

have undiagnosed HSIL, possibly reflecting small HSIL lesions not visualized on HRA.

This study has several limitations. First, the composite endpoints created in this study were based on cross-sectional data and not on prospective follow-up. As our follow-up of men with composite endpoints accrues, we will be able to make more definitive conclusions about the accuracy of the composite-HSIL diagnoses and their natural history. Second, the small sample size in some cytology–histology combinations limits the statistical power of the analysis. However, the statistically significant pattern of HPV16 detection supports the validity of the findings, and our report is almost double the size of the largest previous report of a combined cytology–histology endpoint. Third, although our anoscopists were well trained (16), most were relatively new to the field and it is possible that their performance in diagnosing HSIL at HRA will improve further with time. However, when we stratified baseline study visits by the degree of experience of the anoscopist, we found that the additional HSIL prevalence afforded by the composite endpoint was virtually identical between most- and lesser experienced anoscopists (data not presented). In a Dutch study, it has been reported that the diagnostic yield of HRA increases until an operator has performed approximately 200 HRAs (32). Fourth, it is important to note that HPV genotyping was performed on the ThinPrep aliquot collected before cytologic processing, and not on lesional tissue. Hence, in some instances, if the anal cytology missed an HSIL lesion during sampling, the associated (causative) HPV type may have also been missed. Given the high burden of HPV in this population, including the presence of multiple HPV types (33) and the concurrent presence of multiple lesions (of which the highest grade was taken as the endpoint), it cannot be inferred that the diagnosed lesions were definitely caused by the HPV types detected.

SPANC is one of only a small number of cohort studies globally and the largest published thus far, to perform anal cytology and HRA screening, as well as HPV genotyping at the same visit on all participants, with no limitation on the number of biopsies allowed. In addition, recruitment for the study was mostly from community-based settings with broad inclusion criteria, making the results more generalizable to a target screening population. Biopsy reporting was performed in accordance with the LAST Project recommendations (16, 18), limiting potential misclassification of histologic HSIL. There was a very high degree of inter-rater reliability and intra-rater repeatability in histologic diagnosis in the study (34).

This study demonstrates that epidemiologic studies of HSIL may underestimate the prevalence of HSIL if the results of anal cytology and HRA are not combined, and highlights the limitations of both techniques. Studies on the performance of anal cytology in detecting histologically proven HSIL in homosexual and bisexual men demonstrate that the procedure clearly underestimates the true prevalence of HSIL (35). HRA is also prone to considerable sampling and measurement error, and is much more technically demanding than cervical colposcopy (10). However, it has to be recognized that performing anal cytology and HRA on all participants, in epidemiologic studies and in clinical practice, will often not be practical, because HRA is an invasive and resource-intensive procedure. Research should focus on optimizing anal cytology sampling protocols to improve HSIL detection. Incorporating HPV and molecular biomarkers may prove useful in increasing the yield and diag-

nostic accuracy of anal cytology and targeting HRA to those most likely to have HSIL. It is also important to note, while composite endpoints may be useful in studies of HSIL prevalence to identify cases which are missed at HRA, they are not a substitute for HRA performed by well-trained individuals. Advanced training in HRA and attention to quality assurance programs, similar to those in cervical colposcopy (36) is likely to improve the diagnostic yield of HRA. When HRA is performed by the most experienced hands, composite endpoints may provide smaller additional benefit.

In summary, diagnosis of ASIL has been hindered by the limited sensitivity of both anal cytology sampling and HRA used alone. As a result, many published studies of cytology-only and histology-only ASIL endpoints likely underestimate the true prevalence of HSIL. This study has demonstrated that combining the results of anal cytology and HRA leads to the diagnosis of more biologically relevant disease. Until data on biomarkers that will improve diagnostic accuracy of cytology are available, epidemiologic studies of anal HSIL risk should, where possible, include data on composite cytology and histology endpoints in order to improve the sensitivity of ASIL detection.

