

26TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE & CLINICAL AND PUBLIC HEALTH WORKSHOPS

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Abstracts selected by Mauro Romero L Passos, Felipe DL Passos, Marc Steben

APRESENTAÇÃO / PRESENTATION

Human papillomavirus (HPV) is the most frequent sexually transmitted infection causing 5% of all cancers in human. HPV is the source of various ano genital genital such as cervical, anal, vulvar, vaginal, penile as well as of oropharyngeal cancers. HPV also causes non neoplastic anogenital and respiratory track papillomatosis. Both high risk and low risk HPV now represent, at least partially, preventable burden on the health care system and global loss of productivity.

The 26th International Papillomavirus Conference and its two workshops were held under the theme of "Sharing Knowledge for Global Health". Four commitments were made by the local organizing committee and we made sure to keep them to the International Papillomavirus Society: 1) to foster integrative research, 2) to enhance developing countries' capacities, 3) to inspire young researchers and 4) to offer an affordable learning experience.

There were 1978 participants at the conference. Two 2-day Workshops, Public Health Workshop & Clinical Workshop, preceded the main conference. During the conference we had 12 plenary sessions, 29 parallel Oral Communications Sessions, eight Early Morning Workshops (for young researchers and addressing capacity building in emerging countries), 18 Satellite Symposia (12 academic and 6 industries sponsored), 642 regular Posters and 70 e-posters.

The organisers followed very consistently an environment-friendly policy avoiding printed paper documents, increases the distribution of CD-ROM with all conference abstracts, free web-cast dissemination of the presentations and the book of abstracts through www.hpv2010.org. To recognize the strength and the variety of researchers in our society, awards were given for both oral and poster presentation in all of our four group of interest: basic sciences, clinical and laboratory sciences, epidemiology and public health as well as capacity building. Awards for young

researchers and from developing countries were also given. The current paper contains short summaries of award recipients. Readers interested in the details of certain sessions or presentations are invited to consult www.hpv2010.org.

Held under the theme: sharing knowledge for global health, the conference continues the tradition started last year in Malmö where every presentations were made available free to anyone joining the website, after an identification process, anyone can look or download presentations from the conference or the clinical or the public health workshop. This generosity from our speakers makes it possible to access the high quality of science and methods to all and not only to those able to make it to Montréal. This certainly helped us guarantee that in fact we delivered on our theme of sharing knowledge for global health and this unique collaboration with the Brazilian Journal of STD is a vibrant show of the interest our brazilian colleagues have spread the knowledge on HPV to their scientific community of Brazil and the editor has to be applauded for his vision and help in sharing the highest quality of knowledge!

Obrigado,

MARC STEBEN, MD

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WINNING ABSTRACTS IPV 2010 (TRABALHOS PREMIADOS)

HPV 2010 - Abstract Award Recipients (by Category)

Basic Science, Best Poster Presentation by a Student

P-289: DIFFERENCES IN EARLY ENTRY EVENTS BETWEEN HIGH-RISK NATIVE PAPILLOMAVIRUSES

Linda Cruz¹, Michael J. Conway¹, Craig Meyers¹

Studies using pseudovirions (PsV) have implicated the minor capsid protein L2 in early events of the viral life cycle including the entry of virions into the cell, escape of the viral genome from endosomes after viral uncoating, and bringing the viral genome into the nucleus. In HPV16 PsV, L2 is cleaved by a proprotein convertase, furin and/or PC5/6, at a consensus site in the N-terminal region during infection. We were interested in whether native virions (NV) produced under physiologically relevant conditions of differentiating host tissue would show the same dependence on cleavage by a cellular proprotein convertase to undergo a successful infection. Human foreskin keratinocytes infected with HPV16, HPV18, HPV31, and HPV45 were cultured to produce infectious virus. Surprisingly, infection of HaCat keratinocytes, Chinese hamster ovary cells, as well as primary foreskin keratinocytes with HPV16, was not dependent on cleavage by furin or PC5/6 as demonstrated by infection in the presence of a furin peptide inhibitor. In addition, infection with HPV16 produced from differentiated human cervical keratinocytes was insensitive to the furin inhibitor. HPV45 showed the same resistance to inhibition as HPV16. Interestingly, early studies suggest that the HPV16 NV L2 N-terminus may be cleaved during virion morphogenesis in the differentiating tissue. Further, this virus appears to be independent of primary binding to a heparan sulfate attachment receptor. However, infections with HPV18 and HPV31 suggest that the furin and/or PC5/6 enzyme is required during infection with these viruses.

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Basic Science, Best Poster Presentation from an Emerging Country Participant

P-264: HIGH VIRAL LOAD AND E7 EXPRESSION AMONG CaCx CASES HARBORING INTACT E2: SOME NOVEL INSIGHTS ON DISEASE PATHOGENESIS

Damayanti Das¹, Bornali Bhattacharjee², Laikangbam Premi¹, Sharmila Sengupta¹

Introduction: HPV16 is the major viral isolate identified in cervical cancer (CaCx) cases in India. Other than the significant role of viral E2 gene-disruption, viral DNA load is also likely to influence the CaCx risk.

Objectives: To investigate the role of HPV16 viral load and onco-gene expression in cervical cancer pathogenesis.

Methods and Materials: A case-control study (152 CaCx cases and 87 controls, all HPV16 positive) was undertaken to estimate viral load and E7 and E4 expressions were studied by APOT assay combined with quantitative Real Time PCR (TAQMAN).

Results: Median HPV16 viral load (Mann-Whitney U test) was significantly higher among cases (2.99×10^7) compared to controls (1.92×10^4), irrespective of viral E2-status ($p < 0.001$). Logistic regression of log viral load against disease status confirmed the finding both irrespective of E2-status [OR_{age-adjusted} = 1.261; 95% CI = 1.161-1.369; $p < 0.001$] and with E2-status as a co-variate of disease-risk [OR_{age-adjusted} = 1.333; 95% CI = 1.207-1.472; $p < 0.001$]. mRNA expression was studied on a subset of 40 cancer cases. APOT cum TAQMAN analysis identified 23 intact and 17 disrupted cases based on E7 expression in both intact and disrupted while E4 expression in only intact cases. The median viral load significantly varied ($p = 0.011$; Mann-Whitney U test) between intact (2.55×10^7) and disrupted (9.96×10^5) cases. Both E7_{CT}/β-actin_{CT} and E2_{CT}/β-actin_{CT} were negatively correlated with viral load ($p = 0.016$ and $p = 0.002$, respectively) among intact cases. No association was found between viral load and E7_{CT}/β-actin_{CT} within disrupted cases. A significant difference ($p < 0.001$; t-test) was observed in the mean E7_{CT} between intact (6.69 ± 8.94) and disrupted (25.69 ± 14.31) cases, but no such difference was present in β-actin expression.

Conclusion: Thus association of high viral load with HPV16 positive cases, specifically those having intact E2, and higher E7 expression among E2-intact cases provide novel insights into disease pathogenesis.

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Basic Science, Best Overall Oral Presentation

LACK OF DNA DAMAGE CHECKPOINT REGULATION FOR HPV DNA REPLICATION: A REASON FOR INTEGRATION?

Thomas Melendy^{1,2}, Lauren E. King¹, John C. Fisk², Edward S. Dorman¹, Mary M. Donaldson¹, Iain M. Morgan¹

A hallmark of cervical cancer is integration of the human papillomavirus genome into the host genome such that the viral oncoproteins are overexpressed, promoting genomic instability and progression to cancer. While this is a well-characterized observation, what remain less clear are the factors that promote viral integration. Integration of the HPV genome into that of the host would presumably occur through the non-homologous end joining pathway, which would act via a double strand DNA break (DSB) in the viral genome. Replicating DNA structures are prone to DSBs, particularly when replication forks collide with DNA damage. Indeed, the hallmarks of the cellular DNA damage checkpoint response are to stop DNA replication and mitosis/cell division when cells are subjected to DNA damage. Hence, we investigated whether DNA damage results in a checkpoint-dependent block to HPV E1- and E2-mediated DNA replication. Our results demonstrate that both in vitro and in vivo HPV E1/E2-dependent DNA replication is not arrested following DNA damage, while SV40 DNA replication is arrested; and that the SV40 arrest is dependent on PIKK-dependent checkpoint pathways. We also demonstrate that the SV40 replication protein, large T-antigen, is a target for one or more of the PIKK DNA damage signaling kinases, while HPV E1

is not. We propose that the failure of E1 to be targeted by PIKKs may allow HPV DNA replication in the presence of DNA damaging agents. Unabated DNA replication in the presence of DNA damage results in DSBs, and we propose that such DSBs in the HPV genome promote HPV integration and cervical cancer.

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Basic Science, Best Overall Poster Presentation

P-243: REGULATION OF ERK SIGNALING BY THE HPV E6/hSCRIB/PP1C SIGNALLING COMPLEX

Kazunori Nagasaka¹, Lawrence Banks¹

High risk HPV E6 oncoproteins are characterized by having PDZ binding motifs at their extreme carboxy termini. Through these motifs they interact with a variety of cellular proteins that harbour PDZ domains. Whilst these interactions appear important for E6's ability to contribute to malignancy, the mechanism by which this occurs is unclear. Numerous cellular targets are reported to bind E6 through this motif, and these include the tumour suppressor proteins, hDlg and hScrib. The cell polarity regulator hScrib is a potential tumour suppressor whose loss is a frequent event in late stage cancer development. Little is yet known about the mode of action of hScrib, although recent reports suggest a role in the regulation of cell signaling. We now show that hScrib is itself a major regulator of the ERK signaling cascade. Loss of hScrib expression activates ERK and enhances nuclear ERK translocation. We also show that this is a result of direct interaction between hScrib and ERK and most likely involves recruitment of the protein phosphatase PP1c, by hScrib which subsequently de-phosphorylates ERK, thereby turning off the signaling pathway. Interestingly, hScrib is also phosphorylated by ERK and PKA in this complex, the consequences of which are enhanced susceptibility to HPV E6 induced degradation. These results show that HPV E6 can contribute to ERK activation through a variety of independent mechanisms.

¹ ICGEB, Trieste, Italy.

Basic Science, Best Oral Presentation by a Student

FA-DEFICIENT MICE ARE PREDISPOSED TO HPV-ASSOCIATED CANCERS

*Jung Wook Park¹, Henry Pitot¹, Nicole Spardy³, Stefan Duen-
sing³, Markus Grompe², Paul Lambert¹*

Objective: Fanconi anemia (FA) is a rare, heterogeneous, recessive genetic disorder. Patients with FA are highly predisposed to cancers including squamous cell carcinomas arising at multiple anatomical sites including the head/neck and anogenital regions. Human papillomaviruses (HPVs), particularly HPV type 16 (HPV16), are associated with a subset (~20%) of head and neck cancers and > 99% of cervical cancers in the general population. Some investigators found that the majority of head and neck cancers in FA patients are HPV-positive. Biological interactions also have been

observed between HPV, in particular the viral E7 oncoprotein, and the FA pathway, a DNA response pathway deficient in FA patients. Based on these studies, it was hypothesized that deficiency in the FA pathway contributes to an accumulation of DNA damage induced by HPV16 E7. This hypothesis could explain, at least in part, why FA patients are predisposed to HPV-associated cancers.

Methods: To determine the importance of the FA pathway in modulating E7's oncogenic abilities, we crossed *K14E7* transgenic and *fancD2* knockout mice (*FancD2*^{-/-}) to establish *K14E7/FancD2*^{-/-} and *K14E7/FancD2*^{+/+} mice and monitored their susceptibility to HPV-associated cancers treated with co-factors. *fancD2*, one of 13 FA genes, plays a key role in the FA pathway. Prior studies in mice indicated that E7 is the dominant HPV oncogene in HPV-associated cancers of the cervix and the head and neck region.

Results: *K14E7/FancD2*^{-/-} mice had a significantly higher incidence of HPV-associated cancers compared with *K14E7/FancD2*^{+/+} mice, and this difference correlated with increases in proliferative indices and in the abrogation of normal cellular responses to DNA damage. These animal studies support the hypotheses that FA patients have increased susceptibility to HPV-associated cancers and that the FA pathway normally attenuates the oncogenic potential of HPV16 E7.

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Basic Science, Best Oral Presentation by a Student

CONSTRUCTION OF A COMPLETE TRANSCRIPTION MAP OF HPV18 IN PRODUCTIVE VIRAL INFECTION

Xiaohong Wang¹, Craig Meyers², Zhi-Ming Zheng¹

HPV18 is the second most common oncogenic HPV genotype, responsible for 15.8% of cervical cancers worldwide. Although an early promoter of HPV18 was identified two decades ago, a transcription map of HPV18 has not been completed yet. In this study, we constructed a complete HPV18 transcription map in HPV18-infected raft tissues derived from primary human vaginal keratinocytes (HVK). HPV18 infection and viral gene expression in the raft tissues were confirmed by detection of E6, L1, L2 and E4 mRNAs with RT-PCR and/or northern blot and of L1 protein expression with western blot. We mapped by using 5'-rapid amplification of cDNA ends (RACE) the HPV18 start site for early transcripts to nt 102 position in the virus genome, close to p105 position as reported. A transcription start site for viral late gene expression was also mapped mainly to nt 814 in the E7 ORF of the virus genome, where both HPV16 and HPV31 late promoters are positioned in their corresponding genome. Further investigation showed that HPV18 late promoter p814 functioned highly in differentiated keratinocytes and its activity depended on viral replication origin in a counter-clockwise manner. HPV18 polyadenylation cleavage sites for early and late transcripts were mapped to nt 4270 and mainly to nt 7306 in the virus genome, respectively, by using 3'RACE. Although all early transcripts were cleaved at a single cleavage site, HPV18 late transcripts contained multiple other minor cleavage

sites for RNA polyadenylation. HPV18 splice sites/splice junctions for both early and late transcripts were identified by 5' RACE and primer walking techniques. Five 5' splice sites and 6 3' splice sites were identified in the HPV18 genome and are highly conserved in other papillomaviruses. Collectively, a complete HPV18 transcription map constructed from this report will lead us to further understand HPV18 gene expression and virus oncogenesis.

