the E6 and E7 viral oncogenes [4]. Although never confirmed by *in vivo* or *in vitro* experimentation, there is some indication that smoking facilitates the acquisition and stabilization of the virus thus favouring persistence of HPV infections. It is known from the early studies that tobacco components reduce the number and affect the function of Langerhans cells and CD4+ lymphocytes in cervical tissues, and also decrease in the activity of natural killer cells [5-7]. It is still unclear, however, which of these two mechanisms has a major impact on the increased risk of developing CIN or CC among smokers.

In addition to these direct biological effects of tobacco carcinogens, the increased risk of cervical neoplasia among smokers may also be attributed to epidemiological risk factors (e.g. risk sexual behaviour) augmenting the probability of contracting HR-HPV infections among the women smokers [8]. Indeed, recent data implicates that the prevalence of HR-HPV infections is higher among current smokers, and the strength of this association seems to be dose-dependent (i.e., number of cigarettes smoked) [9]. Similarly, in a large multi-centre screening trial in three New Independent States (NIS) of the former Soviet Union (Russia, Belarus and Latvia), smoking proved to be an independent risk factor for HR-HPV infections (but not prevalence of CIN), but unfortunately, this study did not examine the effect of smoking on the incident CIN [10].

Like many epidemiological studies, also those on smoking and CC are subject to bias and confounding, and there are frequent concerns with the population-based studies on HPV, CIN and CC [11-13]. One of these uncertainties is the enormous variability of the HPV type-distribution across the world and even within large countries, e.g. like Brazil [14,15]. There is a clear indication that different HPV types may have distinct epidemiological and biological properties, making results inconsistent and sometimes even conflicting [16]. Another reservation repeatedly presented against these population-based HPV studies is the unequivocal fact that epidemiological, economical, behavioural and geographical dissimilarities of different populations preclude the generalisation of the results from one geographical region to another [16].

To enable unbiased estimation of the smoking as a risk factor of CIN and CC, study designs need to control for the concurrent acquisition/persistence of HR-HPV infections and development of incident high-grade CIN (CIN2+). This is appropriately done in our LAMS (the Latin American Screening) Study testing eight optional screening tools in a cohort of over 12,000 women in Brazil and Argentina, and providing prospective follow-up data on disease outcome [17]. In the present study, we examined the role of cigarette smoking as a predictor of two main outcomes; 1) acquisition of HR-HPV infections, and 2) development of CIN2+ during a prospective follow-up of over 1,000 women of the LAMS study cohort.

Subjects and methods

Study design

The Latin American Screening (LAMS) study is a multi-center population-based cohort study testing optional screening strategies and assessing risk factors of cervical disease in a cohort of over 12,000 women enrolled between January 2002 and November 2003

in Brazil and Argentina. A total of 12,114 consecutive women from the cities of Campinas, São Paulo and Porto Alegre (Brazil) as well as Buenos Aires (Argentina) were enrolled in the cohort to undergo screening with conventional Pap smear, HR-HPV testing by Hybrid Capture 2 (HC2), visual inspection with acetic acid (VIA) or Lugol iodine (VILI), cervicography and screening colposcopy [17]. All centers performed conventional Pap smear, HC2 and VIA, whereas Porto Alegre performed VILI, Buenos Aires did screening colposcopy and only Campinas did cervicography [18,19]. The study protocol has been approved by the local Ethics Committees of all participating clinics. All enrolled women gave their agreement to participate by signing the Informed Consent Forms, written in their native language.

Women were considered eligible, if they met all of the following criteria: a) age between 15 to 60 years; b) no previous surgical procedure of the cervix or uterine corpus; c) had no history of abnormal Pap test in the past year; d) free of diagnosed (=prevalent) genital wart (external or cervical condyloma), CIN or CC; e) had no sexual intercourse during the three days prior to the examination; f) did not have any confirmed or clinically suspect immunosuppression (HIV, or other conditions).

Study centers and their demographics

The study design and features of the clinical centers as well as the demographics of their female populations were described in detail recently [18,19]. In brief, Campinas and São Paulo are two large south-western Brazilian cities, located only 100km apart, with equivalent standards of living. CC is the fourth major cause of cancer death among women, accounting for 3.3% of all female cancer deaths [20]. The third Brazilian partner is from Porto Alegre, located south of the country. This region offers the best quality of life in Brazil, and CC is the sixth major cause of cancer death, accounting for 6.1% of all female

cancer deaths [20]. The Argentine partner is from Buenos Aires. The country has an overall CC mortality rate of 7.6/100,000 women [21], but most of the national statistics pertain to Buenos Aires city only.

Diagnostic setting

All women were subjected to thorough pelvic examination in this sequence: i) collection of the Pap smear, ii) collection of HC2 sample and iii) VIA. In Porto Alegre, most women were subjected to VILI shortly after VIA. All women, who had at least one of these examinations abnormal, were referred for colposcopic examination. In Buenos Aires and Campinas, women were subjected to screening colposcopy even when their exams were negative. Abnormal colposcopy prompted punch biopsies of the cervix, and women with high-grade cytological abnormalities (HSIL) were treated by conization.

Women had their second visit scheduled one month and a half (average 45 days), to become informed about their examination and/or biopsy results and to be allotted to either A) the treatment- or B) the follow-up group. Treatment was offered to all women who had high-grade lesion (CIN2+) confirmed in the cervical biopsy. Altogether, 32 cases of invasive CC were diagnosed during the recruitment phase and all those were treated according to each institution's standard protocols.

Follow-up (FU)

Patients who did not require treatment for CIN2+ and who had 1) abnormal Pap smear, and/or 2) HR-HPV infection, and/or 3) CIN1 at baseline were selected for follow-up. In the original study design, four such follow-up visits were scheduled (optimally at 6-, 12-, 18- ad 24-months). At each FU-visit, women responded to a brief

questionnaire addressing any relevant gynecological events and epidemiological features changed since the previous control (e.g. sexual partners, smoking). The FU-phase of the study was concluded in September 2006, with the longest FU-times exceeding 50 months, but the bulk of the data covers approximately 36 months (median FU time = 24.4 mo; 90% central range = 6.8 to 32.2 mo).

Cervical cytology (Pap smear)

Conventional Pap smears were taken using the Ayre spatula and endocervical brush, fixed in 95% ethanol and stained by the modified Papanicolaou method. Final cytological diagnoses were issued using the Bethesda System [22] and were classified as normal/inflammatory, ASC, atypical glandular cells (AGC), LSIL, HSIL or cancer.

Hybrid Capture 2 (HC2)

The specimens for HC2 were tested with probe B for high-risk HPV (HR-HPV) types: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) [23] and the tests were classified positive at the relative light unit/positive control (RLU/CO) ratio of 1pg/ml or greater. These RLU/CO ratios provide a semi-quantitative estimate of the amount of HPV DNA in the specimens, i.e., the viral load in the sample. Storage of reagents and specimens as well as their processing were carried out in manufacturer-certified laboratories, under the responsibility of the investigators, following the manufacturer's instructions (*Digene Diagnostics Inc., USA*). São Paulo and Buenos Aires processed their own HC2 samples in-house, whereas Campinas and Porto Alegre had their HC2 specimens processed at Campinas University hospital laboratory.

Retrieval of the smoking data

The information concerning patients' smoking attitudes was gathered during the baseline interview. Patients were asked the following questions: 1) Do you smoke? 2) If you smoke, how many cigarettes do you smoke on a daily basis? 3) Have you smoked in the past? 4) How many cigarettes per day did you smoke in the past? 5) How many years since you stopped smoking? Based on questions 1 and 3, patients were allotted to three study groups: a) current smokers (n=2,706), b) smokers in the past (n=1,871), and c) never smokers (n=7,499).

Statistical analysis

Comparison of the epidemiological variables across the three groups was performed with Chi-Square Test or Fisher's Exact Test for categorical variables (tables 2X2 required Continuity Correction) and with Kruskal-Wallis Test for continuous variables. Risk associates for abnormal cytology (HSIL, LSIL cut-offs), HR-HPV and CIN2+ lesions were first calculated in univariate regression, with crude Odds Ratio (OR) and Confidence Intervals (95%CI). All significant risk factors were entered in a multivariate regression model (together with smoking variables), and the adjusted ORs (95%CI) were calculated for two separate outcome variables: HR-HPV infections and CIN2+. In all tests, the values $p<0.05$ were regarded statistically significant.

Univariate survival (Kaplan-Meier) analysis was used to calculate the survival curves (in the whole cohort) for accumulation of incident HR-HPV infections in women with negative HPV tests at baseline, and for incident CIN2+ during the FU. The curves were compared using log-rank (Mantel-Cox) statistics. Hazard ratios (HR) for the risk associates were calculated with a multi-variate Cox regression model, with 95% CI. All calculations were performed using the R package for statistical computing [24].

Results

Altogether, smoking data were available from 12,076 women participating in the study. **Table 1** depicts the key epidemiological characteristics of the women in the three study groups. Except for the number of deliveries (p=0.094), number of abortions (p=0.420) and history of previous CIN (p=0.474), all other variables recorded differed significantly across the three groups. The most important significant differences were the higher prevalence (21.7%) of HR-HPV infections among current smokers as compared to women who never smoked (16.5%) or those smoking in the past (13.5%). Current smokers also had higher HPV loads (p=0.0004), higher prevalence of abnormal Pap smear and CIN (p<0.0001). Table 1 also describes time-related characteristics of the smoking habits: on average, current smokers had been smoking for 15.89±10.33 years (M±SD), consuming an average of 11.32±8.47 cigarettes per day. Past smokers recalled of having being smokers for 10.26±8.09 years, but referred having abandoned the smoking habit quite recently (8.09 years ago, on average).

Table 2 lists the odds ratios for having HR-HPV infection or CIN2+ at baseline. The odds were adjusted with a multivariate regression model, with all the listed variables being entered in the model. The following factors were significantly associated with a positive HR-HPV test at baseline: the histological diagnosis of CIN2+ or worse (OR=9.15; 95%CI=4.74 to 17.66), age<35 years (OR=1.69; 95%CI=1.39 to 2.04), LSIL pap (OR=12.40; 95%CI=7.15 to 21.51), being single (OR=1.55; 95%CI=1.28 to 1.87) and having multiple sex partners (OR=1.74; 95%CI=1.39 to 2.17). Being current smoker was also significantly (p=0.002) associated with HR-HPV detection in this model (OR=1.36; 95%CI=1.12 to 1.66). Only three significant predictors of baseline CIN2+ were disclosed in the multivariate analysis: testing HR-HPV positive at baseline (OR=9.69; 95%CI=5-18.79), HSIL in baseline Pap smear (OR=40.52; 95%CI = 8.52-192.6) and history of no previous Pap

smear (OR=2.65; 95%CI=1.21-5.79). Importantly, being a current smoker did not increase the risk of CIN2+ at baseline (OR=0.86; 95%CI=0.45-1.46).

Years of smoking among current and past smokers were displayed in relation to the detection of HR-HPV, CIN2+ and Pap smear abnormalities in **Table 3**. In this analysis, years of being smoker had no statistically significant association with the detection of HR-HPV, CIN2, HSIL or LSIL at the baseline examination. The only borderline association was disclosed for HSIL Pap and years of smoking among current smokers, women with HSIL Pap having been smokers longer than women with no HSIL (p=0.074). Within the group of past smokers, the time (months) since the smoking habit had ceased had no relation to the baseline detection of HR-HPV, CIN2, HSIL or LSIL.

Of the total cohort (n=12,114), 1,011 women were allotted to the FU group, and every woman completed at least one FU-visit. The outcomes of HR-HPV infections and clinical disease (measured by Pap test and biopsy) in the three groups of women are summarized in **Table 4**. There was no significant difference between the three groups in their baseline HPV/PAP status (ASC cut-off) (p=0.083). Similarly, the outcome (incident, persistent, cleared) of HR-HPV infection in the three groups was practically identical, with only minor differences in some of the outcome categories, e.g. higher proportion of persistent HR-HPV among current smokers. The outcome of Pap test abnormalities was different in the three groups (p=0.017). Most notably, current and past smokers seem to deviate from never smokers in most of the outcome categories, particularly in their higher proportion of persistent abnormal Pap test. On the other hand, the three groups were similar as to disease outcome monitored by biopsy, with no statistical significance to disease progression from CIN1 to CIN2+ (p=0.622). However, among women with baseline abnormal Pap (and/or

HR-HPV+), progression to CIN2+ was substantially more frequent in current (5.8%) and past smokers (4.8%) than in never smokers (1.7%).

Table 5 lists the HR for the development of incident Pap abnormalities during FU as related to the baseline HPV/Pap status and outcome of HR-HPV infection. Testing HR-HPV+ at baseline significantly increased the risk of incident abnormal Pap, irrespective of the smoking status, HR being highest (HR=11.17) for current smokers. Similarly, persistent HR-HPV infection during FU posed a significant risk for incident Pap abnormalities for both smokers and never smokers (not computable for past smokers). On the other hand, acquisition of HR-HPV infection during FU was not associated with a significant risk of incident Pap abnormalities, however.

Table 6 summarizes the HR for incident HR-HPV infection and biopsy-confirmed CIN2+ in multivariate analysis including the smoking status as a co-factor. These calculations are based on a sample size of 689 (out of 1,011) women, who attended at least one FU visit and who had both baseline cytology and HC2 results available. Additional 219 were excluded because of baseline ASC (atypical squamous cells) cytology or CIN1+ in their baseline cervical biopsy. Being a current smoker was a significant predictor of incident HR-HPV during the follow-up (HR =1.44; 95%CI 1.03-2.04). For incident CIN2+, baseline HR-HPV+ (HR=10.07; 95%CI 1.32-76.49), being a past smoker (HR=3.61; 95%CI 1.06-12.33) and current smoker (HR=3.51; 95%CI 1.21-10.14) were the three significant independent predictors. The incidence rates (per 1000 women months at risk, WMR) of CIN2+ in the three smoking categories (n=1,011) are compared at the bottom of the table. Compared with never smokers (0.75/1000 WMR), the incidence of CIN2+ was significantly higher among past- (3.19/1000 WMR) and current smokers (3.01/1000 WMR) as

calculated by the incidence rate ratio test, p=0.01 and p=0.005, respectively, whereas no difference was found between the past- and current smokers.

Univariate (Kaplan-Meier) survival analysis showing the cumulative incidence of CIN2+ among baseline HR-HPV+ women stratified according to their smoking status is shown in **Figure 1**. Current and past smokers had a significantly increased risk of incident CIN2+. Survival curves deviate from each other starting after the second FU visit, when smokers start rapidly accumulating incident CIN2+ (log-rank; p=0.00105).

Discussion

Epidemiological data on the role of smoking as a risk factor of CC and on the potential confounding of HPV are emerging only recently [9,10]. The early studies examining the development of CIN among smokers were limited by methodological approach because the analyses were invariably restricted to HPV-positive women [4, 25, 26]. This limitation precluded the assessment of the smoking on HPV acquisition, which has been addressed in more detail only recently. The pooled analysis on HPV Prevalence Surveys, undertaken by IARC, casts further light on the possible mechanisms responsible for the increased prevalence of CIN and CC among smokers [1]. In this pooled analysis, there was a consistent association between smoking and HPV infection, and this association seems to be dependent on the smoking intensity: the OR adjusted for several other known epidemiological co-factors, was 1.21 (95%CI 0.95-1.54) for women who smoked <5 cigarettes per day, but 2.01 (95%CI 1.32-3.08) for those smoking >=15 cigarettes daily [9].

The IARC pooled analysis included studies from Vietnam, Thailand, Spain, Mexico, Argentina, Chile, Colombia and Nigeria, thereby covering a wide spectrum of epidemiological differences in HPV type and prevalence, with socio-economic variability. In

the LAMS study, the study cohort is composed predominantly of urban populations from Brazil and Argentina, currently undertaking the demographic transition towards more developed living standards, but still facing economic and health-related constraints. In this predominantly urban population, the OR for current smokers to test HR-HPV positive at baseline (after adjustment for several well-known epidemiological risk factors) was 1.36 (95%CI 1.11-1.66) (Table 2), which is practically identical with the OR reported in the IARC pooled analysis [9]. This similar risk across different geographic regions suggests that the effects of smoking on the acquisition of HPV may not be restricted to HPV type distribution (varying from one region to another), nor be positively or negatively affected by ethnical, nutritional or behavioural characteristics of the women. Further confirmation is provided by another major cohort study (n=3,187 women) from New Independent States (NIS) of the former Soviet Union [10], in which current smokers had OR=1.52 (95%CI 1.09-2.14) for testing HR-HPV positive at baseline. Together with the present results and the IARC survey, these data implicate that cigarette smoking has a universal and independent effect on the risk of contracting HR-HPV.