Disclosure of Potential Conflicts of Interest

J.M. Roberts and A. Farnsworth report receiving commercial research support from Hologic. S.M. Garland is the chief investigator in an HPV vaccine trial by GSK, investigator in a CSLBio Investigator-initiated grant, reports receiving commercial research grant from Merck Investigator initiated grant, has received speakers bureau honoraria from Merck, and is a consultant/advisory board member for Merck Global. C.K. Fairley has ownership interest (including patents) in shares in CSL Biotherapies. A.E. Grulich has received speakers' bureau honoraria from Merck. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The views expressed in this publication do not necessarily represent the position of the Australian Government.

Authors' Contributions

Conception and design: D.A. Machalek, I.M. Poynten, F. Jin, R.J. Hillman, J.M. Roberts, S.M. Garland, A. Farnsworth, A.E. Grulich

Development of methodology: D.A. Machalek, I.M. Poynten, F. Jin, R.J. Hillman, J.M. Roberts, S.N. Tabrizi, S.M. Garland, A. Farnsworth, C.K. Fairley, A.E. Grulich

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R.J. Hillman, D.J. Templeton, C. Law, J.M. Roberts, S.N. Tabrizi, A. Farnsworth

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D.A. Machalek, I.M. Poynten, F. Jin, R.J. Hillman, D.J. Templeton, J.M. Roberts, S.N. Tabrizi, S.M. Garland, A. Farnsworth, A.E. Grulich

Writing, review, and/or revision of the manuscript: D.A. Machalek, I.M. Poynten, F. Jin, R.J. Hillman, D.J. Templeton, C. Law, J.M. Roberts, S.N. Tabrizi, S.M. Garland, A. Farnsworth, C.K. Fairley, A.E. Grulich

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.N. Tabrizi, S.M. Garland, A. Farnsworth

Study supervision: S.N. Tabrizi, A.E. Grulich

Other (director and supervisor of all molecular work performed in the Molecular Microbiology Department at Royal Women's Hospital): S.M. Garland

Acknowledgments

The SPANC study team includes Brian Acraman, Marjorie Adams, Andrew Carr, Susan Carroll, David Cooper, Alyssa Cornall, Leonie Crampton, Deborah Ekman, Kit Fairley, Annabelle Farnsworth, Lance Feeney, Eddie Fraissard, Suzanne Garland, Andrew Grulich, Richard Hillman, Kirsten Howard, Fengyi

Machalek et al.

Jin, Carmella Law, Matthew Law, Dorothy Machalek, Kirsten McCaffery, Ross McDonald, Patrick McGrath, Robert Mellor, Richard Norris, Matthew O'Dwyer, Susan Pendlebury, Kathy Petoumenos, Samuel Phillips, I. Mary Poynten, Garrett Prestage, Adele Richards, Jennifer Roberts, Lance Schema, Daniel Seeds, Eva Segelov, Sepehr Tabrizi, Dave Templeton, Julia Thurloe, Winnie Tong, and Rick Varma.

Grant Support

The SPANC study was funded by a National Health and Medical Research Council Program Grant (Sexually transmitted infections: Causes, consequences and interventions Grant #568971; to A.E. Grulich and C.K. Fairley) and a Cancer Council New South Wales Strategic Research Partnership Program Grant (preventing morbidity and mortality from anal cancer grant

#13-11; to A.E. Grulich, I.M. Poynten, F. Jin, R.J. Hillman, D.J. Templeton, S.N. Tabrizi, S.M. Garland, A. Farnsworth, and C.K. Fairley). Cytologic testing materials were provided by Hologic Pty Ltd. The Kirby Institute is affiliated with the Faculty of Medicine, University of New South Wales, and funded by the Australian Government of Health and Ageing.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 27, 2015; revised April 19, 2016; accepted April 19, 2016; published OnlineFirst April 27, 2016.