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Clinical Science, Best Oral Presentation by a Student

DEVELOPMENT OF GENITAL WARTS AFTER INCIDENT DETECTION OF HPV INFECTION IN YOUNG MEN

Yuzo Arima¹, Rachel Winer¹, Qinghua Feng³, James Hughes², Shu-Kuang Lee², Michael Stern¹, Sandra O'Reilly¹, Laura Koutsky¹

Background: Determining the type-specific rate at which men develop genital warts after incident detection of HPV infection will provide important information for the design of prevention strategies.

Objectives: To determine 1) the cumulative incidence of genital warts following incident detection of specific HPV types and 2) the time between first detection of HPV infection and genital warts among those who developed warts.

Methods: 418 sexually-active male university students 18-21 years old were followed between 2003 and 2009 in Seattle, USA. Subjects completed a biweekly sexual behavior diary and genital samples (shaft, glans, scrotum, and urine) were collected for HPV genotyping by PCR-based assay (37 types) every 4 months, with a maximum follow-up of 3 years. Incident HPV infection was defined as first detection of a specific HPV type at any genital site after > 1 visit where that type was not detected. Kaplan-Meier methods were used to estimate the cumulative incidence of genital warts after incident HPV infection. Separate estimates of cumulative wart incidence were determined for men with (1) incident HPV-6/11 infection, (2) incident infections other than HPV-6/11 infection, and (3) no HPV infection.

Results: The 24-month cumulative wart incidence was 57.9% (95% confidence interval [CI] 38.1%-79.1%) among 46 men with incident detection of HPV-6/11, 2.0% (95% CI 0.5-7.9%) among 161 men with incident detection of other HPV types, and 0.7% (95% CI 0.2%-2.8%) among 331 men who were negative for HPV at enrollment. Among the 18 men with incident HPV-6/11 infection who developed warts, the median time to wart development was 11 months (interquartile range 0-16.1 months).

Conclusions: Genital warts were common after incident HPV-6/11 infection and rare after infection with other low-risk HPV types. Time to wart development was about 3 times longer (11 months vs. 3 months) than was reported for a similar cohort of young women.

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Clinical Science, Best Poster Presentation from an Emerging Country Participant

P-416: CERVICAL INFECTION BY MULTIPLE HUMAN PAPILLOMAVIRUS (HPV) GENOTYPES: CORRELATION WITH COLPOSCOPY AND HISTOPATHOLOGY

Neerja Bhatla¹, Lalit Dar², Sandeep Mathur³, Venkateswaran Iyer³, A. Rajkumar Patro², Arti Gulati¹, Alka Kriplani¹, Keerti Shah⁴, Patti Gravitt⁵

Objective: To correlate the severity of cervical lesions as assessed by colposcopy and histopathology with the type and number of HPV infections.

Methods: A cross-sectional study of 512 women with persistent vaginal discharge, irregular or postcoital bleeding or unhealthy cervix. All women donated cervical samples for conventional cytology and HPV DNA by the Reverse Line Blot Assay (Roche®), followed by same day colposcopy. Biopsies were taken from all lesions. Women diagnosed with CIN were managed according to standard-of-care. Where LEEP/cone biopsy was done, the more severe diagnosis was taken into consideration.

Results: The median age was 36 years. Majority (47%) of women belonged to lower socioeconomic class. Mean age at first coitus was 19.0 years. Colposcopy-guided biopsy was taken in 315 (61.5%) women. Colposcopic diagnosis was normal in 280 (54.6%) women, inflammation in 124 (24.2%), low grade CIN in 65 (12.6%), high grade CIN/Ca in 40 (%) women. Histological examination showed CIN1 in 26, CIN2/3 in 32 and cervical cancer in 8 women. Ninety five (18.75%) women were found positive for any HPV type while 73 (14.3%) were found positive for HR-HPV. Among women with CIN 1, HPV DNA was detected in 42.3% and infection with high-risk types accounted for 81.8%. Among women with CIN 2/3, HPV DNA was detected in 87.5% and infection with high-risk types accounted for all of these. All women with invasive cancer were associated with high risk HPV. Twenty seven HPV types were seen in all, HPV16 was the single most frequent genotype found in 43.7% cases followed by HPV18 in 5.2% and HPV33 in 4.1%. The proportion of multiple infections increased from 12.8% women diagnosed as normal and 2.4% of inflammation to 17.6% of CIN1 and 22.5% of high CIN/Ca.

Conclusion: The frequency of infection with multiple HPV types increased with grade of cervical lesion.

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Clinical Science, Best Overall Oral Presentation

CLINICAL PERFORMANCE OF A BROAD-SPECTRUM RNA ASSAY IN A HIGH-PREVALENCE DISEASE SETTING

Kate Cuschieri¹, Henry Kitchener², Andrew Horne³, Camille Busby-Earle³, Alison Hardie¹, Linsey Nelson², Andrew Bailey⁴, Jennifer Rowan³, Catriona Graham⁵, Heather Cubie²

Objectives: Detection of oncogenic E6/E7 transcripts may be a better predictor of clinically significant cervical HPV infection compared with DNA based assays. However more clinical data are necessary to evaluate this. Consequently, we present an evaluation of the clinical sensitivity and specificity of the APTIMA® HPV RNA based assay (AHPV, Gen-Probe Incorporated) in comparison to the Hybrid Capture 2 DNA based assay (HC2, Qiagen Ltd) in a clinical setting.

Methods: Women attending two NHS colposcopy clinics (for all indications) in two city hospitals in the UK were invited to participate. Liquid based cytology (LBC) samples were collected and tested via the 2 HPV assays described. Biopsies were taken where clinically indicated and relative sensitivity and specificity of each assay for disease (defined as CIN2 or worse) were calculated.

Results: Approximately 1,500 women have been recruited to the study so far. At time of abstract submission, 1008 LBC samples have been tested by both assays and have associated, confirmed pathology results. A total of 276 cases of CIN2+ were detected and sensitivity and specificity of the AHPV for CIN2+ were 93.8%, (95% CI of 90.3,95.4) and 49.9%, (95% CI of 46.2,53.5) respectively. By comparison, sensitivity and specificity of the HC2 for CIN2 or worse were 94.2%, (95% CI of 90.8, 96.7) and 44.7%, (95% CI of 41.0, 48.4) respectively. When analysis was confined to women referred to colposcopy for cytologically defined low grade disease the sensitivity and specificity of the two assays were equivalent.

Conclusions: These preliminary data suggest that AHPV assay shows equivalent sensitivity and slightly higher specificity for the detection of CIN2 or worse (in a population with a high prevalence of disease). Further data including performance of the assays within a post treatment subset will be presented (with associated 95% CI's).

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Clinical Science, Best Poster Presentation by a Student

P-415: DNA METHYLATION PROFILING ACROSS THE SPECTRUM OF HPV-ASSOCIATED ANAL SQUAMOUS NEOPLASIA

Jonathan Hernandez¹, Erin Siegel², William Grady³, Steven Eschrich⁴, Domenico Coppola⁵, Abul Elahi¹, Bridget Riggs², Anna Giuliano², David Shibata¹

Introduction: There is growing evidence to suggest that changes in host genome DNA methylation patterns are among the critical molecular alterations associated with HPV-related carcinogenesis in squamous cell cancers (SCC) of the cervix and oropharynx. However, there is very little known about the epigenetic changes associated with the development of anal SCC. We sought to characterize HPV genotype and broad methylation profiles across the spectrum of anal squamous neoplasia.

Methods: Thirty-one formalin-fixed paraffin embedded samples from 25 patients were evaluated and included normal anal mucosa (NM; n = 4), SCC-in situ (SCC-IS n = 11) and inva-

sive SCC (n = 16). SFP10 LiPA HPV-typing system was used to determine the HPV status. Bisulfite-modified DNA was interrogated for methylation at 1,505 CpG loci representing 807 genes using the Illumina GoldenGate Methylation Assay.

Results: Our population was comprised of 13 women and 12 men with a median age of 48 years (range 26-81). Five patients were immunocompromised either by HIV or chemotherapy. All patients demonstrated infection with at least one high-risk HPV subtype, with HPV 16 noted in 15/16 patients with SCC. There was a trend towards increasing percentages of total CpG loci methylated with histologic progression; $56 \pm 4\%$ for NM, $61 \pm 4\%$ for SCC-IS and $63 \pm 1\%$ for SCC (p = NS). Fourteen gene loci were found to be significantly and differentially methylated (Kruskal-Wallis p < 0.01) across the 3 groups, with nine genes associated with disease progression (**Figure 1**).

Conclusion: In HPV-associated anal neoplasms, we have identified a panel of methylated genes associated with the progression from anal NM to SCC. To our knowledge, this is the first reported application of broad high-throughput methylation analysis to anal neoplasia. Our findings have future implications for advances in the understanding of HPV-associated carcinogenesis as well as for anal SCC screening, diagnosis and treatment.

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Clinical Science, Best overall Poster Presentation

P-411: RECURRENCE OF HIGH GRADE ANAL INTRAEPITHELIAL NEOPLASIA AND CANCER IN PATIENTS TREATED WITH COMBINED MODALITY THERAPY FOR ANAL CANCER

Naomi Jay¹, J. Michael Berry², Teresa Darragh³, Joel Palefsky¹

Objectives: Combined modality therapy (CMT) consisting of chemotherapy and radiation is the standard of care for anal cancer treatment, with recurrence rates reported to be > 16%. Identification of high-grade anal intraepithelial neoplasia (HGAIN) precursor lesions through high resolution anoscopy (HRA)-guided biopsy followed by ablation may prevent recurrent anal cancer in CMT-treated patients and early-stage recurrent cancer may possibly be treated with local excision. Follow-up of patients' post-CMT may therefore be important. This study sought to determine prevalence of HGAIN and cancer following completion of CMT for anal cancer.

Methods: Retrospective chart review of anal cancer patients diagnosed since 1999 and treated with CMT, referred to the University of California San Francisco for post-CMT follow-up.

Results: 52 men and 14 women completed CMT for anal cancer and were evaluated with HRA post-treatment. The average age at cancer diagnosis was 53.2 years (range 37-66). Nineteen (29%) patients returned for one visit and 47 (71%) returned for on-going HRA every 4-6 months (**Figure**). HGAIN was diagnosed in 14 (21%) patients (average 29 months post-CMT) and cancer in 9 (14%) patients (average 28 months post-CMT). 54%

of HGAIN and 78% of cancers were diagnosed at the first post-CMT evaluation (range 7–47 months). Twelve of 14 HGAIN were treated; 5 of the 12 treated lesions progressed to cancer despite treatment. Seven of 9 patients with recurrent cancers were treated surgically, half with colostomy-sparing local resection; one was lost to follow-up and one died with the cause of death unknown.

Conclusions: One-third of patients had HGAIN or cancer post-CMT treatment. These were more common in men suggesting they be targeted for post-CMT HRA evaluation. It is unclear if detection and treatment of HGAIN will prevent cancer recurrence. Early follow-up and intervention may lead to better outcomes, including the possibility of curative local colostomy-sparing resection.

	N	Post-CMT HGAIN N (%)	Post-CMT Cancer N (%)
HIV-positive men	39	8 (21%)	7 (18%)
HIV-negative men	12	5 (42%)	2 (17%)
HIV status unknown, male	1	0	0
HIV-positive women	0	0	0
HIV-negative women	14	1 (7%)	0
Total	66	14 (21%)	9 (14%)

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Clinical Science, Best Overall Oral Presentation

A SPECIFIC HPV TYPE FOR EACH CIN LESION

W.G.V. Quint¹, A. Molijn¹, L. Struijk¹, B. Colau², M. van de Sandt¹, D. Jenkins¹

Background: In 20–40% of CIN and in 4–8% of cervical carcinoma biopsy specimens multiple HPV genotypes are detected by PCR using DNA isolated from whole tissue sections. This technique works well in ascribing causality when only a single HPV type is detected. However, when multiple HPV types or multiple lesions are present, a direct causality cannot be determined.

Aims: To determine whether Laser capture micro-dissection combined with HPV genotyping (LCM-PCR) could accurately recover type-specific HPV DNA from epithelial cells in CIN and normal cervical epithelium of women with HPV and whether one or more viruses are present in one lesion.

Materials and Methods: Histologically selected 5000–100,000 square micrometer samples of CIN and normal epithelium were isolated by LCM (Zeiss P.A.L.M.) and analyzed by the HPV SPF10 PCR/ LiPA25 HPV genotyping system version 1 for 25 genotypes (1*). HPV genotypes detected in 536 areas of CIN (1, 2 or 3) from 60 biopsies by LCM/PCR were compared to results in whole tissue sections with single or multiple HPV types.

Results: When a single HPV type was detected in a cervical biopsy, that type was almost always (99%) recovered from CIN by LCM, confirming accuracy of causal association. When multiple HPV

types were present, their distribution could be mapped clearly by LCM-PCR in several patterns related to CIN and normal epithelium. 94% of HPV types found by LCM-PCR were associated with a discrete lesion or in a collision of lesions. 62% of the HPV types found in the whole section could be confirmed by LCM on selected regions.

Conclusions: The LCM/PCR technique is very accurate for high-resolution HPV genotyping and assigning an individual HPV type to an area of CIN. At LCM level in multiple HPV infections the relation between HPV types and CIN lesions is often complex. Independent of whether single or multiple HPV types are detected in whole sections, each HPV type found in CIN is associated with an independent CIN lesion, supporting the hypothesis of - one virus, one lesion.

(1*) SPF10 HPV LiPA 25, version 1 and SPF10 HPV DEIA are manufactured by Labo Biomedical Products (Rijswijk, The Netherlands) based on licensed INNOGENETICS SPF10 technology.

¹DDL Diagnostic Laboratory, Voorburg, The Netherlands; ²GlaxoSmithKline Biologicals, Rixensart, Belgium.

Epidemiology & Public Health, Best Oral Presentation by a Student

CERVICAL HPV PREVALENCE IN FIVE CONTINENTS: META-ANALYSIS ON ONE MILLION WOMEN WITH NORMAL CYTOLOGY

Laia Bruni¹, Mireia Diaz¹, Elena Ferrer¹, Xavier Castellsagué^{1,2}, F. Xavier Bosch¹, Silvia de Sanjosé^{1,2}

Context: Baseline information on HPV prevalence and type distribution is highly desirable to monitor HPV circulation and risk, to introduce HPV-based cervical cancer screening as well as to evaluate the impact of HPV vaccines in the near future.

Objective: To update previous meta-analyses including data from under-studied regions and providing more robust estimates on the burden of HPV infections in women with normal cytology (WNC) regionally and worldwide.

