ELIANE REGINA ZAMBELLI MESQUITA DE OLIVEIRA

ATIPIAS DE CÉLULAS GLANDULARES DO COLO UTERINO E DETECÇÃO DE *PAPILOMAVÍRUS HUMANO* DE ALTO RISCO ONCOGÊNICO

TESE DE DOUTORADO

ORIENTADORA: Profª. Drª. SOPHIE FRANÇOISE MAURICETTE DERCHAIN
CO-ORIENTADOR: Prof. Dr. LUIZ CARLOS ZEFERINO

UNICAMP
2005

ELIANE REGINA ZAMBELLI MESQUITA DE OLIVEIRA

ATIPIAS DE CÉLULAS GLANDULARES DO COLO UTERINO E DETECÇÃO DE *PAPILOMAVÍRUS HUMANO* DE ALTO RISCO ONCOGÊNICO

TESE DE DOUTORADO APRESENTADA À PÓS-GRADUAÇÃO DA FACULDADE DE CIÊNCIAS MÉDICAS DA UNIVERSIDADE ESTADUAL DE CAMPINAS PARA OBTENÇÃO DO TÍTULO DE DOUTORA EM TOCOGINECOLOGIA, ÁREA DE TOCOGINECOLOGIA.

ORIENTADORA: Profª. Drª. SOPHIE FRANÇOISE MAURICETTE DERCHAIN
CO-ORIENTADOR: Prof. Dr. LUIZ CARLOS ZEFERINO

UNICAMP
2005

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

Ol 4a

Oliveira, Eliane Regina Zambelli Mesquita de
Atipias de células glandulares do colo uterino e detecção de papilomavirus humano de alto risco oncogênico. / Eliane Regina Zambelli Mesquita de Oliveira. Campinas, SP : [s.n.], 2005.

Orientadores : Sophie Françoise Mauricette Derchain, Luiz Carlos Zeferino
Tese (Doutorado) Universidade Estadual de Campinas. Faculdade de Ciências Médicas.

1. Citologia. 2. Adenocarcinoma. 3. Histopatologia. 4. DNA. 5. Biologia molecular. 6. Colo uterino – câncer. I. Derchain, Sophie Françoise Mauricette. II. Zeferino, Luiz Carlos. III. Universidade Estadual de Campinas. Faculdade de Ciências Médicas. IV. Título.
(slp/fcm)

BANCA EXAMINADORA DA TESE DE DOUTORADO

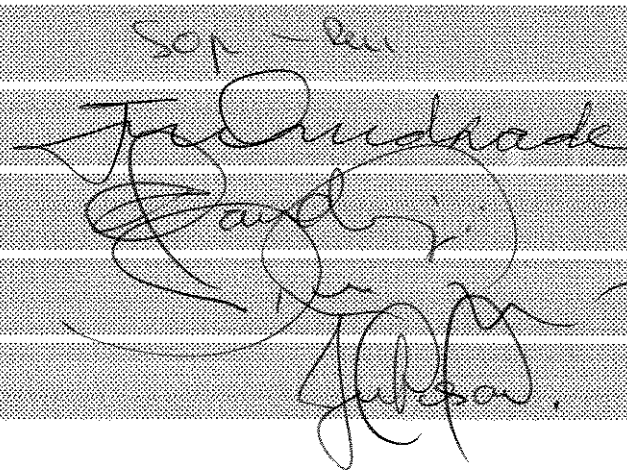
Aluno: ELIANE REGINA ZAMBELLI MESQUITA DE OLIVEIRA

Orientadora: Profª. Drª. SOPHIE FRANÇOISE MAURICETTE DERCHAIN

Co-Orientador: Prof. Dr. LUIZ CARLOS ZEFERINO

MEMBROS:

1.
2.
3.
4.
5.



CURSO DE PÓS-GRADUAÇÃO EM TOCOGINECOLOGIA DA FACULDADE DE CIÊNCIAS MÉDICAS DA UNIVERSIDADE ESTADUAL DE CAMPINAS

DATA: 12/08/2005

Dedico este trabalho...

... aos meus pais, Aristides e Nilva,
que sempre me apoiaram, com amor e dedicação,
e ajudaram a tornar meus sonhos uma realidade.

...ao meu marido Regis,
pela paciência e compreensão, pelo amor e carinho,
e pelo significado em minha vida.

...aos meus filhos Pedro e Ana Luisa,
pela alegria e pelos momentos mágicos
de nossas vidas.

...aos meus irmãos Helder e Elaine,
pelo carinho, apoio
e companheirismo.

AGRADECIMENTOS

À Profa. Dra. Sophie Françoise Mauricette Derchain, a quem eu muito admiro e estimo, agradeço pela paciência, incentivo, dedicação na orientação deste estudo e pelo exemplo de espírito científico.

Ao Prof. Dr. Luiz Carlos Zeferino, pela co-orientação na realização deste trabalho.

Ao Prof. Dr. Júlio César Teixeira, pelo incentivo e sugestões para a realização deste estudo e pelo exemplo de dedicação e responsabilidade.

Aos Profs. Drs. Paulo César Giraldo e César Cabello dos Santos pelo rico debate e sugestões para o aperfeiçoamento desta tese.

À Dra. Maria Cristina do Amaral Westin e Dra.Silvia Helena Rabelo dos Santos, pela revisão e leitura de todas as citologias, bem como pelas idéias compartilhadas que contribuíram para a minha formação.

Á bióloga, Elisabete Ap. Campos pela realização da captura híbrida II.

Á Profa. Dra. Liliana Aparecida Lucci de Ângelo Andrade patologista responsável pela leitura de todos os exames histológicos.

Ao Dr. Luis Otávio Zanatta Sarian, pelo auxílio, sabedoria e amizade.

À Margarte Donadon, da secretaria de Pós-graduação do Departamento de Tocoginecologia, pelo apoio e colaboração em todos os momentos.

Às amigas Cirbia Teixeira, Nilvana Gomes Felipe Carmo e Simone Pollini Gonçalves, pelo apoio e incentivo.

Aos amigos do Serviço de Ginecologia e Obstetrícia do Hospital Estadual de Sumaré pela boa convivência cotidiana e pelo rico aprendizado da prática médica.

Às pacientes que gentilmente concordaram em participar deste estudo.

A todos os amigos, professores, médicos e funcionários que auxiliaram na realização deste trabalho e cujos nomes deixo de citar, mas que ao lerem estas palavras incorporarão estes sinceros agradecimentos.

A toda minha família pelo apoio e carinho.

"Existem horas em que a gente não sabe o que dizer, e momentos em que não precisamos dizer nada; o olhar, a emoção e o calor humano nos traduzem tudo o que meras palavras não poderiam expressar. Apenas quebrariam o encanto e a magia. Mas não poderíamos deixar de dizer muito obrigado".

"Por meio do trabalho as mãos dos homens podem dar forma à vontade divina, construir a beleza e a harmonia, mover-se com graça e perfeição, ser instrumentos de espíritos criadores sublimes".

Trigueirinho

Este estudo foi parcialmente financiado por:

Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP), processo número 00/06394-0 e 04/09309-5

Conselho de Desenvolvimento Científico e Tecnológico (CNPq), processo número CNPq n° 307252/2004-3

ESTRUTURA DA TESE

Esta tese está sendo apresentada no formato alternativo de teses de doutorado da Universidade Estadual de Campinas (Unicamp) e de acordo com o disposto em *Normas, procedimentos e orientações para publicações de dissertações e teses da Faculdade de Ciências Médicas* (2005).

Inclui uma introdução ao tema, os objetivos do projeto de pesquisa, três artigos originais sendo o primeiro publicado no *Diagnostic Cytopathology* (2004), o segundo publicado no *Gynecology Oncology* (2005) e o terceiro aceito com modificações para publicação no *International Journal of Gynecologic Cancer* (junho de 2005). Os métodos e os resultados obtidos estão apresentados em cada artigo. Em seguida, a tese apresenta uma discussão geral, as conclusões e as referências bibliográficas. Nos anexos foram incluídos os termos de consentimento livre e esclarecido. O resumo e o abstract apresentados no inicio da tese abordam os três artigos

A coleta de dados foi realizada no ambulatório de Patologia Cervical do Centro de Atenção Integral à Saúde da Mulher (CAISM) da Unicamp.

O estudo teve a participação dos Departamentos de Tocoginecologia e Anatomia Patológica da Faculdade de Ciências Médicas da Unicamp, do Laboratório de Citologia e Laboratório Clínico Especializado do CAISM.

SUMÁRIO

SÍMBOLOS, SIGLAS E ABREVIATURAS

ACG	Atipia de células glandulares
AGC	Atypical glandular cells
AGUS	Atypical glandular cells of undetermined significance (Atipia de células glandulares de significado indeterminado)
AIS	In situ adenocarcinoma Adenocarcinoma in situ
ASCCP	American Society for Colposcopy and Cervical Pathology (Sociedade Americana de Colposcopia e Patologia Cervical)
ASC	Atypical squamous cells (Atipia de células escamosas)
ASC-H	Atypical squamous cells not excluded high grade lesion (Atipia de células escamosas, não podendo excluir lesão de alto grau)
ASC-US	Atypical squamous cells indetermined significance (Atipia de células escamosas de significado indeterminado)

BHU	Basic health units
CAF	Cirurgia de alta freqüência
CAISM	Centro de Atenção Integral a Saúde da Mulher
CH II	Captura Híbrida II
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in situ
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CO	Cut off
DNA	Ácido desoxirribonucléico
et al.	E outro(s), e outra(s)
FAPESP	Fundação de Apoio à Pesquisa do Estado de São Paulo
HC II	Hybrid capture II
HE	Hematoxylin and eosin
HPV	Papilomavírus humano
H-R	High risk
HSIL	High grade squamous intraepithelial lesion (Lesão escamosa intra-epitelial de alto grau)
LEEP	Loop electrosurgical excision procedure
LSIL	Low grade squamous intraepithelial lesion (Lesão escamosa intra-epitelial de baixo grau)
NIC	Neoplasia intra-epitelial cervical

NOS	Not otherwise specified
OR	Odds ratios
PC	Positive control
PCR	Polymerase chain reaction
	(Reação em cadeia de polimerase)
RLU	Relative Light Unit
RNA	Ácido ribonucléico
SCC	Squamous cells carcinoma
SIL	Squamous intraepithelial lesion
	(Lesão escamosa intra-epitelial)
TBS	Bethesda System
UNICAMP	Universidade Estadual de Campinas
USA	United State of America
WHO	World Health Organization

RESUMO

Objetivos: Avaliar a detecção de *Papilomavírus humano* (HPV) de alto risco oncogênico pelo teste de Captura Híbrida II (CH II) em mulheres com resultado da colpocitologia oncológica sugestiva de atipias de células glandulares (ACG). **Sujeitos e métodos:** Estes três trabalhos foram realizados através de um estudo de corte transversal com mulheres encaminhadas com ACG ou Adenocarcinoma *in situ* (AIS) no exame de rastreamento de câncer cervical. Para o terceiro objetivo, estas mulheres foram comparadas com um grupo de pacientes atendidas por lesões escamosas de alto grau (HSIL). O estudo foi realizado no Ambulatório de Patologia Cervical e no Laboratório de Citologia do Centro de Atenção Integral à Saúde da Mulher (CAISM) da Universidade Estadual de Campinas (UNICAMP) no período de Novembro de 2001 a Março de 2004. As mulheres foram submetidas à coleta de amostra cervical para citologia convencional e teste de CH II para detecção do DNA-HPV de alto risco oncogênico, e avaliação colposcópica. Todas mulheres com anormalidades citológicas persistentes ou alterações colposcópicas foram submetidas à biópsia cervical colpodirigida, conização com alça ou a frio.

Resultados: No primeiro artigo, foram incluídas 91 mulheres encaminhadas por citologia com ACG, sendo que o resultado da segunda citologia foi normal em 28 casos (31%), com ACG em 17 casos (19%) e 24 casos com HSIL ou lesões mais graves (26%). O teste de CH 2 foi positivo em 36% dos casos, sendo o DNA-HPV detectado em 87% das mulheres com HSIL, em 100% das mulheres com AIS, 24% das mulheres com ACG e em apenas 11% das mulheres sem anormalidades citológicas. No segundo artigo, foram avaliadas 146 mulheres encaminhadas por citologia com ACG, ACG associadas a HSIL e AIS, e a prevalência total de DNA-HPV de alto risco oncogênico foi de 38%. O DNA-HPV foi detectado em 93% das mulheres com ACG associado a HSIL e em 71% daquelas citologias com AIS. Já, as mulheres com ACG puras tiveram uma prevalência de 29% na detecção do DNA-HPV. Quarenta e cinco mulheres (30,8%) tinham NIC 2 ou lesões mais graves. O DNA-HPV de alto risco foi detectado em apenas 16% das mulheres que não apresentaram lesões histológicas, em contraste com 96% das mulheres com NIC 2 ou NIC 3 e 75% das mulheres com AIS. As mulheres com carcinoma cervical invasivo, escamoso ou glandular, apresentaram DNA-HPV de alto risco detectável em 85% dos casos. A detecção do DNA-HPV esteve significativamente associada com o diagnóstico histológico de NIC 2 ou lesões mais graves. No terceiro artigo, foram avaliadas 247 mulheres que apresentavam na análise histológica 38 (15%) cervicites, 194 (75%) lesões escamosas e 15 (9%) de neoplasia glandular. Apenas 30% das pacientes com ACG e HPV negativo tiveram alguma lesão glandular ou escamosa em comparação com 76% das mulheres com ACG e HPV positivo. Nas mulheres com ACG e HSIL, a maioria das lesões

histológicas, NIC 2 ou mais graves, foi associada ao teste de HPV positivo. A maioria das lesões (95%) encontradas nas pacientes com ACG e HSIL foi de natureza escamosa, e a CH II não contribuiu para a diferenciação de lesões glandulares. **Conclusões:** A segunda citologia associada ao teste para detecção do DNA-HPV de alto risco oncogênico pode melhorar a conduta nas mulheres com ACG detectado na citologia de rastreamento. A detecção do DNA-HPV esteve fortemente associada com a gravidade da lesão histológica cervical nas mulheres com citologia com ACG ou AIS. O teste de HPV pôde auxiliar na identificação de lesões significativas escamosas ou glandulares, porém não foi capaz de discriminar as lesões glandulares das escamosas.

SUMMARY

Objectives: Detection of high-risk human papillomavirus (HPV) DNA by hybrid capture 2 (HC 2) in women referred due to atypical glandular cells (AGC) in the primary screening. **Subjects and Methods:** A cross-sectional study was conducted on women referred due to AGC or adenocarcinoma in situ (AIS) in the primary screening. These women were compared with a group that had high-grade squamous intraepithelial lesion (HSIL). The study was conducted at the Cervical Patology Ambulatory and Cytology Laboratory of the Centro de Atenção Integral à Saúde da Mulher at the Universidade Estadual de Campinas from November 2001 to March 2004. Cervical sample had been collected for conventional cytology and HPV testing by HC II. The colposcopy had been performed and all women with persistent cytology abnormalities or colposcopy abnormalities were submitted a cervical biopsy or conization. **Results:** In 91 women included in the first paper, a second Pap smear was taken and HPV-DNA test was performed using HC II. The second Pap smear showed no abnormalities in 28 (31%) cases, ACG in 17 (19%) cases and HSIL or worse in 24 (26%). HC II test was positive in 36% of the altogether cases. Considering the second Pap smear diagnosis, HPV-DNA was detected in 87% of

the women with HSIL, 100% of women with AIS, 24% of women with AGC and only in 11% of the women with no abnormalities. In the second paper, the overall prevalence of HPV-DNA was 38%. HPV-DNA was detected in 93% of the women with HSIL associated with AGC and in 71% of women with AIS at Pap smear, being significantly higher when compared with the prevalence (29%) in women with AGC alone. Forty-five women (30.8%) had clinically significant histological lesions (CIN 2 or worse). High-risk HPV-DNA was detected in only 16% of the women without significant abnormalities in biopsy, in contrast to 96% of those who had CIN 2 or CIN 3 and 75% of women with AIS. Women with invasive carcinoma (squamous cells or adenocarcinoma) had over 75% of HPV-DNA positivity. The last paper histological analysis disclosed 38 (15%) cervicitis, 194 (75%) squamous lesions and 15 (9%) glandular neoplasia. Only 30% of AGC-HPV negative patients have a pathologically proven cervical lesion, whereas 76% of women with AGC-HPV positive have been diagnosed with some squamous or glandular lesion. In women with AGC-HSIL, the proportion with significant histological lesion was higher when HPV test was positive. Most (95%) of the lesions in patients with AGC-HSIL were of squamous nature, and HPV do not contribute its differentiation from glandular. **Conclusion:** The use of the second Pap smear combined with HPV-DNA may improve the management of women with AGC detected in the primary screening. HPV-DNA detection was significantly associated with the severity of cervical lesion (CIN 2 or worst) in women referred for AGC or AIS in their Pap smear. HPV test should help to identify significant squamous or glandular lesion among women referred due to glandular or squamous abnormalities at Pap smear, however the test are unable to discriminate glandular from squamous lesion.

1. INTRODUÇÃO

O Sistema de Bethesda foi desenvolvido para reportar os resultados das citologias cervicais a fim de uniformizar as terminologias e fornecer uma orientação para as condutas clínicas. Em 1988, foi proposta para a classificação colpocitológica, uma categoria chamada de atipia glandular de significado indeterminado (AGUS) como uma subcategoria diagnóstica de anormalidades no epitélio glandular. Esta categoria era baseada na presença de células com alterações além das encontradas em processos reativos benignos, porém insuficientes para diagnóstico de adenocarcinoma invasivo. Incluía tanto a possibilidade de processo reativo benigno como adenocarcinoma *in situ* (AIS) (National Cancer Institute Workshop, 1989; International Academy Of Cytology, 1993). Em 1991, esta classificação sofreu modificações, sendo recomendado à categoria AGUS uma qualificação de acordo com a possível origem anatômica da célula: endocervical ou endometrial, e uma especificação para atipias de células endocervicais de acordo com sua possível natureza: reativa ou neoplásica e

atipias de células glandulares não especificada de outra maneira (National Cancer Institute Workshop, 1993).

O termo "significado indeterminado" contido na categoria AGUS geralmente induz a pensar em algo sem importância, podendo-se negligenciar os processos encontrados nesta categoria. Portanto, no simpósio de Bethesda 2001, o termo "AGUS" foi eliminado e substituído por uma classificação mais definida de atipias de células glandulares (ACG), a qual depois foi dividida em "atipia de células glandulares" e "atipias de células glandulares favoráveis à neoplasia" (Jeng et al., 2003). Assim, para os casos que não apresentam as características suficientes para serem interpretados como AIS, existe esta categoria intermediária de "atipia de células glandulares favorável à neoplasia" (Solomon et al., 2002).