Due to the cross-sectional and prospective cohort design of the LAMS study [17], we were able to assess the effect of smoking on both prevalence of HR-HPV infections and CIN2+, but also on the outcome of HR-HPV infections (persistence, clearance, acquisition) as well as on the development of incident CIN2+ during the prospective FU of over three years. The entire cohort of 12,114 women was stratified according to their smoking status as never smokers, past smokers and current smokers. These three groups were significantly different in the majority of their demographic data and epidemiological variables that are known risk factors of HPV, CIN and CC (Table 1). Current smokers had the highest prevalence of HR-HPV infections at baseline, and they also had the highest prevalence of

HSIL (LSIL, ASCUS) and biopsy-confirmed CIN lesions. Without further analysis, this would suggest that current smokers have an increased risk of HR-HPV and CIN, if not controlled for obvious confounders.

When this was done using multivariate models with baseline HR-HPV and baseline CIN2+ as outcomes, the results are interesting (Table 2). Indeed, there were several co-variates that were associated with prevalent HR-HPV much stronger than smoking, e.g. marital status, >5 sexual partners, age <35 years, as well as the surrogate markers LSIL and CIN. Importantly, smoking was not an independent risk factor of prevalent CIN2+, which was independently predicted by HR-HPV (OR=9.69) and history of no previous Pap smear (OR=2.65). These results are fully consonant with the data reported in the NIS cohort, where smoking was not an independent predictor of CIN2+, but the effect was mediated by HR-HPV, of which smoking was an independent predictor, exactly as in the present cohort [10].

This possibility that the effect of smoking on the risk for HR-HPV and CIN might be masked by the sexual behaviour of the women has been addressed to some extent recently. In their analysis, Vaccarella et al. [27] showed that the number of lifetime sexual partners was positively associated to either the prevalence of HPV and the number of cigarettes smoked. In the present study, both factors were entered in the multivariate model, to control whether this association is confounded or not. We observed that only the number of sexual partners (but not number of cigarettes) was significantly associated with HR-HPV (but not CIN2+) at baseline. It must be pointed out, however, that in this case, the statistical approach might be insufficient to overcome the effect of i) reporting and ii) recall biases. For instance, if women underreport their number of lifetime partners (thereby masking part of their risk of having HPV infection), the regression coefficients generated by the adjustment with

the number of cigarettes will be compromised. The reverse is, of course, also true. It is known from several epidemiological studies that reporting and recall bias are particularly strong concerning sexual and smoking habits [1, 4, 9]. This makes it important to regard the relationship between the number of cigarettes and number of sexual partners with caution. Equally important is to consider the confounding due to HR-HPV in all studies assessing the role of smoking as a risk factor for CIN and CC [1].

We next assessed, whether there is a dose-response relationship of smoking to detection of HR-HPV, Pap smear abnormalities or biopsy-confirmed CIN lesions at baseline, by measuring the years being smoker (current or past)(Table 3). There was no difference in the years of smoking as related to detection of HR-HPV+, CIN2+, HSIL or LSIL abnormalities. In the present study, we were unable to adequately measure the influence (if any) of time passed since the cessation of smoking on the detection of HR-HPV. However, it sounds feasible to infer that there must be a time-related decline in the risk of having an HPV infection after ceasing smoking, but a large cohort is likely to be necessary in order to evaluate this trend and determine the time period after which the smoking effect on the prevalence of HPV drops to non-smoker level. The major studies on the subject are cross-sectional and therefore do not provide this information [9].

The prospective cohort designed enabled us to assess, whether the smoking status has any impact on the outcome of HR-HPV infections or Pap smear abnormalities. Women in the three smoking categories did not differ in their baseline HPV/Pap status (Table 4). Similarly, there was no difference between the groups in the outcome (persistence, clearance, acquisition) of HR-HPV during the follow-up. This confirms the observations reported in the NIS cohort [10]. On the other hand, incident abnormal Pap were more common among past smokers, while persistent abnormal Pap were most frequent among

current smokers, making the Pap smear outcome significantly different between the three categories (p=0.017). The outcome of biopsy-confirmed histology was borderline different only among baseline Pap+ and/or HC2+ women with no CIN, in that progression to CIN2+ was more common among current smokers (p=0.037).

The issue becomes even more complex when incident Pap smear abnormalities are related to baseline HPV/Pap status and outcome of HR-HPV infections (Table 5). Smoking status had no effect on incident Pap abnormalities, which significantly increased among baseline HR-HPV+ women, irrespective of their smoking status. Similarly, smoking had no effect on incident Pap among women who had persistent HR-HPV during the follow-up. Interestingly, incident HR-HPV did not significantly increase the risk of abnormal Pap in never smokers and current smokers. This combination of increased prevalence of HR-HPV in the baseline assessment and lack of difference in outcome during follow-up might have several explanations. It might be that the difference may be only seen in the long run, and examining a much larger cohort. It can also suggest that the effects of smoking are temporary, because past smokers are comparable in risk of having HR-HPV infection at baseline to that of never smokers (Table 1). The same was true for CIN2+, which was equally common among past smokers and never smokers. In addition, it seems to be the persistent HR-HPV infections that are more important determinants of incident Pap abnormalities rather than incident HR-HPV, which did not increase the risk (Table 5).

Finally, it was of importance to assess, whether smoking status is an independent risk factor of incident HR-HPV infections or incident CIN2+ during the follow-up (Table 6). Indeed, smoking status (being a current smoker, but not being past smokers) did increase the risk of incident HR-HPV, albeit not dramatically (OR=1.44). On the other hand, smoking

increased the risk of incident CIN2+, despite the fact that it was not an independent predictor of baseline CIN2+ (Table 2). However, the baseline HR-HPV was a far more powerful predictor of incident CIN2+ than being past- or current smoker. When calculated per 1000 woman months at risk, the incidence rate of CIN2+ among past- and current smokers is 3-fold higher than among never smokers. This might implicate that smoke components speed up the transformation process of epithelial cells, once infected by oncogenic HPV that remains persistent.

In the present analysis, different predictors were disclosed for baseline HR-HPV and baseline CIN2+ and the same was true with incident HR-HPV and incident CIN2+. The number of incident CIN2+ cases (n=30) was not particularly large, however, and one needs to consider the possibility that the different sets of predictors for HR-HPV and CIN2+ could be related to the lower prevalence of CIN2+ as compared to that of HR-HPV. This has been encountered in other population-based studies, suggesting that the much lower prevalence of CIN and CC makes HR-HPV more amenable to epidemiological risk assessments. Indeed, this has prompted most studies to limit their analyses to HPV-positive women [1, 4, 25], which hampers controlling for the confounding effect of HPV. Similarly, studies analyzing the effect of smoking in speeding up the progression of low-grade CIN to high-grade CIN or cancer are few; actually, there is only one consistent study on this subject, and their results have never been reproduced [28].

The present results implicate that being current smoker increases the prevalence of baseline HR-HPV infections. Past-, current- and never smokers differ in the majority of key epidemiological risk factors of HPV, CIN and CC, most notably those associated with risk sexual behaviour. Together with several of these risk factors, current smoking is an independent predictor of baseline HR-HPV infections, but not baseline CIN2+, for

which HR-HPV is the single most powerful predictor. Persistent HR-HPV infections increase the risk of abnormal Pap independent on the smoking status, and HR-HPV is also the single most powerful predictor of incident CIN2+. Being past- or current smoker are the other two independent predictors of incident CIN2+, the incidence among these women being 3-fold higher than among never smokers. The most feasible explanation of these data implicates that smoking-related high-risk sexual behaviour increases the risk of contracting HR-HPV infection, making current smokers with persistent HR-HPV+ true high-risk patients for disease progression (OR=16.2; 95%CI 3.72-70.74 for progression to CIN1+; for CIN2+, no cases of progression among HR-HPV-negative never smokers). Similar incidence and predictive power of incident CIN2+ among past- and current smokers (Figure 1; Table 6) might suggest that this increased risk associated with cigarette smoking persists several years after cessation of smoking.

References

1. Plummer M, Herrero R, Franceschi S et al. Smoking and cervical cancer: pooled analysis of the IARC multi-centric case-control study. Cancer Causes and Control 2003; 14(9): 805-814.
2. Procopzyk B, Cox JE, Hoffman D, Waggoner SE. Identification of tobacco-specific carcinogen in the cervical mucus of smokers and nonsmokers. Journal of the National Cancer Institute 1997; 89(12):pp. 868-873.
3. Assieh A, Melikian A, Sunb Peng et al. Identification of benzo[a]pyrene metabolites in cervical mucus and DNA adducts in cervical tissues in humans by gas chromatography-mass spectrometry. Cancer Letters 1999; 146(2):127-134.

4. Tolstrup J, Munk C, Thomsen BL, et a. The role of smoking and alcohol intake in the development of high-grade squamous intraepithelial lesions among high-risk HPV-positive women. Acta Obstet Gynecol Scand 2006; 85:1114-1119.
5. Poppe WA, Ide PS, Drijkoningen MP, et al. Tobacco smoking impairs the local immunossurveillance in the uterine cervix. Gynecol Obstet Invest 1995; 39:34-8.
6. Poppe WA, Peters R, Drijkoningen MP, et al. Cervical cotinine and macrophage-Langerhans cell density in the normal uterine cervix. Gynecol Obstet Invest 1996; 41:253-59.
7. Ferson M, Edwards A, Lind A, Milton GW, Hersey P. Low natural killer-cell activity and immunoglobulin levels associated with smoking in human subjects. Int J Cancer 1979; 23:603-9.
8. Herrero R, Brinton LA, Reeves WC et al. Invasive cervical cancer and smoking in Latin America. J Natl Cencer Inst 1989; 8:205-11.
9. Vaccarella S, Herrero R, Snidijers PJ, et al. Smoking and papillomavirus infection: pooled analysis of the International Agency for Research on Cancer HPV Cancer Prevalence Surveys. Int J Epidemiol 2008; 1-11.
10. Syrjänen K, Shabalova I, Petrovichev N, et al. Smoking is an independent risk factor for oncogenic human papillomavirus (HPV) infections but not for high-grade CIN. Eur J Epidemiol 2007; 22:723-735.
11. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006; 24S1: S1/4-S1/15.
12. Castellsague X, Munoz N. Chapter 3: Cofactors in human papillomavirus carcinogenesis--role of parity, oral contraceptives, and tobacco smoking. J Natl Cancer Inst Monogr 2003; 31:20-28.

13. Campo, S. (ed). Papillomavirus Research: From Natural History to Vaccines and Beyond. Caister Academic Press 2006, Norwich, UK, pp. 1-424.
14. Cavalcanti SM, Zardo LG, Passos MR, Oliveira LH. Epidemiological aspects of human papillomavirus infection and cervical cancer in Brazil. J Infect 2000; 40:80-87.
15. Reeves WC, Rawls WE, Brinton LA. Epidemiology of genital papillomaviruses and cervical cancer. Rev Infect Dis 1989; 11:426-439.
16. Bosch X, de Sanjosé S. The epidemiology of human papillomavirus infection and cervical cancer. Dis Markers. 2007; 23(4):213-27. Review
17. Syrjänen K, Naud P, Derchain S. Comparing PAP smear cytology, aided visual inspection, screening colposcopy, cervicography and HPV testing as optional screening tools in Latin America. Study design and baseline data of the LAMS study. Anticancer Res. 2005; 25:3469-80.
18. Longatto-Filho A, Erzen M, Branca M, Roteli-Martins C, et al. Human papillomavirus testing as an optional screening tool in low-resource settings of Latin America: experience from the Latin American Screening study. Int J Gynecol Cancer 2006; 16:955-962.
19. Sarian LO, Derchain SF, Naud P, et al. Evaluation of visual inspection with acetic acid (VIA), Lugol´s Iodine (VILI), cervical cytology and HPV testing as cervical screening tools in Latin America. This report refers to partial results from the LAMS study. J Med Screen 2005; 12:142-9.
20. Ministério da Saúde – Instituto Nacional do Câncer – Atlas de Mortalidade por Câncer no Brasil 1979-1999. INCA, 2002. Available online (only Portuguese) at http://www.inca.gov.br/atlas/docs/Atlas_completo.pdf

21. Argentina, Ministry of Health. Estadísticas vitales. Información básica año 2005. Buenos Aires: Dirección de Estadísticas e formación de Salud, 2006.
22. 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. JAMA. 2002; 287:2114-9.
23. Lörincz A T, Castle P E, Sherman M E, et al. Viral load of human papillomavirus and risk of CIN 3 or cervical cancer. Lancet 2002; 360:288-9.
24. R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-Project.org.
25. McIntyre-Seltman K, Castle PE, Guido R, Schiffman M, Wheeler CM. Smoking is a risk factor for cervical intraepithelial neoplasia grade 3 among oncogenic human papillomavirus DNA-positive women with equivocal or mildly abnormal cytology. Cancer Epidemiol Biomarkers Prev 2005; 14:1165-70.
26. Matos A, Moutinho J, Pinto D, Medeiros R. The influence of smoking and other cofactors on the time to onset to cervical cancer in a southern European population. Eur J Cancer Prev 2005; 14:485-91.
27. Vaccarella S, Franceschi S, Herrero R et al. Sexual behaviour, condom use, and human papillomavirus infection: pooled analysis of the IARC human papillomavirus prevalence surveys. Cancer Epidemiol Biomarkers Prev 2006; 15:326-33.
28. Ho GY, Kadish AS, Burk RD, et al. HPV 16 and cigarette smoking as risk factors for high-grade cervical intra-epithelial neoplasia. Int J Cancer 1998; 78:281-285.