Methods: Systematic review of the literature between January 1995 and May 2009 of studies using PCR or HC2. Adjusted HPV prevalences were estimated by weighted regression models with logit-transformation of the prevalences which were further standardized by the country-specific population sizes.

Results: 194 studies comprising 1,016,719 WNC were included. Global estimated HPV prevalence was 11.7% (95% CI = 11.6–11.7%). Country-specific adjusted HPV prevalences ranged from 1.6–41.9%. Sub-Saharan Africa (24.0%), Eastern Europe (21.4%) and Latin-America (16.1%) showed higher HPV prevalences than the rest of European regions (ranging from 8.8–10.0%), Asia (9.4%), Northern Africa (9.2%) and North-America (4.7%). Age distribution of cervical HPV infection showed a first peak at younger ages (< 25 years), followed by a lower prevalence plateau at middle ages and, in the Americas and Africa a rebound at older ages (45–55+ years). HPV-16 was the most common type globally (3.2%) as well as in each World region. HPV-18 ranked the second worldwide (1.4%) except in Europe and Africa where it ranked as the third.

Conclusions: The presence of HPV in women without cytological signs of infection is substantially high and varies significantly across world regions. HPV-16 is the predominant type in all regions followed by HPV-18, -31, -52 and -58. Two highly oncogenic types, HPV-45 and HPV-33 are rarely detected in WNC suggesting a different virulence. This information should be useful to guide the introduction of HPV-based cervical cancer prevention strategies.

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Epidemiology & Public Health, Best Overall Oral Presentation

A NATIONAL OUTCOME FOR QUADRIVALENT HPV VACCINATION: DECLINING RATES OF GENITAL WARTS IN AUSTRALIA

Basil Donovan^{1,2}, Neil Franklin¹, Rebecca Guy¹, Andrew Grulich¹, David Regan¹, Handan Wand¹, Christopher Fairley³

Background: The quadrivalent human papillomavirus (HPV) vaccine has demonstrated high efficacy in clinical trials but no reports to date have described the vaccine's effect at a national level. From mid-2007 Australia was among the first countries to fund a universal vaccination program for all females between 12 and 26 years and coverage rates of ~80% have been achieved.

Objectives: We established a national surveillance network to determine trends in clinical presentations for genital warts.

Methods: Eight sexual health services located around Australia provided data on all new patients (n = 110,073) between 2004 and 2009. Data were collected on new diagnoses of genital warts (n = 6,387), demographics, sexual behaviour, and HPV vaccination status.

Results: Up to mid-2007, when the vaccination program began, there had been a gradual increase in the proportion of new patients diagnosed with genital warts. Thereafter there was a marked and ongoing decline in the proportion of women up to the age of 27 years diagnosed with warts: the decline to the end of 2009 was greater among Australian resident women (60%) than traveling/migrant women (47%, $p < 0.001$). There was no decline in warts among women older than 27 years or among men who have sex with men ($p = 0.23$), while heterosexual men experienced a lesser (31%) but significant ($p < 0.001$) decline. By 2009, 75.3% of resident women up to 27 years, 29.7% of traveling/migrant women up to 27 years, and 18% of women over 27 years reported having had a previous quadrivalent or an unknown HPV vaccine.

Conclusions: High coverage by the vaccination program has had a large population-level impact on the incidence of genital warts in young Australian women. A more moderate

impact for heterosexual men has presumably resulted from herd immunity.

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Epidemiology & Public Health, Best Oral Presentation from an Emerging Country Participant

SECULAR TRENDS OF HPV DISTRIBUTION IN INVASIVE CERVICAL CANCER, COLOMBIA 1950-2000

Gustavo Hernandez-Suarez¹, Luis Eduardo Bravo², Nubia Muñoz¹

Background: Up to 70% of cervical cancer cases are attributed to HPV 16 and 18. This proportion is based on studies including only cases occurring in the last 2 decades. Scant information is available in Latinamerica on how much this proportion did change over time.

Aim: To assess the HPV type distribution in paraffin embedded tissue of cervical neoplasias diagnosed between 1950 and 2000 in the city of Cali, Colombia.

Methods: Paraffin blocks were retrieved from the pathology laboratory archives at the University Hospital in Cali. Information on age and year of diagnosis was collected. A highly sensitive method for HPV detection (i.e., SPF 10/LIPA25) was used for HPV genotyping. Four paraffin sections were obtained for each block. If diagnosis of cancer could be done in the first and last sections, in between sections were considered suitable for HPV/DNA testing.

Results: 564 squamous cell carcinomas (SCC) were retrieved and evaluated and age ranged between 22 and 90 years. 556 were considered as suitable for HPV/DNA testing. Out of these, only 425 (76%) tested HPV positive. This figure changed slightly over time, showing an upward trend since 1960s (**Figure 1**). Among HPV positives, HPV 16 was detected around 60% through all decades, while HPV18 was around 6% (**Figure 2**). Types 45 and 52 represented no more than 5% during all the study period. Only 7% of the infections were multiple over time.

Conclusion: Distribution of HPV 16 and 18 in SCC were as ex-

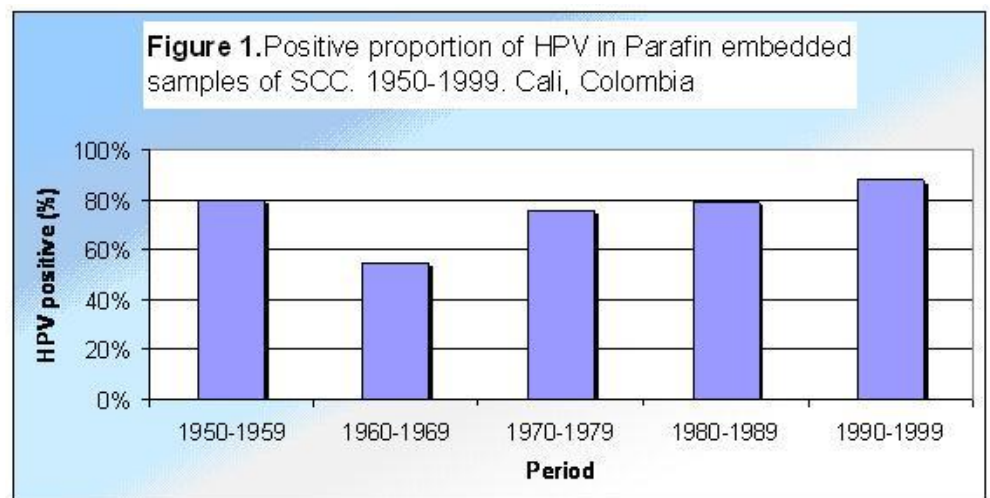
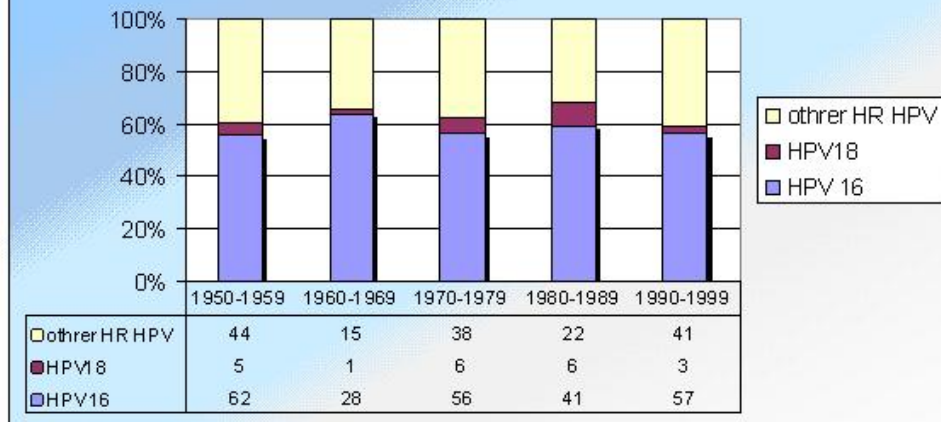


Figure 2. Cumulative proportion of HPV 16, 18 and other types in SCC tested HPV positive. Cali Colombia 1950-1999



pected and rather stable over time. Although almost 99% of blocks were considered suitable for HPV testing, the overall positive rate (76%) was lower than expected and changed over time. In the future, other parameters to evaluate suitability of paraffin blocks should be considered, regarding the relevance of HPV distribution in SCC after introduction of prophylactic HPV vaccines.

¹ Epidemiology Research Group, National Cancer Institute of Colombia, Bogota, Colombia; ² Cancer Registry of Cali City, Universidad del Valle, Cali, Colombia.

Epidemiology & Public Health, Best Poster Presentation from an Emerging Country Participant

P-800: PREDICTORS OF INVASIVE CERVICAL CANCER IN WOMEN BELOW 40 YEARS IN A SCREEN AND TREAT PROGRAM IN ZAMBIA

Sharon Kapambwe^{1,3}, Kristin King², Mulindi Mwanahamuntu^{1,3,4}, Carla Chibwesa^{3,4}, Groesbeck Parham^{1,3,5}, Krista Pfaendler⁶, Jeff Stringer^{1,5}

Background: Although cervical cancer typically occurs in women of middle and older ages, it may also affect reproductive age women. HIV-infected women are at increased risk of persistent HPV infection which is well established as the underlying etiology in the majority of cervical cancers.

Methods: We conducted a cross-sectional analysis on women enrolled in a “screen and treat” cervical screening program in Lusaka, Zambia. We examined predictors of invasive cervical cancer in a cohort of reproductive age women 40 years of age.

Results: Between January of 2006 and December 2008, 21,538 women were enrolled in our “screen and treat” cervical cancer prevention program and 21,010 underwent screening. The mean age of women screened was 33 years (SD ± 9.8). Seventy-eight percent of women were < 40 years old. We detected 266 cases of invasive cervical cancer; 157 (59%) were in < 40 years old. Among women < 40 years, 55% of those with invasive cervical cancer were HIV-infected while only 31% of those without invasive cancer were HIV-infected (p-value < 0.01). In an adjusted mul-

tivariate analysis, the odds of being HIV-infected were 2.5 times higher for women < 40 years with invasive cervical cancer compared to women < 40 years without cervical cancer (95% CI: 1.78, 3.59). This association was not observed in older women. Additionally, women < 40 years old with invasive cancer of the cervix had been sexually active for a longer time (mean 15 years; SD ± 4.98) and with a higher number of lifetime sexual partners (median 3; IQR 2) compared to women < 40 years old without cancer.

Conclusion: The risk of invasive cervical cancer appears elevated in HIV-infected women in their reproductive years. This finding underscores the importance of integrating

HIV testing and reproductive health services into cervical cancer prevention services.

¹ Centre for Infectious Disease Research in Zambia, Lusaka, Zambia; ² University of Michigan School of Public Health, Ann Arbor, MI, USA; ³ University Teaching Hospital, Ministry of Health, Lusaka, Zambia; ⁴ University of Zambia School of Medicine, Lusaka, Zambia; ⁵ University of Alabama at Birmingham School of Medicine, Birmingham, AL, USA; ⁶ Health Alliance, Cincinnati, OH, USA.

Epidemiology & Public Health, Best Overall Poster Presentation

P-748: COST FEASIBILITY STUDY OF FIVE CERVICAL CANCER SCREENING LABORATORY TECHNOLOGY MODELS IN MANITOBA

Linda DeRiviere¹, Shelley Stopera², Paul Van Caesele³, Robert Lotocki⁴

Background: The current diagnostic system in Manitoba for routine cervical screening is deteriorating rapidly and is unsustainable. Currently, traditional Pap tests are performed at seven private and public laboratories since there is no one facility for centralized testing.

Objective: To describe the costing framework (i.e., laboratory costs and savings categories) for the gradual implementation of five new cervical cancer screening technology models in Manitoba.

Methods: Using the tools of cost analysis, this study emphasizes a practical and program context-specific value for money technique, which focuses on the public budget aspects of health resource use. It outlines the steps and data requirements in conducting a costing exercise for health program managers with minimal experience in conducting cost analysis. The cost analysis builds on a baseline model of cervical screening, the traditional Pap test. The direct health care costs in the baseline model are compared to two newer technology platforms, liquid-based cytology (LBC) and HPV testing, which have been approved for use by Health Canada and the FDA. A cost feasibility technique illustrates the ways in which the various approaches to screening have cost implications.

Results: A key finding of the cost analysis is that the adoption of HPV testing as a primary screening model for women aged 30 and over, represents the most cost-savings strategy. LBC would be used for routine screening of women under 30 years of age and to triage women 30 and over whose results are HPV positive.

Conclusions: There are efficiencies to be gained by implementing the new high throughput technology platforms (i.e., HPV testing). The biggest cost savings result from reduced labor, reduced rate of colposcopy referrals and increased length of routine screening intervals. Cervical cancer incidence would be reduced by 50% using HPV testing/LBC screening model relative to conventional cytology.

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Epidemiology & Public Health, Best Poster Presentation by a Student

P-818: HPV TYPE REPLACEMENT: AN UNLIKELY PHENOMENON

Joseph E. Tota^{1,2}, Agnihotram Ramana-Kumar V¹, Francois Coutlee³, Luisa L. Villa⁴, Eduardo L. Franco^{1,2}

Objectives: An important concern regarding HPV vaccination is type replacement, i.e., the potential for the distribution of HPV types to change as a reflection of the vacated ecologic niches following the elimination of HPVs 16 and 18. Although no epidemiologic evidence exists to support this hypothesis, policymakers have used this argument to justify deferring deploying vaccination programs. We address the potential for HPV type replacement using data available from four of our studies conducted in Canada and Brazil.

Methods: HPV DNA testing and patient information came from: a) the Ludwig-McGill Cohort Study (n = 2,462), b) the Biomarkers Cervical Cancer Research (BCCR) study (n = 1,500), c) the HPV Infection and Transmission among Couples through Heterosexual activity (HITCH) cohort study (n = 503), and the Canadian Cervical Cancer Screening Trial (CCCaST) (n = 10,154). In all studies HPV DNA was assessed using the well-established PGMV PCR protocol coupled with the linear array method to permit genotyping of 37 genital types.

We evaluated the joint prevalence of HPV types 16 and 18 with 36 other HPV types using baseline and one-year period prevalence information. For each pair combination, we calculated the expected frequency of co-occurrence and compared this with the observed fre-

quency. If the Standardized Incidence Ratio (SIR) calculated as the observed/expected ratio was < 1 with an associated 95% CI that excluded the null value, this would suggest that the HPV type being considered should remain under suspicion for replacement.