Além desta nova nomenclatura para a antiga categoria AGUS, criou-se também uma categoria distinta para AIS, a qual estava incluído na classificação de AGUS favorável a processo neoplásico segundo o Sistema de Bethesda de 1991. No entanto, há considerável coincidência morfológica entre AIS e adenocarcinoma invasivo bem diferenciado e, portanto uma porcentagem de casos interpretados como AIS apresentam invasão na avaliação histológica (Solomon et al., 2002).

As células endometriais são também reconhecidas e embora, a identificação de tais células seja de natureza benigna, a presença das mesmas na menopausa pode indicar risco para anormalidades endometriais (Keating & Wang, 2001). A diferenciação entre atipia de células endometriais e endocervicais é importante, já que um terço das mulheres com atipia endometrial têm lesões

uterinas significativas, como hiperplasia e carcinoma. Portanto, esta diferenciação nos auxilia em uma abordagem mais adequada segundo a origem da atipia celular (Cangiarella & Chhieng, 2003).

Atualmente, a maioria dos estudos mostra que o achado de ACG é incomum na citologia ginecológica, sendo encontrado uma prevalência menor de 0,5% (Zweizig et al., 1997; Burja et al.,1999; Ronnet et al.,1999, Chhieng et al., 2000b; Davey et al., 2000; Geier, Wilson e Creasman, 2001 Hammoud et al., 2002; Nasuti, Fleisher e Gupta, 2002). Contudo, estes estudos também mostram que uma grande porcentagem de pacientes com ACG apresenta lesões uterinas histológicas escamosas ou glandulares em até um ano após a coleta do exame com o diagnóstico inicial de ACG (Veljovich et al., 1998; Verdiani et al., 2003). A avaliação de pacientes com ACG revela a presença de lesões histológicas uterinas sugestivas de neoplasia intra-epitelial cervical (NIC) 2 ou 3, AIS ou câncer invasor em 17-80%, sendo lesões glandulares em 0-30% (Cangiarella & Chhieng, 2003).

Entre as diferentes populações, a prevalência de ACG é maior naquelas com alta prevalência de atipias escamosas de significado indeterminado e lesões escamosas intra-epiteliais. Contudo, populações com grande porcentagem de pacientes na pós-menopausa, podem também mostrar um aumento na incidência de ACG secundário a maior freqüência de lesões endometriais (Chhieng et al., 2001a).

Histologicamente, entretanto, não foram identificados aspectos morfológicos que possam representar uma lesão precursora de AIS, ou seja, as lesões glandulares endocervicais são classificadas apenas em AIS ou adenocarcinoma

invasor (Solomon et al., 2002). O AIS endocervical é considerado como precursor do adenocarcinoma invasivo (Boon et al., 1981; Tase et al., 1989). Entretanto, a relação entre as atipias glandulares endocervicais e o aparecimento de AIS e invasivo permanece não esclarecido. Ambos, adenocarcinomas *in situ* e invasivo, bem como as atipias glandulares são freqüentemente associadas com lesão neoplásica escamosa, indicando a possibilidade de um fator etiológico comum (Brown & Wells, 1986; Lawrence, 1991).

Apesar de ser encontrado um aumento de sua freqüência nos últimos anos, o AIS do colo uterino, é uma entidade rara. Se a etiologia e história natural das lesões precursoras escamosas do colo uterino são hoje bem conhecidas, as alterações glandulares ainda não estão adequadamente descritas do ponto de vista morfológico, em relação a sua evolução e, da mesma forma, se o tratamento conservador com conização seria adequado como é aceito para o carcinoma escamoso *in situ* (CIS) (Krummins et al., 1977; Ostör et al., 1984; Ayer et al., 1987; Bertrand, Lickrish e Colgan, 1987; Muntz et al., 1992; Poynor, Barakat e Hoskins, 1995).

Considera-se que o melhor protocolo para o seguimento das mulheres que apresentam citologia cervical com ACG depende, primeiramente, em conhecer e entender a história natural da atipia glandular e a taxa de progressão para lesões malignas escamosas e/ou glandulares. Muitos trabalhos têm discutido a questão da história natural das atipias de células escamosas (Ostor, 1993; Raab, Bishop e Zaleski, 1999; Holowaty, et al., 1999) e, comparativamente, a avaliação da

evolução da atipia glandular para câncer invasor, não apresenta estudos conclusivos (Ioffe et al., 2002).

Muitos fatores contribuem para a dificuldade em estudar a história natural e progressão das atipias glandulares nas citologias ginecológicas. O primeiro é a relativa raridade de ocorrência das ACG. Não há também, uma lesão endocervical pré-neoplásica bem definida quando comparada com as lesões escamosas. Outro fator é que as ACG são uma entidade heterogênea formada por uma grande variedade de lesões escamosas, endocervicais e endometriais. Finalmente, a freqüência de lesões clínicas é variável, não somente na população de risco para desenvolvimento de câncer cervical, mas também na distribuição por idade entre diferentes populações de pacientes (Chhieng et al., 2001a; Zaino, 2002; Ioffe et al., 2002).

De acordo com as recomendações publicadas pela Sociedade Americana de Colposcopia e Patologia Cervical (ASCCP) para as condutas das pacientes com resultados anormais da citologia cervical, mulheres com ACG deveriam ser investigadas intensivamente pela falta de sensibilidade na repetição da citologia na detecção das lesões uterinas de alto grau e o alto risco de tais lesões nestas mulheres. Pacientes com diagnóstico de ACG deveriam ser encaminhadas a colposcopia e colhido uma amostra endocervical como uma avaliação inicial. Mulheres com mais de 35 anos, aquelas com sangramento vaginal anormal, e mulheres com ACG de provável origem endometrial sem levar em conta a idade, também deveriam ser encaminhadas para uma amostra endometrial (Wright et al., 2002).

A ASCCP recomenda também que as pacientes com resultado de ACG na citologia de rastreamento, sem evidência de lesões pré-neoplásicas ou neoplásicas a colposcopia, tenham o seguimento com a repetição da citologia cervicovaginal num intervalo de quatro a seis meses até quatro resultados consecutivos negativos, após os quais as mulheres poderão retornar ao controle citológico de rotina. Se algum resultado de lesão escamosa de baixo grau (LSIL) ou atipia de células escamosas (ASC) for obtido durante o seguimento, deverá ser realizada uma colposcopia. Já o achado de lesão escamosa de alto grau (HSIL) ou ACG em algum resultado de citologia durante o seguimento autoriza um procedimento diagnóstico excisional (Wright et al., 2002).

Em relação à etiologia do câncer do colo uterino e das lesões precursoras, os dados da literatura mostram que o *Papilomavírus humano* (HPV) de alto risco oncogênico está presente em mais de 99% das neoplasias cervicais invasoras, tanto em carcinoma de células escamosas como em adenocarcinomas (Walboomers et al., 1999). Alguns tipos de HPV, tal como o 16/18 e 31/33 são freqüentemente associados com NIC 2 ou 3 e carcinoma escamoso invasivo (Brown & Wells, 1986; Yun, Molenaar e Wilkins, 1989).

A detecção do DNA-HPV, através de várias técnicas, tem sido muito estudada como um instrumento associado à citologia oncológica no rastreamento, diagnóstico e seguimento das pacientes com NIC. Embora o HPV seja consistentemente detectado em mais de 90% das lesões precursoras de células escamosas, a prevalência do HPV em AIS é variável, dependendo da população

estudada e do método utilizado (Lee, Chang e Wu, 1998; Skyldberg et al., 1999; Pirog et al., 2000; Bosch et al., 2000). Os tipos de HPV encontrados em AIS e adenocarcinoma invasivo são similares (Farnsworth, Laverty e Stoler, 1989; Okagaki et al., 1989; Andersson et al., 2001), sendo o HPV 18, o tipo predominante quando comparado com o HPV 16 (Syrjänen & Syrjänen, 2000). Porém, a associação entre a detecção do DNA-HPV ou a carga viral com o desenvolvimento de lesões glandulares foram avaliadas em poucos estudos (Ronnett et al., 1999).

Dois tipos de testes são freqüentemente utilizados para detecção de HPV. Um é baseado na detecção do HPV depois da amplificação com a técnica de PCR (*Polymerase Chain Reaction*) e o outro é a Captura Híbrida II (CH II), sistema baseado na detecção direta do DNA-HPV usando sinais de amplificação. Ambos são considerados adequados e úteis em estudos de rastreamento porque podem ser utilizados de uma forma simplificada e rápida (Meijer et al., 2000). Comparando os métodos de PCR e CH II, estudos mostram uma concordância de 84-86% na detecção de HPV de alto risco oncogênico (Farthing, 1994; Nindl, 1995).

A CH II é uma técnica simples que pode ser utilizada em larga escala. Tem um risco muito pequeno de contaminação levando a poucos resultados falsos positivos, apesar de apresentar uma sensibilidade um pouco menor quando comparada com o método de PCR (Syrjänen & Syrjänen, 2000). A CH II utilizando um *probe* B, é uma hibridização capaz de detectar HPV de alto risco oncogênico (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 e 68). Uma das vantagens dessa técnica é trabalhar com sondas de

RNA longas, com alta estringência para hibridização, o que aumenta a sensibilidade do teste (Clavel et al., 1998).

A CH II é um teste de biologia molecular, que amplifica o sinal dos híbridos formados (RNA/DNA), os quais são detectados através de reação enzima-substrato e sua leitura se faz por quimioluminescência. O material para análise passa por cinco procedimentos: desnaturação, hibridização, captura dos híbridos, reação dos híbridos com o conjugado e detecção dos híbridos por quimioluminescência.

A desnaturação é uma etapa na qual o DNA é submetido a altas temperaturas e pH ácido, deixando as bases nitrogenadas (A-T-C-G) livres para a hibridização. Para a realização da hibridização, as sondas de RNA são diluídas em diluente próprio, aliquotadas em microtubos e colocadas em banho-maria à 65° C durante uma hora. Depois de hibridizado, o material é transferido para uma microplaca que tem suas paredes recobertas por anticorpos monoclonais anti-RNA:DNA, que irão reagir com os híbridos formados anteriormente. A partir da captura, com a formação do complexo anti-RNA:DNA-híbrido, passa-se para a fase de detecção onde será adicionado um anti-anti-RNA:DNA conjugado a fosfatase alcalina que irá reagir com o complexo ligado à parede da microplaca. Adiciona-se o substrato Emerald, que será degradado pela fosfatase alcalina. O grau de degradação do substrato dependerá da quantidade de enzima ligada ao complexo, o qual produzirá diferentes intensidades de cor, lidas por quimioluminescência, em equipamento apropriado.

À medida que o substrato é separado pela fosfatase alcalina, a luz é emitida proporcionalmente à carga de DNA no material coletado e medida em unidades (*Relative*

Light Unit - RLU) em um quimioluminômetro, sendo que a intensidade da luz emitida é proporcional à carga de DNA-HPV. Para classificar o resultado da captura de híbridos e quantificar a carga viral, utiliza-se um valor de corte diário "*cut off*", sendo que amostras com emissão de luz maior que o ponto de corte são consideradas positivas e aquelas com emissão de luz menor são consideradas negativas (Syrjänen & Syrjänen, 2000).

Até o momento, os resultados de estudos para o uso de testes na detecção de DNA-HPV em mulheres com ACG ou AIS são insuficientes (Wright et al., 2002). Alguns estudos preliminares sugeriam que o uso dos testes para a detecção do DNA-HPV poderia melhorar a conduta clínica nos casos das mulheres com AGUS (Ronnett et al., 1999). Um estudo utilizando o método de CH II para identificação de HPV de alto risco oncogênico em citologias com AGUS, encontrou este DNA-HPV em 92% das mulheres com biópsias sugestivas de NIC 2 ou 3 e em 100% daquelas com AIS. Já a detecção do DNA-HPV foi positiva em apenas 16% das mulheres com ausência de lesão histológica (Ronnett et al., 1999).

Assim como o carcinoma cervical de células escamosas e suas lesões precursoras, o adenocarcinoma cervical está altamente associado com a infecção pelo HPV de alto risco oncogênico (Smotkin et al., 1986; Pirog et al., 2000). Contudo, enquanto o teste para detecção do HPV de alto risco oncogênico é um método muito estudado para conduzir mulheres com resultado citológico de atipias escamosas, este modelo não está claramente estabelecido para os casos de atipia de células glandulares (Wright et al., 2002). O teste para detecção do HPV de alto risco oncogênico em citologia com ACG foi investigado em dois estudos

publicados com resultados promissores (Ronnett et al., 1999; Krane et al., 2004). Estes estudos sugerem que a utilização dos testes para detecção do DNA-HPV de alto risco oncogênico possam ajudar a entender a fisiologia das atipias de células glandulares de uma maneira mais segura, embora o papel da CH II na assistência clínica às mulheres com ACG, seja ainda desconhecido.

Atualmente, a maior parte do uso dos testes moleculares para detecção de HPV é restrita para pesquisa. Mulheres com infecção persistente pelo HPV podem ser de alto risco para o desenvolvimento subseqüente de neoplasia cervical, quando comparada com mulheres cuja infecção não seja persistente. Importante também é a detecção da carga viral, já que alguns estudos mostram que a doença clínica pode estar relacionada com a alta carga viral persistente (Syrjänen & Syrjänen, 2000).

Assim, a taxa de concordância entre os resultados citológicos e histológicos em mulheres atendidas por ACG, é limitada na prática clínica, e cria um desafio tanto para citopatologistas e patologistas como para ginecologistas. Existe uma necessidade de se diferenciar entre todas as mulheres com ACG, aquelas que apresentam lesões escamosas ou glandulares de alto grau. O uso de exames para detecção de HPV de alto risco oncogênico poderá auxiliar: 1) na diferenciação da gravidade dentro da categoria citológica de ACG; 2) na compreensão do papel do HPV na etiologia das lesões histológicas associadas; 3) na seleção da propedêutica adequada para cada mulher em função da previsão da gravidade histológica da lesão cervical. Para este fim, inicialmente, foi avaliada a relação entre a detecção viral e os achados morfológicos citológicos em mulheres atendidas por resultado da citologia de rastreamento sugestiva

de ACG. Em uma segunda fase foi avaliada a contribuição da CH II no diagnóstico histológico das lesões. E finalmente, foram avaliados os resultados histológicos e da CH II em mulheres atendidas por ACG, utilizando-se um grupo de mulheres com atipias escamosas para comparação. Para tanto, dividiu-se esta pesquisa em três publicações.

2. OBJETIVOS

2.1. OBJETIVO GERAL

Detecção de HPV de alto risco oncogênico pela CH II em mulheres com resultado da citologia de rastreamento sugestiva de ACG.

2.2. OBJETIVOS ESPECÍFICOS

- **ARTIGO 1:**

Comparar o resultado da citologia coletada no momento da colposcopia com o resultado da CH II em mulheres atendidas por ACG no exame de rastreamento de câncer cervical.

- **ARTIGO 2:**

Avaliar a associação entre a detecção do DNA-HPV de alto risco oncogênico e o diagnóstico histológico em mulheres encaminhadas por ACG ou AIS na citologia oncológica.

- **ARTIGO 3:**

1. Comparar o papel da CH II em mulheres com atipias celulares escamosas ou glandulares na previsão do diagnóstico histológico.

2. Avaliar a detecção de DNA-HPV de alto risco oncogênico e a carga viral nas lesões cervicais histológicas glandulares ou escamosas.

3. PUBLICAÇÕES

ARTIGO 1

Detection of High-Risk Human Papillomavirus (HPV) DNA by Hybrid Capture II in Women Referred Due to Atypical Glandular Cells in the Primary Screening.

Eliane RZM Oliveira, Sophie FM Derchain, Silvia H Rabelo-Santos, Maria Cristina A Westin, Luiz Carlos Zeferino, Elisabete A Campos, Kari J Syrjanen.

Publicado no **Diagnostic Cytopatology, 31(1):19-22, 2004.**

ARTIGO 2

Human Papillomavirus DNA Detection and Histological Findings in Women Referred for Atypical Glandular Cells or Adenocarcinoma in Situ in their Pap Smears.

Sophie FM Derchain, Silvia H Rabelo-Santos, Luis Otávio Sarian, Luiz Carlos Zeferino, Eliane RZM Oliveira, Maria Cristina A Westin, Liliana ALA Andrade, Kari J Syrjanen.

Publicado no **Gynecologic Oncology, 95(3):618-23, 2004.**

ARTIGO 3

Prediction of high-grade cervical disease with HPV detection in women with glandular and squamous cytological abnormalities

Eliane RZM Oliveira, Sophie FM Derchain, Luis Otávio Sarian, Renata C Gontijo, Silvia Helena Rabelo-Santos, Adriana Yoshida, Liliana de Angelo Andrade, Luiz Carlos Zeferino

International Journal of Gynecologic Cancer (aceito com modificações em 26 de junho de 2005).

3.1. ARTIGO 1

DETECTION OF HIGH-RISK HUMAN PAPILLOMAVIRUS (HPV) DNA BY HYBRID CAPTURE II IN WOMEN REFERRED DUE TO ATYPICAL GLANDULAR CELLS IN THE PRIMARY SCREENING

Eliane RZM Oliveira[1], Sophie FM Derchain[1], Silvia H Rabelo-Santos[1,2], Maria Cristina A Westin[1], Luiz Carlos Zeferino[1], Elisabete A Campos[1], Kari J Syrjanen[3].

[1]Department of Obstetrics and Gynecology Universidade Estadual de Campinas (UNICAMP), Rua Alexander Fleming 101, CEP:13083-970, Campinas, SP, Brazil; [2]Faculty of Pharmacy, Universidade Federal de Goiás (UFG), Goiânia, GO, Brazil; [3]Cytopathology Unit, Laboratory of Epidemiology, National Institute of Health (ISS), Rome, Italy.

Grant sponsor - Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Grant number - FAPESP number 00/06394-0 and 00/697-7 and CNPq number 300354/01-0

Corresponding author.

Sophie F. M. Derchain, MD, PhD

Rua Antônio Hossri, 629 – Cidade Universitária

13083-370 – Campinas – SP.

Fone: +55-19-37889305 / Fax: + 55-19-3289 5935

E-mail: derchain@supernet.com.br

Running headline: atypical glandular cells and HPV

Diagnostic dilemmas.