References

- Silverberg MJ, Lau B, Justice AC, Engels E, Gill MJ, Goedert JJ, et al. Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America. *Clin Infect Dis* 2012;54:1026–34.
- Edgren G, Sparen P. Risk of anogenital cancer after diagnosis of cervical intraepithelial neoplasia: a prospective population-based study. *Lancet Oncol* 2007;8:311–6.
- Moscicki AB, Darragh TM, Berry-Lawhorn JM, Roberts JM, Khan MJ, Boardman LA, et al. Screening for anal cancer in women. *J Low Genit Tract Dis* 2015;19:S27–42.
- Jay N, Berry JM, Hogeboom CJ, Holly EA, Darragh TM, Palefsky JM. Colposcopic appearance of anal squamous intraepithelial lesions: relationship to histopathology. *Dis Colon Rectum* 1997;40:919–28.
- Darragh TM, Winkler B. Anal cancer and cervical cancer screening: key differences. *Cancer Cytopathol* 2011;119:5–19.
- Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. *Lancet Oncol* 2012;13:487–500.
- Forman D, de Martel C, Lacey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L, et al. Global burden of human papillomavirus and related diseases. *Vaccine* 2012;30 Suppl 5:F12–23.
- Park IU, Palefsky JM. Evaluation and management of anal intraepithelial neoplasia in HIV-negative and HIV-positive men who have sex with men. *Curr Infect Dis Rep* 2010;12:126–33.
- Mathews WC, Agmas W, Cachay E. Comparative accuracy of anal and cervical cytology in screening for moderate to severe dysplasia by magnification guided punch biopsy: a meta-analysis. *PLoS ONE* 2011;6:e24946.
- Palefsky JM. Practising high-resolution anoscopy. *Sex Health* 2012;9:580–6.
- Mathews WC, Cachay ER, Caperna J, Sitapati A, Cosman B, Abramson I. Estimating the accuracy of anal cytology in the presence of an imperfect reference standard. *PLoS ONE* 2010;5:e12284.
- Palefsky JM, Holly EA, Ralston ML, Jay N. Prevalence and risk factors for human papillomavirus infection of the anal canal in human immunodeficiency virus (HIV)-positive and HIV-negative homosexual men. *J Infect Dis* 1998;177:361–7.
- Schwartz LM, Castle PE, Follansbee S, Borgonovo S, Fetterman B, Tokugawa D, et al. Risk factors for anal HPV infection and anal precancer in HIV-infected men who have sex with men. *J Infect Dis* 2013;208:1768–75.
- Berry JM, Palefsky JM, Jay N, Cheng SC, Darragh TM, Chin-Hong PV. Performance characteristics of anal cytology and human papillomavirus testing in patients with high-resolution anoscopy-guided biopsy of high-grade anal intraepithelial neoplasia. *Dis Colon Rectum* 2009;52:239–47.
- Nahas CS, da Silva Filho EV, Segurado AA, Genevcius RF, Gerhard R, Gutierrez EB, et al. Screening anal dysplasia in HIV-infected patients: is there an agreement between anal pap smear and high-resolution anoscopy-guided biopsy? *Dis Colon Rectum* 2009;52:1854–60.
- Machalek DA, Grulich AE, Hillman RJ, Jin F, Templeton DJ, Tabrizi SN, et al. The Study of the Prevention of Anal Cancer (SPANC): design and methods of a three-year prospective cohort study. *BMC Public Health* 2013;13:946.
- Darragh TM, Birdsong GG, Luff RD, Davey DD. Anal-rectal cytology. In: Solomon D, Nayar R, editors. *The Bethesda System for reporting cervical cytology*. New York, NY: Springer; 2004. p. 169–75.
- Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, et al. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *J Low Genit Tract Dis* 2012;16:205–42.
- Tabrizi SN, Stevens M, Chen S, Rudland E, Kornegay JR, Garland SM. Evaluation of a modified reverse line blot assay for detection and typing of human papillomavirus. *Am J Clin Pathol* 2005;123:896–9.
- Kreuter A, Potthoff A, Brockmeyer NH, Gambichler T, Swoboda J, Stucker M, et al. Anal carcinoma in human immunodeficiency virus-positive men: results of a prospective study from Germany. *Br J Dermatol* 2010;162:1269–77.
- Critchlow CW, Surawicz CM, Holmes KK, Kuypers J, Daling JR, Hawes SE, et al. Prospective study of high grade anal squamous intraepithelial neoplasia in a cohort of homosexual men: influence of HIV infection, immunosuppression and human papillomavirus infection. *AIDS* 1995;9:1255–62.
- Palefsky JM, Holly EA, Hogeboom CJ, Ralston ML, DaCosta MM, Botts R, et al. Virologic, immunologic, and clinical parameters in the incidence and progression of anal squamous intraepithelial lesions in HIV-positive and HIV-negative homosexual men. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998;17:314–9.
- Sahasrabudhe VV, Castle PE, Follansbee S, Borgonovo S, Tokugawa D, Schwartz LM, et al. Human papillomavirus genotype attribution and estimation of preventable fraction of anal intraepithelial neoplasia cases among HIV-infected men who have sex with men. *J Infect Dis* 2013;207:392–401.
- Darragh TM, Tokugawa D, Castle PE, Follansbee S, Borgonovo S, LaMere BJ, et al. Interrater agreement of anal cytology. *Cancer Cytopathol* 2013;121:72–8.
- Wentzensen N, Follansbee S, Borgonovo S, Tokugawa D, Schwartz L, Lorey TS, et al. Human papillomavirus genotyping, human papillomavirus mRNA expression, and p16/Ki-67 cytology to detect anal cancer precursors in HIV-infected MSM. *AIDS* 2012;26:2185–92.
- De Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. *Int J Cancer* 2009;124:1626–36.
- Ostor AG. Natural history of cervical intraepithelial neoplasia: a critical review. *Int J Gynecol Pathol* 1993;12:186–92.
- Hillman RJ, Garland SM, Gunathilake MP, Stevens M, Kumaradevan N, Lemech C, et al. Human papillomavirus (HPV) genotypes in an Australian sample of anal cancers. *Int J Cancer* 2014;135:996–1001.
- Sherman ME, Castle PE, Solomon D. Cervical cytology of atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion (ASC-H): characteristics and histologic outcomes. *Cancer* 2006;108:298–305.
- Shield PW, Finnimore J, Cummings M, Wright RG. Performance measures for Australian laboratories reporting cervical cytology: a decade of data 1998–2008. *Pathology* 2010;42:623–8.