Results: Most SIRs were > 1 for the 36 candidate types and none of the pair combinations with an SIR < 1 were statistically significant (95% CI included null). Baseline and period prevalence results were similar.

Conclusions: These preliminary results provide empirical evidence against the argument for type replacement.

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Capacity Building, Best Oral Communication

PREVENTING CERVICAL CANCER IN RURAL INDIA: A NGO/ TRUST COLLABORATION

Shobha Krishnan¹

India has the highest number of cervical cancer cases in the world. Nearly a quarter of all cases occurs in this country with about 130,000 women developing this disease annually and around 200 dying from it on a daily basis. Most women present for treatment in very advanced stages of the disease. However, early detection would decrease the number of advanced cervical cancer cases, reduce financial burden of treating advanced cases and minimize loss of life from this disease. Lack of knowledge, dysfunctional governments, poor infrastructure, and limited finances make progress in the foreseeable future questionable. Hence, creative models, where people empower people and societies help solve societies' problems, with or without help from the government, will also be necessary for successful control of this disease.

With these goals in mind, an innovative grassroots effort is being developed in a small remote district in Gujarat. With collaboration between an American-Indian physician, local NGOs, academic institutions and researchers/interns from the U.S., this program has been initiated to reduce the burden of cervical cancer. So far, six pairs - each composed of a dai (midwife) and one Health Worker - have been trained to conduct mass screenings with the

Name of Village	Nº of Women Tested	Nº of Positive VIA/ VILI	Nº of Women Returning to Gyn/ Camp	Nº of Biopsies	Results of Biopsy	Procedure Required
Bibbar	56	20	15	3	1 mild dyspl	Cryo
Vayor	25	8	5	1	0 dysplasia	
Janakpur	54	13	13	3	0 dysplasia	
Bada	9	2	2	0		
Bhojav	8	1	1	1	1 mild dyspl	Cryo
Bhadli	26	6	5	4	1 mild dyspl	Cryo
Vithon	22	4	4	2	2 mild dyspl	Cryo

VIA/ VILI. Additionally, each pair trains four more pairs of Health Workers and dais from neighboring villages. In this fashion, nearly 100 villages will be covered by the end of 2010. As facilities to initiate the «one stop screen and treat program» are not available, gynecological camps staffed by physicians are held every two months to treat women who test positive and appropriate treatment/referral instituted.

This project is designed for capacity building. The progress accomplished thus far, numerical and programmatic results, pros and cons of the current model and challenges and opportunities encountered in this low resource setting will be discussed in the presentation.

¹ Barnard Health Services, Columbia University, New York, NY, USA.



SELECTED ABSTRACTS **RESUMOS SELECIONADOS**

CERVICAL CANCER SCREENING IN DEVELOPING COUNTRIES JULY 6, 2010

Dr R. Sankaranarayanan, MD

Cervical cancer continues to be a major public health problem in low- and medium-resourced countries of sub-Saharan Africa, South and Central America and South and South East Asia due to lack of effective cervical cancer screening and prevention programmes. Cervical cancer is caused by persistent infection of the genital tract by one of the 15 or so cancer causing human papillomavirus (HPV) types. This knowledge has opened up new avenues of cervical cancer prevention such as preventing HPV infection by vaccination and identifying women with persistent HPV infection by HPV testing. Those with persistent HPV infection are at increased risk for developing precancerous changes in the cervix. A proportion of women with precancerous changes can progress to frank cervical cancer over a 5-20 year period. The early detection of the precancerous changes by screening followed by effective treatment can prevent progression of such changes to frank cervical cancer and thus prevent deaths from cervical cancer.

Approximately four fifths of the global burden of new cases (N = 530,000) and cervical cancer deaths (N = 275,000) are experienced in these countries and the absolute number of cases and deaths due to cervical cancer will register an increase in the future due to population growth unless effective control measures are initiated at the earliest and sustained in the long-term. There are effective cervical cancer screening tests such Pap smears, HPV testing and visual

screening using 3-5% acetic acid (VIA). Although repeated Pap smear screening, at 3-5 year intervals, of women above the age of 25 or 30 years and effective treatment of those with precancerous changes has largely been responsible for the 50-80% reduction in cervical cancer cases and deaths in the developed countries of Europe, North America and Australia, such programmes in many Latin American countries such Brazil, Argentina, Cuba and Mexico, among others, have had only very limited success in reducing cervical cancer cases due to the difficulties in covering large number of women with screening, poor quality of testing and inadequate coverage of women precancerous changes with treatment.

In recent years there has been significant decline in cervical cancer cases and deaths in countries such as Chile, Costa Rica, Singapore, South Korea, Hong Kong SAR and Taiwan due to reorganization of Pap smear screening and improvements in treatment facilities resulting in large number of women receiving Pap smears and treatment.

However, it is not feasible to introduce large scale Pap smear screening in many developing countries, particularly those in sub-Saharan Africa and South Asia, due to resource constraints. Recent research has shown that screening tests such as VIA and HPV testing can accurately detect precancerous lesions and can lead to reduced death rates from cervical cancer, implying these can be effective alternative tests to Pap smear. HPV testing seems to be a more promising approach as compared to Pap smear or VIA due to its objectivity (since the test is done mechanistically as opposed to the subjective human reading associated with Pap smear or VIA) and its higher accuracy in the early detection of biologically significant precancerous changes more likely to progress to cancer if untreated.

Although the currently available HPV testing methods may not be widely feasible in developing countries, a more rapid and affordable HPV test, suitable for use in developing countries, will

soon be available. Since effective treatment of precancerous changes is critical for preventing cervical cancer, strategies that combine screening tests, diagnosis and/or treatment of women with precancerous changes in the same day have been evaluated in developing countries and have been found to be safe, acceptable and effective. Strategies such as screening women aged 30-59 years or 35-49 years at least once in a life time, in order to cover a large proportion of eligible women, have been found to be effective in reducing cervical cancer cases and deaths in experimental, population-based studies. Political commitment and sincere investments in health services are essential to translate the encouraging research findings into effective public health policies and interventions in developing countries.

Cancer Screening Group, International Agency for Research on Cancer.

**THE NATURAL HISTORY OF PAPILLOMAVIRUS DISEASE:
FROM SILENT AND PRODUCTIVE INFECTIONS
TO NEOPLASIA AND LATENCY**

JULY 7, 2010
John Doorbar

Key Points

Papillomaviruses are widespread in the general population, but there are many different types. The different types cause different types of epithelial (skin) lesions and have different modes of transmission.

Most papillomaviruses cause only benign lesions such as warts. Many papillomavirus-associated lesions are not noticeable, and infected individuals may not realise they are infected. These benign lesions will usually regress as a result of the bodies natural immune response.

A small number of human papillomavirus types cause lesions that may progress to cancer, although in most individuals, these HPV types are cleared by the bodies immune response, just as for warts.

Progression to cancer can occur when a high-risk HPV type infects an epithelial site where its normal gene expression patterns are disrupted. A key problem site is the transformation zone of the cervix.

Our current thinking suggests that at these problem sites, certain viral genes are over expressed. The viral E6 and E7 genes are critical for cancer progression as their over expression affects normal cell regulatory pathways and leads to the accumulation of genetic errors and eventually to cancer.

When expressed at the 'correct' level and at the appropriate position in the epithelium, these viral proteins play an important role in ensuring that viral replication occurs in the upper epithelial layers. Although E6 and E7 are called 'oncogenes', they have an important role during productive virus synthesis that is nothing to do with cancer.

The idea that certain viral genes, when overexpressed, can predispose to cancer is well known in the field of Tumour Virus biology. HPV's are unusual in that this overexpression can occur in their natural host, albeit at particular sites.

We can now model the life cycle of many HPV types in the laboratory, and can study the productive life cycle of the virus as well a neoplasia and events leading to cancer. This work provides a rational basis for the selection of biomarkers for diagnosis and to improve cervical screening.

Our increasing understanding of the molecular mechanisms by which the virus subverts the normal differentiation program of the cell is starting to provide opportunities for the development of antivirals or other therapeutic interventions.

Additional work is needed to understand how HPV-associated lesions form and regress, and why they are sometimes persist either as active or latent infections. A clearer understanding of why some types are associated with cancers, while other types are not is needed. This will require strong links between scientist with different backgrounds, including molecular biologists, immunologists, clinicians and epidemiologists. Some such links do exist, but there is a need for continued effort to develop such interdisciplinary research in order to answer key questions in the field.

Summary: Human papillomaviruses complete their life cycle in the epithelium, but the different HPV types have different strategies depending on their mode of transmission and their epithelial tropism. In general, it is thought that lesion formation requires infection of an epithelial stem cell, which for the Beta viruses may be at the base of the hair follicle, and for high-risk Alpha types, may be the reserve cells that are involved in the formation of the cervical transformation zone.

Current thinking suggests that viral gene expression can vary depending on the nature of the infected cell, on epigenetic modifications within the viral genome, and on the presence of external signalling molecules such as growth factors, hormones and chemokines present in the extracellular milieu.

This can result in the over expression of viral genes involved in cell cycle entry and cell proliferation, and the accumulation of chromosomal abnormalities and cellular mutations that predispose to the development of cancer. The high-risk Alpha HPV types are associated with the majority of cancers at cervical and anal transformation zone sites, as well as other sites such as the tonsils and at the base of the tongue, with certain high-risk types being more problematic than others.

Under certain circumstances, Beta HPV infections are also associated with de-regulated patterns of gene expression and predispose to the development of skin cancer in some individuals. Most HPV infections do not however lead to cancer. In these cases, the virus expresses its genes in a defined order and at appropriate levels as the infected cell is pushed through S-phase-like and G2-like-phases before entering into true differentiation towards the epithelial surface.

These changes in cellular environment regulate and coordinate viral and cellular gene expression at the level of transcription and translation, and regulate protein function through post-translational modification, leading eventually to virus assembly and release at the epithelial surface. Productive infections are usually cleared by the immune system, which appears to shut down viral gene expression, possibly through cytokine signalling, allowing viral genomes to persist at low copy number in the basal layers. Latent infections

following immune regression, and silent infection following low titre infection represent may be activated under certain circumstances which are not yet well understood.

National Institute for Medical Research, Mill Hill, London, United Kingdom.

MOLECULAR BASIS OF PV ONCOGENICITY JULY 7, 2010

Magnus von Knebel Doeberitz

- Certain types of human papilloma viruses (HPV) can cause cervical and various other cancers. Two genes (E6 and E7) of these viruses were found to be responsible for its cancer causing role and it is quite well understood how they work.

- HPV-Infections are wide spread in the genital tract among men and women. They initially cause only minor epithelial benign lesions. These early lesions produce progeny virus (we therefore refer to them as productive HPV infections). They usually regress spontaneously in most cases.

- Virus production in these lesions requires maturing squamous epithelial cells. Although the virus infects the deeper layer of the epithelium, there is no virus production in the more immature deeper layers of the genital epithelium, whereas there is active production of the virus in the more matured superficial squamous cells. Thus, the differentiation of the infected epithelial cells is important for the multiplication of HPVs. This requires a well orchestrated expression of distinct viral genes during the maturation of the epithelial cells. Our research now elucidated for the first time, that the DNA of the virus is chemically modified (i.e., adding or removing methyl-groups to the DNA) during the epithelial maturation process, thereby helping to orchestrate the expression of the right genes in the right order epithelial cells. This includes a well balanced expression of the cancer causing genes E6 and E7.

- In rare instances this orchestrated order of alterations of the DNA in a particularly sensitive part of the viral genome may fail. This can result in strong activation of the cancer causing genes (E6 and E7) in immature basal epithelial cells. If this happens, the affected cells start to proliferate and to grow out into pre-neoplastic lesions (high grade dysplasia, high grade cervical intraepithelial neoplasia (CIN)) that often to progress to frank cancers if they are not treated early enough. These “transformed” cells strongly express a cellular biomarker referred to as p16^{INK4a}, that is now a well accepted biomarker to identify these dangerous HPV transformed cells.

- Taken together the data presented at this conference for the first time reveal a molecular mechanism that controls the complex and well orchestrated order of expression of viral genes during the normal HPV life cycle. If this mechanism fails and the viral oncogenes E6 and E7 become released from that molecular control “HPV infected cells” shift into “HPV-transformed cells” that are characterized by strong overexpression of the biomarker p16^{INK4a}. The understanding of the chemical nature of the involved regulatory control processes now allow to develop new drugs that interfere with the chemical modification of the viral DNA and that may allow to treat HPV-induced lesions including cancer without necessarily requiring surgical excision.

Department of Applied Tumor Biology, Institute of Pathology, University of Heidelberg.

SEXUAL BEHAVIOUR AND HPV INFECTION: WHAT'S SEX GOT TO DO WITH IT? JULY 8, 2010

Ann Burchell

Abstract: The sexually-transmitted nature of mucosal genotypes of human papillomavirus has been well documented in countless studies. A ubiquitous finding is that the risk of HPV infection is greater among persons with multiple sex partners. However, many details about the precise role of sexual behaviours for HPV transmission remain unresolved. In this presentation, I will discuss current theories and evidence for the importance of sexual networks and partner effects on HPV transmission on the population level (i.e., how HPV enters a partnership in the first place). Next, I will explore the contribution of specific sexual activities on transmission (i.e., how HPV transmits from one partner to the other and from one anatomic site to the other). Finally, I will discuss the influence of time on the relation between sexual behaviour and transmission, including the duration of the sexual partnership and evidence for re-infection through sexual exposure to an infected partner. The latter is particularly important for our understanding of the re-appearance of HPV in older women, and whether this represents true re-acquisition or reactivation of a previously latent infection. Gaps in evidence will be highlighted and future directions for research will be suggested.

Main messages: How does HPV enter a partnership in the first place? The strongest predictor is the number of previous partners both partners have had. Theoretically, having more than one partner at a time and/or having short gaps between partners should also fuel HPV spread in a population. These influences might become even more important following the introduction of vaccination if coverage is low.

How does HPV transmit from one partner to the other and from one anatomic site to the other? Vaginal and anal intercourse are the most efficient routes of person-to-person transmission. Other non-penetrative sexual activities are probably less efficient, but plausible routes of person-to-person transmission; they may play a larger role in transmission between anatomic sites (e.g., genital to oral). Recent data suggest that auto-inoculation may also be important for site-to-site transmission.