Summary

We assessed the detection of high-risk Human Papillomavirus DNA (HPV-DNA) in women examined by the second Pap smear due to atypical glandular cells (AGC) detected in their screening Pap smear. In 91 women included in the study, a second Pap smear was taken and HPV-DNA test was performed using Hybrid Capture II (HC II). The second Pap smear showed no abnormalities in 28 (31%) cases, ACG in 17 (19%) cases and high-grade squamous intraepithelial lesions (HSIL) or worse in 24 (26%). HC II test was positive in 36% of the altogether cases. Considering the second Pap smear diagnosis, HPV-DNA was detected in 87% of the women with HSIL, 100% of women with in situ adenocarcinoma and only in 11% of the women with no abnormalities. The use of the second Pap smear combined with HPV-DNA may improve the management of women with AGC in the primary screening.

Key words: Atypical glandular cells, Adenocarcinoma in situ, Human Papillomavirus, Hybrid Capture II, Bethesda System

Introduction

Glandular cell abnormalities are a challenging diagnostic category in gynecological cytology, because of their frequent association with underlying biopsy-confirmed high-grade disease[1-5]. Firstly classified as atypical glandular cells of undetermined significance (AGUS) in the 1991 Bethesda System (TBS)[1], this category was revised in TBS 2001 and renamed as atypical glandular cells (AGC). This diagnostic category of AGC consists of a wide spectrum of cervical pathology,

including both pre-invasive and invasive squamous and glandular lesions[6]. Cervical glandular pre-invasive lesion, known as adenocarcinoma *in situ* (AIS) as well as micro-invasive adenocarcinoma are thought to be intermediate steps in the development of frankly invasive cervical adenocarcinoma[7].

In the 1991 TBS, the cytological features of AIS were included into the "AGUS probably neoplastic" category, but subsequent studies have clearly documented reproducibility and predictive value for the cytological criteria of AIS, if properly applied[8,9]. Endocervical AIS is therefore now a separate category in the new TBS[10], reflecting a reappraisal of the strengths and weaknesses of cytoloy in assessing these abnormalities.

Cervical adenocarcinoma and its precursor lesions have recently been included in the growing list of Human Papillomavirus (HPV) associated genital tumors[11-14]. Walboomers *et al.* [15] showed that HPV is present in 95% of the patients with invasive adenocarcinoma, when polymerase chain reaction (PCR)-based assays or serology were used. However, cervical glandular precursor lesions that may be the cause of AGC, have shown significantly lower HPV detection rates than AIS or cervical adenocarcinoma, strongly supporting a non-neoplastic etiology of many such cases.[13,14]

To cast further light on this AGC-HPV relationship, we compared the results of the second Pap smear and H-R (high-risk) HPV-DNA detection by Hybrid Capture II (HC II) in women referred for AGC in routine cervical cancer screening.

Material and methods

The study was conducted at the Cytology Laboratory of the Women Hospital, School of Medicine, Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil. The Institutional Review Board approved the study protocol and all participants gave a written informed consent prior to entry in the study. Women were excluded from the study if a) they had a previous history of CIN or cervical, vaginal or vulvar cancer, b) had clinical immunossuppression or c) were pregnant.

A total of 91 women referred for the Colposcopy Clinic due to AGC in their Pap smears taken in cervical cancer screening program, were enrolled in the study from November 2001 until November 2002. Samples were taken from the endocervix using a Dacron brush. From this material, a second conventional Pap smear was first done and the brush was placed in 1.0ml of Specimen Transport Medium (*Digene corporation*) for HPV-DNA testing using Hybrid Capture II (HC II) test. All women were subjected to colposcopic examination, and biopsies were taken from any colposcopically abnormal area. The colposcopy and biopsy results are not included in this analyses.

All the referral- and second Pap smears were processed at the Cytology Laboratory and the diagnosed by the same cytopathologist according to the 2001 TBS[10]. The interval between the referral and the second cytology varied case by case, and was determined from the documents. The cytological features used to determine the diagnosis of glandular atypia included endocervical cells crowded in clusters with anisonucleosis, nuclear enlargement and nuclear hyperchromasia[5]. Other suspect features include increased cellularity, presence of isolated atypical cells, fragments,

cellular over position and appolar cells, higher than normal nuclear:cytoplasmic ratio, decreased cytoplasm, irregular nuclear membranes, or thick membranes, smudged chromatin, pleomorphism and atypical stripped nuclei. In differential diagnosis to AIS, we also considered the presence of sheets and clusters of tightly packed glandular cells, overlap of nuclei, pseudostratified strips of columnar epithelial cells, epithelial rosettes, nuclear protrusion at group margins, nuclear enlargement with pleomorphism and nuclear irregularity, elongation of nuclei, distinctive coarsely granular chromatin pattern and nucleoli not typically prominent, as previously described[16] (Figure 1).

The specimen for HC II were tested for probe B (high-risk HPVs: types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and the test was classified as positive when the relative light unit (RLU) ratio (RLU of specimen/mean RLU of two positive controls - HC index) was 1pg/ml or greater. The storage of the specimens and all reagents as well as conduction of the tests took place at the Medical School Hospital Laboratory, following the manufacturer's instructions (*Digene Diagnostics Inc., USA*).

All statistical analysis were done using the SAS software, version 8.0. Odds ratios (OR) with 95% confidence interval (95% CI) were used to evaluate the associations between HPV test and Pap smear results.

Results

Altogether, 17 (19%) women had pure ACG in their second Pap smear. However, 4 (4%) women showed AGC associated with atypical squamous cells that cannot exclude high-grade squamous intraepithelial lesion (ASC-H). In addition, 9 (10%) cases had AGC associated with high-grade squamous intraepithelial lesions

(HSIL) and 16 (16%) showed HSIL or worse. Finally, 28 (31%) women presented with completely negative second Pap smear.

The detection of high-risk types HPV-DNA was significantly associated with more severe abnormalities in the second Pap smear, as shown in Table 1. HC II was positive in 36% of the cases. HPV-DNA was detected in 87% of the cases whith HSIL in the second Pap smear and in 100% of women with AIS. In contrast, only 11% of the women who had negative second Pap smear, 21% of those who had ASC-US and 24% of those with AGC had a positive HCII test for H-R HPV. Three cases suggesting endometrial adenocarcinoma were all HC II negative.

Discussion

The prevalence of glandular abnormalities in the Pap smears is expected to increase due to the increased use of endocervical sampling techniques in this country. Because the diagnosis of AGC is still ambiguous, it is important to have the tools to make distinction between benign and/or reactive glandular cells and pre-malignant and malignant glandular lesions, as well as to differentiate truly glandular lesions from their squamous cell counterparts[2,5,14]. Several recent studies include exhaustive and detailed descriptions on the cytological criteria which is essential to improve the sensitivity and specificity of the Pap smear in detecting glandular disease of the uterine cervix[2-5,8,9].

However, sensitivity and specificity of cytology in detecting glandular pathology remain modest even in the hands of experts[17-21]. Unlike squamous cell abnormalities, glandular cell atypia is far more rare and, consequently less experience has been gained with the diagnostic category of AGC and AIS [4-7]. This can explain at least in part,

the substantial proportion of negative Pap smears in the follow-up of patients with diagnostic of AGC, reported in several studies.

In this study HSIL, associated or not with AGC, was the major diagnosed abnormality in the second Pap smear. A number of studies have shown that it is often difficult to differentiate between AGC and squamous metaplasia in the Pap smear and HSIL involving the transformation zone or endocervical glands[22,23]. Some atypia of the squamous cells, especially the high-grade, may look like columnar cells, due to scant, nondescript cytoplasm or even some feathering[22]. Squamous lesions involving endocervical glands may show architectural arrangements that resemble those of endocervical cells and groups. They may also acquire some of the nuclear and cytoplasmic features that are more characteristic to the cells of glandular origin, and thus may be misclassified as AGC[24].

High-risk HPV has been found consistently associated with glandular atypia when strict cytological criteria have been used to make diagnosis of AIS[25]. In the present study, the diagnostic of HSIL, AIS and SCC was strongly associated to detection of H-R HPV-DNA in these women with AGC in their primary Pap smear. The detection rate of HPV-DNA presented by Ronnet et al. [14] in women referred for AGUS was 28% which is close to the figures (36%) observed in the present study. These authors also showed that women with histological diagnostic of CIN III and AIS were HC II positive in 92% and 100% respectively.[14] This is in full agreement with our data, where 83-88% of HSIL and/or HSIL/AGC cases and 100% of AIS were H-R HPV positive (Table 1).

To conclude, the present data suggest that the use of the second Pap smear analyzed using the refined cytological criteria for glandular cell abnormalities and combined with HPV-DNA testing by HC II may lead to improve management of women with AGC, including appropriate referral to colposcopy and use of diagnostic cone to confirm the diagnosis in suspicious cases. Further studies are needed to confirm the safety and cost-effectiveness of this approach, however.

Acknowledgements

To Denise Pita and Lucia Fagian for technical assistance in HPV detection.

References

1. Kurman, RJ. & Soloman, D. The Bethesda System for reporting cervical/vaginal diagnoses. Bethesda, Springer-Verlag, New York, Inc., 1994. 81p.

2. Raab, SS, Snider, TE, Potts, SA et al. Atypical glandular cells of undetermined significance. Diagnostic accuracy and iterobserver variability using select cytologic criteria. Am J Clin Pathol 1997;107:299-307.

3. Burja, IT; Thompson SK; Sawyer, WI; Shurbaji, MS – Atypical glandular cells of undetermined significance on cervical smears. A study with cytohistologic correlation. Acta Cytol 1999;43:351-356.

4. Raab, S.S. Can Glandular Lesions Be Diagnosed in pap Smear Cytology?. Diagnostic Cytopathology 2000;23 (2);127-133.

5. Chieng, DC.; Elgert, PA.; Cangiarella, JF.; Cohen, JM. – Clinical significance of atypical glandular cells of undetermined significance. A follow-up study from na academic medical center. Acta Cytol 2000a;44:557-566.

6. Chieng, DC, Elgert, PA, Cangiarella, JF, Cohen, JM. –Variation in the incidence of. AGUS Between Different Patient Populations. Acta Cytol 2000b;45:287-299.

7. DeMay, RM. The Pap Smear. In: The Art and the Science of Cytopathology.: Chicago: ASCP press, 1993. p 61-185.

8. Lee, KR, Manna, EA, Jones, MA. Comparative cytologic features of adenocarcinoma in situ of uterine cervix. Acta Cytol 1991;35; 117-126.

9. Biscotti, CV, Gero, MA, Toddy, SM, Fischier, DF, Easley, KA. Endocervical adenocarcinoma in situ: an analyses of cellular features. Diagn Cytopathol 1997;17:326-332

10. Solomon, D, Davey, DD, Kurman, RJ, et al._ The 2001 Bethesda System - Terminology for Reporting Results of Cervical Cytology. JAMA 2002;287; 2114-2119

11. Anciaux D, Lawrence WD, Gregoire, L. Glandular lesions of the uterine cervix: prognostic implications of human papillomavirus status. Int J Gynecol Pathol 1997;16:103-110.

12. Hörding, U, Daugaard, S, Visfeldt, J. Adenocarcinomas of the cervix and adenocarcinoma of the endometrium: distintion with PCR-mediated detection of HPV-DNA. APMIS 1997;105: 313-316.

13. Andersson, S, Rylander E, Larsson B. et al.. The role of human papillomavirus in cervical adenocarcinoma carcinogenesis. Eur J cancer 2001;37:246-250.

14. Ronnet, BM, Manos, MM, Ransley, JE et al. Atypical glandular cells of undetermined significance: cytopathologic features, histopathologic results, and human papillomavirus DNA detection. Hum. Pathol 1999;30(7): 816-825

15. Walboomers JM, Jacobs MV, Manos MM, et al.. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J. Pathol* 1999;189: 12-19.

16. National Cancer Institute Workshop –Bethesda System 2001 avaible in site "http.www.bethesda2001.cancer.gov".

17. Wilbur, DC. Endocervical glandular atypia: A "new" problem for the cytopathologist. Diagn Cytopathol 1995;13: 463-469.

18. Raab, SS.; Geisinger KR, Silverman, JF, Thomas, PA, Stanley, MW. Interobserver variability of a Papanicolaou smear diagnosis of atypical glandular cells of undetermined significance. Am J Clin Pathol 1998;110:653-659.

19. Diaz-Rosario, LA & Kabawat, SE. Cell Block Preparation by Inverted Filter Sedimentation is Useful in the Diferential Diagnosis of Atypical Glandular Cells of Undetermined significance in ThinPrep Specimes. Cancer 2000;90(5):265-272.

20. Soofer, SB. & Sidawy, MK. Atypical glandular cells of undetermined significance: clinically significant lesions and means of patient follow-up. Cancer 2000;90:207-214.

21. Geier, CS.; Wilson, M.; Creasman, W. Cinical evaluation of atypical glandular cells of undetermined significance. Am. J. Obstet. Gynecol 2001;184(2):6469.

22. Lee, KR, Manna, EA, St Jonh T. Atypical endocervical glandular cells: accuracy of cytologic diagnosis. Diagn cytopathol 1995;13:202-208.

23. Raab, SS.; Bishop, NS & Zaleski, MS. Effect of cervical disease history on outcomes of women who have a pap diagnosis of atypical glandular cells of undetermined significance. Gynecol. Oncol 1999;74:460-464.

24. Wilbur, D. Citology of Endocervix, Endometrium, and upper femalr genital tract. In: Bonfiglio, TA & Erozan, YS editor. Gynecologic Cytopathology: Philadelphia: Lippincott-Raven Publishers, 1997. p 107-169.

25. Solomon, D, Frable, WJ, Vooijs, GP, et al. ASCUS and AGUS criteria. Intenacional Academy of Cytology Task Force summary. Diagnostic Cytology Towards the 21st Century: An International Expert Conference and Tutorial. Acta Cytol 1998;42; 16-24.

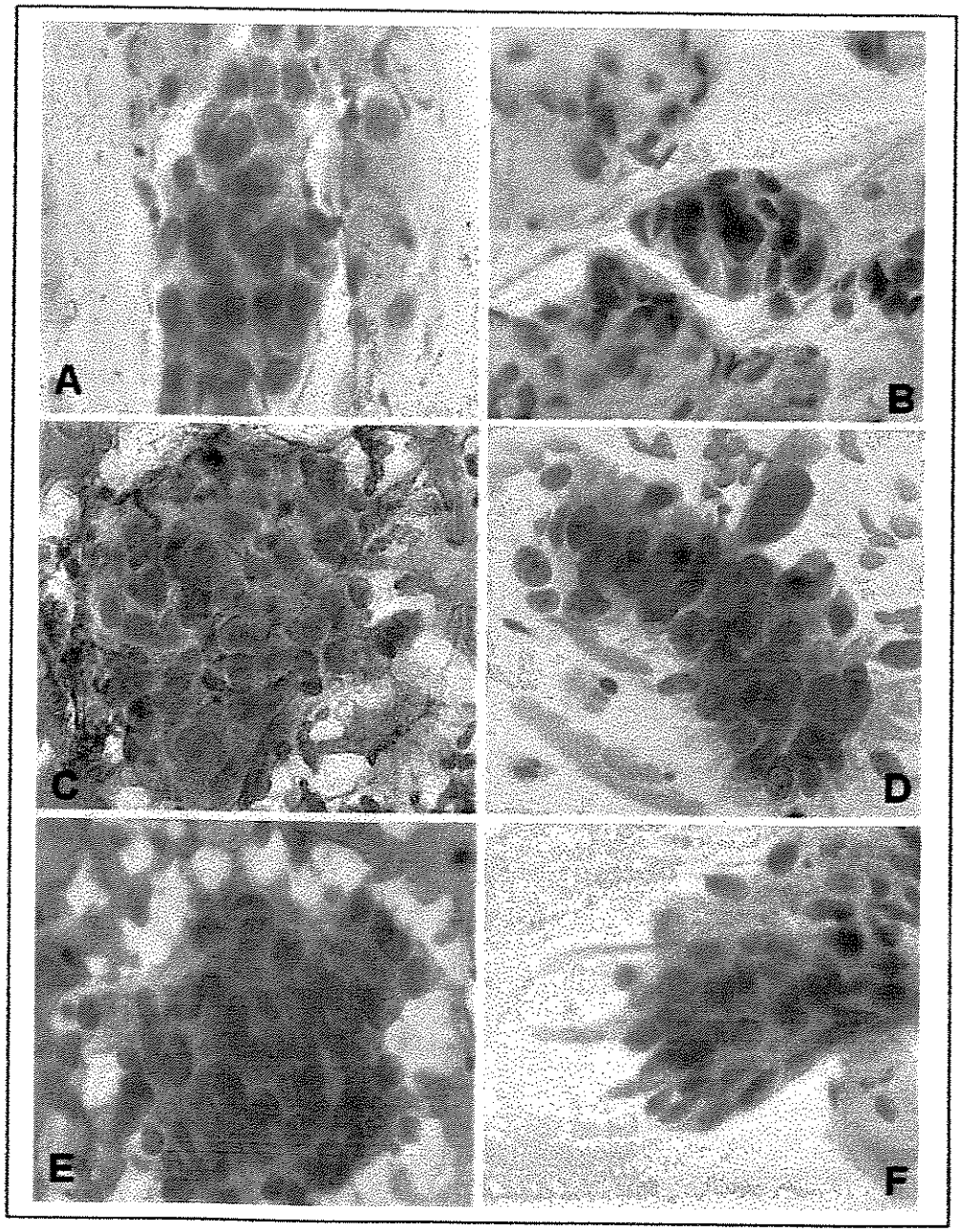


Figure 1

A - Case HPV positive diagnosticated as AGC in the second Pap smear (B)
C - Case HPV positive diagnosticated as AIS in the second Pap smear (D)
E - Case HPV positive diagnosticated as CIN 3 in the second Pap smear (F)

Table 1. Results of the Second PAP Smear and Hybrid Capture II test in Women Originally Diagnosed as Having AGC

Second PAP smear	n	(%)	HPV pos (%*)	OR (95%CI)
Negative	28	(31)	3 (11)	Reference
ASC-US	14	(15)	3 (21)	2.27 (0.30-17.6)
AGC	17	(19)	4 (24)	2.56 (0.40-17.6)
ASC-H	4	(4)	2 (50)	5.00 (0.57-48.0)
ASC-H e AGC	4	(4)	1 (25)	
HSIL	6	(7)	5 (83)	54.1 (6.37-667.1)
HSIL/AGC	9	(10)	8 (88)	
AIS	5	(6)	5(100)	-
SCC	1	(1)	1 (100)	-
Endometrial Adenocarcinoma	3	(3)	-	-
Total	**91**	**(100)**		

* % of HPV positive cases in each diagnostic category

3.2. ARTIGO 2

HUMAN PAPILLOMAVIRUS (HPV) DNA DETECTION AND HISTOLOGICAL FINDINGS IN WOMEN REFERRED FOR ATYPICAL GLANDULAR CELLS (AGC) OR ADENOCARCINOMA IN SITU (AIS) IN THEIR PAP SMEARS.