Table 1. Key epidemiological characteristics of the women stratified by their smoking status

Characteristic	Never smoked (N=7499)	Smoked in the past (N=1871)	Current smoker (N=2706)	Significance p-value
Age (M±SD)#	37.20±11.40	41.13±10.44	37.42±10.67	<0.0001
Years of Education (M±SD)#	8.63±4.07	8.31±4.29	8.94±4.27	<0.0001
Marital Status – Single*	2285/7492 (30.5%)	550/1868 (29.4%)	971/2702 (35.9%)	<0.0001
HPV positive (HCII test)*	444/2699 (16.5%)	96/709 (13.5%)	215/990 (21.7%)	0.001
HPV index (pg/ml; M±SD)#	33.34±203.88	24.66±168.54	56.11±266.94	0.00047
PAP Smear:				
HSIL or worse*	73/7492 (1.0%)	14/1870 (0.7%)	62/2704 (2.3%)	<0.0001
LSIL or worse*	157/7492 (2.1%)	42/1870 (2.2%)	105/2704 (3.9%)	<0.0001
ASCUS or worse*	355/7492 (7.1%)	88/1870 (4.7%)	192/2704 (7.1%)	<0.0001
Final Screening Diagnosis:				
CIN3 or Cancer*	57/7419 (0.8%)	14/1851 (0.8%)	47/2677 (1.8%)	<0.0001
CIN2 or worse*	92/7499 (1.2%)	19/1871 (1.0%)	64/2706 (2.4%)	<0.0001
Any Lesion*	267/7499 (3.6%)	52/1871 (2.8%)	160/2706 (6.0%)	0.0005
Ever been pregnant*	5869/7497 (78.3%)	1641/1871 (87.7%)	2198/2706 (81.2%)	<0.0001
Number of deliveries (M±SD)#	1.39±1.78	1.56±1.84	1.45±1.71	0.094
Ever had abortions*	2170/7497 (28.9%)	723/1871 (38.6%)	1001/2706 (37.0%)	<0.0001
Number of abortions (M±SD)#	0.44±0.88	0.62±1.05	0.62±1.10	0.420
Age at first sexual intercourse#	18.80±4.10	18.59±3.94	17.63±3.32	<0.0001
Sexually active (last sexual intercourse < 12 months)*	6777/7497 (90.4%)	1620/1870 (86.6%)	2408/2705 (89.0%)	<0.0001
Currently, only one sex partner	6483/7497 (86.5%)	1558/1870 (83.3%)	2231/2705 (82.5%)	<0.0001
No. partners during previous 12 months (M±SD)#	0.96±0.49	0.92±0.52	1.08±2.76	<0.0001
No. partners since the first intercourse	2.38±2.85	3.10±4.99	3.51±5.32	<0.001
Ever had STD*	484/7495 (6.5%)	202/1870 (10.8%)	241/2704 (8.9%)	<0.0001
Partner ever had STD*	505/7495 (6.7%)	219/1870 (11.7%)	239/2705 (8.8%)	<0.0001
Ever taken Pap smear*	6656/7499 (88.2%)	1750/1871 (93.5%)	2417/2704 (89.4%)	<0.0001
No. of life-time Pap smears	6.82±6.12	8.29±6.77	7.05±6.28	<0.0001
Time since the last Pap test (months; M±SD)#	22.80±23.91	22.81±23.31	26.09±26.40	<0.0001
History of skin- or genital warts*	133/7499 (1.8%)	43/1871 (2.3%)	74/2705 (2.7%)	0.008
History of previous CIN*	94/7499 (1.3%)	30/1871 (1.6%)	38/2705 (1.4%)	0.4746
If current smoker, for how long (yrs; M±SD)	-	-	15.89±10.33	-
Number of cigarettes per day currently (M±SD)	-	-	11.32±8.47	-
If smoked in the past, for how long (yrs; M±SD)	-	10.26±8.09	-	-
Time since stopped smoking (yrs; M±SD)	-	8.09±7.81	-	-
Number of cigarettes per day in the past (M±SD)	-	11.14±10.60	-	-

#Kruskal-wallis test; * Pearson Chi-Square

Table 2. Predictors of HR-HPV infection and CIN2+ at baseline

Co-variates	HR-HPV infection		High-grade CIN (CIN2+)	
	*Adjusted OR (95% CI)	P	*Adjusted OR (95% CI)	P
HR-HPV+ at baseline			9.69 (5-18.79)	<0.0001
High-Grade CIN (CIN2 and above)	9.15 (4.74-17.66)	<0.0001		
Age <35 years	1.69 (1.39-2.04)	<0.0001	1.42 (0.76-2.67)	0.271
HSIL Pap	1.00 (0.41-2.43)	0.998	40.52 (8.52-192.6)	<0.0001
LSIL Pap	12.4 (7.15-21.51)	<0.0001	1.08 (0.25-4.78)	0.916
Hormonal Contraception	1.22 (0.95-1.58)	0.118	1.01 (0.42-2.45)	0.983
Other Contraception	0.96 (0.75-1.22)	0.727	1.46 (0.65-3.29)	0.361
Early onset of sexual activity (=14 yo)	1.2 (0.91-1.58)	0.198	0.97 (0.41-2.31)	0.944
No previous Pap	1.16 (0.88-1.54)	0.289	2.65 (1.21-5.79)	0.015
Marital status (single)	1.55 (1.28-1.87)	<0.0001	1.61 (0.89-2.93)	0.115
>5 partners	1.74 (1.39-2.17)	<0.0001	1.51 (0.78-2.91)	0.224
Partner with STD	1.24 (0.87-1.77)	0.233	1.05 (0.35-3.14)	0.937
History of STD	0.77 (0.54-1.11)	0.161	1.46 (0.52-4.1)	0.471
Ever Been Pregnant	0.79 (0.63- 1.00)	0.045	1.32 (0.63-2.79)	0.467
Current Smoker**	1.36 (1.12-1.66)	0.002	0.81 (0.45-1.46)	0.488

*Adjusted for all other variables in the table; **Ever smoker or Smoked in the past were not statistically associated with HR-HPV infection or CIN2+

Table 3. Years of being smoker related to baseline HR-HPV, CIN2+ and Pap smear abnormalities among current and past smokers

Co-variates	Current smokers			Past smokers					
	Time of smoking (Years)			Time of smoking in the Past (Years)			Time since stopped smoking (Years)		
	Yes	No		Yes	No		Yes	No	
	Mean ±SD	Mean ±SD	P*	Mean ±SD	Mean ±SD	P*	Mean ±SD	Mean ±SD	P*
HR-HPV at baseline	14.5 ±±10.2	16.7 ±10.3	0.87	8.5 ±7.4	10.3 ±8.8	0.49	6.5 ±6.1	7.9 ±7.6	0.45
CIN2+ at baseline	16.9 ±9.4	15.8 ±10.4	0.59	7.9 ±7.7	10.3 ±8.8	0.18	4.8 ±5.3	8.1 ±7.8	0.43
HSIL Pap at baseline	18.4 ±9.4	15.8 ±10.4	0.07	7.6 ±5.5	10.3 ±8.8	0.56	5.4 ±4.2	7.9 ±7.6	0.96
LSIL Pap at baseline	15.9 ±9.6	15.8 ±10.4	0.29	8.6 ±6.8	10.3 ±8.8	0.22	5.1 ±4.9	8.2 ±7.8	0.86

*Adjusted for age, number of cigarettes smoked per day, marital status, number of partners, history of STD (patient and partner), number of pregnancies; **Adjusted for age, number of cigarettes smoked per day, marital status, number of partners, history of STD (patient and partner), number of pregnancies and time elapsed since quit smoking

Table 4. Outcome of HR-HPV infections and clinical disease monitored by Pap test and biopsy

	Never smoked	Smoked in the past	Current smoker	*P#*
Baseline Status	Per Cent (Number)	Per Cent (Number)	Per Cent (Number)	
HPV-/PAP-	24.8% (120/195)	23.8% (46/195)	23.1% (29/195)	
HPV-/PAP+	14.9% (72/108)	16.4% (16/108)	8.1% (20/108)	0.083
HPV+/PAP-	46.2% (223/375)	46.7% (95/375)	47.7% (57/225)	
HPV+/PAP+	14.1% (68/126)	13.1% (42/126)	21.1% (16/126)	
Outcome of HPV infection				
Always negative	35.1% (122/193)	37.9% (33/193)	27.5% (38/193)	
New infection	3.7% (13/19)	3.4% (3/19)	2.2% (3/19)	
Persistence	16.7% (58/106)	17.2% (15/106)	23.9% (33/106)	0.549
Cleared	41.7% (145/237)	39.1% (34/237)	42.0% (58/237)	
Fluctuation	2.9% (10/18)	2.3% (2/18)	4.3% (6/18)	
Outcome of Pap smear				
Always negative	55.9% (337/547)	47.3% (71/547)	54.9% (139/547)	
New abnormal PAP	12.1% (73/132)	18.7% (28/132)	12.3% (32/132)	
Persistent abnormal PAP	2.3% (14/39)	5.3% (8/39)	6.7% (17/39)	0.017
Cleared abnormal PAP	27.0% (163/256)	24.7% (37/256)	24.7% (56/256)	
Fluctuation (pos-neg-pos)	2.7% (16/32)	4.0% (6/32)	4.0% (10/32)	
Outcome of baseline CIN 1				
Progressed to CIN2+	3.0% (4/7)	7.7% (2/7)	1.6% (1/7)	
Persisted	9.8% (13/22)	3.8% (1/22)	13.1% (8/22)	0.622
Persisted and then regressed	12.8% (17/27)	7.7% (8/27)	13.1% (2/27)	
Regressed	74.4% (99/164)	80.8% (21/164)	72.1% (44/164)	
Outcome of baseline PAP+ and/or HPV+ cases				
Progressed to CIN2+	1.7% (8/25)	4.8% (6/25)	5.8% (11/25)	
Progressed to CIN1	6.1% (29/50)	4.8% (6/50)	7.9% (15/50)	0.037
No progression to CIN lesion	92.2% (435/710)	90.3% (112/710)	86.2% (163/710)	

#Pearson Chi-Square

Table 5. Risk of incident Pap smear abnormalities as related to baseline status and outcome of HR-HPV infections

	Never smoked	Smoked in the past	Current smoker
Baseline HPV#/PAP Status	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
HPV-/PAP-*	Ref	Ref	Ref
HPV-/PAP+	2.02 (0.84-4.81)	4.19 (0.99-17.6)	2.33 (0.65-8.33)
HPV+/PAP-	3.43 (1.68-6.97)	6.11 (1.80-20.7)	2.70 (1.08-6.70)
HPV+/PAP+	5.74 (2.70-12.17)	3.92 (1.01-15.2)	11.17 (4.22-29.50)
Outcome of HPV infection			
ALWAYS NEGATIVE	Ref	Ref	Ref
NEW INFECTION	2.75 (0.76-9.85)	NC	3.68 (0.71-19.06)
PERSISTENCE	7.51 (3.75-15.02)	NC	4.94 (1.76-13.83)
CLEARED	3.01 (1.51-5.96)	NC	2.26 (0.79-6.47)
FLUCTUATION	3.42 (0.95-12.25)	NC	2.94 (0.56-15.32)

Hybrid Capture II assay; *LSIL threshold; Ref = reference; NC = not computable; HR = hazard ratio

Table 6. Predictors of incident HR-HPV infection and CIN2+ in multivariate model as well as incidence rates of CIN2 among the three smoking categories

Co-variates	Hazard Ratio (95% CI)	
	Outcome During Prospective Follow-up	
	HR-HPV infection	CIN2+
Positive hr-HPV at baseline		10.07 (1.32-76.49)
Age <35 yrs	1.16 (0.80-1.69)	1.81 (0.86-3.79)
Baseline HSIL Pap	NC	0.91 (0.12-6.80)
Other contraception	0.75 (0.46-1.24)	0.79 (0.32-1.95)
Oral contraception	1.25 (0.77-2.02)	1.70 (0.46-6.34)
Ever been pregnant (Y/N)	0.96 (0.64-1.43)	0.69 (0.34-1.39)
No. of deliveries	0.98 (0.89-1.09)	0.85 (0.65-1.13)
Recent partner	1.03 (0.87-1.22)	0.89 (0.54-1.45)
Early onset of sexual activity	0.96 (0.91-1.01)	0.98 (0.89-1.08)
Previous Pap normal	0.88 (0.55-1.42)	2.63 (0.89-7.74)
Smoker in the past	**1.05 (0.68-1.64)**	**3.61 (1.06-12.33)**
Current smoker	**1.44 (1.03-2.04)**	**3.51 (1.21-10.14)**

		Differences in CIN2+ Incidence				
Smoking status	**N**	**No of Cases**	**WMR**	**Incidence rate/1000 WMR**	**Incidence Rate Ratio (95%CI)**	**Significance**
Never Smoked	608	10	13332	0.75	Reference	
Smoked in the past	150	8	3337	2.39	3.19 (1.32-7.70)	p=0.0103
Current smoker	253	12	5301	2.26	3.01 (1.35-6.70)	p=0.0058

NC = not computable; WMR, woman months at risk; RR between smokers and past smokers (RR=0.944; 95%CI 0.386-2.309)(p=0.445)

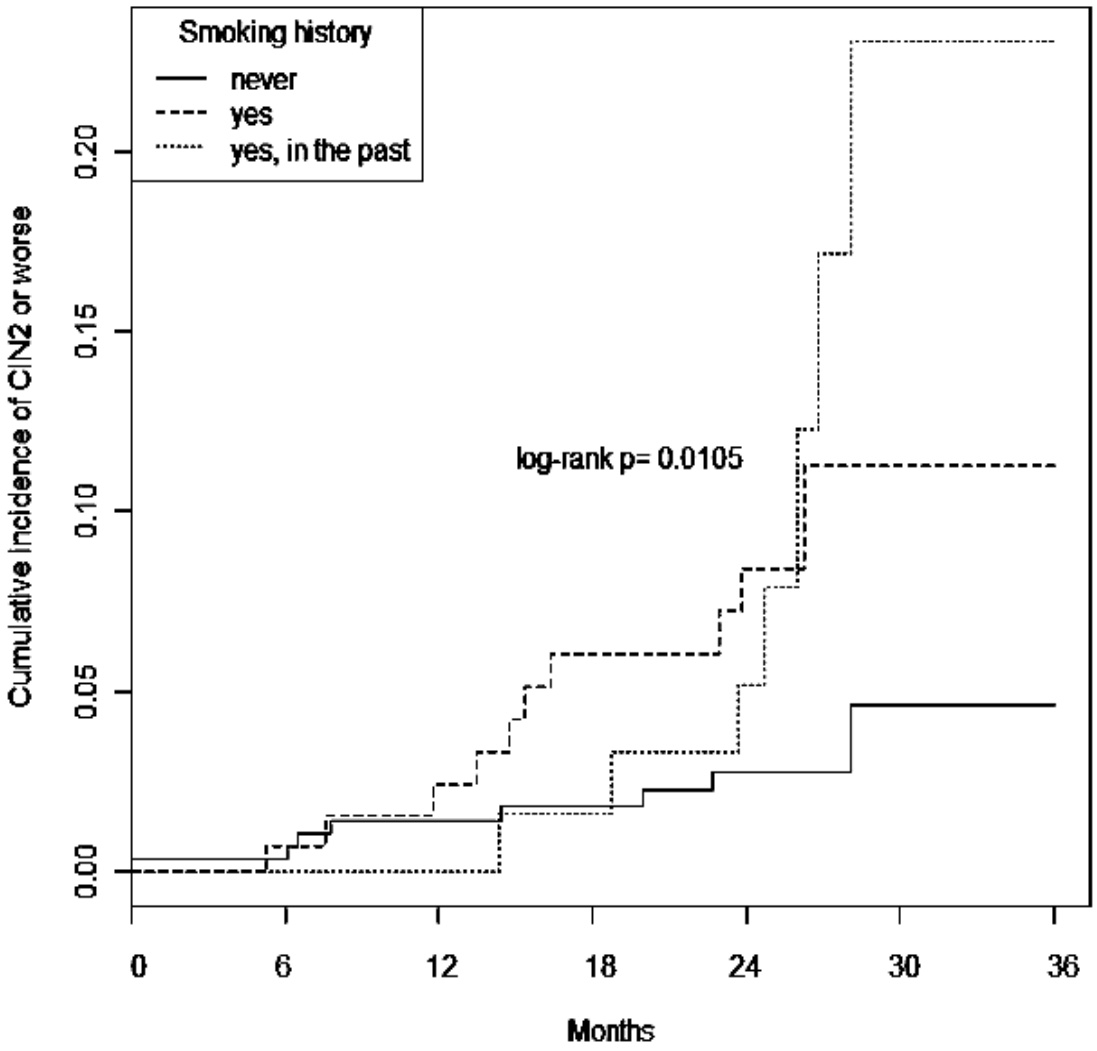


Figure 1. Cumulative incidence of CIN2+ among baseline HR-HPV+ women stratified by their smoking history in univariate Kaplan-Meier analysis

3.2. Artigo 2

---------- Cabeçalho original ----------

De: onbehalfof@scholarone.com
Para: sarian@terra.com.br
Cópia:
Data: Sat, 28 Jun 2008 19:47:22 -0400 (EDT)
Assunto: Diagnostic Cytopathology – Article submitted

28-Jun-2008

Dear Prof. Sarian,

This is to acknowledge receipt of your manuscript entitled "Smoking contributes additional risk for cervical intraepithelial neoplasia in women with normal colposcopy and atypical squamous cells (ASC) or low-grade squamous intraepithelial lesion (LSIL)" at Diagnostic Cytopathology - Manuscript Central. The manuscript will be checked for compliance with the journal's instructions to authors and once accepted a tracking number will assigned.

Account details follow:

Site URL: http://mc.manuscriptcentral.com/dc
Your User ID: sarian@terra.com.br
Your Password: Your Password:
Your Full Name: Prof. Luis Sarian
Your Address: hermantino coelho 501
Campinas
Sao Paulo
Brazil
13087-500
Your Degree:
Your E-Mail Address: sarian@terra.com.br
Your Cc E-Mail Address:
Your Institution: Universidade Estadual de Campinas
Your Department: Obstetrics and Gynecology

Thank you.

