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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 aliquat 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 diagnostic 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.

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

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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

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

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