Sophie Françoise Mauricette Derchain[1], Silvia Helena Rabelo-Santos[1,3], Eliane Regina Zambelli Mesquita de Oliveira[1], Luis Otávio Sarian[1], Maria Cristina do Amaral Westin[1], Liliana Aparecida Lucci de Ângelo Andrade[2], Luiz Carlos Zeferino[1], Kari Juhani Syrjänen[4].

[1]Department of Obstetrics and Gynecology and [2]Department of Pathology Universidade Estadual de Campinas (UNICAMP), Rua Alexander Fleming 101, CEP:13083-970, Campinas, SP, Brazil; [3]Faculty of Pharmacy, Universidade Federal de Goiás (UFG), Goiânia, GO, Brazil; [4]Cytopathology Unit, Laboratory of Epidemiology & Biostatistics, National Institute of Health (ISS), Rome, Italy.

Support: Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP number 00/697-7) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq number 300354/01-0)

Corresponding author: Sophie F. M. Derchain, MD, PhD
Rua Antônio Hossri, 629 – Cidade Universitária
13083-370 – Campinas – SP.
Fone: +55-19-37889305 / Fax: + 55-19-3289 5935
E-mail: derchain@supernet.com.br

Running headline: atypical glandular cells and cervical histology

Summary

Objective: To evaluate the association between high-risk papillomavirus (HPV) DNA detection and histological diagnosis in women referred for atypical glandular cells (AGC) or adenocarcinoma in situ (AIS) at Pap smear

Methods: In this cross-sectional study, 146 women referred for AGC (124), AGC with high-grade squamous intraepithelial lesion (HSIL) (15) or AIS (7), were tested for HPV-DNA using Hybrid Capture II (HC II). All women underwent colposcopic examination, and cervical biopsy was performed for 95 patients. Fifty-one women referred due to AGC with normal colposcopy and normal second Pap smear, were scheduled for control visits every 4 months.

Results: The overall prevalence of HPV-DNA was 38%. HPV-DNA was detected in 93% of the women with HSIL associated with AGC and in 71% of women with AIS at Pap smear, being significantly higher when compared with the prevalence (29%) in women with AGC alone. Forty-five women (30.8%) had clinically significant histological lesions (CIN 2 or worse). High-risk HPV-DNA was detected in only 16% of the women without significant abnormalities in biopsy, in contrast to 96% of those who had CIN 2 or CIN 3 and 75% of women with AIS. Women with invasive carcinoma (squamous cells or adenocarcinoma) had over 75% of HPV-DNA positivity. HPV-DNA detection was significantly associated with the severity of cervical lesions (CIN 2 or worst) with an OR=51.8 (95%CI 14.3-199.9).

Conclusion: HPV-DNA detection was significantly associated with the severity of cervical lesion (CIN 2 or worst) in women referred for AGC or AIS in their Pap smear. These data implicate the use of HPV testing in triage of women with AGC Pap smears.

Key words: atypical glandular cells, adenocarcinoma *in situ*, human papillomavirus, Hybrid Capture II, CIN

Introduction

The introduction of the Pap test as a screening tool allowed early detection of cervical lesions, and endocervical brush usage has improved the detection of glandular cell abnormalities from the endocervical canal. However, only the cytological criteria for adenocarcinoma in situ (AIS) are clearly defined, and a broad spectrum of other benign and dysplastic glandular changes remains less well characterized. To better classify these abnormalities, the term AGUS or *atypical glandular cell of undetermined significance* was introduced at the 1988 Bethesda System (TBS), which was significantly revised in 2001 and finally replaced with the term *atypical glandular cells* or AGC. AGC is subclassified as not otherwise specified (NOS) or favor neoplasia. Endocervical AIS is a separate category in the new TBS, reflecting a reappraisal of the strengths and weaknesses of cytology in assessing these abnormalities[1,2].

Glandular abnormalities in cervical smears pose more clinical problems than do squamous cell abnormalities because of the poor correlation between the smear result and final histopathology [3, 4, 5]. A diagnosis of AGC is uncommon, being found in less than 0,5% in the majority of studies, and consequently the natural history of glandular abnormalities is less well understood[3, 6-12]. The clinical importance of AGC is due to the high percentage of cases associated with underlying high-grade disease[1, 3, 4, 13-14]. These clinically significant lesions comprise a wide spectrum of pathologic findings and include preinvasive and invasive squamous and glandular lesions[8]. The remainders of AGC cases usually represent benign conditions that mimic these clinically significant lesions [15].

Cervical adenocarcinoma and its precursor lesions have recently been included in the growing list of Human Papillomavirus (HPV) associated genital tumors[7,16,17-18]. Walboomers et al. [19] showed that HPV is present in 95% of the patients with invasive adenocarcinoma, when polymerase chain reaction (PCR)-based assays or serology were used. However, the finding of mild cervical glandular lesions that may appear as AGC in Pap smear, have shown significantly lower HPV detection rates than AIS or cervical adenocarcinoma, strongly supporting a non-neoplastic etiology of many such cases [7, 18].

HPV infection is the main risk factor for the development of both squamous and adenocarcinoma, and HPV test should help identifying the patients at increased risk of clinically significant cervical lesions among women followed-up for AGC cytology (with or without SIL). The objective of this study was to evaluate the association between high- risk HPV-DNA detection and histological diagnosis in women referred for AGC or AIS in their Pap smear.

Methods

Patients

A series of 146 women were included, who were referred for AGC or AIS in their Pap smear collected at cervical cancer screening, between November 2001 and October 2003. The Institutional Review Board approved the study protocol and all participants gave a written informed consent prior to their entry in the study. Women were excluded from the study if a) they had a previous history of CIN or cervical, vaginal or vulvar cancer, b) had clinical immunossuppression or c) were pregnant.

Procedures

Of these 146 women, 124 were referred for the Colposcopy Clinic due to AGC, 15 due to AGC associated with high-grade squamous intra-epithelial lesion (HSIL) and 7 due to AIS. From all women, cytological samples were taken from the endocervix using a Dacron swab. A second conventional Pap smear was collected with the brush and stored in 1.0ml of Universal Transport Medium (*Digene corporation*) for HPV-DNA testing using Hybrid Capture II (HC II) assay. All women undertook a colposcopic examination and 95 had cervical biopsies or conization. Fifty-one women who were referred due to AGC in their screening Pap smear but with normal colposcopy and second Pap smear results, were scheduled for visits every 4 months.

Cytopathology

All slides were processed in the same cytology laboratory and diagnosed by the same cytopathologist and cytologist (MCAW and SHRS) according to the 2001 TBS[1]. The cytological features used to determine the diagnosis of glandular atypia included endocervical cells crowded in clusters with anisonucleosis, nuclear enlargement and nuclear hyperchromasia[4]. Other suspect features included increased cellularity, presence of isolated atypical cells, fragments, cellular overlapping and apolar cells, higher than normal nuclear/cytoplasmic ratio, decreased cytoplasm, irregular nuclear membranes, or thick membranes, smudged chromatin, pleomorphism and atypical stripped nuclei (Figure 01 A B, D). In differential diagnosis to AIS, we also considered the presence of sheets and clusters of tightly packed glandular cells, overlap of nuclei, pseudostratified strips of columnar epithelial cells, epithelial rosettes, nuclear protrusion at

group margins, nuclear enlargement with pleomorphism and nuclear irregularity, elongation of nuclei, distinctive coarsely granular chromatin pattern and nucleoli not typically prominent, as previously described[20] (Figure 01 F, G, H). These criteria represent features for the endocervical adenocarcinoma better differentiated. With increasing grade, the nuclei increase in size, resulting in high N/C ratios. As the grade increases, nucleoli may become more prominent and multiple, but usually remain round. In high-grade cancer, the chromatin may become paler, finer, and irregularly distributed. Also, with increasing grade, the cells tend to lose their well-formed columnar shape and cell borders become indistinct granularity of the cytoplasm becomes more apparent, and vacuolization diminishes[21].

HPV-DNA testing

The specimens for HC II were tested with probe B (high-risk HPVs: types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68)[22] and the tests were classified positive at the relative light unit/cutoff (RLU/CO) ratio (RLU of specimen/mean RLU of two positive controls) of 1pg/ml or greater. These RLU/CO ratios also provide a semi-quantitative estimate of the amount of HPV-DNA in the specimens, i.e., the viral load in the sample. The storage of the specimens and all reagents as well as conduction of the tests took place at the Medical School Hospital Laboratory, following the manufacturer's instructions (*Digene Diagnostics Inc., USA*).

Histopathology

Biopsy specimens were stained with hematoxilin and eosin (HE), reviewed according to the WHO [23]criteria and classified as: 1) significant clinical lesions including CIN 1, CIN 2, CIN 3, cervical or endometrial in situ adenocarcinoma and invasive carcinoma or 2) benign lesions including cervicites, squamous metaplasia, tubal metaplasia, microglandular hyperplasia and polyps. Histological specimens were considered as the gold standard. All the histological analyses were processed in the Laboratory of Pathology and diagnosed by the same pathologist (LAA), unaware of the cytological diagnoses.

Data Analysis

All statistical analysis was done using the SAS software, version 8.0. Odds ratios (OR) with 95% confidence interval (95% CI) were used to evaluate the associations between HPV test and histological results.

Results

The overall prevalence of HPV-DNA detection was 38%. HPV-DNA was detected in 93% of the women with Pap smear showing HSIL associated with AGC and in 71% of women with AIS. This prevalence of HPV-DNA detection was significantly higher than that (29%) in women with AGC (**Table 1**).

Forty one per cent of the women referred due to AGC were not subjected to cervical biopsy or conization because their second Pap smear and colposcopy were normal. Among the 73 women with AGC who were biopsied, 24 (33%) presented clinically

significant histological lesions (CIN 2 or higher). Among women with AGC associated with HSIL, 100% presented high grade CIN or worse. One woman with AIS Pap smear presented with cervicitis and micro-glandular hyperplasia. Four cases showed well-differentiated invasive adenocarcinomas (**Table 2**).

High-risk HPV-DNA was detected in only 16% of the women without significant biopsy, in contrast with 96% of those with CIN2 or CIN3 and 75% in AIS cases. Women with invasive carcinoma, irrespective whether glandular or squamous, had more than 75% of HPV-DNA positivity. HPV-DNA detection was significantly associated with the severity of cervical lesion (CIN 2 or worse), with an OR=51.8 (95%CI 14.3 to 199.9)(**Table 3**).

Discussion

In agreement with previous studies, a large variety of diagnoses were disclosed in the subsequent examination of AGC diagnoses, ranging from HPV/CIN I to cervical carcinomas. Of major importance in this study was the observation that 20% of AGC cases were associated with underlying high-grade intraepithelial lesion (CIN3) or invasive carcinoma. This is a clear indication that an accurate recognition and classification of AGC cells in the Pap smear remains problematic. This also emphasizes that careful control of every single such smear on colposcopy and biopsy is mandatory.

Infections with oncogenic HPV cause virtually all cases of cervical cancer and precancer lesions[24, 25]. Detection of high-risk HPV-DNA was significantly

associated with morphological abnormalities in the cervical biopsies of women referred for AGC associated with HSIL and/or AIS in this study. On the other hand, the HPV-DNA and histological lesions were less frequent in women referred by pure AGC, emphasizing the challenging nature of this diagnostic category [5].

Ronnet et al. (1999)[7] detected high-risk HPV-DNA in 28% of the 137 women tested, including 92% of those with biopsy-confirmed HSIL, 100% with AIS and 56% with LSIL. We detected high-risk HPV-DNA in 38% of the 146 women, including 96% of the women with biopsy-confirmed CIN 2 or 3 and 83% with AIS or invasive cervical adenocarcinoma. We found HPV-DNA also in 13% of the women with HPV and/or CIN 1 in biopsy, i.e., a detection rate equal to that of histologically negative women.

Lack of well-defined cytomorphologic criteria and a high degree of interobserver variability in the interpretation of the AGC specimens are some of the most common contributing factors to this diagnostic variability[9, 13, 26, 27]. Unlike the squamous abnormalities, atypia in glandular cells is an infrequently encountered diagnosis. Consequently, far less experience has been acquired with the diagnostic category of AGC and AIS [4].This fact can partially explain the considerable proportion of negative Pap smears in the follow-up of AGC patients, reported in several studies, and also shown in the present one. The most common benign confounding findings include reactive/reparative changes, tubal metaplasia, microglandular hyperplasia, endometrial cell groups [21, 27, 28].

On the other hand, the majority of AGC diagnoses during the follow-up have been found to be squamous in nature. Certainly, squamous and endocervical lesions may coexist in 50-70% of the cases of AIS, but it is far more common for a squamous cell lesion to represent the ultimate diagnostic outcome in a case containing atypical endocervical cells that are not specifically diagnostic of AIS[29]. CIN 3 was the most frequent histopathological diagnosis. A number of studies have shown that it is often difficult to differentiate between AGC and squamous metaplasia in the Pap smear, or HSIL involving the transformation zone or endocervical glands [30, 31].

Squamous lesions involving endocervical glands or with glandular extension may show architectural arrangements that resemble those of endocervical cells and groups. They may also acquire some of the nuclear and cytoplasmic features that are more characteristic to the cells of glandular origin, and thus may be misclassified as AGC[27, 32]. Such characteristics include grouped round cells with ill-defined peripheral outlines as well as nuclear pseudostratification, even some other feathering, mimicking glandular abnormality[33]. In previous studies, AGC diagnosis has been associated with abnormalities in approximately 50 to 60% of the cases and this was also the case in our study.

We have found true glandular lesions in only 14 cases; two of them were diagnosed as endometrial adenocarcinoma. Seven women with AIS cytological diagnosis showed one AIS histological diagnosis and four well-differentiated adenocarcinomas, including two with extensive area of AIS. Four women had AIS

histological diagnosis, of them two with previous AGC, one with AIS and one AGC and HSIL in the Pap smear. It is frequently impossible to distinguish between in situ and invasive endocervical adenocarcinoma.[27] There is considerable morphological overlap or features indistinguishable between AIS and well-differentiated invasive endocervical adenocarcinoma; thus, inevitably a proportion of cases interpreted as AIS will demonstrate invasion on histological evaluation[1, 21].

In summary as suggested by Krane et al. (2004)[34] HPV positive AGC has a high positive predictive value for significant cervical disease. Our data suggest that a negative HPV-DNA test provides good reassurance that a patient with AGC does not have HSIL, AIS or carcinoma. One clearly justified indication for the clinical use of HPV-DNA test could be in the triage of women with AGC Pap smears.

References

1. Solomon D, Davey DD, Kurman RJ, et al: The 2001 Bethesda System – Terminology for Reporting Results of Cervical Cytology. JAMA 287:2114-2119, 2002.

2. Levine L, Lucci III JA, van Dinh T: Atypical glandular cells: New Bethesda Terminology and Management Guidelines. Obstet and Gynecol Survey 58(6): 399-406, 2003.

3. Burja IT, Thompson SK, Sawyer WI, Shurbaji MS: Atypical glandular cells of undetermined significance on cervical smears. A study with cytohistologic correlation. Acta Cytol 43:351-356, 1999.

4. Chhieng DC, Elgert PA, Cangiarella JF, Cohen JM: Clinical significance of atypical glandular cells of undetermined significance. A follow-up study from an academic medical center. Acta Cytol 44:557-566, 2000.

5. Hare AA, Duncan AR, Sharp AJ: Cytology suggestive of glandular neoplasia: outcomes and suggested management. Cytopathology 14:12-18, 2003.

6. Zweizig S, Noller K, Reale F, Collis S, Resseguie L: Neoplasia associated with atypical glandular cells of undetermined significance on cervical cytology. Gynecol Oncol 65:314-18, 1997.

7. Ronnet BM, Manos MM, Ransley JE et al.: Atypical glandular cells of undetermined significance: cytopathologic features, histopathologic results, and human papillomavirus DNA detection. Hum Pathol 30(7): 816-825, 1999.

8. Chhieng DC, Elgert PA, Cangiarella JF, Cohen JM: Variation in the incidence of. AGUS Between Different Patient Populations. Acta Cytol 45:287-299, 2000.

9. Davey DD, Woodhouse S, Styer J, Mody D: Atypical epithelial cells and specimen adequacy: current laboratory practices of participants in the college of American pathologists interlaboratory comparison program in cervicovaginal cytology. Arch Pathol Lab Med 124:203-11, 2000.

10. Geier CS, Wilson M, Creasman W: Clinical evaluation of atypical glandular cells of undetermined significance. Am J Obstet Gynecol 184(2):64-69, 2001.

11. Nasuti JF, Fleisher SR, Gupta PK: Atypical glandular cells of undetermined significance (AGUS): clinical considerations and cytohistologic correlation. Diagn Cytopathol 26:186-190, 2002.

12. Hammoud MM, Haefner HK, Michael CW, Ansbacher r: Atypical glandular cells of undetermined significance. Histologic findings and proposed management. J Reprod Med 47(4):266-170, 2002.

13. Raab SS, Snider TE, Potts SA et al.: Atypical glandular cells of undetermined significance. Diagnostic accuracy and interobserver variability using select cytologic criteria. Am J Clin Pathol 107:299-307, 1997.

14. Raab SS: Can glandular lesions be diagnosed in Pap smear cytology?. Diagn Cytopathol 23(2):127-133, 2000.

15. Diaz-Rosario LA, Kabawat SE: cell block preparation by inverted filter sedimentation is useful in the differential diagnosis of atypical glandular cells of undetermined significance in Thinprep specimens. Cancer 90(5):265-272, 2000.

16. Anciaux D, Lawrence WD, Gregoire, L. Glandular lesions of the uterine cervix: prognostic implications of human papillomavirus status. Int J Gynecol Pathol 16:103-110, 1997.

17. Hördin U, Daugaard S, Visfeldt J: Adenocarcinomas of the cervix and adenocarcinoma of the endometrium: distinction with PCR-mediated detection of HPV-DNA. APMIS 105:313-316, 1997.

18. Andersson S, Rylander E, Larsson B. et al.: The role of human papillomavirus in cervical adenocarcinoma carcinogenesis. Eur J Cancer 37:246-250, 2001.

19. Walboomers JM, Jacobs MV, Manos MM, et al.: Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189:12-19, 1999.

20. National Cancer Institute Workshop –Bethesda System 2001 available in site "http.www.bethesda2001.cancer.gov".

21. DeMay RM: The Pap Smear. In The Art and the Science of Cytopathology.: Chicago: ASCP press, 1993; p 61-185.

22. Lorincz AT: Screening for cervical cancer: new alternatives and research. Salud Publica Mex 45(Suppl 3):S376-387, 2003

23. Scully RE, Bonfiglio TA, Kurman RJ, Silverberg SG, Wilkins EJ: Histological typing of female genital tract tumors. – Wordl Health Organization – International histological classification of tumors, 2th ed., - 1994 Springer-Verlag, Berlin.