Sincerely,

Diagnostic Cytopathology Editorial Office

Smoking contributes additional risk for cervical intraepithelial neoplasia in women with normal colposcopy and atypical squamous cells or low-grade squamous intraepithelial lesion.

Authors:
Renata Guarisi[1],M.D.
Luis Otavio Sarian[1], M.D, PhD.
Luciano Serpa Hammes[2] , M.D., PhD.
Adhemar Longatto-Filho[3,4], M.Sc, PhD, PMIAC.
Sophie FM Derchain[1], M.D., PhD.
Cecília Roteli-Martins[5], M.D., PhD.
Paulo Naud[2], M.D., PhD.
Mojca Eržen[6], M.D., PhD, MIAC.
Margherita Branca[7], M.D., PhD., MIAC.
Sílvio Tatti[8], M.D., PhD.
Jean Carlos de Matos[2], M.D.
Renata Gontijo[1], M.D.
Marina YS Maeda[4], M.Sc.
Temístocles Lima[5], M.D.
Silvano Costa[9], M.D., PhD,
Stina Syrjänen[10], D.D.S., PhD.
Kari Syrjänen[11], M.D., PhD, FIAC.

[1]Universidade Estadual de Campinas, Brazil
[2]Hospital de Clinicas de Porto Alegre, Brazil
[3]Life and Health Sciences Research Institute, School of Health Sciences, University of Minho, Braga, Portugal
[4]Instituto Adolfo Lutz , São Paulo, Brazil
[5] Hospital Leonor Mendes de Barros (HLMB), São Paulo, Brazil
[6]SIZE Diagnostic Center, Ljubljana, Slovenia
[7]Unit of Cytopathology, National Centre of Epidemiology, Surveillance and Promotion of Health, National Institute of Health (ISS), Rome, Italy
[8]First Chair Gynecology Hospital de Clinicas, BA, Argentina
[9]Department of Gynecological, Obstetric and Pediatric Science, S.Orsola-Malpighi Hospital, Bologna, Italy
[10]Department of Oral Pathology, University of Turku, Finland
[11]Department of Oncology & Radiotherapy, Turku University Hospital, Finland

Address for correspondence:
Luís Otávio Sarian

Alexander Fleming, 848, Nova Campinas, Campinas, São Paulo – Brazil

sarian@terra.com.br

Abstract

Objective: To examine the effect of tobacco smoking on the incidence of low- and high-grade CIN in women with baseline atypical squamous cells (ASC) or low-grade squamous intraepithelial lesion (LSIL). **Methods:** This study reports on a subset of 1,000 cohort of the Latin American Screening (LAMS) study, composed of 150 women, prospectively followed up (FU) as part of the (LAMS) Study in São Paulo, Campinas, Porto Alegre and Buenos Aires, who had baseline cytology ASC/LSIL and normal colposcopy. Two were formed according to the women's smoking history: women that never smoked or that smoked in the past (n=83); current smokers (n=67). These women were controlled at 6-month intervals with colposcopy, HC2 and Pap to assess the cumulative risk of incident low- and high-grade CIN. **Results:** In a multivariate Cox analysis, the only factor related to an increased risk of developing CIN was the positive hr-HPV status (HR = 3.42; 95%CI 1.11 to 9.43). The smoking history was not associated to the risk of developing CIN (HR = 0.73; 95%CI 0.40 to 1.33). A total of 21 cases of incident CIN were detected during FU. Of these, 11 appeared in the group of 67 smokers and 10 among the 83 nonsmoker women (log-rank p = 0.33). While restricting the analysis to high-grade CIN, the probability of developing the disease was significantly higher for smokers (p= 0.04). Eleven cases of high-grade CIN were detected during FU, being 7 in smokers versus only 3 in the group of nonsmoker women. The probability (Kaplan-Meyer) curves clearly deviate from each other from semester 4 onwards. **Conclusions:** These data clearly implicate that smoking contributes additional risk for developing high-grade CIN in women with atypical squamous cells or low-grade squamous intraepithelial lesion and normal colposccopy.

Key Words: Cervical cancer, smoking, follow-up, cervical intraepithelial neoplasia

Introduction

Recent epidemiological lines of evidence demonstrate that tobacco usage is a potential contributor to the risk of developing cervical abnormalities (Castellsague 2003, Plummer 2003). It is still unknown, however, whether this effect should be ascribed solely to the carcinogens present in tobacco or to the combined injunctions from these carcinogens and the reduced immune response in the cervix of smoker women (Procopzyk 1997, Melikian 1999). It is also known that women with a screening cytology = ASC (atypical squamous cells) or LSIL (low-grade squamous intraepithelial lesion) form a group at increased risk of developing low- or high grade cervical intraepithelial neoplasia (CIN), and therefore should receive intensified screening attention. For this group of women, the ASC/LSIL Triage Study provided solid epidemiological evidence that helped plan the necessary special screening strategies (Cox 20003, Guido 2003, Walker 2006). However, the current guidelines make no distinction between smokers and non-smokers.

There is firm epidemiological and laboratorial substrate to justify a special analysis of the long-term outcomes of women with ASC/LSIL cytology according to their smoking history (Ho 1998). For instance, it is speculated that the tobacco carcinogens may contribute additional DNA damage to epithelial cells already infected by oncogenic (high-risk, HR) Human papillomavirus (HPV). The E6 and E7 oncogenes are known to disrupt the p53 and pRb gene pathways, leading to uncontrolled cell proliferation and failure to repair the damaged DNA (Trottier 2006). The DNA harms caused by tobacco carcinogens may therefore be amplified by the disrupted repair mechanisms, accelerating the carcinogenic process (Poppe 1995, Poppe 1996, Ferson 1979). There is unequivocal indication that women with ASC/LSIL most likely harbor a HPV infection, which indicates that they are a group of women for which tobacco smoking may be specially threatening (McIntyre

2005). It is also speculated that smoking facilitates the acquisition and stabilization of HPV thus favoring persistence of HPV infections. In this case, the clearance of the HPV infection, expected in most (specially young) women with mild HPV-related cervical abnormalities, would less likely occur. The immunological surveillance is also essential for the spontaneous regression of low-grade CIN, which may be thus impaired in smokers.

Behavioral habits more prevalent in smokers may also contribute additional risk to the development of CIN. It is known from large epidemiological studies that women who smoke tend to have more liberal sexual habits compared to nonsmokers (Herrero 1989, Vaccarela 2006). This behavior may lead to an augmented risk of contracting multiple type HPV infections, which in turn are a known risk factor for CIN (Castellsague 2003, Campo 2006).

The large dataset constructed from the LAMS (Latin American Screening Study), testing eight optional screening tools in a cohort of over 12,000 women in Brazil and Argentina, enabled the prospective analysis of a large cohort, formed of over 1000 women in Brazil and Argentina (Syrjänen 2005). From this cohort, it was possible to examine women with different baseline statuses. In the present study, we address the long-term effects of smoking on women with baseline ASC/LSIL. This approach yielded further insight into the potential contributory role of tobacco smoking to the risk of developing CIN in a group of women with an already increased potential of an unfavorable outcome.

Patients and methods

Study design

The Latin American Screening (LAMS) study is a multi-center population-based cohort study testing optional screening strategies and assessing risk factors of cervical disease in a cohort of over 12,000 women enrolled between January 2002 and November 2003

in Brazil and Argentina. A total of 12,114 consecutive women from the cities of Campinas, São Paulo and Porto Alegre (Brazil) as well as Buenos Aires (Argentina) were enrolled in the cohort to undergo screening with conventional Pap smear, HR-HPV testing by Hybrid Capture 2 (HC2), visual inspection with acetic acid (VIA) or Lugol iodine (VILI), cervicography and screening colposcopy (Syrjänen et al. 2005). All centers performed conventional Pap smear, HC2 and VIA, whereas Porto Alegre performed VILI, Buenos Aires did screening colposcopy and only Campinas did cervicography (Longatto 2006, Sarian 2005). The study protocol has been approved by the local Ethics Committees of all participating clinics. All enrolled women gave their agreement to participate by signing the Informed Consent Forms, written in their native language.

Women were considered eligible, if they met all of the following criteria: a) age between 15 to 60 years; b) no previous surgical procedure of the cervix or uterine corpus; c) had no history of abnormal Pap test in the past year; d) free of diagnosed (=prevalent) genital wart (external or cervical condyloma), CIN or cervical cancer (CC); e) had no sexual intercourse during the three days prior to the examination; f) did not have any confirmed or clinically suspect immunosuppression (HIV, or other conditions).

Study centers and their demographics

The study design and features of the clinical centers as well as the demographics of their female populations were described in detail recently (Longatto 2006, Sarian 2005). In brief, Campinas and São Paulo are two large south-western Brazilian cities, located only 100 km apart, with equivalent standards of living. CC is the fourth major cause of cancer death among women, accounting for 3.3% of all female cancer deaths (Ministério da Saúde 2002). The third Brazilian partner is from Porto Alegre, located south of the country. This region

offers the best quality of life in Brazil, and CC is the sixth major cause of cancer death, accounting for 6.1% of all female cancer deaths (Ministério da Saúde 2002). The Argentine partner is from Buenos Aires. The country has an overall CC mortality rate of 7.6/100,000 women (Argentina 2006), but most of the national statistics pertain to Buenos Aires city only.

Diagnostic setting

All women were subjected to thorough pelvic examination in this sequence: i) collection of the Pap smear, ii) collection of HC2 sample and iii) VIA. In Porto Alegre, most women were subjected to VILI shortly after VIA. All women, who had at least one of these examinations abnormal, were referred for colposcopic examination. In Buenos Aires and Campinas, women were subjected to screening colposcopy even when their exams were negative. Abnormal colposcopy prompted punch biopsies of the cervix, and women with high-grade cytological abnormalities (HSIL) were treated by conization.

Women had their second visit scheduled one month and a half (average 45 days), to become informed about their examination and/or biopsy results and to be allotted to either A) the treatment or B) the follow-up group. Treatment was offered to all women who had high-grade lesion (CIN2+) confirmed in the cervical biopsy. Altogether, 32 cases of invasive CC were diagnosed during the recruitment phase and all those were treated according to each institution's standard protocols.

Follow-up (FU)

A total cohort of 1,011 women completed at least one follow-up (FU) visit. In the present study, however, we analyzed the FU data derived from a cohort of 659 women who had both baseline cytology and HC2 results available. This group was further

reduced to 150, because only women with baseline ASC (n=98) or LSIL (n=52) were included. Therefore, the study sample in the present study consists of 150 women with ASC/LSIL baseline cytology and normal colposcopy and/or normal cervical biopsies, who attended at least one FU visit.

The FU workup for women considered "normal" after baseline assessment was similar in all study centers, with FU visits being scheduled at six months intervals. The study protocol determined that women should be re-examined four times (optimal moments should have been 6, 12, 18 ad 24 months; see below). At each FU visit, women responded to a brief questionnaire addressing any relevant gynecological events and epidemiological features changed since the previous control (e.g. sexual partners, smoking). The total FU time encompassed in this report approaches 50 months, but the bulk of the data covers approximately 36 months (median FU time = 24.4 mo; 90% central range = 6.8 to 32.2 mo).

Cervical cytology (Pap smear)

Conventional Pap smears were taken using the Ayre spatula and endocervical brush, fixed in 95% ethanol and stained by the modified Papanicolaou method. Final cytological diagnoses were issued using the Bethesda System (Solomon 2002) and were classified as normal/inflammatory, ASC, atypical glandular cells (AGC), LSIL, HSIL or cancer.

Hybrid Capture 2 (HC2)

The specimens for HC2 were tested with probe B for high-risk HPV (HR-HPV) types: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) (Lörincz 2002) and the tests were classified positive at the relative light unit/positive control (RLU/CO) ratio of 1pg/ml or greater. These RLU/CO ratios provide a semi-quantitative estimate of the amount of

HPV DNA in the specimens, i.e., the viral load in the sample. Storage of reagents and specimens as well as their processing were carried out in manufacturer-certified laboratories, under the responsibility of the investigators, following the manufacturer's instructions (*Digene Diagnostics Inc., USA*). São Paulo and Buenos Aires processed their own HC2 samples in-house, whereas Campinas and Porto Alegre had their HC2 specimens processed at Campinas University hospital laboratory.

Retrieval of the smoking data

The information concerning patients' smoking attitudes was gathered during the baseline interview. Patients were asked the following questions: 1) Do you smoke? 2) If you smoke, how many cigarettes do you smoke on a daily basis? 3) Have you smoked in the past? 4) How many cigarettes per day did you smoke in the past 5) How many years since you stopped smoking? Based on questions 1 and 3, patients were allotted to two study groups: a) current smokers (n=67) and b) non-smokers (n=83). This classification differs from the original design of the questionnaire, which gives three answer options: current, past or never smokers. The rearrangement was performed for two reasons; one epidemiological and the other, statistical. The epidemiological reason relies on the fact that the main analysis of the LAMS's smoking data (awaiting publication) revealed that, in the present sample, past smokers are closer to current smokers than to never-smokers in terms of the behavior of HR-HPV infection and risk for CIN. The statistical reason is that using the original three-part distribution in this relatively small subset of women with ASC/LSIL would dramatically reduce the power of the analysis, therefore preventing the correct evaluation of risk for CIN as a function of the smoking history.

Statistical analysis

Comparison of the epidemiological variables across the two groups was performed with a multivariate regression model (together with smoking variables), and the adjusted ORs (95%CI) were calculated for CIN. In all tests, the values $p<0.05$ were regarded as statistically significant.

Univariate survival (Kaplan-Meier) analysis was used to calculate the survival curves (in the whole cohort) for accumulation of incident CIN 1+ and CIN2+ during the FU. The curves were compared using log-rank (Mantel-Cox) statistics. Hazard ratios (HR) for the risk associates were calculated with a multi-variate Cox regression model, with 95% CI. All calculations were performed using the R package for statistical computing (R environment 2008).

Results

Table 1 presents the distribution of women as a function of their smoking history and epidemiological characteristics. Women aged <30 years presented a significantly higher odds ratio for smoking (OR = 2.81; 95%CI 1.29 to 6.10). The baseline HPV status, age at first intercourse, number of lifetime sex partners, use of hormonal contraceptives and history of previous CIN were not related to the smoking history in this sample.

Table 2 describes the cytological results obtained at each of the FU visits in smokers and non-smokers. At the second visit, one woman presented HSIL cytology, whereas 90 women had a normal exam. Twenty-six women maintained ASC/LSIL. There was no significant difference in the proportion of cytological diagnoses comparing smokers to non-smokers at this visit (p=0.67). By contrast, at the third and last visits, a

disproportionately higher of smokers developed HSIL (p=0.01 for third and p<0.01 for the last visit). There were no cases of HSIL among non-smokers in this sample.

Table 3 lists the HR for developing CIN during the 36-month follow-up, calculated with multivariate Cox Proportional Hazards. In this analysis, the only factor related to an increased risk of developing CIN was the positive hr-HPV status (HR = 3.42; 95%CI 1.11 to 9.43). The smoking history was not associated to the risk of developing CIN (HR = 0.73; 95%CI 0.40 to 1.33).

Figures 1 and 2 describe graphically the probability of developing CIN1 or worse or CIN2 or worse during follow-up according to the women's smoking histories. Statistically equivalent curves were found for CIN1 or worse (log-rank p = 0.33). A total of 21 cases of incident CIN were detected during FU. Of these, 11 appeared in the group of 67 smokers and 10 in the 83 nonsmoker women. While restricting the analysis to high-grade CIN, the probability of developing the disease was significantly higher for smokers (p= 0.04). Eleven cases of high-grade CIN were detected during FU, being 7 in smokers versus only 3 in the group of nonsmoker women. The curves clearly deviate from each other from semester 4 onwards.