How does time influence the relation between sexual behaviour and transmission? Recent data suggests that HPV transmits quickly between partners, and that reinfection with the same HPV type is possible.

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PSYCHOSOCIAL EFFECTS OF SCREENING JULY 8, 2010

Gregory D. Zimet

DST - J bras Doenças Sex Transm 2010; 22(2): 84-106

Key Points

HPV DNA testing is increasingly used as part of cervical cancer screening strategies. However, positive HPV test results often lead to:

- Shame, stigma, sense of isolation, anger at partner.
- Confusion about meaning of terms like “High-Risk HPV Positive”, fear of cancer, worries about future pregnancies.
- Negative psychosocial effects appear generally to decrease somewhat over time.

But, common elements underlying these reactions are poor understanding of HPV infection and inadequate communication by health care providers about test results

Health care providers can minimize the negative impact of an HPV diagnosis by encouraging questions, not minimizing distress, and taking time to explain clearly the meaning of such test results, including an emphasis on the high prevalence of HPV infection and the difficulty identifying when infection first occurred.

Conclusions: Both case example and research identify frequent negative psychosocial sequelae to testing positive for HPV, including confusion, anger, anxiety, shame, concerns about fertility, worries about cancer, and mistrust of sexual partners. Common elements underlying these reactions are a poor understanding of HPV infection and inadequate communication by health care providers about test results.

Implications: The availability of HPV vaccines and the attendant publicity and provision of information about HPV and vaccination has led to increases in knowledge. However, relatively poor understanding about HPV infection persists among both women and men. In order to minimize negative psychosocial reactions to a positive HPV test, health care providers will need to take time to explain clearly the meaning of such test results, including an emphasis on the ubiquity of HPV infection and the difficulty identifying when infection first occurred. Given the high level of complexity associated with HPV-related information, future research will need to identify effective strategies for information delivery.

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HPV 6, 11, 16, AND 18 IMMUNOGENICITY OF FOUR MULTI-VALENT HPV VACCINE FORMULATIONS IN NON-HUMAN PRIMATES

Christine C. Roberts¹, Michelle K. Brownlow¹, Judith F. Smith¹, Rose Kowalski¹, Ryan Swoyer¹, Janine T. Bryan¹

Preclinical studies in non-human primates were performed to evaluate the effect of various formulations of HPV vaccines containing VLPs from four, eight or nine different HPV types on HPV 6, 11, 16 and 18 type-specific immunogenicity. The quadrivalent (HPV 6/11/16/18), 8-valent (HPV 6/11/16/18/31/45/52/58) and two variations of a 9-valent (HPV 6/11/16/18/31/33/45/52/58) recombinant HPV L1 VLP vaccine produced in yeast and formulated with a proprietary amorphous aluminum hydroxyphosphate sulfate adjuvant (AAHS) were administered by intramuscular injection into non-human primates at Day 0, week 8 and week 24. The total

amount of VLPs received per dose for the quadrivalent group was 24 ug; the 8-valent group: 40 ug; and the two 9-valent groups: A, 44 ug and B, 54 ug. Serum samples were collected and evaluated for Day 0 and Weeks 4, 8, 12, 24, and 28. Immunogenicity was assessed by competitive Luminex Immunoassay (cLIA) for the 4 HPV types common to all administered vaccines. All animals seroconverted to HPV 6, 11, 16 and 18 after the first immunization and mounted an anti-HPV response in a typical prime/boost response. The highest titers were typically reached post-dose three. The HPV 6, 11, 16 and 18 type-specific antibody titers reached by the quadrivalent, 8-valent and both formulations of 9-valent HPV VLP vaccines were, in general, within one log of one another with overlapping confidence intervals and considered comparable. The level of antibody response required for protection from HPV infection and disease is currently unknown. However, these data support the hypothesis that increasing the number of different VLP antigens from quadrivalent to 8- or 9-valent will continue to elicit similar levels of type-specific neutralizing antibodies which will provide HPV protection from infection and disease related to the vaccine HPV types in human clinical trials.

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CHIMERIC HPV 16L1-16L2 VIRUS-LIKE PARTICLES (RG1-VLP) CAUSE ENDURING CROSS-NEUTRALIZATION ANTIBODIES AGAINST MUCOSAL AND CUTANEOUS HPV

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Licensed HPV L1 VLP vaccines induce persisting, high-titer and primarily type-restricted antibody responses and protection. In contrast immunization with several L2 peptides can induce low-titer cross-neutralizing antibodies. Specifically the highly conserved motif HPV 16L2 aa 17-36 (RG1) has been characterized as a broad-spectrum cross-neutralization epitope. We have previously generated genetically engineered chimeric HPV 16 L1 VLP that display the RG1 epitope within the DE-surface loop of L1 (RG1-VLP). In pseudovirion-based assays antisera raised against RG1-VLP showed high-titer neutralization to HPV 16 (similar to wt HPV 16 L1 VLP vaccination), and in addition broad cross-neutralization to mucosal high-risk HPV 18 / 31 / 45 / 52 / 58, mucosal low-risk HPV 6 / 11, and beta-skin HPV 5 (titers 50-100,000). Cross-neutralization was induced in rabbits and mice using Alum-MPL as adjuvant, which is applicable for human use.

The objective was to more completely characterize the spectrum and robustness of L2-based cross-neutralization. Using newly established pseudovirion assays, rabbit antisera to RG1-VLP additionally cross-neutralized mucosal high-risk HPV 33, 68, 76, alpha-skin type HPV 3, and HPV 32 causing Heck's disease (titers 50 to 1,000). Assays for additional skin-HPV of genus alpha, gamma and mu are currently being established. To determine neutralization titers over time, two rabbits were immunized with RG1-VLP plus Alum-MPL at 0, 4, 6, 8 weeks and sera analyzed 10 months following the third boost. Similar to the decline of 16L1 antibodies, L2-mediated cross-neutralizing antibodies were detectable with a

10 to 100-fold decrease of titer. Importantly, additional RG1-VLP boost at month 10 increased titers to former levels at the minimum. These data indicate a functional B-cell memory response to the cross-neutralization epitope RG1. Immunization with chimeric HPV 16L1-RG1 VLP in adjuvant applicable for human use can induce long-lasting broad-spectrum antibody responses to mucosal high-risk, low-risk and divergent cutaneous alpha and beta papillomaviruses.

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HPV 16/18 L1 VLP VACCINE INDUCES CROSS-NEUTRALIZING ANTIBODIES THAT MAY MEDIATE CROSS-PROTECTION

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Neutralizing antibodies are thought to be the primary immune mechanism for the prophylactic HPV 16/18 L1 VLP vaccine-induced protection against persistent HPV 16/18 infection. Though efficacy studies have demonstrated partial cross-protection against non-vaccine, related HPV types, these studies have not shown that vaccination generates antibodies against cross-reactive HPV types, or that antibodies are the correlates of protection against infection.

Here, we evaluated neutralizing antibody responses to HPV types related to HPV 16 (HPV 31, HPV 52, and HPV 58) and HPV 18 (HPV 45), as well as a control (BPV) in sera from a subset of 50 HPV 16/18 VLP vaccinated women from the NCI-sponsored Costa Rican Vaccine Trial, collected at Month 0, Month 1, and Month 12 after vaccination. Antibody titers were determined using a pseudovirion (PsV)-based neutralization assay (SeAP). We assessed the correlation between anti-HPV 16/18 antibody titers induced by vaccination and respective cross-related HPV types. HPV 16/18 L1 VLP vaccination induced cross-neutralizing titers for HPV types for which evidence of vaccine efficacy has been demonstrated (HPV 31/45) but not for other types (HPV 52/58).

In addition, HPV 31/45 cross-neutralizing titers showed a significant increase with number of doses (HPV 31, $p < 0.001$; HPV 45, $p < 0.001$) and correlated with HPV 16/18 neutralizing titers, respectively. This is the first demonstration that sera from individuals vaccinated with the HPV 16/18 Cervarix (TM) vaccine can cross-neutralize two phylogenetically-related HPV types (HPV 31 and HPV 45) for which vaccine efficacy has been demonstrated but not other types for which Cervarix (TM) vaccination does not appear to confer protection. Our findings suggest that cross-neutralizing antibodies are part of the effector mechanism in the recently reported cross-protection observed for the HPV 16/18 L1 VLP vaccine. This project has been funded in whole or in part by the NCI Intramural Research Program, the National Institutes of Health Office for Research on Women's Health (ORWH), and with federal funds from the National Cancer Institute, National Institutes of Health Contract No. HHSN261200800001E.

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L2 VACCINE-INDUCED ANTIBODIES HAVE GREATER POTENCY IN VIVO THAN PREDICTED FROM IN VITRO ASSAYS

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L2-based vaccination typically induces lower levels of in vitro neutralizing antibodies than L1 VLP vaccination, yet both vaccines can induce strong protection from experimental challenge. In fact, in our murine cervicovaginal challenge model, we observed complete protection from HPV pseudovirus challenge after L2 vaccination in animals whose sera contain no measurable neutralizing antibodies, as determined in a standard in vitro pseudovirus-based neutralization assay. To assess whether antibody-independent mechanisms contributed to protection after L2 vaccination, we passively transferred rabbit hyper-immune sera raised against an L2 vaccine composed of the amino acids 11-88 of HPV types 1, 5, 6, 16 and 18 into naïve mice and challenged them with HPV 16 and HPV 45 pseudoviruses. Strong protection was observed against both types. Microscopic examination of capsids in the genital tract indicated that they initially bound the basement membrane (BM) at sites of trauma but that the passively transferred antibodies prevented translocation to the epithelium. Indistinguishable results were obtained in L2-vaccinated mice, establishing that antibody-mediated mechanisms were responsible for protection following L2 vaccination. Interestingly, protection after passive transfer was observed with substantially lower doses of L2 antibodies than L1 VLP antibodies, relative to in vitro neutralizing titers. We speculate that in vitro neutralizing assays underestimate the protective potential of L2 vaccines because in cultured cells both the heparan sulfate primary receptor and the secondary receptor responsible for capsid internalization are on the cell surface; their proximity provides only a brief opportunity for L2 neutralization after exposure of the L2 epitope prior to endocytosis. In vivo, because L2 exposure occurs on the BM, rather than on the cell surface, there is ample opportunity for L2 antibodies to bind capsids and inhibit transfer to the keratinocyte, thereby preventing infection.

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TRIAGE OF PAP NEGATIVE, HPV POSITIVE SCREENING TEST RESULTS USING p16/Ki-67 DUAL-STAINED CYTOLOGY

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Background: Co-testing for Pap cytology and HPV has been proposed as an approach to increase the sensitivity for the detection of high-grade CIN (HGCIN) in women aged 30 years and older. To increase sensitivity over Pap cytology testing, HPV co-testing requires the follow-up of women tested negative for Pap abnormalities, but positive for HPV.

Objectives: As the amount of underlying HGCIN within the group of Pap negative, HPV positive results still is relatively low, we analyzed the potential of a new immuno-cytochemical dual staining approach that detects the co-localization of p16 and Ki-67 expression as an indicator of cell cycle deregulation, to efficiently triage Pap negative, HPV positive screening results.

Methods: Out of a group of 7, 976 women enrolled into a prospective cervical cancer screening study for Pap cytology and HPV co-testing in 2007/2008 in the Wolfsburg/Germany area, a total of 427 Pap negative, HPV positive cases were retrospectively analyzed for the presence of cells showing immuno-cytochemical co-expression of both p16 and Ki-67. Positive test results were correlated to the presence of HGCIN confirmed during colposcopy and histology follow-up.

Results: 109 out of 427 (25.5%) of Pap negative, HPV positive women tested positive for the p16/Ki-67 Dual stain. Sensitivity of baseline Dual stain testing for the detection of CIN2+ during follow-up (mean follow-up time period of > 12 months) within the group of Pap negative, HPV positive women was 91.9% for CIN2+ (34/37 cases), and 96% for CIN3+ (27/28 cases). Negative p16/Ki-67 Dual stain test results at baseline had a negative predictive value of 99.1% for the development of HGCIN during the study follow-up period.

Conclusion: The detection of co-localization of p16/Ki-67 expression in cervical cells identifies women that may benefit from immediate colposcopy, whereas negative Dual stain test results may exclude HGCIN with a high NPV.

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CLINICAL PERFORMANCE OF A BROAD-SPECTRUM RNA ASSAY IN A HIGH-PREVALENCE DISEASE SETTING

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Objectives: Detection of oncogenic E6/E7 transcripts may be a better predictor of clinically significant cervical HPV infection compared with DNA based assays. However more clinical data are necessary to evaluate this. Consequently, we present an evaluation of the clinical sensitivity and specificity of the APTIMA® HPV RNA based assay (AHPV, Gen-Probe Incorporated) in comparison to the Hybrid Capture 2 DNA based assay (HC2, Qiagen Ltd) in a clinical setting.

Methods: Women attending two NHS colposcopy clinics (for all indications) in two city hospitals in the UK were invited to participate. Liquid based cytology (LBC) samples were collected and tested via the 2 HPV assays described. Biopsies were taken where clinically indicated and relative sensitivity and specificity of each assay for disease (defined as CIN2 or worse) were calculated.

Results: Approximately 1, 500 women have been recruited to the study so far. At time of abstract submission, 1008 LBC samples

have been tested by both assays and have associated, confirmed pathology results. A total of 276 cases of CIN2+ were detected and sensitivity and specificity of the AHPV for CIN2+ were 93.8%, (95% CI of 90.3, 95.4) and 49.9%, (95% CI of 46.2, 53.5) respectively. By comparison, sensitivity and specificity of the HC2 for CIN2 or worse were 94.2%, (95% CI of 90.8, 96.7) and 44.7%, (95% CI of 41.0, 48.4) respectively. When analysis was confined to women referred to colposcopy for cytologically defined low grade disease the sensitivity and specificity of the two assays were equivalent.

Conclusions: These preliminary data suggest that AHPV assay shows equivalent sensitivity and slightly higher specificity for the detection of CIN2 or worse (in a population with a high prevalence of disease). Further data including performance of the assays within a post treatment subset will be presented (with associated 95% CI's).

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IMPACT OF PERFORMING MULTIPLE BIOPSIES ON THE DETECTION OF PRECANCER IN THE NCI-OUHSC BIOPSY STUDY

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Background: A single colposcopic examination detects only 50-60% of prevalent precancers but taking multiple biopsies increases the sensitivity.