24. Munoz N, Bosch FX, de Sanjose S, et al.: Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 348(6):518-527, 2003

25. Schiffman M, Castle PE: Human papillomavirus: epidemiology and public health. Arch Pathol Lab Med 127(8):930-934, 2003

26. Soofer SB, Sidawy MK: Atypical glandular cells of undetermined significance: clinically significant lesions and means of patient follow-up. Cancer 90:207-214, 2000.

27. Syrjänen, K. HPV and cervical adenocarcinoma. Chapter 8. In: Syrjänen, K. and Syrjänen, S. Papillomavirus Infections in Human Pathology. J. Wiley & Sons, New York, 2000: pp. 189-206.

28. Kurman RJ, Solomon D: The Bethesda System for reporting cervical/vaginal diagnoses. Bethesda, Springer-Verlag, New York, Inc., 1994. 81p.

29. Moritani S, Ioffe, OB, Sagae S, Dahmoush L, Silverberg SG, Hattori T: Mitotic activity and apoptosis in endocervical glandular lesions. Int J Gynecol Pathol 21(2): 125-133, 2002

30. Lee KR, Manna EA, St Jonh T: Atypical endocervical glandular cells: accuracy of cytologic diagnosis. Diagn Cytopathol 13:202-208, 1995.

31. Raab SS, Bishop NS, Zaleski MS: Effect of cervical disease history on outcomes of women who have a pap diagnosis of atypical glandular cells of undetermined significance. Gynecol Oncol 74:460-464, 1999.

32. Bonfiglio TA, Erozan YS. Gynecologic Cytopathology: Philadelphia: Lippincott-Raven Publishers, 1997, p 107-169.

33. Mattosinho de Castro Ferraz M da G, Focchi J,.Stavale JN, Niclolau SM, Rodrigues de Lima G, Baracati EC: Atypical glandular cells of undermined significance. Cytologic predictive value for glandular involvement in high grade squamous intraepithelial lesions. Acta Cytol 47(2):154-158, 2003

34. Krane JF, Lee KR, Sun D, Yuan L, Crum CP: Atypical glandular cells of undetermined significance. Outcomes predictions based on human papillomavirus testing. Am J Clin Pathol 121(1):87-92, 2004.

Table 1: HPV-DNA Detection Related to Cytological Diagnosis

Cytological Diagnosis	HPV-DNA		OR (95% IC)
	Negative	Positive	
Pure AGC (n=124)	88 (71%)	36 (29%)	Ref
AGC and HSIL (n=15)	1 (7%)	14 (93%)	34.2 (4.4 to 723.1)
AIS (n=7)	2 (21%)	5 (71%)	6.1 (1.0 to 47.9)
TOTAL (n=146)	91 (62%)	55 (38%)	

Table 2: Cervical Pathology Related to Cytological Diagnosis

Histological results	Pure AGC		AGC + HSIL		AIS	
	N	(%)	n	(%)	n	(%)
Without neoplasia (no biopsy)	51	(40)	-	-	-	-
Cervicites/endometrial polyp	34	(27)	-	-	1	(14)
HPV/CIN 1	15	(12)	-	-	-	-
CIN 2 or 3	14	(11)	12	(79)	1	(14)
AIS	2	(2)	0	-	1	(14)
Squamous invasive carcinoma*, **	3	(3)	2	(14)	-	-
Cervical adenocarcinoma	3	(3)	1	(7)	4	(58)
Endometrial adenocarcinoma	2	(2)	0	-	-	-
TOTAL	**124**	**(100)**	**15**	**(100)**	**7**	**(100)**

AGC:Atypical glandular cells, HSIL: high grade squamous intraepithelial lesion
AIS:adenocarcinoma in situ
*One case with AIS component
** Four cases were microinvasive squamous carcinoma

Table 3: HPV-DNA Detection Related to Cervical Histology

Histological result	HPV-DNA		Total
	Negative n (%)	Positive n (%)	
Without histology	46 (90%)	5 (10%)	**51**
Cervicites/polips	26 (74%)	9 (26%)	**35**
HPV/CIN 1	13 (87%)	2 (13%)	**15**
CIN 2 or 3	1 (4%)	26 (96%)	**27**
Adenocarcinoma *in situ*	1 (25%)	2(75%)	**3**
Invasive cervical squamous cells carcinoma	1 (20%)	4 (80%)	**5**
Invasive cervical adenocarcinoma	1 (12%)	7 (88%)	**8**
Adenocarcinoma endometrial	2 (100%)	0 (0%)	**2**

OR=51.8 (95%CI 14.3 to 199.9)(HPV-DNA detection in women with CIN 2 or worse compared with those without histology, cervicites, polyps and CIN 1, excluding endometrial carcinoma)

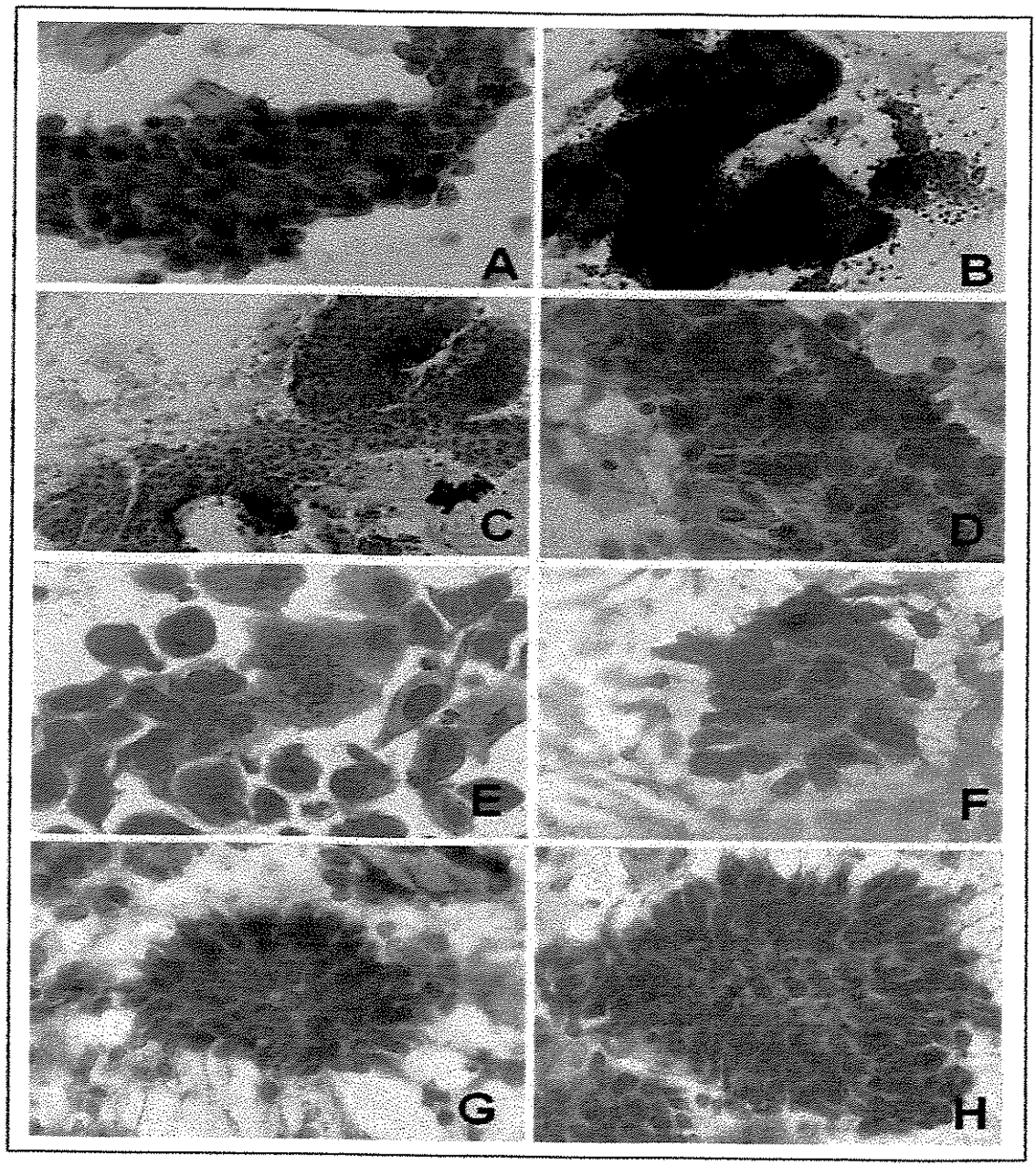


Figure 1:

A - B – Case categorized as AGC; the histological results showed adenomyosis
C – D - Case categorized as AGC associated with HSIL, cone biopsy specimen showed CIN 3 involving endocervical glands
E - F - Case categorized as AGC with HSIL, cone biopsy specimen showed microinvaisve squamous cells, grade 1A1 in association with adenocarcinoma in situ
G - H- Case categorized as AIS, the histological results showed well differentiated invasive adenocarcinoma

3.3. ARTIGO 3

PREDICTION OF HIGH-GRADE CERVICAL DISEASE WITH HPV DETECTION IN WOMEN WITH GLANDULAR AND SQUAMOUS CYTOLOGICAL ABNORMALITIES

Authors:
Eliane RZM de Oliveira
Sophie FM Derchain
Luis Otávio Z Sarian
Silvia Helena Rabelo-Santos
Renata C Gontijo
Adriana Yoshida
Liliana A L A Andrade
Luiz Carlos Zeferino

Universidade Estadual de Campinas (UNICAMP), Obstetrics and Gynecology Department, Campinas, São Paulo, Brazil.

Clinical study

This study has been funded by Fundação de Amparo a Pesquisa do Estado de São Paulo and Conselho Nacional de Desenvolvimento Científico e Tecnológico (FAPESP). (Grants # 00/697-7 and 04/09309-5 and CNPq 307252/2004-3)

Corresponding author: Sophie F. M. Derchain, MD, PhD
Rua Antônio Hossri, 629 – Cidade Universitária
13083-370 – Campinas – SP.
Fone: +55-19-37889305 / Fax: + 55-19-3289 5935
E-mail: derchain@supernet.com.br

Running headline: HPV detction and high-grade cervical disease

Abstract

Objective: To assess whether HPV detection with Hybrid Capture II (HC II) can help to predict the presence and the nature - glandular or squamous – of histological cervical lesions in women referred due to atypical glandular cells (AGC) or high-grade squamous intra-epithelial lesion (HSIL). **Method:** 247 women were included. Referral Pap smears comprised: AGC (51 cases); AGC plus HSIL (28 cases); *in situ* adenocarcinoma (AIS) (10 cases) and HSIL (158 cases). All patients were tested for high-risk HPV with HC II and had a histological assessment of their cervix. **Results**: Histological analysis disclosed 38 (15.3%) cervicitis, 194 (75.5%) squamous and 15 (9.2%) glandular neoplasia. The overall rate of high-risk HPV detection was 77%. Almost 70% of AGC-HPV negative patients did not have a pathologically proven cervical neoplasia, whereas 76% of women with AGC-HPV positive were diagnosed with a squamous or glandular neoplasia. Most (95%) of the lesions in patients with AGC-HSIL were of squamous nature, and HPV detection did not contribute to their differentiation from glandular lesions. **Conclusion:** In women with AGC, HPV positivity strongly correlated with the presence of glandular or squamous cervical lesion, but did not help distinguishing women with squamous from those with glandular neoplasia.

Key words: AGC, HSIL, Pap smear, Human papillomavirus, Hybrid Capture II, cervical intraepithelial neoplasia, cervical adenocarcinoma

Introduction

Squamous cell carcinoma and adenocarcinoma are the two most frequent malignancies of the uterine cervix. The former still accounts for almost 85% of the cases, but widespread use of cervical screening allowed substantial control of the disease over the past five decades. Conversely, glandular cancers have been increasing in relative and absolute incidence as a result of better endocervical sampling, through endocervical brushes [1-3]. Both squamous and glandular neoplasias are curable in their early phases, especially if detected in their pre-invasive forms, respectively cervical intraepithelial neoplasia (CIN) and adenocarcinoma *in situ* (AIS).

Conservative treatment options are available for CIN and AIS. Loop electrosurgical excision procedure (LEEP), also known as diathermic conization of the cervix, has been established as the mainstay treatment for CIN, allowing complete removal of the disease in up to 90% of the cases [4, 5]. LEEP can be safely and inexpensively performed with local anesthesia and in an ambulatory setting. Unfortunately, because AIS is usually topographically located up in the cervical canal [6], LEEP is not suited for its treatment. Failure (i.e. specimens with involved margins) is reported to occur in 75% of the cases [7]. For AIS, the conservative treatment option is cold-knife conization, with several disadvantages when compared to LEEP: requires hospitalization and spinal block, or general anesthesia; recovery time is prolonged; cervical scarring is almost universal.

Most diagnoses of squamous and glandular cervical neoplasias are obtained with cervical cytology (Pap smears). While squamous abnormalities in Pap smear

strongly correlate with histopathological squamous histological findings, glandular abnormalities pose significant clinical problems because of their poor correlation with final histopathology [8-10]. Atypical Glandular Cells (AGC), an uncommon cytological diagnosis defined in the last revision of the Bethesda System (2001), has its clinical significance still in debate. A high percentage of AGC cases is representative of underlying high-grade disease, but these clinically significant lesions comprise not only neoplasia of glandular nature. Pre-invasive and invasive squamous lesions in patients harboring AGC are far from uncommon [8, 9, 11-14]. By the other hand, many glandular lesions are detected in women diagnosed with LSIL or HSIL in Pap smears. Given the desproportionate prevalence of squamous cytologies over glandular, the number of *in situ* and invasive adenocarcimas detected because of a cytological finding rendered as "squamous" in not negligible.

The fact that a "glandular" diagnosis in Pap smear may be ultimately representative of a squamous lesion poses the physician a dilemma. Patients subjected to cold-knife conization, or even hysterectomy, that had been found to harbor CIN in their histological specimens, most likely had been over treated. Conversely, performing LEEP in patients with glandular cytological abnormalities poses the risk of having to perform another surgical treatment if AIS with involved margins is found. Therefore, a technique that could help differentiating patients with squamous lesions among those with a Pap smear showing glandular abnormalities will certainly have a significant clinical application.

HPV detection, through a variety of techniques, has been studied to a great extent as a possible substitute of Pap smear, or an adjuvant test, and in the screening of CIN. Although HPV is consistently detected in more than 90% of squamous cells carcinomas, the reported prevalence of HPV in adenocarcinoma varies widely depending on the population studied and detection method used [15-18]. Similar HPV types are found in both AIS and invasive adenocarcinoma [19-21], but the association between HPV detection and viral load with the development of glandular lesions has been studied with commercially available Hybrid Capture 2 (HC II) only to a very limited extent [22].

Management of women referred with AGC in their screening Pap smears has been addressed by this same study group in previous reports [23,24]. HPV detection was strongly associated with high-grade morphological changes (squamous and/or glandular) at follow-up citology, collected at the colposcopy visit [23]. Furthermore, analyzing the association between HC II results and histological diagnosis in women with AGC or AIS, a negative HPV test has been observed to provide good reassurance that women do not have CIN, AIS or cancer [24]. In these previous reports, only women with glandular cytological abnormalities have been included. However, many glandular histological lesions are detected while investigating women referred to colposcopy because of a high-grade squamous abnormality in Pap smear. Therefore, the investigators decided to extend the analysis to women referred not only due to glandular cytological changes, but also squamous abnormalities. The present study has been carried out to assess

whether HPV detection with HC II, combined with Pap smear, can help predict the presence and the nature - glandular or squamous – of histological cervical lesions.

Subjects and methods

Patient selection

Campinas State University has a Teaching Hospital dedicated to care for women. It is a referral center for almost four hundred basic health units (BHU), covering a region with approximately one million women. BHU refer patients with abnormal Pap smears to the University colposcopy clinics, when specialized investigation or treatment is deemed necessary. The sample of the present study was made up of 247 women, referred due to Pap smear abnormalities of glandular or squamous nature. The investigators included only patients that, after cervical examination made by well-trained colposcopists, had been elected to undertake punch biopsies, diathermic or cold-knife conization, and therefore had a histological evaluation of their uterine cervix. Referral Pap smears comprised: AGC (51 cases); AGC associated to HSIL 28 cases); cyto-morphologic changes associated AIS (10 cases) and pure HSIL (158 cases). The local Ethics Committee has approved the study protocol. All enrolled women gave their agreement to participate by signing the Informed Consent Forms.

All women were tested for HPV with HC II, with samples collected before performing biopsy or conization. Enrollment has been carried out between March 2002 and March 2004.

Hybrid Capture II

The specimens for HC II were tested with probe B (high-risk HPVs: types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) [25] and the tests have been classified at the relative light unit/positive control (RLU/PC) ratio (RLU of specimen/mean RLU of two positive controls) of 1pg/ml or greater. These RLU/PC ratios provided an estimate of the amount of HPV-DNA in the specimens, i.e. the viral load in the sample. The storage of the specimens and all reagents as well as conduction of the tests took place at the Campinas State University Medical School Hospital Laboratory, following the manufacturer's instructions (*Digene Diagnostics Inc., USA*).

Histology

Clinical judgment guided the colposcopists' decision to sample the cervix. When possible, biopsy and treatment of the cervical lesions was accomplished with the same procedure, i.e. diathermic or cold-knife conization. When invasive carcinoma was suspected, only punch biopsies have been performed. Insufficient colposcopy, or a cervix too shallow to safely perform a diathermic or cold-knife conization, coupled with high-grade referral Pap smear prompted the researchers to perform hysterectomies in two cases. Histological samples consisted of 230 diathermic conization specimens, 11 cold-knife, four punch biopsies and two hysterectomy specimens. Biopsy, conization and hysterectomy specimens were fixed in 10% phosphate buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin (HE). Biopsies were analyzed according to the World Health

Organization criteria's [26] and classified as normal/cervicitis, CIN1, CIN2, CIN3, invasive squamous cell carcinoma, AIS, and invasive adenocarcinoma. For statistical purposes, CIN2 and CIN3 were grouped under the CIN2/3 label.

Statistical analysis

Data were stored in OpenOffice Calc® spreadsheet files. Shapiro-Wilk's test for normality has been used to rule out normality in the viral load distribution, achieving statistical significance. Therefore, overall differences in the viral load distribution, according to histological diagnosis, have been assessed trough the Kruskall-Wallis test. In order to identify specific viral load differences in selected pairs of histological groups, two-by-two analysis has been carried out with the Wilcoxon rank sum test. All statistical calculations have been performed with the *R Environment* [27] statistical software package, set to 95% confidence intervals (95%CI).