Discussion

The present analysis was able to demonstrate an increased risk for the medium-term (36 months) development of high-grade CIN in smoker women with baseline ASC/LSIL cytology compared to nonsmokers. It is known that up to 25% of women with ASC/LSIL cytology may ultimately be found to harbor CIN3 or CIN2, as demonstrated by the ASCUS/LSIL Triage Study (ALTS) (COX 2003 and GUIDO 2003). In the ALTS trial, however, the first round (baseline) of colposcopic examination

diagnosed almost 70% of the cases, leaving the remainder for the 2-year follow-up. In our case, we excluded from the analysis women with CIN at baseline, deliberately examining a cohort with a normal cervix at baseline at therefore estimating the risk of disease development over time (excluding, of course, the few expected false-negatives from colposcopy).

In our series, of the 150 women prospectively followed-up, 22 (14%) ultimately developed CIN (11/67 smokers and 10/83 nonsmokers). However, a significant difference in incidence between smoker and nonsmoker women was found for high-grade CIN (11 cases during follow-up; 7 of these in smokers). The incidence of low- and high grade CIN in the present sample was substantially higher than that reported in ALTS trial (Walker 2006; Cox 2003; Guido 2003), because, as mentioned earlier, we excluded from the analysis women with abnormal colposcopy at baseline. Importantly, however, the previous studies on the risk of further development of CIN in women with low-grade cytological abnormalities do not give consideration to the smoking history of the women. As detected by our analysis, the 3-year incidence of high-grade CIN ranged from 3/83 (3.6%) in nonsmoker women to as high as 7/67 (10.4%) in smokers, a highly significant (log-rank $p = 0.04$) leap.

As studies on the epidemiological effects of smoking on HPV and CIN/cervical cancer accumulate in the literature, the analysis of the epidemiological data becomes more focused on detail. The first studies to consistently examine the development of CIN in smokers were limited in methodological terms because the analyses were invariably restricted to HPV-positive women (Tolstrup 2006, McIntre 2005; Matos 2005). This limitation precluded the assessment of the tobacco effects on the HPV acquisition, which have only recently been addressed with sufficient proficiency. The pooled analysis on HPV Prevalence Surveys, undertaken by IARC, was released in the beginning of 2008, and is now

contributing to the understanding of the causative factors behind the increased incidence of CIN and cervical cancer in smokers. The IARC pooled analysis demonstrated that there is a consistent association between smoking and HPV infection. According to the IARC, this association is dependent on the smoking intensity: the ORs, adjusted fo several other known epidemiological co-factors, were 1.21 (95%CI 0.95-1.54) for women that smoked <5 cigarettes per day, but 2.01 (95%CI 1.32-3.08) for those reporting to smoke >= 15 cigarettes on a daily basis (Vaccarella 2008). The IARC pooled analysis included studies from Vietnam, Thailand, Spain, Mexico, Argentina, Chile, Colombia and Nigeria, thereby covering a wide spectrum of epidemiological differences in HPV type and prevalence, with socio-economic variability. Our study examines a Latin-American urban population, currently facing the demographic transition towards more elevated standards of living. This population is therefore closer to the ALTS sample in terms of socio-economic descriptors than to African, Asian or Indian populations examined by other large epidemiological trials. This fact encourages the use of ALTS data as a comparative basis to our results.

The 2001 American Society for Colposcopy and Cervical Pathology (ASCCP) management guidelines have been accepted by the American College of Obstetricians and Gynecologists and by several other societies worldwide (Wright 2002; Wright 2003). These guidelines recognize that women who have LSIL or HPV-positive ASC at a given baseline assessment remain at an increased risk for more severe disease for at least a period of two years. Follow-up is recommended by either repeat cytology at 6 and 12 months, or high-risk HPV testing at 12 months (Wright 2002). No attention to other epidemiological factors is given in these guidelines, and smoking history is not mentioned in the entire document. However, most of the firm epidemiological evidence relating smoking to the risk of acquiring an HPV infection and the further development

of cervical neoplasia surfaced in more recent years (Plummer 2003, Vaccarella 2008). Only recently it has been firmly demonstrated that current tobacco smoking may increase the prevalence of HPV infection, and that the strength of this effect may vary as a function of the number of cigarettes smoked (Vaccarela 2008). Another large multi-factorial analysis on 3,187 women living in Russia, Belarus and Latvia, part of them prospectively followed-up, suggested that smoking may be an independent risk factor for HPV infections, although this study did not demonstrate an effect of tobacco usage on the incidence of cervical intraepithelial neoplasia (CIN) (Syrjänen 2007). Although never confirmed with *in vivo* or *in vitro* experimentation, it seems that smoking facilitates the acquisition and stabilization (thereby favoring persistence) of HPV infections. It is known from early studies on immunology that tobacco components reduce the number and hampers the function of Langerhans cells and CD4 lympocytes in cervical tissues, and decrease in the activity of natural killer cells (Poppe 1995, Poppe 1996 and Ferson 1979). Therefore, given the novelty of the epidemiological evidence linking smoking to the risk for cervical cancer, it seems justifiable that the current management guidelines have not yet been adapted to the fact that smokers deserve special attention in terms of triaging attention.

The present report brings important new information on the effects of smoking for the epidemiology of HPV and CIN in women with ASC/LSIL. This is a special group of women, because they are at already increased probability of developing CIN, even if they have a normal colposcopy at the time when ASC/LSIL appears in cytology. The incidence of CIN or worse was unequivocally higher for smokers compared to nonsmokers in this 3-year follow-up. The present findings, combined with the information given by previous large studies, may help to tailor more appropriate management strategies for women at

increased risk of developing CIN. The current management guidelines generally omit most of the known epidemiological factors linked to CIN, and smoking is not an exception. The risk of acquiring a HPV infection may be higher for smokers than for non-smokers, and the epidemiological implications of this fact should be moderate, but HPV-positive women that smoke face a significant likelihood of developing CIN in the short to medium-term, which is of utmost importance in women with ASC/LSIL.

Acknowledgements

This report is a part of the LAMS (Latin American Screening) study, entitled: IMPROVING HEALTH SYSTEMS TOWARDS EQUALITY-BASED CONTROL OF CERVICAL CANCER IN LATIN AMERICA, and supported by the INCO-DEV Program of the European Commission (Project# ICA4-CT-2001-10013). The generous contribution of DIGENE Inc. (USA) providing the HC2 tests at our disposal is gratefully acknowledged.

References

1. Argentina, Ministry of Health. Estadísticas vitales. Información básica año 2005. Buenos Aires: Dirección de Estadísticas e formación de Salud, 2006.
2. Castellsague X, Munoz N. Chapter 3: Cofactors in human papillomavirus carcinogenesis--role of parity, oral contraceptives, and tobacco smoking. J Natl Cancer Inst Monogr 2003;31:20-28.
3. Cox JT, Schiffman M, Solomon D, for the ALTS Group. Prospective follow-up suggest similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy. Am J Obstet Gynecol 2003; 188: 1406-12.

4. Ferson M, Edwards A, Lind A, Milton GW, Hersey P. Low natural killer-cell activity and immunoglobulin levels associated with smoking in human subjects. Int J Cancer 1979;23:603-9.

5. Guido R, Schiffman M, Solomon D, for the ALTS Group. Post colposcopy management strategies for women referred with low-grade squamous intraepithelial lesions or human papillomavirus DNA-positive atypical squamous cells of undetermined significance: a two-year prospective study. Am J Obstet Gynecol 2003; 188:1383-92.

6. Herrero R, Brinton LA, Reeves WC et al. Invasive cervical cancer and smoking in Latin America. J Natl Cencer Inst 1989;8:205-11.

7. Ho GY, Kadish AS, Burk RD, et al. HPV 16 and cigarette smoking as risk factors for high-grade cervical intra-epithelial neoplasia. Int J Cancer 1998;78:281-285.

8. International Collaboration of Studies of Cervical Cancer. Carcinoma of the cervix and tobacco smoking: collaborative of individual data on 13,541 women with carcinoma of the cervix and 23,017 women without carcinoma of the cervix from 23 epidemiological studies. Int J Cancer 2006;118:1481-95.

9. Longatto-Filho A, Erzen M, Branca M, Roteli-Martins C, et al. Human papillomavirus testing as an optional screening tool in low-resource settings of Latin America: experience from the Latin American Screening study. Int J Gynecol Cancer 2006;16:955-962.

10. Lörincz A T, Castle P E, Sherman M E, et al. Viral load of human papillomavirus and risk of CIN 3 or cervical cancer. Lancet 2002;360:288-9.

11. Matos A, Moutinho J, Pinto D, Medeiros R. The influence of smoking and other cofactors on the time to onset to cervical cancer in a southern European population. Eur J Cancer Prev 2005;14:485-91.

12. McIntyre-Seltman K, Castle PE, Guido R, Schiffman M, Wheeler CM. Smoking is a risk factor for cervical intraepithelial neoplasia grade 3 among oncogenic human papillomavirus DNA-positive women with equivocal or mildly abnormal cytology. Cancer Epidemiol Biomarkers Prev 2005;14:1165-70.

13. Melikian A, Sun P, Prokopczyk B et al. Identification of benzo[a]pyrene metabolites in cervical mucus and DNA adducts in cervical tissues in humans by gas chromatography-mass spectrometry. Cancer Letters 1999;146(2):127-134.

14. Ministério da Saúde – Instituto Nacional do Câncer – Atlas de Mortalidade por Câncer no Brasil 1979-1999. INCA, 2002. Available online (only Portuguese) at http://www.inca.gov.br/atlas/docs/Atlas_completo.pdf

15. Plummer M, Herrero R, Franceschi S et al. Smoking and cervical cancer: pooled analysis of the IARC multi-centric case-control study. Cancer Causes and Control 2003; 14(9): 805-814.

16. Poppe WA, Ide PS, Drijkoningen MP, et al. Tobacco smoking impairs the local immunossurveillance in the uterine cervix. Gynecol Obstet Invest 1995;39:34-8.

17. Poppe WA, Peters R, Drijkoningen MP, et al. Cervical cotinine and macrophage-Langerhans cell density in the normal uterine cervix. Gynecol Obstet Invest 1996;41:253-59.

18. Procopzyk B, Cox JE, Hoffman D, Waggoner SE. Identification of tobacco-specific carcinogen in the cervical mucus of smokers and nonsmokers. Journal of the National Cancer Institute 1997; 89(12):pp. 868-873.

19. R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-Project.org.

20. Sarian LO, Derchain SF, Naud P, et al. Evaluation of visual inspection with acetic acid (VIA), Lugol´s Iodine (VILI), cervical cytology and HPV testing as cervical screening tools in Latin America. This report refers to partial results from the LAMS study. J Med Screen 2005;12:142-9.

21. 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. JAMA. 2002; 287:2114-9.

22. Syrjänen K, Naud P, Derchain S. Comparing PAP smear cytology, aided visual inspection, screening colposcopy, cervicography and HPV testing as optional screening tools in Latin America. Study design and baseline data of the LAMS study. Anticancer Res. 2005;25:3469-80.

23. Syrjänen K, Shabalova I, Petrovichev N, et al. Smoking is an independent risk factor for oncogenic human papillomavirus (HPV) infections but not for high-grade CIN. Eur J Epidemiol 2007;22:723-735.

24. Tolstrup J, Munk C, Thomsen BL, et a. The role of smoking and alcohol intake in the development of high-grade squamous intraepithelial lesions among high-risk HPV-positive women. Acta Obstet Gynecol Scand 2006;85:1114-1119.

25. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006; 24S1: S1/4-S1/15.

26. Vaccarella S, Franceschi S, Herrero R et al. Sexual behaviour, condom use, and human papillomavirus infection: pooled analysis of the IARC human papillomavirus prevalence surveys. Cancer Epidemiol Biomarkers Prev 2006;15:326-33.

27. Vaccarella S, Herrero R, Snidijers PJ, et al. Smoking and papillomavirus infection: pooled analysis of the International Agency for Research on Cancer HPV Cancer Prevalence Surveys. Int J Epidemiol 2008; 1-11.

28. Walker JL, Wang SS, Schiffman M, Solomon D, for the ASCUS LSIL Triage Study (ALTS) Group. Predicting absolute risk of CIN3 during post-colposcopic follow-up: Results from the ASCUS-LSIL Triage Study (ALTS). Am J Obstet Gynecol 2006; 195:341-8.

29. Wright TC, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ. 2001 ASCCP-sponsored Consensus Conference: 2001 Consensus Guidelines for the management of women with cervical cytological abnormalities. JAMA 2002; 287: 2120-9.

30. Wright TC, Cox JT, Massad LS, Carlson J, Twiggs LB, Wilkinson EJ. 2001 ASCCP-sponsored Consensus Workshop: 2001 Consensus Guidelines for the management of women with cervical intraepithelial neoplasia. Am J Obstet Gynecol 2003; 189:295-394.

Table 1: Association of smoking history with relevant epidemiological and clinical characteristics of the women

Characteristic	Smoking history			
	Current or past smokers (n=67)	Never smokers (n=83)	OR*	95%CI*
	n(%)	n(%)		
Baseline HPV				
Positive	42 (62.7)	44 (53.0)	0.62	(0.28 to 1.35)
Negative	25 (37.3)	39 (47.0)		Ref.
Age				
< 30	21 (31.3)	41 (49.4)	2.81	(1.29 to 6.10)
≥ 30	46 (68.7)	42 (50.6)		Ref.
First intercourse				
< 18 years	42 (62.7)	48 (57.8)	0.67	(0.33 to 1.36)
≥ 18 years	25 (37.3)	35 (42.2)		Ref.
Lifetime sexual partners				
≥ 2	49 (74.2)	47 (56.6)	0.47	(022 to 1.02)
1	17 (25.8)	36 (43.4)		Ref.
Contraceptive methods				
Hormonal	23 (34.3)	37 (44.6)	1.44	(0.70 to 2.96)
No method or non-hormonal	44 (65.7)	46 (55.4)		Ref.
History of previous CIN				
Yes	6 (9.0)	5 (6.0)	0.77	(0.20 to 2.86)
Never	61 (91.0)	78 (94.0)		Ref.

*Adjusted with logistic regression; Ref: reference

Table 2: Cytological abnormalities at each FU visit according to the smoking history.