Objectives: We created the NCI-OUHSC Biopsy Study to systematically evaluate the benefit of taking multiple biopsies and to study cervical precancer on the lesion level.

Methods: Previously-untreated women referred to the University of Oklahoma colposcopy clinic for abnormal screening results were eligible for participation. Before colposcopy, a specimen was taken for liquid-based cytology, HPV genotyping, and biomarker studies. During colposcopy, a digital image of the cervix was taken and annotated for observed lesions and biopsy sites. Up to four biopsies were taken from distinct lesions; if less than four targeted biopsies were obtained, a random biopsy was added. All biopsies were ranked by severity based on visual impression and evaluated individually in histology.

Results: To date, 337 women have been enrolled in the study; 248 with histological results available. As worst diagnoses, 26/248 women (10.5%) had CIN3, 72 (29.0%) had CIN2, 82 (33.1%) had CIN1, and 68 (27.4%) had benign changes or normal results. 199/337 women (59.1%) had four, and 280/337 women (83.1%) had at least three biopsies. 47/335 women (14.0%) with a low grade or benign colposcopic impression had CIN2+ in one of the biopsies. Conversely, 61/335 (18.2%) with a high grade colposcopic impression had < CIN2 in their worst biopsy result. In 66.7% of women with CIN3, the worst lesion was detected in the first biopsy, in 25.9% it was found at the second

biopsy and in 7.4% it was detected in the third or fourth biopsies. **Discussion:** We confirm the limitations of colposcopy even if performed at highest standards with extensive quality control. We demonstrate that taking multiple biopsies substantially increases the detection of CIN3. Future analyses will use ROC-type methods to explore the optimal number of biopsies.

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CERVICAL CANCER EPIDEMIOLOGY AMONG HIV-INFECTED WOMEN IN NORTH AMERICA

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Background: Initial studies suggest immunosuppression may be associated with the increased rates of pre-cancerous cervical lesions observed in HIV-infected compared with HIV-uninfected individuals. To characterize the incidence of cervical cancer among HIV-infected women in the HAART-era, we examined data from the NA-ACCORD HIV cohort collaboration of IeDEA. **Materials and Methods:** This analysis includes data from 13 North American cohorts of HIV-infected women that collected clinically confirmed or cancer registry-linked data on invasive cervical cancer. Cervical cancer-free women were followed from the first HAART-era CD4+ measurement until the earliest of: cancer diagnosis, lost to follow-up, death, or 2007. Incidence rate overall, by calendar period, and by first CD4+ cell count after 1995 (baseline) were standardized for age using the 2000 US standard population. **Results:** Among the 16,467 HIV infected women free of disease at baseline, 102 cases of invasive cervical cancer were reported, yielding an age-standardized incidence rate of 114 per 100,000 person-years (95% CI: 88-139). Among 13,716 HIV-negative women free of disease in these cohorts there were 10 invasive cervical cancers for an incidence of 12.3 per 100,000 person-years (95% CI 6.6 -23), similar to the age-adjusted SEER population incidence of 8.2 per 100,000 person-years. In the HAART era the age-standardized cervical cancer incidence among HIV-infected women remained similarly high in 1996-99, and 2000-03, with a non-significant decrease in 2004-07 (133, 152, and 87 per 100,000 respectively). The age-standardized incidence rates increased with decreasing baseline CD4+ categories of > 350, 200-350 and <200 cells/ μ L (68, 113 and 185 respectively). There was no clear trend in incidence was not associated with age. **Conclusions:** In this large collaboration of North American HIV cohorts, cervical cancer incidence was ten-fold higher among HIV-infected than uninfected women. Stable rates over calendar time suggest improvements in HIV-therapies may have not impacted rates.

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PREVALENCE INCIDENCE AND PERSISTENCE OF GENITAL HPV INFECTION IN WOMEN BETWEEN THE AGES OF 25 AND 45

Xavier Castellsague¹

Background: A prophylactic quadrivalent HPV vaccine could benefit adult women if they are susceptible to incident genital HPV infections and are acquiring new infections with vaccine HPV types. This report presents baseline and prospective data from a randomized, double-blind, placebo-controlled trial of the safety, immunogenicity and efficacy of the quadrivalent HPV (type 6/11/16/18) vaccine in women ages 24 to 45.

Methods: We present the results of an epidemiologic analysis of 3,730 women enrolled in a quadrivalent HPV vaccine efficacy trial between 6/18/2004 and 4/30/2008. Subjects were enrolled from 7 countries (Colombia, France, Germany, Philippines, Spain, Thailand, and the United States) through community and academic health centers and primary health care providers.

Results: Average baseline prevalence of anogenital infection and/or seropositivity was 32.8% for ≥ 1 vaccine HPV types, and 0.3% for all vaccine HPV types (vaccine and placebo arms). Incidence of anogenital infection with any vaccine HPV type was $\sim 10.5\%$ (placebo arm). The rate of persistent infection was $\sim 5\%$ over a 30-month period among women in the placebo arm naïve to the relevant type at baseline. Predictors of incident infection included younger age, marital status other than first marriage, higher number of lifetime and recent sex partners and Chlamydia/gonorrhea infection at baseline.

Conclusions: These findings indicate that women up to age 45 could benefit from administration of the quadrivalent HPV vaccine, as they are acquiring anogenital infections with vaccine HPV types. Future study concerning incident and prevalent HPV infection among women over age 25 would be warranted.

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THE PSYCHOLOGICAL IMPACT OF ANAL CANCER SCREENING ON HIV+ MEN

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Background: Human papillomavirus is the sexually transmitted precursor for cervical and anal cancer. Anal cancer occurs at rates of up to 137/100,000 in HIV + men who have sex with men (MSM). Anal cancer screening is not currently standard of care for HIV + MSM in Australia, but is suggested in some guidelines. Studies of cervical screening have shown psychological impacts including increased anxiety, reduced quality of life and

diminished sexual well-being. There are no comparable studies in men. Therefore we initiated the first known study of the psychological impact of anal cancer screening on HIV + MSM. **Methods:** Self-report measures were used in a prospective, multi-wave longitudinal study of 291 men undergoing anal screening. Using previously validated methods, we determined baseline psychological well-being (worry, depression, anxiety, stress and sexual interest) and changes to well-being after cytological and histological sampling procedures and receipt of results. **Results:** Three groups were identified. The "Low Threat" group received cytology results of negative or LSIL and no further investigation. Both the "Reassured" and "High Threat" groups received ASC-H, ASCUS or HSIL cytology results and were referred for High Resolution Anoscopy (HRA). At HRA, the Reassured group were either not biopsied (negative HRA) or were given LSIL histology results. The High Threat group received histology results of HSIL. The Reassured and High Threat groups differed significantly to the Low Threat group in four areas: more worry about cancer, worse anal and future health and less interest in sex. These results are similar to cervical studies. **Conclusion:** Men were psychologically impacted by the anal cancer screening processes, particularly in terms of worry about cancer, both by referral to and results from HRA investigation. The introduction of any screening program needs to consider the psychological impact of the procedures and offer appropriate psychosocial support.

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RISK OF CERVICAL AND VULVAR CANCERS ASSOCIATED WITH VARIATION IN IMMUNE RESPONSE AND HPV RELATED GENES

Margaret Madeleine¹, Lisa Johnson¹, Mari Malkki¹, Denise Galloway¹, Effie Petersdorf¹, Stephen Schwartz¹

Variation in genes related to immune response (CD28, CTLA4, and ICOS) and HPV susceptibility or mechanism (EVER1/2, ERAP1, TERT, UBE2C, and UBE3A) may affect development of HPV-related cancers. We examined the risk of cervical and vulvar cancers in case-control studies conducted in Seattle. DNA from women with cervical (n = 1,000) and vulvar cancer (n = 545) and population-based controls (n = 951) was genotyped for tagging SNPs. Odds ratio (OR) estimates under a log-additive model for minor alleles were calculated from age-adjusted logistic regression. Increased risk of cervical cancer was associated with CD28 rs10932017 (OR 1.2, 95% CI 1.1-1.4) and both cervical and vulvar cancers were associated with rs3181096 (OR 0.9, 95% CI 0.7-1.0). Vulvar cancer was associated with CTLA4 rs733618 (OR 0.8, 95% CI 0.6-1.0). Several ERAP1 variants were associated with cervical adenocarcinoma: rs998509 (OR 1.3, 95% CI 1.1-1.6), rs3734016 (OR 1.5, 95% CI 1.1-2.0), and rs27639 (OR 1.3, 95% CI 1.1-1.7), rs12517900 (OR 0.7, 95%

CI 0.5-0.9), and rs149078 (0.8, 95% CI 0.7-1.0). In contrast, a different set of SNPs in ERAP1 was associated with vulvar cancer, including a non-synonymous coding SNP rs10050860 (OR 1.3, 95% CI 1.1-1.6), rs2032890 (OR 1.3, 95% CI 1.1-1.5), rs27039 (OR 0.8, 95% CI 0.7-1.0), rs27619 (OR 0.8, 95% CI 0.7-1.0), 3'UTR rs28096 (OR 1.2, 95% CI 1.0-1.4), and 3'UTR rs7063 (OR 1.3, 95% CI 1.1-1.5). For TERT, risk of vulvar cancer was associated with rs2736100 (OR 0.8, 95% CI 0.7-1.0), rs2736118 (OR 1.2, 95% CI 1.1-1.5), rs2853690 (OR 1.3, 95% CI 1.1-1.6), rs33961405 (OR 0.8, 95% CI 0.7-1.0), and rs7726159 (OR 0.8, 95% CI 0.7-1.0). There were no significant associations with tagSNPs in the EVER1/2, ICOS, UBE2C, or UBE3A genes. Our data suggest that common variants of genes important in immune regulation and HPV-related processing, or genes in linkage with them, may influence risk of HPV-related cancer.

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A NATIONAL OUTCOME FOR QUADRIVALENT HPV VACCINATION: DECLINING RATES OF GENITAL WARTS IN AUSTRALIA

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Background: The quadrivalent human papillomavirus (HPV) vaccine has demonstrated high efficacy in clinical trials but no reports to date have described the vaccine's effect at a national level. From mid-2007 Australia was among the first countries to fund a universal vaccination program for all females between 12 and 26 years and coverage rates of ~80% have been achieved. **Objectives:** We established a national surveillance network to determine trends in clinical presentations for genital warts. **Methods:** Eight sexual health services located around Australia provided data on all new patients (n = 110,073) between 2004 and 2009. Data were collected on new diagnoses of genital warts (n = 6, 387), demographics, sexual behaviour, and HPV vaccination status. **Results:** Up to mid-2007, when the vaccination program began, there had been a gradual increase in the proportion of new patients diagnosed with genital warts. Thereafter there was a marked and ongoing decline in the proportion of women up to the age of 27 years diagnosed with warts: the decline to the end of 2009 was greater among Australian resident women (60%) than traveling/migrant women (47%, p < 0.001). There was no decline in warts among women older than 27 years or among men who have sex with men (p = 0.23), while heterosexual men experienced a lesser (31%) but significant (p < 0.001) decline. By 2009, 75.3% of resident women up to 27 years, 29.7% of traveling/migrant women up to 27 years, and 18% of women over 27 years reported having had a previous quadrivalent or an unknown HPV vaccine. **Conclusions:** High coverage by the vaccination program has had a large population-level impact on the incidence of genital

warts in young Australian women. A more moderate impact for heterosexual men has presumably resulted from herd immunity.

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INCREASING BURDEN OF HPV – ASSOCIATED ANAL CANCER IN AUSTRALIA

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Background: Given current clinical trials investigating the prophylactic efficacy of vaccination against HPV 16/18 anal cancer precursors in males, there is a need to quantify the projected burden of anal cancer. In this study we analyse anal cancer incidence and survival in the Australian population, with a focus on the squamous cell histological types, over 90% of which are attributable to HPV 16/18.

Methods: Data were obtained from the Australian National Cancer Statistics Clearing House databases including counts and incidence of squamous cell carcinomas and squamous cell variants of the anus, anal canal and rectum (anal SCC) and anal adenocarcinoma (anus and anal canal) from 1982–2005, and five year relative survival of all anal cancer from 1982–2004.

Results: From 2000–2005 there were ~270 cases of invasive anal cancer per year with annual age-adjusted incidence of 1.35 (95% CI 1.28-1.41)/100,000. SCC was the most common histology - 74% vs. 22% for adenocarcinoma. SCC incidence was higher in females than males (1.10 vs. 0.88/100,000), but adenocarcinoma incidence was lower (0.25 vs. 0.37/100,000). From 1982 - 2005 there was a significant increase in the incidence of SCC, more pronounced in males (3.4% pa 95% CI: 2.5-4.3) than females (1.9% pa 95% CI: 1.2-2.6), compared to a borderline increase in anal adenocarcinoma incidence in males and none in females. Five year relative survival from anal cancer improved from 59% in 1982–1988 to 68% in 1997 - 2004. Survival was higher in females than males (73% vs. 61%).

Conclusion: There has been a marked increase in incidence of HPV-associated anal carcinoma, particularly in males, over the past 25 years in Australia. Projections in anal cancer burden will need to consider changes in behavioral risk factors, the impact of the current female HPV vaccination program and the contribution of MSM.

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PAPILLOMAVIRUS INFECTION REQUIRES γ SECRETASE

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The mechanisms by which papillomaviruses breach cellular membranes and traffic to deliver their genomic cargo to the nucleus are poorly understood. Here we show that infection by a broad range of papillomavirus types requires the intramembrane protease γ -secretase. The γ -secretase inhibitor XXI inhibits infection in vitro by all types of papillomavirus pseudovirions tested (HPV 16, HPV 18, HPV 31, HPV 45, HPV 58 and CRPV) with IC_{50s} of 130-500pM, and regardless of reporter construct. Vaginal application of XXI prevents infection of the mouse genital tract by HPV 16 pseudovirions. Conversely, XXI does not inhibit in vitro infection by pseudovirions derived from the polyomaviruses BK or Merkel Cell polyomavirus. Many γ -secretase substrates are pre-cleaved by sheddases prior to intramembraneous digestion. The γ -secretase inhibitors batimistat, marimistat and TAPI-0 failed to prevent HPV infection in vitro. Nicastrin, APH-1 and Presenilin-1 are essential components of the γ -secretase complex, and mouse embryo fibroblasts deficient in any one of these components were not infected by HPV 16, whereas wild type and γ -secretase (BACE1)-deficient cells were susceptible. Neither the uptake of HPV 16 nor the disassembly of capsid to reveal a buried L1 epitope and BrdU-labeled encapsidated DNA are dependent upon endosomal acidification or γ -secretase activity in HaCaT cells. However, blockade of either endosomal acidification or γ -secretase activity by ammonium chloride or XXI respectively prevent the BrdU-labeled DNA encapsidated by HPV 16 from reaching the ND-10 subnuclear domains. Since L2 is critical for endosomal escape and targeting of the viral DNA to ND-10 and γ -secretase is located in endosomal membranes, our findings suggest that either L2 or a cellular protein involved in infectious entry must be cleaved by γ -secretase for the escape of the papillomavirus L2/genome complex from the endosome and trafficking of the L2-nucleohistone complex to the nucleus.