Results

Patients age ranged 19 to 61 years (mean 33.6 years). Histological analysis of cervical specimens disclosed 38 (15%) women with cervicitis, 194 (76%) with squamous lesions and 15 (9%) with glandular neoplasia. Of women with cervicitis, 26 (68%) had been referred due to AGC and 7 (18%) due to HSIL Pap smears. Most patients with CIN1 (21/30) had been referred due to HSIL, as well as most of those with confirmed CIN2/3 (120/153) (Figure 1). However, 20 patients with histological CIN had only glandular abnormalities detected in their Pap smears, the same occurring in two out of the 11 cases of invasive squamous cell carcinoma. Fifteen women have been detected with lesions of glandular nature, 5 AIS and 10

invasive adenocarcinomas. Two of these women had been referred as having only HSIL in their Pap smears (Table 1).

The overall rate of high risk HPV detection was 77%. Women referred due to AGC were more likely to harbor cervicitis if their HPV test was negative (p=0.001). Almost 70% of AGC HPV negative patients did not have a pathologically proven squamous or glandular lesion, whereas 76% of women with AGC HPV positive have been diagnosed with some cervical lesion. HPV testing did not help differentiating squamous from glandular lesions in women with AGC (p=1). In women with AGC+HSIL referral Pap smears, again the proportion of women without a significant histological lesion was higher when HPV test was negative (p=0.001). All women with AGC+HSIL HPV positive harbored a significant histological lesion, whereas 75% of those HPV negative presented only with cervicitis. Most (95%) of the lesions in patients with AGC+HSIL were of squamous nature, and HPV contribution to its differentiation from glandular was none (p=1). A small fraction (10 out of 247) of the patients was referred due to pure AIS in their Pap smears. HPV testing has been unable to help discriminating women with or without neoplasia (p=0.37) and glandular from squamous lesions (p=1). Women with pure HSIL composed a large part of this study's sample (63%). In this group, proportion of women with significant lesion was higher among those with positive HPV test (p<0.001), being that only one (0.7%) women HPV positive had cervicitis, whereas 29% of those HPV negative were found to have no significant lesion (in spite of their HSIL Pap smears). Only two women with pure HSIL were found to have a glandular lesion, and HPV testing was of no aid in distinguishing them from

their squamous counterparts (p=0.83). Considering Pap results altogether, HPV detection strongly correlated with presence of a significant lesion (p<0.001), but was of no use in distinguishing women with squamous from those with glandular lesions (p=0.83) (Table 2).

Mean HPV viral loads strongly differed between patient with cervicitis and those with any type of squamous (p<0.001) or glandular (p<0.001) lesions. There were no mean viral load differences in the groups of patients with glandular and squamous lesions, regardless of the invasiveness (p=0.94). Mean viral loads did not significantly vary while comparing the groups of patients diagnosed with pure invasive adenocarcinoma and invasive squamous cell carcinoma (p=0.17). HPV-DNA amounts were not significantly different in the groups of patients with CIN2/3 and AIS (p=0.11). By the other hand, while comparing the mean viral load in patients diagnosed with CIN with that encountered among patients with invasive squamous cell carcinoma, the later have been detected with significantly higher viral copy numbers (p=0.03). These difference did not persist when only patients with high-grade CIN are considered for comparison with their counterparts diagnosed with squamous cancer (p=0.05). No viral load differences have been detected between women with *in situ* or invasive adenocarcinoma (p=0.22) (Table 3).

Discussion

Adenocarcinoma of the cervix lacks a firm correlation with glandular cytological abnormalities, because many of these lesions are detected in women with HSIL. Conversely, making a decision on how to manage women with glandular findings in Pap smear is cumbersome and may be a source of anxiety for patients and their physicians. Rare and ill defined, the AGC and, to some extent, AIS Bethesda´s cytological diagnoses do not have a clear relation with an underlying histological entity. Importantly, with the increasing use of endocervical sampling devices, the presence of glandular abnormal findings is expected to continuously rise in the years to come. Additionally, false posive findings in cytology, either glandular or squamous, are not uncommon. Even women presenting with HSIL in their screening cytologies may actually be devoid of any histological lesion, as several factors can affect the Pap smear result, ranging from clinical situations that modify the morphology of the cells (e.g. cervitis, tubal hiperplasia, atrophia) and the subjective component inherent to human interpretation of images. The present study has been an attempt to address these clinical problems, by evaluating a possible adjunct tool, i.e. HPV detection with commercially available HC II, for the differentiation of patients that present with a high likelihood to harbor a clinical significant lesion. Also, because CIN and histological AIS should be treated with different techniques, the investigators have tried to assess whether HPV detection could possibly help distinguishing patients with squamous from those with glandular lesions.

HPV detection, in the present study meaning HC II with >1.0RLU/PC, strongly correlated with the presence of histologically confirmed significant lesions. It is unfortunate that the prediction of the nature of these lesions, glandular or squamous, has not been aided by HC II. A very high prevalence of high risk HPV has been detected by HC II in *in situ* and invasive adenocarcinomas of the cervix, similarly to that reported for cervical squamous cell carcinoma. This conspicuous viral detection, regardless of glandular or squamous origin of the lesion, prevented any possible differentiation based upon HPV positivity.

HPV detection with polymerase chain reaction (PCR) in histollogicaly proven glandular cervical lesions has been the subject of several well-conducted studies. A decade ago, Yamakawa et al. [28] assessed the HPV infection in 64 patients with adenocarcinoma or adenosquamous carcinoma of the uterine cervix using PCR with primers specific for the 6, 11, 16, 18, 31, 33, and 35 types. HPV-DNA was detected in 56% of the adenocarcinomas, and in 91% of adenosquamous carcinoma. The majority of adenosquamous and adenocarcinomas contained HPV 16 or 18. Their results suggested that human papillomaviruses, particularly HPV 16 and 18, play a role in the etiology of cervical adenocarcinoma and adenosquamous carcinoma. Lee et al. [15] examined by PCR 69 tissue blocks of cervical adenocarcinoma and found 31,9% HPV-DNA positive results. Skyldberg et al. [16] found a 60.5% overall prevalence of high-risk types HPV-DNA in 38 women with invasive adenocarcinoma, being types 16 and 18 the most frequent types. HPV-16 had a prevalence of 23.7% (9 of 38), and HPV-18 had a prevalence of 26.3% (10 of 38). Another study using PCR, found 91.1% HPV-DNA positivity in 73 cases of

invasive adenocarcinoma and 23 cases of AIS [17]. And finally, Andersson et al. [21] studied 131 adenocarcinomas and identified 71% of HPV positivity. HPV 16 has been shown to be the most prevalent genotype, irrespective of geographical location, in women with normal, premalignant or cancerous lesions. Nevertheless, considering only women who were positive for HPV 18, the majority of diagnoses of neoplasia consisted of adenocarcinoma [29-31].

The present study has been deliberately designed to assess HPV detection through a commercially available technique. As previously stressed, type-specific detection has been studied extensively, but PCR is not widely available outside research settings. The present study aimed at providing information that could be easily implemented in common practice, by ordinary physicians. HC II probes are designed to detect the most prevailing and clinically relevant viral types, covering the HPV types that had been evaluated in squamous and glandular lesions in the studies mentioned before. Because the previous studies have reported a reduced prevalence of HPV infection in glandular lesions when compared to the almost universal presence of the virus in women harboring squamous neoplasia, the authors of the present study expected a better and distinctive correlation between HPV detection and histological type of the cervical lesions.

AIS and invasive adenocarcinoma of the cervix are usually considered difficult to visualize colposcopically. In fact, the topography of such lesions has been proved to be generally up in the cervical canal [6]. This feature of the disease poses another clinical problem, especially when the physician is confronted with an AGC Pap smear and an insufficient colposcopy. Many such women may have no

cervical neoplasia, as previously reported [8-10] and corroborated in this sample. In the present study, 26 (51%) out of 51 women with AGC were proven to have no clinically significant histological lesion. In this case, HPV detection has been of some importance in pointing-out women at elevated risk to harbor a significant lesion. It is highly sensible to mention, at this point, that a negative HC II result does not preclude a pelvic examination with colposcopy, in women with referral AGC. This discriminating capability has also been confirmed among women with AGC+HSIL and HSIL Pap smears, but not with the same strength: although statistically different, the proportion of women HPV negative and devoid of disease was remarkably lower in the groups with HSIL or AGC+HSIL when compared to that among women with only AGC.

One plausible flaw of the present study, that might have obscured the discriminating power of HPV testing, is the relative small number (15 out of 247) of histologically confirmed glandular lesions. However, more important than the actual number of pathologically confirmed glandular lesions, is the large number of patients with Pap smear containing glandular abnormalities, which has permitted a consistent analysis on the correlation of such findings with actual histological lesions. The association of AGC and AGC+HSIL with CIN was remarkable, although not unexpected [9]. AIS in cytology showed the best correlation with histological glandular lesions of in situ or invasive adenocarcinomas (70%). Of course, these results have an important clinical connotation, especially when choosing the treatment option.

In an ultimate attempt to evaluate a possible role for HC II in the workup for patients with glandular Pap smear abnormalities, the authors have calculated the mean viral load differences between histological groups. HPV detection *per se* had anticipated that the means of viral load would differ when comparing patients harboring only cervicitis with those with glandular or squamous neoplasia. Although little is known about the events involved in the origin of glandular carcinomas, it is assumed that the same mechanism of HPV-related carcinogenesis occurs in cervical glandular epithelium, and the presence of high viral load might play role in the arousal of the disease and its progression. Pirog et al. [17] claimed that the relative difficulty in detecting HPV-DNA in adenocarcinomas, in contrast to squamous cells carcinomas, could be attributed to a lower viral load found in glandular lesions compared to squamous lesions. Glandular epithelium does not support productive viral infection and HPV-DNA in endocervical neoplasias is usually present in the integrated form. This previous findings have led the present investigators to believe that the viral load measurements, provided by HC II, could furnish some clues on whether the cervical lesion was of glandular or squamous nature. Unfortunately again, the patients included in this sample had no detectable differences in the amounts of HPV-DNA according to the nature of the lesions they had. It is important to mention that in the majority of the studies that reported lower HPV detection rates and viral loads in glandular lesions, HPV infection was investigated using methods based on signal amplification, as PCR, accepted as more sensitive and specific for HPV detection and typing than HC II.

Further clarification on the role of glandular Pap smear abnormalities is needed. Currently, several investigators are consistently pursuing an improvement in cytological criteria for glandular abnormalities. Time will tell whether these efforts can be fruitful. Nevertheless, until then, research on the role of HPV testing in differentiating patients with glandular lesions from those with squamous neoplasia should certainly deserve attention. This study has reported that HPV testing can be useful in patients with AGC, because women with a positive HPV test were at a remarkably greater risk of having a significant cervical lesion. For instance, a plausible recommendation is to perform colposcopy in all patients with AGC and follow those with normal pelvic/colposcopy examination and negative HPV test clinically, until their HPV tests turn to positive or the cytological abnormalities subside. Based on the current data, it has not been possible to ascertain a role for HPV testing, by HC II, in determining which patients were more likely to have a glandular lesion, and therefore deserve cold-knife conization as primary treatment.

References

1. Schwartz S, Weiss N. Increased incidence of adenocarcinoma of the cervix in young women in the United States. Am J Epidemiol 1986; 124:1045-7.

2. Sherman ME, Wang SS, Carreon J, Devesa SS. Mortality trends for cervical squamous and adenocarcinoma in the United States. Relation to incidence and survival. Cancer 2005 15; 103(6):1258-64.

3. Zaino RJ. Glandular lesions of the uterine cervix. Mod Pathol. 2000; 13(3):261-74.

4. Prendiville W, Cullimore J, Norman S. Large loop excision of the transformation zone (LLETZ): a new method of management for women with cervical intraepithelial neoplasia. Br J Obstet Gynecol 1989; 96:1054-60.

5. Paraskevaidis E, Koliopoulos G, Malamou-Mitsi V et al. Large loop excision of the transformation zone for treating cervical intraepithelial neoplasia: a 12-year experience. Anticancer Res. 2001; 21:3097-9.

6. Bertrand M, Lickrish GM, Colgan TJ. The anatomic distribution of cervical carcinoma *in situ*: implications for treatment. Am J Obstet Gynecol 1987; 157: 21-5.

7. Soutter WP, Haidopoulos D, Gornall RJ, McIndoe GA, Fox J, Mason WP, Flanagan A, Nicholas N, Barker F, Abrahams J, Lampert I, Sarhanis P. Is conservative treatment for adenocarcinoma in situ of the cervix safe? BJOG. 2001;108(11):1184-9.

8. Sharpless KE, Schnatz PF, Mandavilli S, Greene JF, Sorosky JI.Lack of adherence to practice guidelines for women with atypical glandular cells on cervical cytology. Obstet Gynecol. 2005;105(3):501-6.

9. Chhieng DC, Elgert PA, Cangiarella JF, Cohen JM. Clinical significance of atypical glandular cells of undetermined significance. Follow-up study from an academic medical center. Acta Cytol 2000; 44:557-66.

10. Hare AA, Duncan AR, Sharp AJ. Cytology suggestive of glandular neoplasia: outcomes and suggested management. Cytopathology 2003;14:12-8.

11. Barreth D, Faught W, Schepansky A, Johnson G. The relationship between atypical glandular cells of undetermined significance on Pap smear and a clinically significant histologic diagnosis. J Obstet Gynaecol Can. 2004 Oct;26(10):867-70

12. Chhieng DC, Elgert PA, Cangiarella JF, Cohen JM. Variation in the incidence of AGUS between different patient populations. Acta Cytol 2000; 45:287-99.

13. Raab SS. Can glandular lesions be diagnosed in Pap smear cytology? Diagn Cytopathol 2000; 23(2):127-33.

14. Solomon D, Davey DD, Kurman RJ et al. The 2001 Bethesda system – Terminology for reporting results of cervical cytology. JAMA 2002; 287:2114-9.

15. Lee MF, Chang MC, Wu CH. Detection of human papillomavirus types in cervical adenocarcinoma by the polymerase chain reaction. Int J Gynaecol Obstet. 1998 Dec; 63(3):265-70.

16. Skyldberg BM, Murray E, Lambkin H, Kelehan P, Auer GU. Adenocarcinoma of the uterine cervix in Ireland and Sweden: human papillomavirus infection and biologic alterations. Mod Pathol. 1999 Jul;12(7):675-82.

17. Pirog EC, Kleter B, Olgac S et al. Prevalence of human papillomavirus DNA in different histological subtypes of cervical adenocarcinoma. Am J Pathol. 2000 Oct;157(4):1055-62.

18. Bosch FX, Muñoz N, Chichareon S et al. HPV and cervical adenocarcinoma: an IARC based multicentric case-control study. 18th International Papillomavirus Conference- Program and abstracts book. Barcelona 2000. Available on line: http://www.hpv2000.com

19. Farnsworth A, Laverty C, Stoler MH. Human papillomavirus messenger RNA expression in adenocarcinoma in situ of the uterine cervix. Int J Gynecol Pathol. 1989;8(4):321-30.

20. Okagaki T, Tase T, Twiggs LB, Carson LF. Histogenesis of cervical adenocarcinoma with reference to human papillomavirus-18 as a carcinogen. J Reprod Med. 1989 Sep;34(9):639-44.

21. Andersson S, Rylander E, Larsson B, Strand A, Silfversvard C, Wilander E. The role of human papillomavirus in cervical adenocarcinoma carcinogenesis. Eur J Cancer 2001;37:246-50.

22. Ronnett BM, Manos MM, Ransley JE et al. Atypical glandular cells of undetermined significance (AGUS): cytopathologic features, histopathologic results, and human papillomavirus DNA detection. Hum Pathol. 1999; 30(7):816-25.

23. Oliveira ER, Derchain SF, Rabelo-Santos SH, Westin MC, Zeferino LC, Campos EA, Syrjanen KJ. Detection of high-risk human papillomavirus (HPV) DNA by Hybrid Capture II in women referred due to atypical glandular cells in the primary screening. Diagn Cytopathol. 2004 Jul;31(1):19-22.

24. Derchain SF, Rabelo-Santos SH, Sarian LO, Zeferino LC, de Oliveira Zambeli ER, do Amaral Westin MC, de Angelo Andrade LA, Syrjanen KJ. Human papillomavirus DNA detection and histological findings in women referred for atypical glandular cells or adenocarcinoma in situ in their Pap smears. Gynecol Oncol. 2004; 95(3):618-23.

25. Nindl I, Lorincz A, Mielzynska I et al. Human papillomavirus detection in cervical intraepithelial neoplasia by the second-generation hybrid capture microplate test, comparing two different cervical specimen collection methods. Clin Diagn Virol. 1998 May 1; 10(1):49-56.

26. Scully RE, Bonfiglio TA, Kurman RJ, Silverberg SG, Wilkins EJ. Histological typing of female genital tract tumors. World Health Organization - International histological classification of tumors. 2nd ed. Berlin: Springler-Verlag; 1994.

27. R Development Core Team (2004). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-00-3, URL http://www.R-project.org.

28. Yamakawa Y, Forslund O, Teshima H, Hasumi K, Kitagawa T, Hansson BG. Human papillomavirus DNA in adenocarcinoma and adenosquamous carcinoma of the uterine cervix detected by polymerase chain reaction (PCR). Gynecol Oncol. 1994 May;53(2):190-5.

29. Xi LF, Toure P, Critchlow CW, Hawes SE, Dembele B, Sow PS, Kiviat NB. Prevalence of specific types of human papillomavirus and cervical squamous intraepithelial lesions in consecutive, previously unscreened, West-African women over 35 years of age. Int J Cancer 2003; 1;103(6):803-9.

30. Brestovac B, Harnett GB, Smith DW, Shellam GR, Frost FA. Human papillomavirus genotypes and their association with cervical neoplasia in a cohort of Western Australian women. J Med Virol 2005; 76(1):106-10.

31. Chaturvedi AK, Dumestre J, Gaffga AM, Mire KM, Clark RA, Braly PS, Dunlap K, Beckel TE, Hammons AF, Kissinger PJ, Hagensee ME. Prevalence of human papillomavirus genotypes in women from three clinical settings. J Med Virol 2005; 75(1):105-13.