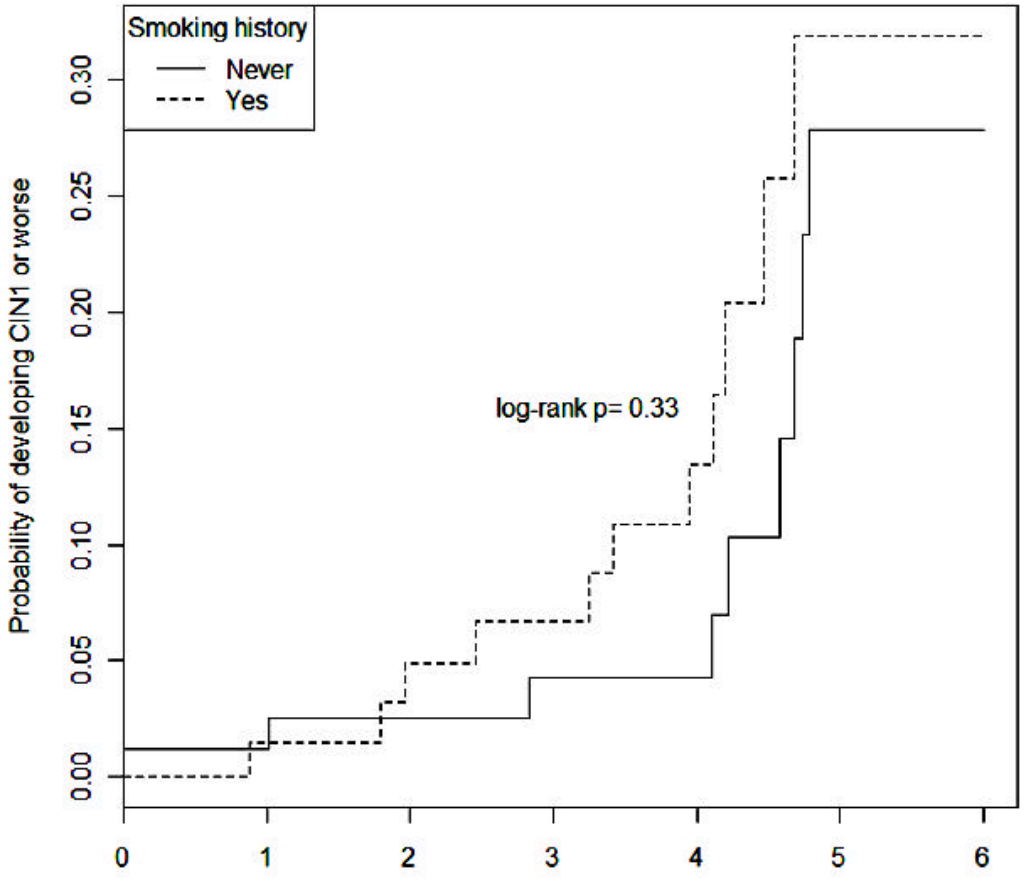
	Visit											
	Second (n= 107)				Third (n= 101)				Last (n= 81)			
	Neg.	ASC	LSIL	HSIL	Neg.	ASC	LSIL	HSIL	Neg.	ASC	LSIL	HSIL
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	N (%)	n (%)	n (%)	n (%)
Smokers	34 (42)	13 (54)	2 (100)	1 (100)	33(40)	5 (55)	4 (50)	2 (100)	30(42)	5 (71)	1 (100)	2 (100)
Non-smokers	46 (58)	11 (46)	0	0	49(60)	4 (45)	4 (50)	0	41(58)	2 (29)	0	0
	p = 0.67				p = 0.01				p < 0.01			

"Neg." = negative (normal)

Table 3: Risk estimates for CIN at the last FU-visit

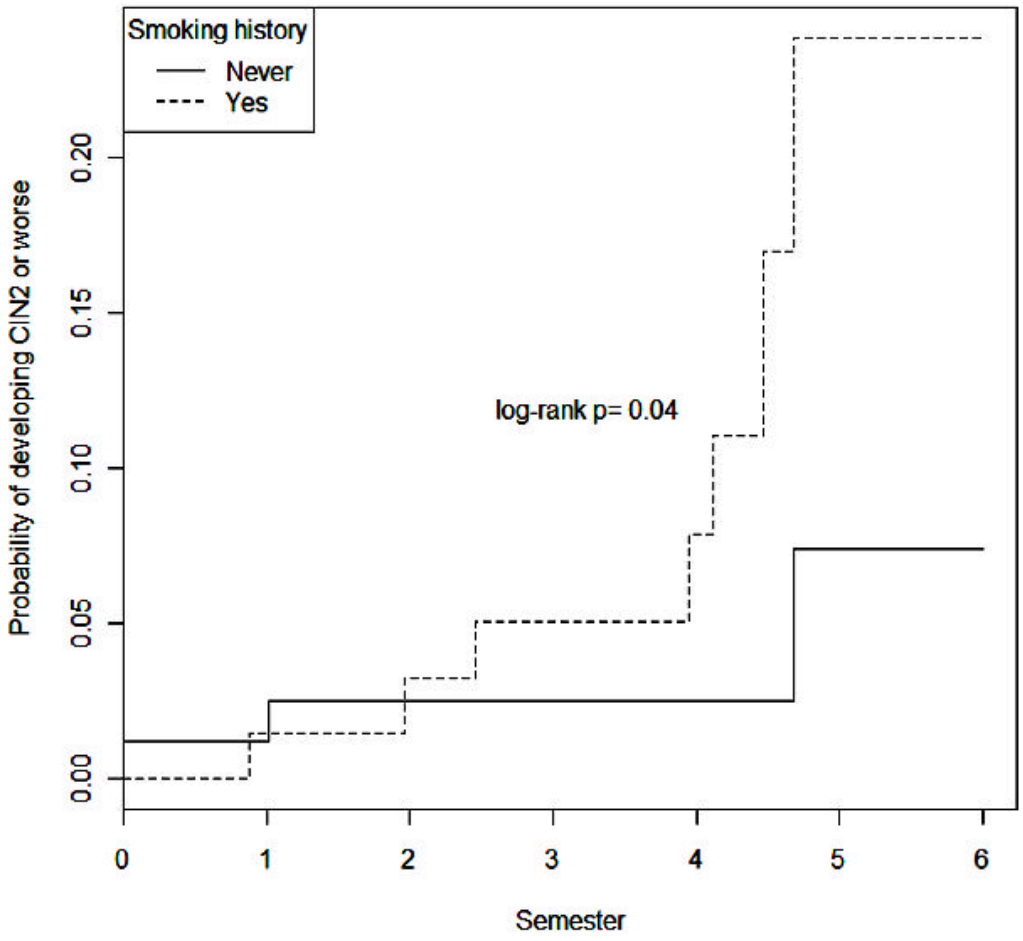
Characteristic	HR	95%CI
Positive baseline hr-HPV	3.42	1.11 to 9.43
Age ≤ 30 years	1.17	0.48 to 2.88
Age at first sexual intercourse <18 years	1.57	0.61 to 4.02
Life time partners ≥ 2	0.85	0.35 to 2.06
Oral hormonal contraception*	2.09	0.84 to 5.18
Smoking history	0.73	0.40 to 1.33

HR = hazard ratio



Smoking	N	Observed	Expected	(O-E)^2/E	(O-E)^2/V
Yes	67	11	8.82	0.538	0.941
Never	83	10	12.18	0.389	0.941

Figure 1 – Kaplan-Meyer representation of the cumulative probability of developing CIN during the follow-up



Smoking	N	Observed	Expected	(O-E)^2/E	(O-E)^2/V
Yes	67	7	4.34	1.63	2.91
Never	83	3	5.66	1.25	2.91

Figure 2 – Kaplan-Meyer representation of the cumulative probability of developing high-grade CIN during the follow-up

4. Discussão

O presente estudo demonstrou, através da avaliação concomitante da infecção pelo HPV e da detecção da NIC, que os efeitos do tabagismo sobre a primeira provavelmente são tênues e ocorrem em longo prazo, enquanto a potencialização dos efeitos neoplásicos da infecção pelo HPV é intensa e ocorre em menor espaço de tempo. Dados epidemiológicos sobre o papel do tabagismo como um fator de risco para câncer cervical e como potencial confundidor do HPV estão vindo à tona recentemente (Syrjänen et. al., 2007; Vaccarela et al., 2008). Os estudos mais atuais que examinaram o desenvolvimento da NIC entre tabagistas foram limitados pelo método escolhido, porque as análises foram invariavelmente restritas apenas a mulheres HPV-positivas (Matos, 2005; McIntre et al., 2005; Tolstrup et al., 2006).

Os resultados deste estudo se somam àqueles da análise combinada de estudos de prevalência, realizados pelo IARC, contribuindo para o entendimento de fatores de risco no aumento da incidência de NIC e câncer cervical em tabagistas (Plummer et al., 2003). As dúvidas em relação à associação consistente entre

tabagismo e infecção pelo HPV parecem desaparecer a cada estudo somado à literatura. De acordo com o IARC, essa associação seria dependente da intensidade do tabagismo (ou seja, número de cigarros ao dia): o OR ajustado para vários co-fatores epidemiológicos conhecidos foi 1,21 (95%CI 0,95-1,54) para mulheres que fumavam menos de cinco cigarros por dia, mas 2,01 (95%CI 1,32-3,08) para aquelas que fumavam 15 ou mais cigarros por dia (Vaccarella et al., 2008). Neste estudo, esta associação não foi confirmada. É importante ressaltar que a análise do IARC incluiu estudos do Vietnã, Tailândia, Espanha, México, Argentina, Chile, Colômbia e Nigéria, cobrindo, com isso, um amplo espectro de diferenças epidemiológicas na prevalência e no tipo de HPV, com grande variabilidade socioeconômica. No estudo LAMS, a coorte foi composta predominantemente por populações urbanas do Brasil e Argentina, que já sofreram transição demográfica e têm um padrão de vida mais próximo daquele de nações desenvolvidas. Essa população é, portanto, mais próxima à amostra do ALTS em termos de descritores socioeconômicos que outras examinadas por grandes estudos epidemiológicos da África, Ásia e Índia. Este fato permite o uso dos dados do ALTS como base comparativa para nossos resultados.

Vale também avaliar a prevalência de tabagismo na população estudada em relação aos dados oficiais sobre o assunto. A prevalência de tabagismo nas mulheres deste estudo foi 22%. Uma recente publicação da Organização Mundial da Saúde (WHO, 2008) mostrou que a prevalência de tabagismo entre as mulheres de Buenos Aires era 26,8%. Dados nacionais mostram uma prevalência de 23% de mulheres tabagistas em Campinas, 22,9% em Porto Alegre e 17,5%

em São Paulo (Brasil, 2006). Levando-se em consideração que a prevalência de tabagismo neste estudo reflete uma média da prevalência encontrada nos quatro centros – São Paulo, Campinas, Porto Alegre e Buenos Aires, consideram-se esses dados concordantes com os da literatura.

No presente estudo, o OR para tabagistas com teste HR-HPV positivo na consulta inicial (depois de ajustado para vários fatores de risco epidemiológicos conhecidos) foi 1,36 (95%IC 1,11-1,66), o qual é praticamente idêntico com o OR reportado na análise do IARC (Vacarella et al., 2008). Este risco similar através de diferentes regiões geográficas sugere que os efeitos do cigarro na aquisição do HPV podem não ser restritos à distribuição do tipo de HPV (variando de uma região para outra), nem ser positiva ou negativamente afetado por características étnicas, nutricionais ou comportamentais das mulheres. Outro grande estudo de coorte (3187 mulheres) dos novos estados independentes da antiga União Soviética (Syrjänen et al., 2007), confirma os achados deste estudo, mostrando que tabagistas tiveram OR=1,52 (95%IC 1,09-2,14) para HR-HPV positivo na consulta inicial. Junto com os presentes resultados e com o estudo do IARC, estes dados implicam que o cigarro tem um efeito universal e independente no risco de contrair HR-HPV.

Devido ao desenho combinando corte-transversal e coorte prospectivo do estudo LAMS (Syrjänen et al., 2005), ficamos habilitados a avaliar os efeitos do cigarro em duas frentes: prevalência de infecção pelo HR-HPV e prevalência/incidência de NIC 2 ou pior. Além disso, pôde-se avaliar também seus efeitos tanto na conseqüência da infecção pelo HR-HPV (persistência, regressão, aquisição) quanto na incidência de NIC2 ou pior durante o seguimento

prospectivo de mais de três anos. A coorte de 12.114 mulheres foi estratificada em três grupos: não tabagista, ex-tabagista e tabagista. Estes três grupos foram significantemente diferentes na maioria das variáveis demográficas e epidemiológicas, que são conhecidos fatores de risco para HPV, NIC e câncer cervical. Tabagistas tiveram prevalência mais alta de infecção pelo HR-HPV na consulta inicial, e ainda prevalência mais alta de citologia HSIL, LSIL e ASCUS, e NIC confirmada por biópsia. Isto sugere que mulheres tabagistas têm um aumento no risco de HR-HPV e NIC se a análise não for controlada por confundidores óbvios. Ao avaliar aquelas mulheres que iniciaram a fase prospectiva do estudo já com ASC/LSIL, foi possível detectar a grande contribuição do tabagismo no aumento de risco para NIC de alto grau, o que, como será analisado mais adiante, pode ter implicações para as condutas a serem adotadas em relação a estas mulheres.

O uso de modelos multivariados permitiu a obtenção de resultados interessantes. Realmente, houve muitas co-variáveis que foram associadas tanto com a prevalência de HR-HPV, mais fortemente que o tabagismo, como, por exemplo, ser solteira, ter mais de cinco parceiros sexuais, ter idade maior que 35 anos, quanto como marcadores de LSIL e NIC. Tabagismo não foi um fator de risco independente para prevalência de NIC2 ou pior, mas foi preditor independente para infecção por HR-HPV (OR=9,69) e história de não ter Papanicolaou prévio (OR=2,65). Esses resultados são plenamente concordantes com os dados reportados por Syrjänen et al. (2007), em que tabagismo não foi um preditor independente de NIC2 ou pior, mas o efeito foi mediado pela

infecção pelo HR-HPV, onde o tabagismo foi um preditor independente, exatamente como na presente coorte.

A possibilidade que o efeito do tabagismo no risco para HR-HPV e NIC poder ser mascarado pelo comportamento sexual das mulheres tem sido aventada ultimamente. Vaccarella et al. (2006) mostraram que o número de parceiros sexuais durante a vida esteve positivamente associado com a prevalência de HPV e o número de cigarros fumados. No presente estudo, ambos os fatores foram colocados na análise multivariada, para controlar se essa associação é confundidora ou não. Observamos que apenas o número de parceiros sexuais (mas não o número de cigarros) foi significantemente associado com o HR-HPV (mas não NIC2 ou pior) na consulta inicial. Isso pode apontar, entretanto, que neste caso, o poder de aproximação estatística seja insuficiente para superar o efeito dos vieses de relação e ordem. Por exemplo, se a mulher relatar número de parceiros sexuais inferior à realidade (com isso mascarando parte do seu risco de infecção pelo HPV), o coeficiente de regressão produzido pelo ajuste com o número de cigarros será comprometido. O inverso, certamente, também é verdade. Este fato mostra que é importante considerar com cautela a relação entre o número de cigarros e o número de parceiros sexuais. Igualmente importante é considerar a confusão devido à HR-HPV em todos os estudos avaliando o papel do cigarro como um fator de risco para NIC e CC (Plummer et al., 2003).

Foi avaliado, a seguir, se há uma relação de dose-dependente do cigarro para detecção de HR-HPV, anormalidades no Papanicolaou ou NIC confirmadas por biópsia na consulta inicial, mensurando os anos de tabagismo (atualmente ou no

passado). Não houve diferença nos anos de tabagismo com relação à detecção do HR-HPV, NIC2 ou pior e anormalidades no Papanicolaou, como HSIL ou LSIL. No presente estudo fomos impedidos de medir adequadamente a influência do tempo passado desde a cessação do hábito de fumar na detecção do HR-HPV. Entretanto, parece possível inferir que deve existir um tempo relacionado ao declínio no risco de ter uma infecção por HPV depois de parar de fumar, mas uma grande coorte é necessária para avaliar esta tendência e determinar o período de tempo depois do qual o efeito do tabagismo na prevalência do HPV cai para o nível de não-tabagista. Os maiores estudos disponíveis são de corte-transversal, e, portanto, não testam esta hipótese (Vaccarella et al., 2008). O estudo LAMS também possui avaliação transversal consideravelmente maior que a longitudinal (corte de >12000 mulheres seguido de coorte com pouco mais de 1000 pacientes).

A coorte prospectiva permitiu investigar se a história do tabagismo tem algum impacto na infecção pelo HR-HPV ou anormalidades no Papanicolaou ao longo do tempo e em mulheres com avaliação inicial normal. Mulheres nas três categorias de tabagismo (fumantes, ex-fumantes e não fumantes) não tiveram diferença no diagnóstico da infecção por HR-HPV na consulta inicial. Similarmente, não houve diferença entre os grupos na persistência, regressão ou aquisição do HR-HPV durante o seguimento. Isto confirma a observação da coorte com mulheres da ex-União Soviética (Syrjänen et al., 2007). Por outro lado, a incidência de Papanicolaou anormal foi mais comum entre ex-tabagistas, enquanto a persistência do Papanicolaou anormal foi mais freqüente entre tabagistas, mostrando o resultado do Papanicolaou significantemente diferente entre as três categorias (p=0,017).

A questão torna-se mais complexa quando a incidência de anormalidades no Papanicolaou está relacionada ao resultado de infecção pelo HR-HPV. O histórico de tabagismo não teve efeito na incidência de anormalidades no Papanicolaou. Essas anormalidades estavam significantemente aumentadas entre mulheres com HR-HPV positivo na consulta inicial, sem levar em conta a história de tabagismo. Similarmente, tabagismo não teve efeito na incidência de anormalidades no Papanicolaou entre mulheres que tiveram persistência de HR-HPV durante o seguimento. Curiosamente, a incidência de HR-HPV não aumentou significativamente o risco de Papanicolaou anormal entre não-tabagistas e tabagistas. Esta combinação de aumento de prevalência de HR-HPV na consulta inicial e a falta de diferença no resultado durante o seguimento pode ter várias explicações. Pode ser que a diferença seja semelhante apenas depois de muito tempo, e examinando uma coorte muito grande. Isso pode ainda sugerir que o efeito do tabagismo é temporário, porque ex-tabagistas são semelhantes às não tabagistas no risco de infecção pelo HR-HPV na consulta inicial. O mesmo foi verdade para NIC2 ou pior, que foi igualmente achado entre ex-tabagistas e não tabagistas. Em adição, parece que a persistência do HR-HPV é um determinante mais importante nas taxas de incidência de anormalidades no Papanicolaou do que a incidência de HR-HPV.