31. Davey DD, Neal MH, Wilbur DC, Colgan TJ, Styer PE, Mody DR. Bethesda 2001 implementation and reporting rates: 2003 practices of participants in the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology. *Arch Pathol Lab Med* 2004;128:1224-9.
32. Richel O, Prins JM, de Vries HJ. Screening for anal cancer precursors: what is the learning curve for high-resolution anoscopy? *Aids* 2014;28:1376-7.
33. Machalek DA, Grulich AE, Jin F, Templeton DJ, Poynten IM. The epidemiology and natural history of anal human papillomavirus infection in men who have sex with men. *Sex Health* 2012;9:527-37.
34. Roberts JM, Jin F, Thurloe JK, Biro C, Poynten IM, Tabrizi SN, et al. High reproducibility of histological diagnosis of human papillomavirus-related intraepithelial lesions of the anal canal. *Pathology* 2015;47:308-13.
35. Roberts JM, Thurloe JK. Comparison of the performance of anal cytology and cervical cytology as screening tests. *Sex Health* 2012;9:568-73.
36. Moss EL, Redman CW, Arbyn M, Dollery E, Petry KU, Nieminen P, et al. Colposcopy training and assessment across the member countries of the European Federation for Colposcopy. *Eur J Obstet Gynecol Reprod Biol* 2015;188:124-8.

Cancer Epidemiology, Biomarkers & Prevention

A Composite Cytology–Histology Endpoint Allows a More Accurate Estimate of Anal High-Grade Squamous Intraepithelial Lesion Prevalence

Dorothy A. Machalek, I. Mary Poynten, Fengyi Jin, et al.

Cancer Epidemiol Biomarkers Prev 2016;25:1134-1143. Published OnlineFirst April 27, 2016.

Updated version Access the most recent version of this article at:
doi:[10.1158/1055-9965.EPI-15-1106](https://doi.org/10.1158/1055-9965.EPI-15-1106)

Supplementary Material Access the most recent supplemental material at:
<http://cebp.aacrjournals.org/content/suppl/2016/04/27/1055-9965.EPI-15-1106.DC1>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link <http://cebp.aacrjournals.org/content/25/7/1134>. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.