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GENOTYPE-SPECIFIC NATURAL HISTORY OF ORAL HPV INFECTIONS AMONG YOUNG MOTHERS FOLLOWED-UP FOR 6-YEARS IN THE PROSPECTIVE FINNISH FAMILY HPV STUDY

Stina Syrjänen¹, Jaana Willberg¹, Jaana Rautava¹, Marjut Rintala², Kari Syrjänen³, Seija Grenman²

Background: Nearly nothing is known about the genotype specific natural history of oral HPV-infection.

Methods: In the Finnish Family HPV Study 329 pregnant women (mean age 25.5yrs) were enrolled at 3rd trimester, and followed-up for 6yrs (median 62.4mo). HPV-genotyping was done with Multimatrix® at each visit. The following outcome patterns of oral HPV-infections (n = 326) were defined: 1. always negative (n = 143), 2. incident HPV (n = 116), 3. type-specific persistence (n = 51), 4. non type-specific persistence (n = 12), 5. fluctuation (n = 33), and 6. clearance = transient (n = 76). Predictors of type-specific acquisition, clearance and persistence of species 7/9-genotypes were determined by generalized estimating equation models.

Results: HPV-detection varied from 15% to 24% during the follow-up. The following single-type infections were found: HPV 6, 11, 16, 18, 45, 56, 58, 59, 66, 70. HPV 16 was the most prevalent, followed by HPV 6, 56, 58 (Figure 1). Multiple-type infections with species 9 were most common, followed by species 6, 10 and 7. Clearance rate was much higher for LR- than HR-types (CR 227-400/1,000 wmr versus 48-60/1,000 wmr). The clearance of species 7/9 was predicted by age, history of atopic reaction, history of STD and second pregnancy. The mean time to clearance for HPV 6 and 11 was shorter than that for HPV 16, 58, 59, 4.6-2.5mo versus 18.8-37mo, respectively. HPV 16, multiple-type infections and HPV 6 persisted most frequently with a mean time of 18-20mo. The predictors of persistent oral HPV infection with the species 9 were mothers being seropositive to LR-HPV s at baseline, use of oral contraceptives, consumption of alcohol and second pregnancy during FU (Figure 2).

Conclusion: HPV 16 and multiple types caused most frequent incident infections, were most likely to remain persistent and least likely to clear. The type spectrum in oral mucosa is more narrow than that reported in the genital tract, but LR-HPV s are more frequent. No association with oral sex was found. Predictors of oral HPV and genital mucosa might interplay in controlling the outcome of female HPV infection.

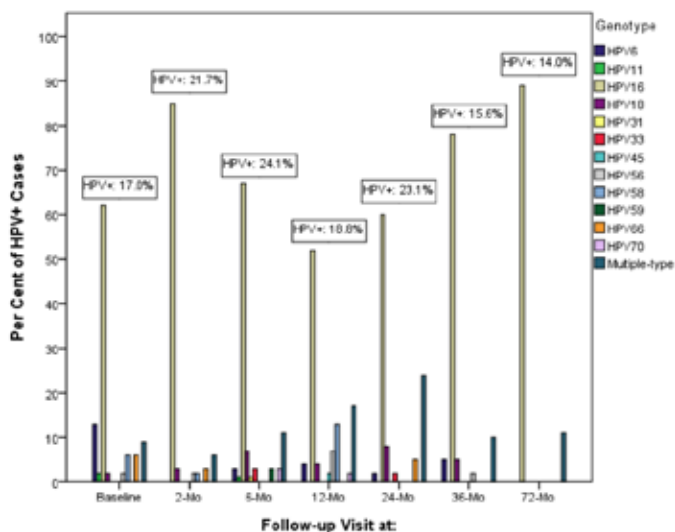


Figure 1 - Per cent distribution of oral HPV genotypes by FU-visits (boxes represent the total detection rate in women at that FU-visit).

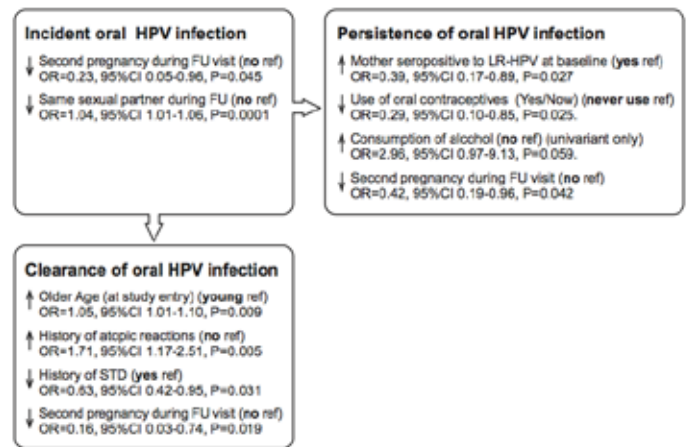


Figure 2 - Predictors of oral HPV infection and its outcome in women.

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VIRAL ONCOGENE EXPRESSION DEFINES OROPHARYNX CARCINOMAS WITH ACTIVE HPV 16 INVOLVEMENT

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Background: A subset of oropharyngeal squamous cell carcinomas (OPSCC) contains HPV 16 DNA and appears to have a better prognosis. However, this subset may be heterogeneous. To better define the group of HPV-driven tumors we included HPV RNA analysis in a large OPSCC series and evaluated the association of viral DNA and RNA status with clinical parameters.

Methods: Fresh-frozen tissues from 199 German OPSCC collected between 1990 and 2008 were subjected to semi-quantitative multiplex papillomavirus genotyping (MPG). HPV 16 DNA positive tumors were quantitatively analyzed for viral oncogene E6*II RNA expression by NASBA amplification and bead-based hybridization.

Results: HPV 16 DNA was present in 97/199 (48.7%) OPSCC, viral load was low (HPV⁺) in 61 (31.1%) and high (HPV⁺⁺) in 36 (18.4%) tumors. Prevalence of HPV 16 was highest in tonsillar carcinomas (60.7% vs. 41.1% in the other oropharyngeal subsites) and increased by 24.4% from 1990 to 2008. HPV 16 E6*II RNA was expressed in 35/37 (94.6%) HPV⁺⁺ tumors but only in 14/60 (23.3%) HPV⁺ tumors. Kaplan Meier analyses showed that viral load and even more viral oncogene RNA were associated with better overall survival (OS) and progression free survival (PFS; OS: p = 0.015 vs. p = 0.035; PFS: p = 0.007 vs. p = 0.030, logrank test). Oncogene RNA positive tumors were associated with never smoking and never drinking and with the absence of distant metastasis, but not with younger age.

Conclusion: This study demonstrates a pronounced

heterogeneity among HPV 16 DNA positive tumors and confirms that DNA genotyping alone is insufficient to define the role of the virus. OPSCC expressing oncogene HPV 16 RNA are truly HPV-driven tumors. OPSCC harboring HPV 16 DNA but negative for E6*II behave like the HPV negative tumors with regard to clinical parameters.

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HPV IN ORAL MUCOSA OF NEWBORN AND THEIR CONCORDANCE WITH HPV TYPES IN MOTHER'S CERVICAL SAMPLE TAKEN BEFORE DELIVERY

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Marjut Rintala², Kari Syrjänen³, Seija Grénman²,
Stina Syrjänen¹

Background: HPV DNA has been found in oral mucosa of newborn but the HPV genotype distribution study is incomplete.

Methods: In Finnish Family HPV Study, 324 pregnant women (median age 26 yrs; range 18-46) were enrolled at 3rd trimester (= baseline visit), and subjected to oral and cervical sampling for HPV testing. At delivery newborn's oral and cord blood samples as well as placental samples were collected. HPV-genotyping was done with Multimetrix® assay. Maternal risk factors predisposing newborn to test HPV-positive were evaluated, and concordance of HPV genotypes between mother's genital and newborn's oral samples was tested and analyzed.

Results: HPV was detected in 22.4% of the oral samples from the newborn and in 16.4% of the mothers' cervical samples. The point prevalence of HPV genotypes is given in **Figures 1 and 2**. In both mothers and newborns, HPV 16, multiple-type combinations and HPV 6 were the most prevalent types. In addition, HPV 45 was detected frequently in mothers. The species-specific concordance between the newborn and the mothers was almost perfect (weighted kappa = 0.988; 95% CI 0.951-0.997), and in pair-wise comparison at genotype level, the mother-newborn pairs were not significantly different (Wilcoxon signed ranks test, $p = 0.753$). Oral HPV carriage in newborn was most significantly associated with HPV detection in the placenta (OR = 13.9; 95% CI 3.7-52.2) and in umbilical cord blood (OR = 4.5; 95% CI 1.3-15.3). At genotype level, 4/5 HPV 6- and 5/7 HPV 16 infections were concomitantly present in the placental and oral samples, similarly as 2/5 HPV 6-, 3/5 HPV 16- and 1/1 HPV 39 infections were detected in the umbilical cord blood and oral mucosa.

Conclusion: HPV detection in oral samples of newborn at delivery is common. Genotype spectrum, however, is narrower when compared with maternal cervical samples shortly before delivery. The high-level maternal-newborn concordance leaves little doubt that infected mother transmits HPV to her newborn via placenta and cord blood.

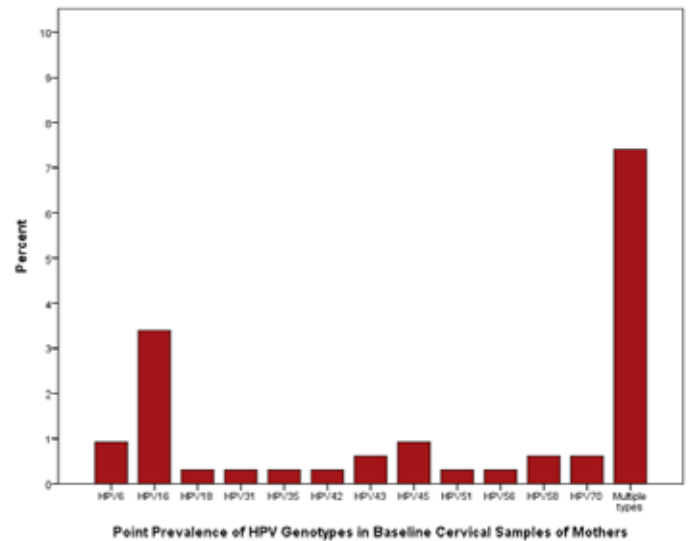


Figure 1 - Point prevalence of HPV genotypes in the pre-term (baseline) cervical samples of mothers.

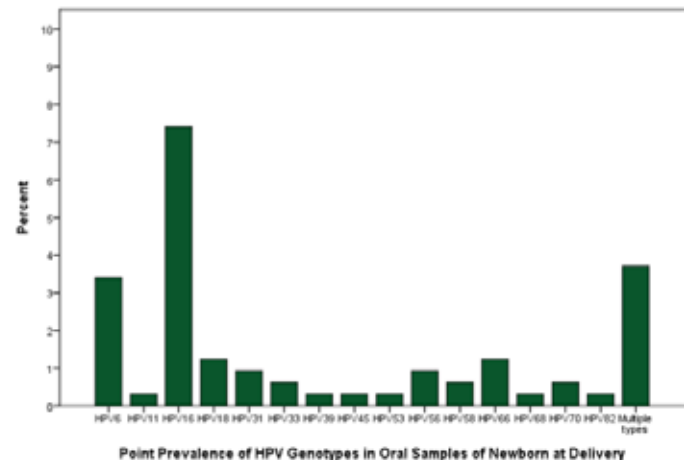


Figure 2 - Point prevalence of HPV genotypes in the oral samples of newborn at delivery.

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ORAL HPV PERSISTENCE AT 6- AND 12- MONTHS AMONG HEALTHY MEN: THE HIM STUDY

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Danelle Smith², Mary Papenfuss², Luisa L. Villa³, Eduardo
Lazcano⁴, Anna R. Giuliano²

Background: HPV causes some head and neck cancers mainly in the oropharynx; yet, oral HPV natural history is poorly understood. The rarity of oral HPV infection among healthy individuals (estimated prevalence ~5%) hinders research in this area.

Methods: Oral rinse/gargle specimens were collected and tested from men aged 18-70 (median age 32 years), participating in the HIM Study, a prospective study of healthy men residing in the United States, Mexico, and Brazil. DNA was extracted using the Qiagen BioRobot Media MDx Automated DNA Purification Protocol; Roche Linear Array (LA) was used to detect 37 HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 were considered carcinogenic). Oral HPV persistence at 6- and 12-months was determined among men who tested HPV positive on their first oral collection.

Results: Oral HPV DNA was detected among 142 of 1618 men (8.8%; HPV 16:0.4%; carcinogenic HPV:1.0%) on first collection, of which 67 were known types on the LA system; 57 of these had at least a 6-month follow-up visit and median follow-up time was 12 months. Overall, type-specific HPV persistence for at least 6-months was present in 49.1% (n = 28 of 57) of men; of these 28 men, 14 had specimens collected at 12+ months and 42.8% (n = 6) of their infections persisted. HPV 16 infections appeared more likely to persist at least 6-months than non-16 carcinogenic HPV infections (75% [3 of 4] vs. 44.4% [4 of 9], respectively). Of note, an unexpectedly high number of HPV 55 infections were detected (n = 16); 68.8% (n = 11) of these infections persisted 6+ months.