Table 1 – Histological findings and referral cytology

Histological diagnosis	AGC n (%)	Cytology AGC + HSIL n (%)	AIS n (%)	HSIL n(%)
Normal/Cervicitis	26 (51)	3* (11)	2** (20)	7*** (5)
Squamous	21 (41)	23 (82)	1 (10)	149 (94)
CIN1	*8 (15)*	*1 (4)*	*0*	*21 (13)*
CIN2/3	*11 (22)*	*21 (75)*	*1 (10)*	*120 (76)*
Squamous cell carcinoma	*2 (4)*	*1 (4)*	*0*	*8 (5)*
Glandular	4 (8)	2 (7)	7 (70)	2 (1)
In situ adenocarcinoma	*2 (4)*	*0*	*2 (20)*	*1 (.5)*
Invasive adenocarcinoma	*2 (4)*	*2 (8)*	*5 (50)*	*1 (.5)*
TOTAL	**51 (100)**	**28 (100)**	**10 (100)**	**158 (100)**

CIN=cervical intraepithelial neoplasia
AGC=atypical glandular cells
AGC + HSIL= atypical glandular cells + high-grade squamous intraepithelial lesion
AIS=adenocarcinoma *in situ*
HSIL= high-grade squamous intraepithelial lesion
*squamous metaplasia (2), microglandular hyperplasia (1)
** erosive chronic cervicitis (1), microglandular hyperplasia (1)
*** immature squamous metaplasia (5), microglandular hyperplasia (2)

Table 2 - Referral cytology and HPV detection in differentiating squamous from glandular disease.

Cytology	HPV	Histology				
		Normal cervicitis n(%)	Squamous n(%)	Glandular n(%)	p 1	p 2
AGC	Negative	21 (81)	8 (38)	1 (25)	0.001	1
AGC	Positive	5 (19)	13 (62)	3 (75)		
AGC+HSIL	Negative	3 (100)	1 (4)	0 (0)	0.001	1
AGC+HSIL	Positive	0 (0)	22 (96)	1 (100)		
AIS	Negative	1 (50)	0 (0)	1 (14)	0.37	1
AIS	Positive	1 (50)	1 (100)	6 (86)		
HSIL	Negative	6 (86)	15 (10)	0 (0)	<0.001	0.64
HSIL	Positive	1 (14)	134 (90)	2 (100)		
All	Negative	31 (82)	24 (12)	2 (14)	<0.001	0.83
All	Positive	7 (18)	170 (88)	12 (86)		

AGC=atypical glandular cells
AGC + HSIL= atypical glandular cells + high-grade squamous intraepithelial lesion
AIS=adenocarcinoma *in situ*
HSIL= high-grade squamous intraepithelial lesion
p 1 (Chi-square or Fisher) = normal/cervicitis X squamous +glandular
p 2 = squamous X glandular

Table 3 - Mean HPV viral load differences by histological pairs

Histology	Significance (p*)
Cervicitis X squamous lesion (invasive and CIN 3)	**<0.001**
Cervicitis X glandular lesion (invasive and AIS)	**<0.001**
Squamous lesion (any) X Glandular lesion (any)	0.94
Invasive adenocarcinoma X Squamous cell carcinoma	0.17
CIN2/3 X AIS	0.11
CIN(1/2/3) X Squamous cell carcinoma	**0.03**
CIN(2/3) X Squamous cell carcinoma	0.05
AIS X Invasive adenocarcinoma	0.22

*p=Wilcoxon rank sum tesLegend to Figure 1.

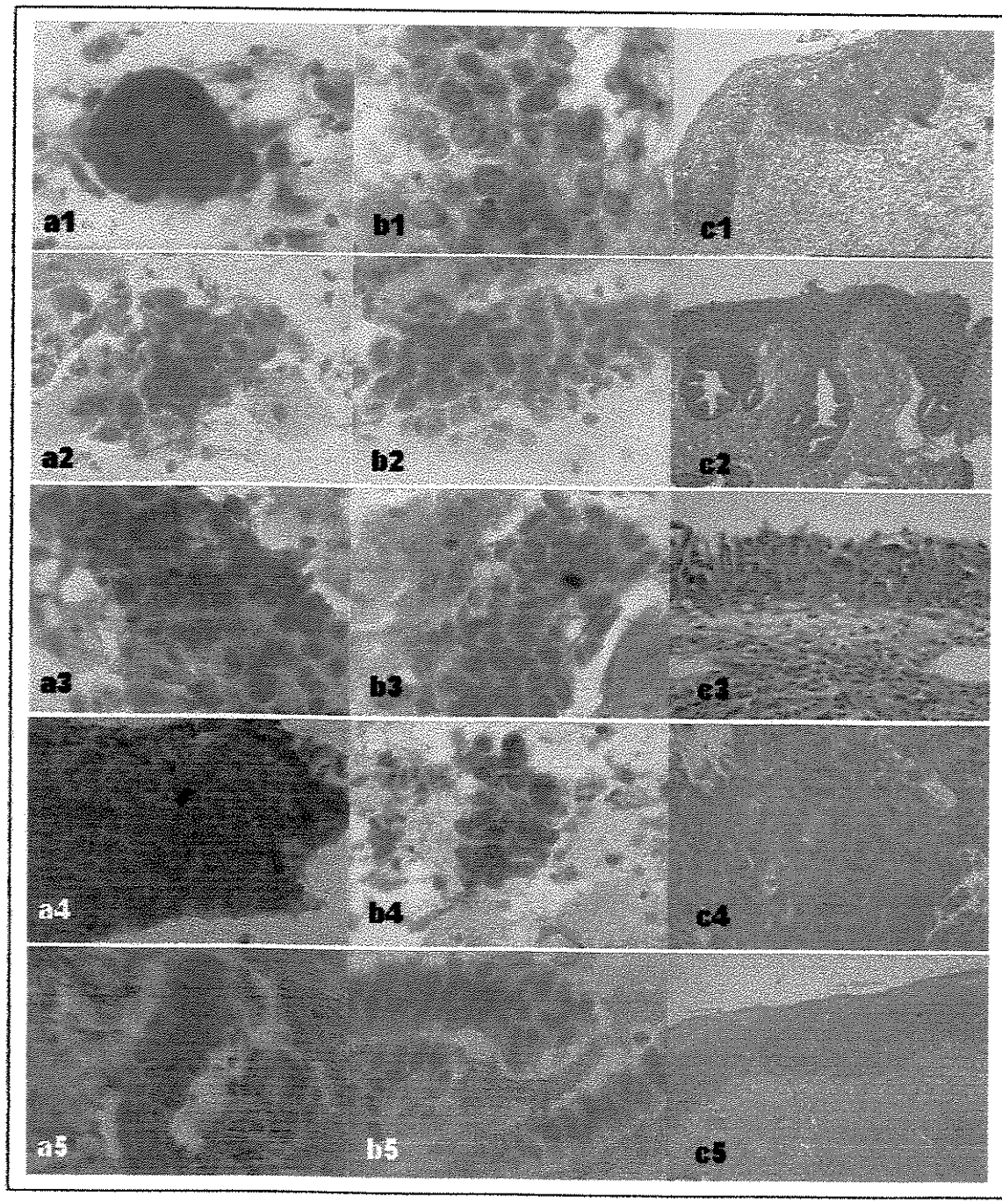


Figure 1 – Discrepant cyto-histological diagnoses. Letters "a" and "b": cytological aspects; letter "c": histology
Case 1: AGC in cytology; histology: CIN2
Case 2: AGC in cytology; histology: CIN3
Case 3: Cytological aspect suggestive of invasive adenocarcinoma; histology: CIN3
Case 4: AGC in cytology; histology: invasive squamous carcinoma
Case 5: AGC in cytology; only chronic cervicitis in histology

4. DISCUSSÃO

Nesta discussão final pretende-se apresentar a interligação das idéias desenvolvidas nos artigos, de maneira que se perceba a coesão e o propósito desta pesquisa como um todo. Ao analisar as mulheres inicialmente considerando a detecção do HPV em relação à segunda citologia, a seguir a relação da CH II com a lesão histológica e finalmente comparando mulheres atendidas por ACG e outras por HSIL, pudemos evidenciar a importância da detecção viral em várias etapas da propedêutica.

Os resultados deste estudo mostraram que, o resultado da CH II esteve significativamente associado ao resultado da segunda citologia. As mulheres atendidas por ACG puras e que apresentavam CH II positiva, também apresentavam alterações mais graves na citologia coletada no momento da colposcopia. Por outro lado, a detecção de DNA-HPV é útil em selecionar as mulheres com ACG que tenham lesão histológica mais grave. Também foi observado que quando, ao exame de rastreamento, quando a lesão citológica consiste em ACG associado a HSIL ou quando é sugestiva de AIS, a HC II não é

necessária, pois a taxa de lesões histológicas significativas é muito alta. E finalmente, a CH II não permite diferenciar lesão histológica glandulares de escamosas é importante ressaltar que todas as mulheres foram conduzidas independentemente do resultado da CH II. A propedêutica complementar foi orientada pela colposcopia e pela citologia coletada nesta consulta. Mesmo para o seguimento das mulheres que não foram submetidas à avaliação histológica, o resultado da CH II não foi levado em consideração. Estas mulheres estão retornando a cada quatro meses para coleta de citologia e colposcopia e serão avaliadas em outra análise. Além disso, vale acrescentar que conforme previamente observado por Verdiani et al. (2003) entre as mulheres com ACG na citologia de rastreamento, algumas apresentaram carcinoma de endométrio. Nestes casos, obviamente, a CH II não tem utilidade, mas deve-se valorizar a anamnese, exame físico e outros exames complementares.

Pelos resultados obtidos, observamos que ainda é precoce identificar o papel da CH II nas atipias glandulares. Nas 51 mulheres com ACG puras foi encontrado na histologia mais de 30% de lesões significativas, ou seja, NIC 2 ou mais graves. Entre estas lesões graves, duas mulheres apresentavam carcinoma escamoso invasor, duas AIS e duas adenocarcinoma invasor. Todas estas lesões são graves e se não tratadas tem uma repercussão clínica inaceitável. Assim, embora tenha uma relação significativa entre os aspectos morfológicos da citologia, o resultado histológico e a detecção do HPV, o falso negativo da CH II a torna inadequada para triagem de mulheres com lesões glandulares (artigo 3).

Uma das grandes vantagens desta pesquisa é o número significativo de mulheres avaliadas por ACG. A seleção dos sujeitos foi estruturada por meio de uma carta convite enviada às unidades básicas de saúde a partir do momento em que apresentaram alteração na citologia de rastreamento. Existem poucos estudos encontrados na literatura que avaliaram um número significativo de mulheres com ACG procurando a associação dos aspectos morfológicos com a detecção viral (Ronnet et al., 1999, Krane et al., 2004). Entretanto, este estudo tem como principal limitação científica, a metodologia utilizada. A CH II indica apenas a presença e carga de HPV de alto risco oncogênico, porém não identifica o tipo viral. O HPV 16 é, no mundo todo, o tipo mais prevalente tanto em lesões glandulares quanto em lesões escamosas, invasoras ou pré-invasoras (Munoz et al., 2003, Xi et al., 2003, Brestovac et al., 2005, Chaturvedi et al, 2005). Porém, ao se considerar apenas mulheres infectadas pelo HPV 18, a maioria dos diagnósticos relacionados, consistem em adenocarcinoma (Walboomer et al., 1999). Atualmente, a tipagem viral com PCR está sendo realizada com o material coletado nestas mesmas mulheres e será objeto de outras publicações.

Concluímos assim que, são necessários mais estudos sobre as anormalidades citológicas glandulares. Atualmente muitos investigadores estão propondo uma melhora nos critérios citológicos para as anormalidades glandulares e pesquisando o papel do teste de HPV tipo específico para identificar pacientes de risco. Nosso estudo demonstrou que o teste de HPV pode ser útil em pacientes com ACG puras, pois as mulheres com HPV detectável são de maior risco para ter alguma lesão cervical significativa. Entretanto, independentemente

do resultado da CH II é recomendável acompanhar por pelo menos dois anos as pacientes com ACG e colposcopia ou biópsia negativa. E finalmente, baseado nestes dados, observamos que a CH II não diferencia lesões glandulares das lesões escamosas e, portanto, continua indicada conização com espécime cirúrgico adequado, nas mulheres com atipias de células glandulares.

5. CONCLUSÕES

- **ARTIGO 1**

A utilização de uma segunda citologia oncológica combinada com o teste de CH II pode melhorar a conduta clínica em mulheres referidas por ACG em exame de rastreamento.

- **ARTIGO 2**

A detecção do DNA-HPV esteve fortemente associada com a gravidade da lesão cervical (NIC 2 ou mais grave) nas mulheres referidas por ACG ou AIS na citologia cervical.

- **ARTIGO 3**

1. A detecção do DNA-HPV esteve fortemente associada com a detecção de lesões histológicas significativas em mulheres com atipias celulares escamosas ou glandulares, porém não ajudou na distinção da natureza histológica das lesões.
2. A avaliação da carga viral entre os diferentes grupos histológicos pode diferenciar cervicites das lesões escamosas e glandulares significativas, sendo que a alta carga viral estaria relacionada à severidade da lesão cervical. Não diferenciou lesões glandulares das escamosas.

6. REFERÊNCIAS BIBLIOGRÁFICAS

Anciaux D, Lawrence WD, Gregoire L, Glandular lesions of the uterine cervix: prognostic implications of human papillomavirus status. ***Int J Gynecol Pathol*** 1997; 16:103-10.

Andersson S, Rylander E, Larsson B, Strand A, Silfversvard C, Wilander E. The role of human papillomavirus in cervical adenocarcinoma carcinogenesis. ***Eur J Cancer*** 2001; 37:246-50.

Ayer B, Pacey F, Greenberg M, Bousfiel S. The cytologic diagnosis of adenocarcinoma in situ of the cervix uteri and related lesions. I. Adenocarcinoma in situ. ***Acta Cyto*** 1987; 31:397-411.

Bertrand M, Lickrish GM, Colgan TJ. The anatomic distribution of cervival carcinoma *in situ*: implications for treatment. ***Am J Obstet Gynecol*** 1987; 157:21-5.

Biscotti CV, Gero MA, Toddy SM, Fischier DF, Easley KA. Endocervical adenocarcinoma in situ: an analyses of cellular features. ***Diagn Cytopathol*** 1997; 17:326-32.

Bonfiglio TA, Erozan YS. Gynecologic Cytopathology: Philadelphia: Lippincott-Raven Publishers 1997; p 107-169.

Boon ME, Baak JP, Kurver PJ, Overdiep SH, Verdonk GW. Adenocarcinoma in situ of the cervix: an underdiagnosed lesion. ***Cancer*** 1981; 48(3):768-73.

Bosch FX, Muñoz N, Chichareon S, Ngelangel C, Cáceres E, Eluf-Neto J, et al. HPV and cervical adenocarcinoma: an IARC based multicentric case-control study. 18th International Papillomavirus Conference- Program and abstracts book. Barcelona 2000. Available on line: http:/www.hpv2000.com.

Brown LJR, Wells M. Cervical glandular atypia associated with squamous intraepithelial neoplasia. A premalignant lesion?. ***J Clin Pathol*** 1986; 39:22-8.

Brestovac B, Harnett GB, Smith DW, Shellam GR, Frost FA. Human papillomavirus genotypes and their association with cervical neoplasia in a cohort of Western Australian women. ***J Med Virol*** 2005; 76(1):106-10.

Burja IT, Thompson SK, Sawyer WI, Shurbaji MS. Atypical glandular cells of undetermined significance on cervical smears. A study with cytohistologic correlation. ***Acta Cytol*** 1999; 43:351-56.

Cangiarella JF & Chhieng DC. Atypical glandular cells-An update. ***Diagn. Cytopathol*** 2003; 29:271-279.

Chaturvedi AK, Dumestre J, Gaffga AM, Mire KM, Clark RA, Braly PS, et al. Prevalence of human papillomavirus genotypes in women from three clinical settings. ***J Med Virol*** 2005; 75(1):105-13.

Chhieng DC, Elgert P, Cangiarella JF, Cohen JM. Clinical significance of atypical glandular cells of undertermined significance. A follow-up study from an academic medical center. ***Acta Cytol*** 2000a; 44:557-66.

Chhieng DC, Elgert PA, Cangiarella JF, Cohen JM. Variation in the incidence of AGUS Between Different Patient Populations. ***Acta Cytol*** 2000b; 45:287-99.

Chhieng DC, Elgert P, Cangiarella JF, Cohen JM. Variation in the Incidence of AGUS between different patient populations. ***Acta Cytol*** 2001a; 45:287-293.

Clavel C, Masure M, Putaud I, Thomas K, Bory JP, Gabriel R, et al. Hybrid capture II, a new sensitive test for human Papillomavirus detection. Comparison with hybrid capture I and PCR results in cervical lesions. ***J Clin Pathol*** 1998; 51(10):737-40.

Davey DD, Woodhouse S, Styer J, Mody D. Atypical epithelial cells and specimen adequacy: current laboratory practices of participants in the college of American pathologists interlaboratory comparison program in cervicovaginal cytology. ***Arch Pathol Lab Med*** 2000; 124:203-11.

Demay RM. The Pap Smear. In: the art and the science of cytopathology.: Chicago: ASCP press, 1993; p 61-185.

Diaz-Rosario LA & Kabawat SE. Cell block preparation by inverted filter sedimentation is useful in the differential diagnosis of atypical glandular cells of undetermined significance in ThinPrep specimens. ***Cancer*** 2000; 90(5):265-72.

Farnsworth A, Laverty C, Stoler MH. Human Papillomavirus messenger RNA expression in adenocarcinoma in situ of the uterine cervix. ***Int J Gynecol Pathol*** 1989; 8(4):321-30.

Farthing A, Masterson P, Mason WP, Vousden KH. Human Papillomavirus detection by hybrid capture and its possible clinical use. ***J Clin Pathol*** 1994; 47:649-652.

Geier CS, Wilson M, Creasman W. Cinical evaluation of atypical glandular cells of undetermined significance. ***Am J Obstet Gynecol*** 2001; 184(2):6469.

Hammoud MM, Haefner HK, Michael CW, Ansbacher R. Atypical glandular cells of undetermined significance. Histology findings and proposed management. ***J Reprod Med*** 2002; 47(4):266-170.

Hare AA, Duncan AR, Sharp AJ. Cytology suggestive of glandular neoplasia: outcomes and suggested management. ***Cytopathology*** 2003; 14:12-18.

Holowaty P, Miller AB, Rohan,T, To T. Natural history of dysplasia of the uterine cervix. ***J Natl Cancer Inst*** 1999; 91(16):1420A-1421.

Hörding U, Daugaard S, Visfeldt J. Adenocarcinomas of the cervix and adenocarcinoma of the endometrium: distinction with PCR-mediated detection of HPV-DNA. ***APMIS*** 1997; 105:313-16.