Finalmente, foi de importância atribuir se a história do tabagismo é um fator de risco independente para infecção pelo HR-HPV ou para incidência de NIC2 ou pior durante o seguimento. Sem dúvida, a história de tabagismo (ser tabagista e não ex-tabagista) aumentou o risco de incidência de HR-HPV, embora não drasticamente (OR=1,44). Por outro lado, fumar aumentou o risco

de incidência de NIC2 ou pior, a despeito do fato de não ser um preditor independente do NIC2 ou pior na consulta inicial. De qualquer forma, o HR-HPV inicial foi o mais poderoso preditor de NIC2 ou pior do que ser tabagista ou ex-tabagista. Quando calculado por 1000 mulheres/mês o risco, a incidência de NIC2 ou pior entre ex-tabagistas e tabagistas é três vezes mais alta que em não tabagistas. Isto pode implicar que os componentes do cigarro aceleram o processo de transformação de células epiteliais, uma vez infectadas por HPV oncogênico, e seus efeitos tornam-se persistentes.

Na presente análise, encontraram-se diferentes preditores de positividade para HR-HPV e NIC2 (ou pior) no início do seguimento, e o mesmo foi verdade com relação à incidência de HR-HPV e NIC2 ou pior ao longo do seguimento. O número de casos de NIC2 ou pior (n=30) não foi particularmente grande, de qualquer forma, é necessário considerar a possibilidade de que diferentes grupos de preditores de HR-HPV e NIC2 ou pior podem estar relacionados a menor prevalência de NIC2 ou pior quando comparado com mulheres sem HR-HPV. Isto tem sido encontrado em outros estudos de base populacional, sugerindo que a prevalência muito menor de NIC e CC torna o HR-HPV mais sensível aos fatores de risco avaliados. Realmente, isto tem levado a maioria dos estudos limitar suas análises a mulheres HPV-positivas (Plummer et al, 2003; McIntre et al, 2005; Tolstrup et al., 2006), o que dificulta o controle dos efeitos confundidores do HPV. Similarmente, estudos que analisam o efeito do tabagismo no aumento da velocidade de progressão de NIC de baixo grau para NIC de alto grau ou CC

são poucos. Atualmente, há apenas um estudo consistente com este objetivo, e seus resultados nunca foram reproduzidos (Ho et al., 1998).

Como comentado anteriormente, ao avaliar os efeitos do cigarro em mulheres com ASC/LSIL na consulta inicial, o presente estudo encontrou um aumento no risco de desenvolver NIC de alto grau em mulheres tabagistas quando comparadas às não tabagistas, ao longo de 36 meses de seguimento. É conhecido que 25% das mulheres com ASC/LSIL podem desenvolver NIC 2 ou 3, como demonstrado pelo estudo ALTS (Cox et al., 2003; Guido et al., 2003). No estudo ALTS, entretanto, 70% dos casos de NIC foram diagnosticados pela colposcopia na consulta inicial, e essas mulheres deixaram o seguimento de dois anos. No caso deste estudo, excluímos da análise mulheres com NIC na consulta inicial, formando uma coorte de mulheres com cérvix normal para então estimar o risco de desenvolver doença ao longo do tempo.

No presente estudo, das 150 mulheres seguidas prospectivamente, 22 (14%) desenvolveram NIC (11/67 tabagistas e 10/83 não tabagistas). Entretanto, uma diferença significante na incidência entre mulheres tabagistas e não tabagistas foi encontrada para NIC de alto grau (11 casos durante o seguimento; 7 deles em tabagistas). A incidência de NIC de baixo e alto grau na presente amostra foi substancialmente maior que a reportada no estudo ALTS (Cox et al, 2003, Guido et al., 2003; Walker et al., 2006), porque, como mencionado anteriormente, foram excluídas as mulheres com colposcopia anormal na consulta inicial. Importante, entretanto, os estudos prévios sobre o risco de desenvolver NIC em mulheres com anormalidades citológicas menores (LSIL/ ASC) não consideraram a

história de tabagismo da mulher. Como detectado na análise deste estudo, a incidência de NIC de alto grau em três anos de seguimento foi de 3/83 (3,6%) em não fumantes e 7/67 (10,4%) em fumantes, um aumento significativo (log-rank p=0,04).

O guia de conduta da Sociedade Americana de Colposcopia e Patologia Cervical tem sido aceito pelo Colégio Americano de Obstetrícia e Ginecologia e por várias outras sociedades do mundo (Wright et al., 2002; Wright et al., 2003). Este guia reconhece que mulheres com LSIL ou ASC e HPV-positivo em um rastreamento têm um aumento no risco de doenças mais graves por um período de dois anos. No seguimento, recomenda-se repetir a citologia em seis e 12 meses, ou realizar o teste para HPV de alto risco em doze meses (Wright et al., 2002). Nenhuma atenção para outros fatores epidemiológicos foi dada por estes guias, e a história de tabagismo não foi mencionada neste documento. Entretanto, a maioria das evidências epidemiológicas confere ao tabagismo o status de importante fator de risco de adquirir uma infecção por HPV e facilitar o desenvolvimento de neoplasia cervical (Plummer et al., 2003; Vaccarella et al., 2008). Portanto, considerando que são novas as evidências epidemiológicas relacionando o tabagismo com o risco de CC, parece justificável que os guias de conduta atuais ainda não estão adaptados ao fato que tabagistas merecem atenção especial em termos de rastreamento.

O presente estudo traz novas informações importantes sobre os efeitos do cigarro na epidemiologia do HPV e NIC em mulheres com ASC/LSIL. Este é um grupo especial de mulheres, porque elas já têm um aumento na probabilidade de desenvolver NIC, mesmo que possuam colposcopia normal no momento em que

apresentaram citologia ASC/LSIL. A incidência de NIC2 ou pior foi inequivocadamente maior para tabagistas comparada a não-fumantes, em três anos de seguimento. Tais achados, combinados com as informações dadas por estudos prévios, podem ajudar a traçar estratégias apropriadas para mulheres com risco aumentado de desenvolver NIC. Os guias de conduta atuais geralmente omitem a maioria dos fatores de risco epidemiológicos associados a NIC, e o tabagismo não é uma exceção. O risco de adquirir uma infecção por HPV pode ser maior para fumantes do que não fumantes, e as implicações epidemiológicas deste fato devem ser ponderadas.

5. Conclusões

– **Artigo 1**: O tabagismo, passado ou presente, esteve associado a maior prevalência de infecção por HPV de alto risco oncogênico. O tabagismo também esteve associado ao aumento de risco de desenvolvimento de NIC2 ou pior em mulheres com infecção por HPV de alto risco oncogênico.

– **Artigo 2:** O tabagismo aumentou o risco de desenvolvimento de NIC 2 ou pior em mulheres com citologia ASC/LSIL e colposcopia normal, em um período de 36 meses.

6. Referências Bibliográficas

Barton SE, Maddox PH, Jenkins D, Edwards R, Cuzick J, Singer A. Effect of cigarette smoking on cervical epithelial immunity: a mechanism for neoplastic change? ***Lancet*** 1988; ii: 652-4.

Borck KE, MacLennan R, Brinton LA, Melnick JL, Adam E, Mock PA, et al. Smoking and infection agents and risk of in situ cervical cancer in Sydney, Australia. ***Cancer Res*** 1989; 49: 4825-928.

Bosch X, de Sanjosé S. The epidemiology of human papillomavirus infection and cervical cancer. ***Dis Markers*** 2007; 23(4): 213-27. Review.

Boyle DCM, Smith JR. Infection and cervical intraepithelial neoplasia. ***Int J Gynecol Cancer*** 1999; 9:177-86.

Brasil. Ministério da Saúde. Indicadores e Dados Básicos – Brasil – 2006. Disponível em: >http://tabnet.datasus.gov.br/cgi/idb2006/d21.htm<

Campo S, ed. Papillomavirus Research: From Natural History to Vaccines and Beyond. ***Caister Academic Press*** 2006, Norwick, UK, 1-424.

Castellsague X, Munoz N. Chapter 3: Cofactors in human papillomavirus carcinogenesis role of parity, oral contraceptives, and tobacco smoking. ***J Natl Cancer Inst Monogr*** 2003; 31: 20-28.

Castle PE, Solomon D, Schiffman M, Wheeler CM, for the ALTS Group. Human Papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. ***J Natl Cancer Inst*** 2005; 97 (14): 1066-71.

Cavalcanti SM, Zardo LG, Passos MR, Oliveira LH. Epidemiological aspects of human papillomavirus infection and cervical cancer in Brazil. ***J Infect 2000***; 40:80-7.

Cox JT, Schiffman M, Solomon D. Prospective follow-up suggest similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy. ***Am J Obstet Gynecol*** 2003; 188:1406-12.

Ferson M, Edwards A, Lind A, Milton GW, Hersey P. Low natural killer-cell activity and immunoglobulin levels associated with smoking in human subjects. ***Int J Cancer*** 1979; 23:603-9.

Gontijo RC, Derchain SFM, Ortiz RT, Guarisi R, Sarian LOZ, Bragança JF, et al. Fatores associados às alteraçöes da colpocitologia oncológica, à inspeçäo visual com ácido acético e à detecçäo de DNA-HPV de alto risco oncológico em mulheres de uma unidade básica de saúde em Campinas. ***DST - J bras Doenças Sex Transm*** 2002; 14(4):4-8.

Guido R, Schiffman M, Solomon D, Burke L. Postcolposcopy management strategies for women referred with low-grade squamous intraepithelial lesions or human papillomavirus DNA-positive atypical squamous cells of undetermined significance: a two-year prospective study. ***Am J Obstet Gynecol*** 2003; 188:1401-5.

Hart KW, Williams MO, Thelwell N, Fiander AN, Brown T, Borysiewicz LK, et al. Novel method for detection, typing, and quantification of human papillomaviruses in clinical samples. ***J Clin Microbiology*** 2001; 39:3204-12.

Hellberg D, Valentin J, Nilsson S. Smoking as risk factor in cervical neoplasia. ***Lancet*** 1983; i: 1-2.

Herrero R, Brinton LA, Reeves WC, Brenes MM, Tenorio F, de Britton RC, et al. Invasive cervical cancer and smoking in Latin America. ***J Natl Cancer Inst*** 1989; 81(3):205-11.

Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Matural history of cervicovaginal papillomavirus infection in young women. ***N Engl J Med*** 1998; 338: 423-8.

Kinney WK, Manos MM, Hurley LB, Ransley JE. Where's the high-grade cervical neoplasia? The importance of minimally abnormal Papanicolaou diagnoses. ***Obstet Gynecol*** 1998; 91:973-6.

Layde PM. Smoking and cervical cancer: cause or coincidence? ***JAMA*** 1989; 261:1631-3.

Matos A, Moutinho J, Pinto D, Medeiros R. The influence of smoking and other cofactors on the time to onset to cervical cancer in a southern European population. ***Eur J Cancer Prev*** 2005; 14:485-91.

McIntyre-Seltman K, Castle PE, Guido R, Schiffman M, Wheeler CM. Smoking is a risk factor for cervical intraepithelial neoplasia grade 3 among oncogenic human papillomavirus DNA-positive women with equivocal or mildly abnormal cytology. ***Cancer Epidemiol Biormarkers Prev*** 2005; 14:1165-70.

Melikian AA, Sun P, Prokopczyk B, El-Bayoumy K, Hoffmann D, Wang X, et al. Identification of benzo[a]pyrene metabolites in cervical mucus and DNA adducts in cervical tissues in humans by gas chromatography-mass spectrometry. ***Cancer Lett*** 1999; 146(2): 127-34.

Moreno V, Bosch FX, Muñoz N, Meijer CJ; Shah KV, Walboomers JM, et al. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multricentric case-control study. ***Lancet*** 2002; 359: 1085-92.

Muñoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS, et al. Role of parity and human papillomavirus in cervical cancer: The IARC multicentric case-control study. Lancet 2002; 359: 1093-101.

Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. ***N Engl J Med*** 2003; 348: 518-27.

Plummer M, Herrero R, Franceschi S, Meijer CJ, Snijders P, Bosch FX, et al. Smoking and cervical cancer: pooled analysis of the IARC multi-centric case--control study. ***Cancer Causes Control*** 2003; 14(9):805-14.

Poppe WA, Ide PS, Drijkoningen MP, Lauweryns JM, Van Assche FA. Tobacco smoking impairs the local immunosurveillance in the uterine cervix. An immunohistochemical study. ***Gynecol Obstet Invest*** 1995; 39(1):34-8.

Poppe WA, Peeters R, Drijkoningen M, Ide OS, Daenens P, Lauweryns JM, et al. Cervical cotinine and macrophage-Langerhans cell density in the normal human uterine cervix. ***Gynecol Obstet Invest*** 1996; 41(4):253-9

Procopzyk B, Cox JE, Joffman D, Waggoner SE. Identification of tobacco-specific carcinogen in the cervical mucus of smorkers and monsmokers. J ***Natl Cancer Inst*** 1997; 89(12): 868-73.

Reeves WC, Rawls WE, Brinton LA. Epidemiology of genital papillomaviruses and cervical cancer. ***Rev Infect Dis*** 1989; 11:426-39.

Sarian LO, Derchain SF, Naud P, Roteli-Martins C, Longatto-Filho A, Tatti S, et al. Evaluation of visual inspection with acetic acid (VIA), Lugol's iodine (VILI), cervical cytology and HPV testing as cervical screening tools in Latin America. This report refers to partial results from the LAMS (Latin AMerican Screening) study. ***J Med Screen*** 2005; 12(3):142-9.

Schiffman M, Solomon D. Findings to date from the ASCUS-LSIL Triage Study (ALTS). ***Arch Pathol Lab Med*** 2003; 127:946-9.

Snijders PJ, Steenbergen RD, Heideman DA, Meijer CJ. HPV-mediated cervical carcinogenesis: concepts and clinical implications. ***J Pathol*** 2006; 208:152-64.

Solomon D, Schiffman M, Tarone R. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline resuls from a randomized trial. ***J Natl Cancer Inst*** 2001; 93: 293-9.

Syrjänen K, Naud P, Derchain S. Comparing Pap Smear cytology, aided visual inspection, screening colposcopy, cervicography and HPV testing as optional screening tools in Latin America. Study design and baseline data of the LAMS study. ***Anticancer Res*** 2005; 25:3469-80.

Syrjänen K, Shabalova I, Petrovichev N, Kozachenko V, Zakharova T, Pajanidi J, et al. Smoking is an independent risk factor for oncogenic human papillomavirus (HPV) infections but not for high-grade CIN. ***Euro J Epimiol*** 2007; 22: 723-35.

Tolstrup J, Munk C, Thomsen BL, Svare E, van den Brule AJ, Gronbaek M, et al. The role of smoking and alcohol intake in the development of high-grade squamous intraepithelial lesions among high-risk HPV-positive women. ***Acta Obstet Gynecol Scand*** 2006; 85(9):1114-9.

Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. ***Vaccine*** 2006; 24S1: S1-15.

Vaccarella S, Franceschi S, Herrero R, Muñoz N, Snijders PJ, Clifford GM, et al. Sexual behavior, condom use, and human papillomavirus: pooled analysis of the IARC human papillomavirus prevalence surveys. ***Cancer Epidemiol Biomarkers Prev*** 2006; 15(2):326-33.

Vaccarela S, Herrero R, Snijders PJ, Dai M, Thomas JO, Hieu NT, et al. Smoking and human papillomavirus infection: pooled analysis of the International Agency for Research on Cancer HPV Prevalence Surveys. ***Int J Epidemiol*** 2008; 37(3):536-46.