Conclusions: Almost half of oral HPV infections persisted for one year; rates of oral HPV persistence may be longer than cervical (~33% persist to 12-months), although small sample size precluded precise estimates. Larger studies with longer follow-up periods are required to estimate rates of type-specific persistence and factors associated with persistence.

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HPV VACCINE INTRODUCTION IN EUROPE: LESSONS LEARNED

Linda Eckert^{1,2}, Liudmila Mosina³, Rebecca Martin³

Objective: The 53 countries of the WHO European Region are diverse in income levels and health care systems. To date, 18 countries have introduced the HPV vaccine, with varying success and documentation of their immunization schedules. However, there has been scant documentation of these successes and the challenges encountered with HPV vaccine implementation in the WHO European Region in a consistent, standardized manner to allow for lessons learned. These experiences could benefit other countries in the Region considering HPV vaccine implementation.

Methods: We conduct a standardized phone interview with 15 of the 18 European countries that have introduced the HPV vaccine. Information regarding 15 items such as implementation strategies, target population, communication, funding, challenges, and coverage was obtained using a standardized data collection questionnaire which the participant received prior to the phone call. Data was aggregated where similar strategies have been used to implement the vaccine.

Conclusions: In the WHO European Region, success of HPV

vaccine introduction has varied widely, with some countries obtaining high rates of coverage and others very low. Financing mechanisms have also varied substantially in this economically diverse WHO region. Information from the interviews will be tabulated and presented. We will present strategies on the decision to use the vaccine and how it was implemented (mass catch-up campaigns or routine immunization programmes, and whether a comprehensive approach was used). We collected and will present samples of vaccine education offered to providers, patients, and media as well as problem solving strategies for handling adverse events and negative publicity. We will address finance mechanisms and cost effectiveness analyses used in this varied region with middle and high income countries. Disseminating the collective experiences of HPV vaccine implementation successes and challenges is vital to enable assistance to countries as they seek guidance towards introduction of the HPV vaccine.

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HIGH-GRADE ANAL INTRA-EPITHELIAL NEOPLASIA IN HOMOSEXUAL MEN: DATA FROM TWO COMMUNITY-BASED COHORTS

Fengyi Jin¹, Marina van Leeuwen², Claire Vajdic³, Leo McHugh¹, Garrett Prestage², Leon Botes¹, Gabriele Medley⁴, Sepehr Tabrizi⁵, Andrew Grulich², Richard Hillman¹

Background: We report the prevalence and risk factors for high grade anal intra-epithelial neoplasia (HG-AIN), the precursor of anal cancer, in two community-based cohorts of HIV-negative and HIV-positive homosexual men in Sydney, Australia.

Methods: A cross-sectional study of consecutive participants in both cohorts in 2005 was performed (204 HIV-negative and 127 HIV-positive men). Anal cell samples collected by the research nurse were used for anal cytological analysis using the ThinPrep procedure. The residuum of PreservCyt vials was tested for HPV using Digene Hybrid Capture 2. Participants with anal squamous cell of undetermined significance (ASCUS) or greater cytological abnormality were offered high resolution anoscopy (HRA).

Results: Seventy-seven men had cytological changes of ASCUS or greater (16.7% HIV-negative and 33.6% HIV-positive men, p < 0.001). However, only 3 (2.3%) HIV-positive men and no HIV-negative men had high grade cytological abnormalities. Of these 77 men, 63 (81.8%) underwent HRA, and 21 (33.3%) of these had histologically confirmed HG-AIN. The prevalence of HG-AIN was significantly higher in HIV-positive men (12.6%) than in HIV-negative men (5.0%, OR = 2.73, 95% CI 1.14-6.53). HG-AIN was not related to age, current smoking status, anal symptoms, or reporting receptive anal intercourse in the last six months. The presence of HG-AIN was strongly associated with the detection of high risk types of anal HPV (OR = 11.20, 95% CI 1.48-84.49), but not with low risk types (OR = 1.80, 95% CI 0.72-4.53).

Conclusion: HG-AIN was prevalent in sexually active

homosexual men across all age groups and almost three times as common in HIV-positive men as in HIV-negative men. The presence of high risk anal HPV was highly predictive of HG-AIN. More studies are needed to better determine the incidence and the natural history of HG-AIN in this population.

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ACCEPTABILITY OF THE HPV VACCINE FOR MALES: A REVIEW OF THE LITERATURE

Julia Hood¹, Nicole Liddon¹, Bridget Wynn¹, Lauri Markowitz¹

Background: A quadrivalent HPV vaccine is available to men in some countries. This vaccine was licensed for use in males in the United States in 2009, but was not recommended for routine use. We reviewed available literature on the acceptability of the HPV vaccine for males.

Methods: PubMed was searched for articles presenting HPV vaccine intention data for males published between 2000 and 2010. Thirty-eight articles met inclusion criteria and were reviewed using a standardized abstraction form. Researchers independently reviewed and summarized each study and discrepancies were reconciled.

Results: Half of the studies were conducted in the United States (n = 19); the remainder were conducted in Canada (4), UK (3), Australia (2), Malaysia (2), Netherlands (2), Brazil (1), El Salvador (1), Italy (1), Sweden (1), Thailand (1), and Turkey (1). The majority (86%) of studies relied upon cross-sectional surveys and convenience samples (60%). HPV vaccine acceptability was high among American male college students (74%-78%) and gay/bisexual men (73%), but lower in samples of primarily heterosexual men from a wider age range (33%-48%). Contradictory findings about 'partner protection' messages were found among men and parents. Parental acceptance varied widely (12%-100%) depending on the question frame, respondents' ethnicity, and study location. Among Latino parents in both the US and El Salvador, acceptability was high (up to 100%). Physicians preferred the quadrivalent vaccine and to recommend to girls and older adolescents. Among studies conducted in the US using nationally representative samples, intention to recommend HPV vaccine to older male patients ranged from 82%-92%. Acceptability was lower in surveys of providers in more localized settings using different methodologies (27%-82%).

Conclusion: HPV vaccine acceptability for males varied substantially, most likely due to variation in study designs, including information provided to participants and question

framing. Findings from this review suggest among whom and under which circumstances HPV vaccine acceptability for men is likely to be high.

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CONTINUED RAPID DECLINE IN WARTS AFTER NATIONAL QUADRIVALENT HPV VACCINE PROGRAM

Christopher Fairley^{1,2}, Jane Hocking¹, Lyle Gurrin¹, Marcus Chen¹, Basil Donovan³, Catriona Bradshaw^{2,4}

Background: Australia provided free quadrivalent human papillomavirus (HPV) vaccine to 12-18 year old girls in a school-based program from April 2007 and to women < 27 years through general practices from July 2007. Coverage rates for three doses of the vaccine are about 70% in both groups.

Methods: The proportion of new clients with genital warts at Melbourne Sexual Health Centre (MSHC) from January 2004 to December 2009.

Results: 44, 256 new clients attended MSHC between 2004-2009 and genital warts were diagnosed in 4, 518 (10.2%; 95% confidence intervals (CI): 9.9-10.5). The proportion of warts in women < 28 years fell from an average of 12.7% before 2008 to 4.4% in the last quarter of 2009 (**Figure 1**).

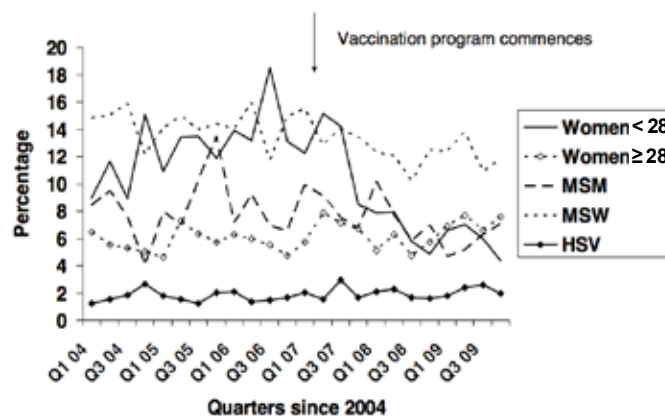


Figure 1 - Presentations with warts. Quarters 2004 to 2009.

The proportion of new clients with genital warts was significantly lower in 2008-9 than 2004-7 for women < 28 years (RR = 0.45 (95% CI 0.39-0.52)), heterosexual men (RR = 0.82 (95% CI 0.75-0.90)) and men who have sex with men (MSM) (RR = 0.80 (95% CI 0.65-0.98)) but not women < 28 (RR = 1.1 (95% CI 0.87-1.3)). The falls in warts in women < 28 and heterosexual men occurred despite significantly higher mean numbers of sexual partners per year in 2008-9 compared to 2004-7 (P < 0.001 for both women < 28, and heterosexual men). In contrast, the fall in warts in MSM was associated with a lower mean number of male partners in 2008-9 (11.5 partners per year) compared to 2004-7 (17.3 male partners per year, P < 0.001), **Figure 2**.

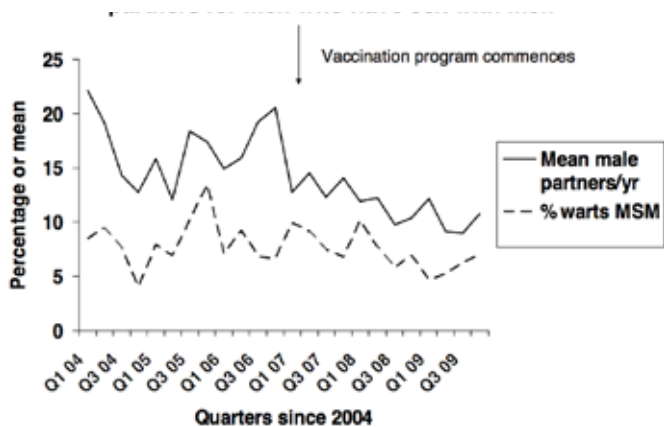


Figure 2 - Presentations with warts (2004-2009) and male partners for men who have sex with men

Conclusions: Our data suggests that the initial rapid and marked reduction in the incidence of genital warts among women < 28 years of age, originally seen in 2008, is continuing in 2009. The reduction in genital wart diagnoses observed in MSM may be due to a lower risk profile of MSM in 2008-9.

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NEW MOLECULAR MARKERS FOR CERVICAL CANCER SCREENING: DISTINGUISHING HYPE FROM HOPE

Paloma Martin¹, Linah Kilany¹, Diego Garcia¹, Ana M López², Josefa Martin-Azaña³, Carmen Bellas⁴

Objective: The aim of this study was to analyze the distribution of type specific HPV infection among women with normal and abnormal cytology and its correlation with grade of lesion.

Methods: From May 2006 to December 2009, a total of nine hundred and forty HPV positive specimens were included in this study. Cases were included if the molecular analysis and cytology were available and if they had no previous HPV study done. HPV DNA detection and genotyping was performed by PCR using the L1 consensus HPV MY09/11 primers, and flow-through hybridization.

Results: There were 290 (31%) women with normal cytology and 650 (69%) with cytologic abnormality. The diagnoses of the abnormal cases (**Figure 1**) were: 253 cases (27%) with atypical squamous cell of undetermined significance (ASCUS), 303 cases (32%) low-grade squamous intraepithelial lesion (LSIL), 62 cases (7%) high-grade squamous intraepithelial lesion (HSIL) and 32 cases (3%) undetermined grade squamous intraepithelial lesion. The seven most common types were: HPV 16 (29%),

HPV 53 (16%), HPV 31 (12%), HPV 52 (9%), HPV 18 (8%), HPV 58 (8%), and HPV 6 (8%). In normal samples we found: HPV 16 (20%), HPV 53 (16%), HPV 31 (11%), and HPV 6 (9%). In ASCUS and LSIL, types 16, 53, and 31, were the three more frequent. In HSIL the proportion of HPV 16 rises to 55%, with HPV 31 being the second (11%). HPV 18 is present in only 3% of HSIL (Figure 2). More than one genotype was found in 314 (33.4%) patients. Co-infections were found in 25% of normal cases, 36% of ASCUS, 43% of LSIL, and 19% of HSIL.

Conclusions: 1. HPV 16 is the most frequent type detected: 20% of cases with normal cytology were positive for HPV 16, and this prevalence was associated with increased grade of lesion, therefore genotyping is a useful strategy for the follow-up of patients. 2. HPV 18 is not common in our series, regardless of the lesion. 3. Co-infections were more frequent in low grade lesions.

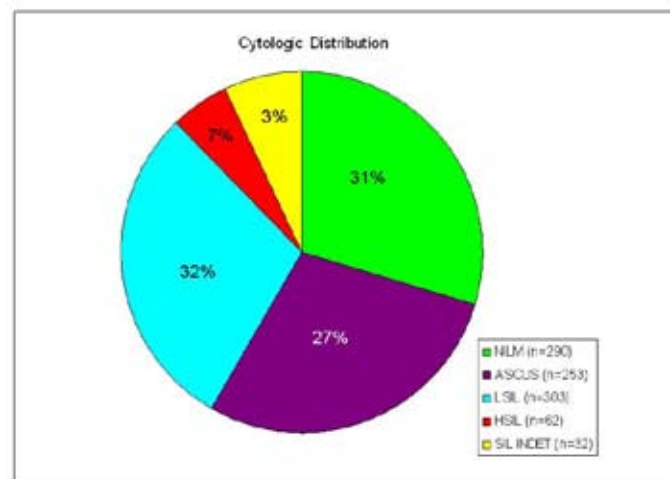


Figure 1 - The proportion of warts in women.

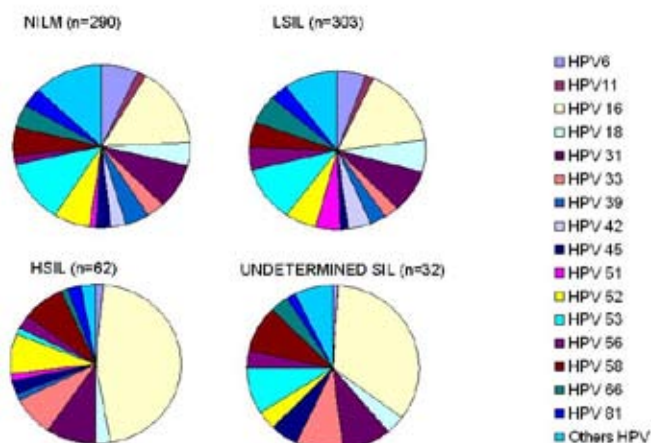


Figure 2 - Distribution of HPV genotypes in cytological lesions.