International Academy of Cytology. The Bethesda system for reporting cervical/vaginal cytologic diagnoses. ***Acta Cytol*** 1993; 37:115-24.

Ioffe OB, Sagae S, Moritani S, Dahmoush L, Chen TT, Silverberg SG. Should pathologists diagnose endocervical preneoplastic lesions "less than" adenocarcinoma in situ?: point. ***Int J Gynecol Pathol*** 2002; 22:18-21.

Jeng CJ, Liang HS, Wang TY, Shen J, Yang YC, Tzeng CR. Cytologic and histologic review of atypical glandular cells (AGC) detected during cervical cytology screening. ***Int J Gynecol Cancer*** 2003;13(4):518-21.

Keating JT, Wang HH. Significance of a diagnosis of atypical squamous cells of undetermined significance for Papanicolaou smears in perimenopausal and postmenopausal women. ***Cancer*** 2001; 93(2):100-5.

Krane JF, Lee KR, Sun D, Yuan L, Crum CP. Atypical glandular cells of undetermined significance. Outcomes predictions based on human papillomavirus testing. ***Am J Clin Pathol*** 2004; 121(1):87-92.

Krummins I, Young Q, Pacey F, Bousfield S. The cytologic diagnosis of adenocarcinoma in situ of the cervix uteri. ***Acta Cytol*** 1977; 21:320-9.

Kurman RJ & Solomon, D. The Bethesda System for reporting cervical/vaginal diagnoses. Bethesda, Springer-Verlag, New York, Inc., 81p, 1994.

Lawrence WD. Advances in the pathology of the uterine cervix. Current concepts in cervical pathology. ***Hum Pathol*** 1991; 22:792-806.

Lee KR, Manna EA, Jones MA. Comparative cytologic features of adenocarcinoma in situ of uterine cervix. ***Acta Cytol*** 1991; 35:117-26.

Lee KR, Manna EA, St Jonh T. Atypical endocervical glandular cells: accuracy of cytologic diagnosis. ***Diagn cytopathol*** 1995; 13:202-08.

Lee MF, Chang MC, Wu CH. Detection of human Papillomavirus types in cervical adenocarcinoma by the polymerase chain reaction. ***Int J Gynaecol Obstet*** 1998; 63(3):265-70.

Levine L, Lucci JA 3rd; Dinh, TV. Atypical glandular cells: New Bethesda Terminology and Management Guidelines. ***Obstet Gynecol Survey*** 2003; 58(6):399-406,.

Lorincz AT. Screening for cervical cancer: new alternatives and research. ***Salud Publica Mex*** 2003; 45(Suppl 3):S376-87.

Mattosinho de Castro Ferraz M Da G, Focchi J, Stavale JN, Nicolau SM, Rodrigues de Lima G, Baracat EC. Atypical glandular cells of undermined significance. Cytologic predictive value for glandular involvement in high grade squamous intraepithelial lesions. ***Acta Cytol*** 2003; 47(2):154-58.

Meijer CJ, Rozendaal R, Verheijen RM, Walboomers JM. Clinical role of HPV testing. ***CME J of Gynecol Oncol*** 2000; 5:26-29.

Moritani S, Ioffe OB, Sagae S, Dahmoush L, Silverberg SG, Hattori T. Mitotic activity and apoptosis in endocervical glandular lesions. ***Int J Gynecol Pathol*** 2002; 21(2):125-33.

Munoz N, Bosch FX, De Sanjose S, Herrero R, Castellsague X, Shah KV, et al. International Agency for Research on Cancer Multicenter Cervical Cancer Study

Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. ***N Engl J Méd***, 2003; 348(6):518-27.

Muntz HG, Bell DZ, Lage JM, Goff BA, Feldman S, Rice LW. Adenocarcinoma in situ of the uterine cervix. ***Obstet. Gynecol*** 1992; 80:935-9.

Nasuti F, Fleisher SR, Gupta PK. Atypical glandular cells of undetermined significance (AGUS): clinical considerations and cytohistologic correlation. ***Diagn Cytopathol*** 2002; 26:186-90.

National Cancer Institute Workshop. The 1988 Bethesda System for reporting cervical/vaginal cytological diagnoses. ***JAMA*** 1989; 262:931-4.

National Cancer Institute Workshop. The Bethesda system for reporting cervical/vaginal cytological diagnoses: revised after the second National Cancer Institute Workshop, April 29-30,1991. ***Acta Cytol*** 1993; 37:115-124.

National Cancer Institute Workshop – Bethesda System 2001 available in site "http.www.bethesda2001.cancer.gov".

Nindl I. Human papillomavirus detection in high-grade squamous intraepithelial lesions: Comparison of hybrid capture assay with a polymerase chain reaction system. ***Diagn Microbiol Infect Dis*** 1995; 23:161-164.

Nindl I, Lorincz A, Mielzynska I, Petry U, Baur S, Kirchmayr R, et al. Human papillomavirus detection in cervical intraepithelial neoplasia by the second-generation hybrid capture microplate test, comparing two different cervical specimen collection methods. ***Clin Diagn Virol*** 1998; 10(1):49-56.

Okagaki T, Tase T, Twiggs LB, Carson LF. Histogenesis of cervical adenocarcinoma with reference to human papillomavirus-18 as a carcinogen. ***J Reprod Med*** 1989; 34(9):639-44.

Östör AG, Pagano R, Davoren RA, Fortune DW, Chanen W, et al. Adenocarcinoma in situ of the cervix. ***Int J Gynecol Pathol*** 1984; 3:179-90.

Östör AG. Studies on 200 cases of early squamous cell carcinoma of the cervix. ***Int J Gynecol Pathol*** 1993; 12(3):193-207.

Paraskevaidis E, Koliopoulos G, Malamou-Mitsi V, Zikopoulos K, Paschopoulos M, Pappa L. et al. Large loop excision of the transformation zone for treating cervical intraepithelial neoplasia: a 12-year experience. ***Anticancer Res*** 2001; 21:3097-9.

Pirog EC, Kleter B, Olgac S, Bobkiewicz P, Lindeman J, Quint WG, et al. Prevalence of human papillomavirus DNA in different histological subtypes of cervical adenocarcinoma. ***Am J Pathol*** 2000; 157(4):1055-62.

Poynor EA, Barakat RR, Hoskins WJ. Management and follow-up of patients with adenocarcinoma in situ of uterine cervix. ***Gynecol Oncol*** 1995; 57:158-64.

Prendiville W, Cullimore J, Norman S. Large loop excision of the transformation zone (LLETZ): a new method of management for women with cervical intraepithelial neoplasia. ***Br J Obstet Gynecol*** 1989; 96:1054-60.

R Development Core Team (2004). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-00-3, URL http://www.R-project.org

Raab SS, Snider TE, Potts SA, McDaniel HL, Robinson RA, Nelson DL, et al. Atypical glandular cells of undetermined significance. Diagnostic accuracy and iterobserver variability using select cytologic criteria. ***Am J Clin Pathol*** 1997; 107:299-307.

Raab SS, Geisinger KR, Silverman JF, Thomas PA, Stanley MW. Interobserver variability of a Papanicolaou smear diagnosis of atypical glandular cells of undetermined significance. ***Am J Clin Pathol*** 1998; 110:653-59.

Raab SS, Bishop NS, Zaleski MS. Effect of cervical disease history on outcomes of women who have a pap diagnosis of atypical glandular cells of undetermined significance. ***Gynecol Oncol*** 1999; 74:460-64.

Raab SS. Can glandular lesions be diagnosed in Pap smear cytology?. ***Diagn Cytopathol*** 2000; 23 (2):127-33.

Ronnet BM, Manos MM, Ransley JE, Fetterman BJ, Kinney WK, Hurley LB, et al. Atypical glandular cells of undetermined significance: cytopathologic features, histopathologic results, and human papillomavirus DNA detection. ***Hum Pathol*** 1999; 30(7):816-25.

Schiffman M, Castle PE. Human papillomavirus: epidemiology and public health. ***Arch Pathol Lab Med*** 2003; 127(8):930-34.

Schwartz S, Weiss N. Increased incidence of adenocarcinoma of the cervix in young women in the United States. ***Am J Epidemiol*** 1986; 124:1045-7.

Scully RE, Bonfiglio TA, Kurman RJ, Silverberg SG, Wilkins EJ. Histological typing of female genital tract tumors. World Health Organization - International histological classification of tumors. 2nd ed. Berlin: Springler-Verlag; 1994.

Skyldberg BM, Murray E, Lambkin H, Kelehan P, Auer GU. Adenocarcinoma of the uterine cervix in Ireland and Sweden: human papillomavirus infection and biologic alterations. ***Mod Pathol*** 1999; 12(7):675-82.

Smotkin D, Berek JS, Fu YS, Hacker NF, Major FJ, Wettstein FO. Human papillomavirus deoxyribonucleic acid in adenocarcinoma and adenosquamous carcinoma of the uterine cervix. ***Obstet Gynecol*** 1986; 68(2):241-4.

Solomon D, Frable WJ, Vooijs GP, Wilbur DC, Amma NS, Collins RJ, et al. ASCUS and AGUS criteria. Intenacional Academy of Cytology Task Force summary. Diagnostic Cytology Towards the 21st Century: An International Expert Conference and Tutorial. ***Acta Cytol*** 1998; 42:16-24.

Solomon D, Davey D, Kurman R, Moriarty A, O'connor D, Prey M, et al. FORUM GROUP MEMBERS;BETHESDA 2001 WORKSHOP. The 2001 Bethesda System – Terminology for Reporting Results of Cervical Cytology. ***JAMA*** 2002; 287:2114-19.

Soofer, S.B.; Sidawy, M.K. Atypical glandular cells of undetermined significance: clinically significant lesions and means of patient follow-up. ***Cancer*** 2000; 90:207-14.

Syrjänen K. HPV and cervical adenocarcinoma. Chapter 8. In: Syrjänen K and Syrjänen, S. Papillomavirus Infections in Human Pathology. J. Wiley & Sons, New York, 2000: pp. 189-206.

Tase T, Okagaki T, Clark BA, Twiggs LB, Ostrow RS, Faras AJ. Human papilloma virus DNA in adenocarcinoma in situ, microinvasive adenocarcinoma of the uterine cervix, and co-existing cervical squamous intra-epithelial neoplasia. ***Int J Gynecol Pathol*** 1989; 8:8-17.

Veljovich DS, Stoler MH, Andersen WA, Covell JL, Rice LW. Atypical glandular cells of undetermined significance: A five-year retrospective histopathologic study. ***Am J Obstet Gynecol*** 1998; 179:382-90.

Verdiani LA, Derchain SF, Schweller M, Gontijo RC, Andrade LA, Zeferino LC. Atipia de células glandulares em esfregaços do colo do útero: avaliação dos métodos propedêuticos. ***Rev Bras Ginecol Obstet*** 2003; 25(3):193-200.

Vesterinen, E.; Forss M.; Nieminen U. Increase of cervical adenocarcinoma: a report of 520 cases of cervical carcinoma including 112 tumors with glandular elements. ***Gynecol Oncol*** 1989; 33:49-53.

Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. ***J Pathol*** 1999; 189:12-9.

Widrich T, Kennedy AW, Myers TM, Hart WR, Wirth S. Adenocarcinoma *in situ* of the uterine cervix: management and outcome. ***Gynecol Oncol*** 1996; 61:304-8.

Wilbur DC. Endocervical glandular atypia: A "new" problem for the cytopathologist. ***Diagn Cytopathol*** 1995; 13:463-69.

Wilbur D. Citology of Endocervix, Endometrium, and upper femalr genital tract. In: Bonfiglio TA & Erozan YS Editors. Gynecologic Cytopathology: Philadelphia: Lippincott-Raven Publishers, p 107-169, 1997.

Wright TCJr, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ. Conference AS-SC. 2001 Consensus Guidelines for the management of women with cervical cytological abnormalities. ***JAMA*** 2002; 287:2120-2129.

Yamakawa Y, Forslund O, Teshima H, Hasumi K, Kitagawa T, Hansson BG. Human papillomavirus DNA in adenocarcinoma and adenosquamous carcinoma of the uterine cervix detected by polymerase chain reaction (PCR). ***Gynecol Oncol*** 1994; 53(2):190-5.

Yun K, Molenaar AJ, Wilkins RJ. Detection of human papilloma virus DNA in cervical lesions by in-situ DNA hybridization. ***Pathology*** 1989; 21:1-4.

Xi LF, Toure P, Critchlow CW, Hawes SE, Dembele B, Sow PS, et al. Prevalence of specific types of human papillomavirus and cervical squamous intraepithelial lesions in consecutive, previously unscreened, West-African women over 35 years of age. ***Int J Cancer*** 2003; 1;103(6):803-9.

Zaino RJ. Glandular lesions of the uterine cervix. ***Mod Pathol*** 2000; 13(3):261-74.

Zaino RJ. Adenocarcinoma in situ, glandular dysplasia, and early invasive adenocarcinoma of the uterine cervix. ***Int J Gynecol Pathol*** 2002; 21:314-26.

Zweizig S, Noller K, Reale F, Collis S, Resseguie L. Neoplasia associated with atypical glandular cells of undetermined significance on cervical cytology. ***Gynecol Oncol*** 1997; 65:314-18.

7. BIBLIOGRAFIA DE NORMATIZAÇÕES

França JL, Borges SM, Vasconcellos AC, Magalhães MHA. **–Manual para normatização de publicações técnico-científicas.** 4ªed., Editora UFMG, Belo Horizonte, 1998. 213p.

Normas e procedimentos para publicação de dissertações e teses. Faculdade de Ciências Médicas, UNICAMP. Ed. SAD – Deliberação CCPG-001/98 (alterada 2005).

8. ANEXOS

8.1. ANEXO 1 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

AGUS E DETECÇÃO DE HPV DE ALTO RISCO ONCOLÓGICO PELO SISTEMA DE CAPTURA HÍBRIDA II

Eu, Sra _____, atendida no Setor de Patologia Cervical do CAISM, fui convidada a participar de uma pesquisa porque o resultado do meu exame preventivo mostrou alterações que podem levar ao câncer do colo do útero. Essa pesquisa tem como objetivo investigar as possíveis causas que levam a esse tipo de câncer e dessa forma ajudar a evitar essa doença. Sei que responderei a um questionário sobre informações pessoais, mas meu nome e endereço não serão divulgados, constarão somente no meu prontuário, pois a ficha da pesquisa terá apenas números de série. Essa ficha ficará de posse dos médicos responsáveis pela pesquisa: Dra. Eliane Regina Zambelli Mesquita de Oliveira e Dra. Sophie Françoise Mauricette Derchain.

Sei que serei submetida a uma investigação que é necessária para esclarecer as alterações encontradas no meu exame preventivo e receber o tratamento que for preciso. Essa investigação consta de nova coleta de exame

preventivo, e da mesma forma, será colhida secreção para descobrir se existe algum vírus que possa estar relacionado com o meu problema. Será realizado também colposcopia, na qual o médico vai olhar o colo do meu útero com lente de aumento, e caso seja encontrada alguma alteração, esta será biopsiada para saber com certeza o que eu tenho. Essa biópsia é feita no ambulatório e o máximo que pode causar é um pequeno sangramento, que logo pára. Caso seja descoberto que eu tenho uma alteração mais grave, ou seja, maior possibilidade de transformar-se em câncer, serei submetida a uma pequena cirurgia feita com anestesia local e que se chama cirurgia de alta freqüência (CAF). Esse procedimento também é simples, normalmente feito no ambulatório. Todos estes exames estão indicados para o diagnóstico da lesão do colo do útero quando o exame de prevenção mostra as alterações que foram encontradas no meu exame. Não serei submetida a exames desnecessários, tudo será feito como normalmente se faz em casos como o meu, de acordo com os programas de prevenção do câncer do colo do útero, e tratamento adequado.

Fui esclarecida quanto ao meu direito de não participar da pesquisa ou sair a qualquer momento, e de ser atendida no ambulatório sempre que necessário e tratada da mesma forma. Em caso de dúvidas ou esclarecimentos, tenho o direito de telefonar para a Dra. Eliane Regina Zambelli Mesquita de Oliveira no número 3788-9305 ou para o Comitê de Ética em Pesquisa da UNICAMP no número 3788-8936. Sei que não serei paga para participar deste estudo.

Campinas, _____ de _____ de 200__

_____

Assinatura da Paciente

_____

Dra. Eliane R. Zambelli Mesquita de Oliveira

8.2. ANEXO 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

PERSISTÊNCIA DO DNA-HPV APÓS A CONIZAÇÃO COM CIRURGIA DE ALTA FREQÜÊNCIA (CAF) EM MULHERES COM NIC 2 OU 3

Eu, Sra _____, portadora do RG_____, atendida no Centro de Atenção Integral à Saúde da Mulher (CAISM) no ambulatório de Patologia Cervical, participo da pesquisa "Persistência do DNA-HPV após a Conização com Cirurgia de Alta Freqüência (CAF) em mulheres com NIC 2 ou 3". Fui convidada a participar de uma continuação desta pesquisa que será realizada com o material que eu já coletei antes (aquela que identifica o vírus). Sei que este estudo tem como objetivo avaliar a presença do HPV de alto risco oncológico no meu colo uterino antes e após a conização por cirurgia de alta freqüência. O exame que foi feito antes identificou se tinha vírus ou não e este novo teste, chamado PCR irá determinar o tipo específico de vírus eventualmente presente no material que eu já coletei.

Fui esclarecida quanto ao meu direito de não participar da pesquisa e a não aceitação na participação no estudo não implicará na perda dos direitos iniciais rotineiramente oferecidos pelo ambulatório. Sei também que a qualquer momento posso desistir de participar da pesquisa, sem nenhum dano para a minha saúde e tratamento. Também fui informada de que não terei custos para participar da pesquisa, pois, o intervalo entre as consultas será o mesmo realizado de rotina no ambulatório. Sei que todas as informações pessoais serão avaliadas somente pelo médico que me atendeu e que as fichas ficarão de posse do(s) Doutore(s) responsáveis pela pesquisa, Doutora Sophie Françoise Mauricette Derchain e Dr Luis Otávio Zanatta Sarian que manterão o sigilo da fonte destas informações. Em caso de dúvidas ou esclarecimento, tenho o direito de entrar em contato com os

Drs. Luís Otávio Zanatta Sarian e Sophie F M Derchain, no telefone 3788-9305 ou com o Comitê de Ética em Pesquisa da UNICAMP no telefone 3788-8936.

Nome da paciente _____

Assinatura da paciente: _____

Campinas, _____ de _____ de 200__.

Nome do pesquisador _____

Assinatura do pesquisador: _____.

Campinas, _____ de _____ de 200__.

Leandro Master
Capa/Editoração
ldromaster@yahoo.com.br
www.leandromaster.v10.com.br