Walboomers JM, Jacobs MM, Manos FX, Bosch JA, Kummer LV, Shah PJ, et al. Human papillomavirus is a necessary cause of invasive cervical câncer worldwide. ***J Pathol 1999***; 189 (1):12-9.

Walker JL, Wang SS, Schiffman M, Solomon D, for the ASCUS LSIL Triage Study (ALTS) Group. Predicting absolute risk of CIN3 during post-colposcopic follow-up: Results from the ASCUS-LSIL Triage Study (ALTS). ***Am J Obstet Gynecol*** 2006; 195 (2):341-8.

Wang SS, Hildesheim A. Chapter 5: Viral and host factors in human papillomavirus persistence and progression. ***J Natl Cancer Inst Monogr*** 2003; 31: 35-40.

WHO. World Health Organization Report on the global tobacco epidemic, 2008. Disponível em: <http://who.int/tobacco/mpower/mpower_report_full_2008.pdg<

Winkelstein W. Smoking and cervical câncer – current status: a review. ***Am J Epidemiol*** 1990; 131 (6): 945-57.

Wright TC, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ. 2001 ASCCP-sponsored Consensus Conference: 2001 Consensus Guidelines for the management of women with cervical cytological abnormalities. ***JAMA*** 2002; 287 (16): 2120-9.

Wright TC, Cox JT, Massad LS, Carlson J, Twiggs LB, Wilkinson EJ. 2001 ASCCP-sponsored Consensus Workshop: 2001 Consensus Guidelines for the management of women with cervical intraepithelial neoplasia. ***Am J Obstet Gynecol*** 2003; 189 (1):295-394.

7. Anexos

7.1. Anexo 1 – Termo de Consentimento Livre e Esclarecido

Título do Estudo: ***Melhoria da qualidade dos programas de controle de câncer de colo uterino, comparando a citologia oncológica (CO), inspeção visual, cervicografia e teste para detecção do papilomavirus humano (HPV), como otimizador de*** **rastreamento na região de Campinas.**

Pesquisadores: Sophie Françoise M. Derchain

Justificativa e objetivo do estudo e planejamento do estudo:

O câncer do colo de útero é a terceira doença maligna mais freqüente entre as mulheres do mundo inteiro. Existe forte correlação entre as infecções genitais causadas por determinados grupos de vírus e o desenvolvimento deste câncer anos mais tarde. Esses vírus, do grupo do papilomavírus humano (HPV), são adquiridos geralmente durante as relações sexuais. Este estudo visa comparar diferentes métodos de diagnóstico do câncer do colo uterino e suas lesões precursoras. Reunirá este tipo de informação através do preenchimento de um questionário sobre a saúde das participantes e coleta de amostras de exames do colo de útero a procura de uma eventual infecção pelo HPV ou lesão precursora no colo do útero.

Descrição dos procedimentos

O primeiro passo será uma entrevista que lhe explicará o estudo. Após esta entrevista, será realizado um exame pélvico interno; o médico fará um Papanicolaou e depois aplicará ácido acético no colo do útero e vai olhar a procura de alguma lesão (ferida). Este exame se chama inspeção visual. Algumas de vocês serão sorteadas para fazer um deste dois exames: um exame

de DNA de HPV (se você não tiver nenhuma experiência a respeito desse exame informe o médico para que ele lhe explique); e Cervicografia onde serão feitas duas fotografias do seu colo de útero, e as fotos depois de reveladas, mostrarão seu colo em grande aumento.

O material da citologia será encaminhado ao Laboratório de Citopatologia do CAISM, e alguns exames serão sorteados e encaminhados para São Paulo e posteriormente para Roma, Itália para o controle de qualidade. O material coletado para captura de híbridos será encaminhado ao Laboratório de Procedimentos Especializados do CAISM, e alguns serão sorteados e posteriormente encaminhados para a Finlândia, também para controle de qualidade. As cervicografias serão reveladas no CAISM, mas a sua leitura será feita na Itália, onde serão lidos os resultados por um avaliador treinado pelo laboratório que patenteou o aparelho.

Riscos associados com o estudo:

Os procedimentos usados para a colheita de amostras são indolores e são normalmente realizados em mulheres sexualmente ativas. No Brasil, o mais utilizado é o exame de Papanicolaou. Os outros exames – captura de híbridos para HPV DNA, cervicografia e a inspeção visual - são métodos que serão utilizados junto com o Papanicolaou. Isto levará a um aumento do tempo do exame ginecológico, mas não trará nenhum outro risco ou desconforto.

Benefícios do estudo:

Você fará um exame de Papanicolaou, cervicografia e um exame para detectar infecção pelo HPV. Você também receberá orientação sobre quaisquer anormalidades que possam vir a serem reveladas pelos resultados dos exames.

Exames aos quais será submetida em conseqüência da pesquisa:

O médico lhe informará os resultados do seu Papanicolaou, da captura de híbridos e da cervicografia dentro de oito semanas em uma nova consulta e por escrito. Se um ou mais dos resultados dos exames coletados na primeira consulta vier alterado, você deverá fazer um outro exame chamado colposcopia, com eventual biópsia (retirada de pequeno pedaço do colo uterino), para verificar se realmente você tem doença. Se todos os seus exames vierem negativos, você poderá ser escolhida para fazer colposcopia. Isto será realizado no mesmo dia em que você vier buscar seus resultados. Se o resultado da colposcopia indicar alguma anormalidade, o médico lhe explicará a importância de tal resultado e você será tratada ou chamada para acompanhamento, de acordo com o grau da sua lesão. Você poderá ser acompanhada anualmente (se tiver lesões chamadas NIC1, de

baixo grau) ou tratada imediatamente (se tiver lesão de alto grau, chamadas NIC 2 ou 3). Depois que este estudo houver terminado, planejamos iniciar um estudo para avaliar a manutenção da ausência de doença e cura espontânea. Se for necessário coletar material para biópsia, será inicialmente avaliado da UNICAMP e alguns casos serão encaminhados para a Finlândia, para avaliação de marcadores biológicos por imunohistoquímica, de proteínas para divisão celular, marcadores biológicos de prognóstico, apoptose, interações HIV-HPV, e carga viral (HPV). Em alguns casos será realizado um exame para ver se o HPV que você tem apresenta alterações genéticas (do DNA do vírus, não do seu). É importante entender que todos este exames não modificam seu tratamento.

Direito a fazer perguntas e/ou retirar-se do estudo:

Você pode fazer perguntas sobre o estudo e tem direito de retirar-se do estudo quando quiser e não estará obrigada a ser submetida a exame do colo do útero com coleta de amostras. Se você tiver alguma pergunta, por favor, entre em contato com: Dra. Sophie Derchain no telefone 3788 9305 das 8:00 às 17:00 horas. A sua participação é voluntária. Negar-se a tomar parte ou continuar o estudo não implica nenhuma penalidade ou perda de benefícios ou de atenção que lhe sejam devidos por seu prestador de saúde. Sua participação será tratada com absoluto sigilo e seu nome não será mencionado nos informes do estudo. Seus dados médicos ou amostras do estudo poderão ser enviados e processados em outros lugares, no Brasil, Argentina e Europa, sempre respeitando as exigências da Diretiva de Proteção de Dados da União Européia (95/46/EC) e/ou a lei equivalente aplicável. Este termo de consentimento foi aprovado pelo Comitê de Ética em Pesquisa da FCM UNICAMP e qualquer recurso ou reclamação poderá ser efetuada pelo **Fone 3788 8936.**

Assinatura da participante: _____

Assinatura do pesquisador: _____

Campinas, _____200_.

7.2. Anexo 2 – Questionário

SCREENING - Dados Demográficos **UNICAMP Código 7**

1. Número ☐ - ☐☐☐☐
2. Data ☐☐ / ☐☐ / ☐☐☐☐ (dia/mês/ano)

DADOS GERAIS

3. Data de nascimento: ☐☐ / ☐☐ / ☐☐☐☐ (dia/mês/ano)
4. Estado conjugal: **(1)** solteira **(2)** vive com parceiro
5. Educação: ☐☐ anos de estudo
6. Cor: **(1)** branca **(2)** preta **(3)** mestiço **(4)** Outro

HISTÓRIA MÉDICA

7. Última menstruação: ☐☐ / ☐☐ / ☐☐☐☐ (dia/mês/ano)
8. Idade à primeira relação sexual: ☐☐ anos de vida
9. Você está grávida ? **(0)** Não **(1)** Sim **(2)** não sabe
10. Número de: ☐ gestações = ☐ partos + ☐ cesáreas + ☐ aborto / morte ante natal
11. Número de: ☐ parceiros sexuais desde primeira relação
12. Número de: ☐ parceiros sexuais nos últimos 12 meses
13. Um dos seus parceiros teve alguma doença sexualmente transmissível agora ou no passado: **(0)** Não **(1)** Sim **(2)** Ignorado
14. Contracepção: **(1)** Hormonal – número total de anos: ☐☐
    (2) Camisinha **(3)** DIU **(4)** Laqueadura **(5)** Outro
15. Você já teve uma destas doenças sexualmente transmissíveis (Tricomonas, gonorréia, HPV, sífilis): **(0)** Não **(1)** Sim **(2)** Ignorado

FATORES DE RISCO

16. CO anterior: **(1)** Sim - Quantos em toda sua vida? ☐☐
Quando foi o último? ☐☐meses
(2) nunca

17. Antecedentes de HPV: **(pacientes com infecção atual devem ser excluídos do estudo)**:
(1) Vulvar **(2)** Anal **(3)** Oral **(4)** NIC **(5)** Carcinoma **(6)** Sem antecedente

18. Tabagismo: **(0)** Nunca
(1) Sim - atualmente
Há quantos anos? ☐☐ Quantos cigarros ao dia? ☐☐
(2) Sim - no passado
Por quantos anos? ☐☐ Quantos cigarros ao dia? ☐☐
Há quantos anos parou? ☐☐

19. Antecedente de uso de drogas: **(0)** Não **(1)** Sim

SCREENING - PHYSICAL EXAMINATION **UNICAMP**

1. Code ☐ - ☐☐☐☐

2. Date ☐☐ / ☐☐ / ☐☐☐☐ (dd/mm/yyyy)

TO BE PERFORMED AT FIRST VISIT - SCREENING

3. PAP SMEAR COLLECTED BY: **(1)** Citobrush **(2)** Ayre **(3)** Citobrush + Ayre

4. PAP SMEAR RESULT: **(0)** Normal - with endocervical cells
(1) Normal – without endocervical cells
(2) LSIL **(3)** HSIL **(4)** ASCUS
(5) AGCUS **(6)** CARCINOMA **(7)**ADENOCARCINOMA
(8) Inadequate, due to _____

5. HYBRID CAPTURE BY PHYSICIAN: **(0)** No**(1)** Yes ✍ **50% OF ALL PATIENTS**

6. HYBRID CAPTURE RESULT: RLU _ _ _ _ _ INDEX _ _ _ _ _ _

7. HYBRID CAPTURE SELF-SAMPLING: **(0)** No **(1)** Yes

8. HYBRID CAPTURE RESULT: RLU _ _ _ _ _ INDEX _ _ _ _ _ _

9. RNA SAMPLING: **(0)** No **(1)** Yes

10. RNA RESULT: **(0)** Normal **(1)** Abnormal

VISUAL INSP. ACETIC ACID

I 1 I Negativa
(1.1) Normal: **I a I** nulípara **I b I** muco cervical **I c I** multípara
I d I DIU **I e I** metaplasia escamosa
(1.2) Atípico: **I a I** ectrópio **I b I** inflamação **I c I** cisto de Naboth
I d I pólipo **I e I** leucorréia

I 2 I Positiva
(2.1) Neoplasia Intra-epitelial **I a I** condiloma **I b I** NIC 1 **I c I** NIC 2 **I d I** NIC3
(2.2) Câncer **I a I** crescimento couve flor **I b I** massa hemorrágica na vagina

11. VISUAL INSP. ACETIC ACID RESULT: **(0)** Normal **(1)** Abnormal **(2)**Suggestive of cancer

12. VISUAL INSP. IODINE SOLUTION: **(0)** No **(1)** Yes

13. VISUAL INSP. IODINE SOLUTION: **(0)** Normal **(1)** Abnormal **(2)** Suggestive of cancer

14. CERVICOGRAPHY: **(0)** No **(1)** Yes - Nº. ☐☐☐☐☐☐-☐☐

15. CERVICOGRAPHY RESULT: **(0)** Technically defected **(1)** Negative **(2)** Positive

If negative: **(1) N1** **(2) N2** **(3) A1** **(4) A2**

If positive: **(1) P1** **(2) P2** **(3) P3**

16. CONDUCT: **(0)** All exams negative – eliminated from study

1) All exams negative – will be re-screened by HCII 24 months after (20% - randomized)

(2) All exams negative – will be submitted to colposcopy (5% - randomized)

(3) All exams negative – will be submitted to colposcopy because of Argentina trial

(4) Lesions at vulva or vagina (independent of exams) – will need colposcopy evaluation

(5) One or more exam altered – will need colposcopy evaluation

Retorno data ☐☐ / ☐☐ / ☐☐☐☐ (dd/mm/yyyy)

Número ☐ - ☐☐☐☐

Data ☐☐ / ☐☐ / ☐☐☐☐ (dia / mês / ano)

TO BE PERFORMED AT FIRST OR SECOND VISIT - COLPOSCOPY

RESULT OF COLPOSCOPY: **(0)** Normal - go to question 19 **(1)** Abnormal

FINDINGS OF COLPOSCOPY AT TRANSFORMATION ZONE:

(0) No findings (1) Aceto-white epithelium (2) Flat epithelium

(3) Micropapilar epithelium (4) Punctation (5) Mosaic

(6) Leukoplakia (7) Iodine negative area (8) Abnormal blood vessels

FINDINGS OF COLPOSCOPY OUT OF TRANSFORMATION ZONE:

(0) No findings (1) Aceto-white epithelium (2) Flat epithelium

(3) Micropapilar epithelium (4) Punctation (5) Mosaic

(6) Leukoplakia (7) Iodine negative area (8) Abnormal blood vessels

OTHER FINDINGS OF COLPOSCOPY

(0) No satisfactory colposcopic findings (squamous columnar junction no visible, inflammation or atrophy)

(1) Colposcopic indicators of invasive carcinoma

(2) Vaginal alterations

(3) Vulvar alterations

21. BIOPSY: **(0)** No - go to question 24 **(1)** Yes

22. BIOPSY RESULT:

(1) Acute colpitis, inflammation, others

(2) Malignant or pre-malignant squamous cells alterations

(3) Malignant or pre-malignant glandular cells alterations

(4) Malignant or pre-malignant connective tissue alterations

23. IF BIOPSY = 2,3,4, INDICATE GRADE:

(1) HPV infection no-CIN (2) Condyloma acuminatum 3) CIN I

(4) CIN II (5) CIN III (6) Microinvasive carcinoma(-5mm)

(7) Macroinvasive carcinoma (+5mm)

(8) VAIN (9) Invasive carcinoma of vagina

(10) VIN (11) Invasive carcinoma of vulva

24. CONDUCT:

(0) No lesion, eliminated from study

(1) No lesion, but HCII positive - no treatment, follow-up 6/6months during 24months with Pap Smear, visual inspection and cervicography (only for Campinas). Hybrid capture to be performed at 12th and 24th month of follow-up.

(2) LSIL – no treatment, follow-up 6/6months during 24months with Pap Smear, visual inspection and cervicography (only for Campinas). Hybrid capture to be performed at 12th and 24th month of follow-up.

(3) HSIL – treatment

25. IF CONDUCT=3, INDICATE FINAL TREATMENT:

(1) Removal (not-conization) by LEEP

(2) Conization by LEEP

(3) Cold-knife conization

(4) Hysterectomy

(5) Hysterectomy and lymphadenectomy

(6) Vulvectomy

(7) Radiotherapy

(8) Chemotherapy

(9) Combination of radio/chemo/surgery

26. Retorno data ☐☐ / ☐☐ / ☐☐☐☐ (dd/mm/yyyy)