Roberts Jennifer (Orcid ID: 0000-0001-7229-8617)
Clarke Megan A (Orcid ID: 0000-0003-0435-7376)
Wentzensen Nicolas (Orcid ID: 0000-0003-1251-0836)
Deshmukh Ashish A. (Orcid ID: 0000-0001-8936-8108)

A Systematic Review and Meta-Analysis of Cytology and HPV-related Biomarkers for Anal Cancer Screening Among Different Risk Groups

Megan A. Clarke[1], Ashish A. Deshmukh[2], Ryan Suk[2], Jennifer Roberts[3], Richard Gilson[4], Naomi Jay[5], Elizabeth A. Stier[6], Nicolas Wentzensen[1]

Affiliations:
[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, Maryland, USA; [2]Center for Health Services Research, Department of Management, Policy and Community Health, UTHealth School of Public Health, Houston, TX, USA; [3]Douglass Hanly Moir Pathology, Sydney, Australia; [4]Centre for Clinical Research in Infection and Sexual Health, University College London, London, UK; [5]Anal Neoplasia Clinic, Research, and Education Center, University of California San Francisco, San Francisco, California, USA; [6]Department of Obstetrics and Gynecology, Boston Medical Center/Boston University School of Medicine, Boston, MA, USA

Correspondence to:
Megan Clarke, PhD, MHS
Clinical Epidemiology Unit
Clinical Genetics Branch
Division of Cancer Epidemiology & Genetics
National Cancer Institute
9609 Medical Center Dr., Rm. 6E552
Rockville, MD 20892
240-276-7823
megan.clarke@nih.gov
Twitter handle: @megan_clarke01

Keywords: Anal cancer, anal precancer, screening, cytology, HPV testing

Running title: Review of anal cancer screening tests

Abbreviations:
AIN2+, anal intraepithelial neoplasia grade 2 or worse
ASC-US+, atypical squamous cells of undetermined significance or worse
CI, confidence interval
CIN2+, cervical intraepithelial neoplasia grade 2 or worse
HIV, human immunodeficiency virus
HPV, human papillomavirus
HRA, high resolution anoscopy
HSIL, high grade squamous intraepithelial lesion
LBC, liquid based cytology
LGTD, lower genital tract disease
LWH, living with human immunodeficiency virus

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ijc.34199

MSM, men who have sex with men
PLWH, people living with HIV

Novelty and Impact: This systematic review and meta-analysis summarizes data from 39 studies evaluating the performance of anal cancer screening tests among different populations. We used a novel approach pooling absolute risk estimates to summarize diagnostic accuracy of different screening tests to inform clinical decision making. These data can be used to support the development of anal cancer screening guidelines using cytology and HPV testing in different populations with elevated risk of anal cancer.

Abstract

To inform optimal approaches for detecting anal precancers, we performed a systematic review and meta-analysis of the diagnostic accuracy of anal cancer screening tests in different populations with elevated risk for anal cancer. We conducted a literature search of studies evaluating tests for anal precancer and cancer (anal intraepithelial neoplasia grade 2 or worse, AIN2+) published between January 01, 1997 to September 30, 2021 in PubMed and Embase. Titles and abstracts were screened for inclusion and included articles underwent full-text review, data abstraction, and quality assessment. We estimated the prevalence of AIN2+ and calculated summary estimates and 95% confidence intervals (CI) of test positivity, sensitivity and specificity, and predictive values of various testing strategies, overall and among population subgroups. A total of 39 articles were included. The prevalence of AIN2+ was 20% (95% CI, 17-29%), and ranged from 22% in men who have sex with men (MSM) living with HIV to 13% in women and 12% in MSM without HIV. The sensitivity and specificity of cytology and HPV testing were 81% and 62%, and 92% and 42% respectively, and 93% and 33%, respectively for cytology and HPV co-testing. AIN2+ risks were similar among those testing positive for cytology, HPV, or co-testing. Limited data on other biomarkers (HPV E6/E7 mRNA and p16/Ki-67 dual stain), suggested higher specificity, but lower sensitivity compared with anal cytology and HPV. Our findings provide important evidence for the development of clinical guidelines using anal cytology and HPV testing for anal cancer screening.

Introduction

Anal cancer incidence and mortality have been increasing over the past decade, with highest incidence rates occurring in people living with HIV (PLWH), particularly men who have sex with men (MSM) (MSM LWH).(1) Other groups with elevated risk include MSM without HIV, women with a history of gynecologic cancers and precancers, and non-HIV immunosuppressed individuals.(1) Most anal squamous cell cancers are caused by carcinogenic human papillomavirus (HPV) infection, particularly HPV16.(2) Like cervical cancer, anal cancer develops through precursors that can be detected by exfoliative sampling and high-resolution anoscopy (HRA) with directed biopsy.(3) Recently, the U.S. Anal Cancer HSIL Outcomes Research (ANCHOR) study demonstrated that treating anal precancers significantly reduces risk of progression to anal cancer among PLWH aged 35 years and older.(4) These results underscore the need to identify approaches for detecting anal precancers that can be treated to prevent invasion. Currently, there are no consensus recommendations for anal screening; some clinics perform anal cytology among populations with elevated risk (i.e., MSM LWH).(5, 6) Like cervical cytology, anal cytology is subjective, lacks sensitivity, and needs to be repeated frequently. Thus, there has been growing interest in evaluating HPV-related biomarkers for anal cancer screening.(7)

The International Anal Neoplasia Society (IANS) assembled a Task Force to develop recommendations for anal cancer screening. Critical components of this process are to understand the prevalence of anal precancer and to evaluate diagnostic tests for anal cancer screening in different populations to make recommendations for clinical use. To inform IANS Task Force recommendations, we conducted a systematic review and meta-analysis of tests for anal cancer screening, pooling estimates of diagnostic accuracy measures and absolute risk to assess clinical performance.

Methods

Study Selection and Data Abstraction

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Supplemental Figure 1) and included observational studies and clinical trials with primary data on the performance of tests for anal precancer and cancer detection in populations with known elevated risk for anal cancer.(1) We searched English-language, peer-reviewed studies published in PubMed or EMBASE from 01/01/1997 through 09/30/2021, using terms listed in the Supplement. At least two reviewers screened titles and abstracts for inclusion and reviewed full-text articles to determine eligibility. We reviewed reference lists of identified articles for additional relevant studies. We excluded studies that did not assess diagnostic performance, and those that reported results at the test (rather than individual) level. For multiple publications from the same study, data from the most recent publication were included. We abstracted data on eligibility criteria, location, design, screening test, diagnostic procedures, referral algorithms, age of participants, and the number of cases and non-cases by test result. Study quality was independently evaluated by N.W. and M.A.C. using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool(8) as described in the Supplemental Material.

Statistical Analysis

To estimate diagnostic accuracy, we abstracted information on the number of true positives, false negatives, false positives, and true negatives at baseline for each test. We calculated pooled sensitivity and specificity with 95% confidence intervals (CI) by fitting a bivariate random effects model using Stata SE (version 16) [21] with metandi (9) analyses of four or more studies. We plotted sensitivity and specificity estimates for each study in a summary receiver operator characteristic (ROC) plot, in which each point on the curve represents a different study, weighted according to sample size. The summary estimate of sensitivity and specificity for meta-analyzed studies and its confidence region are

also plotted. We defined cases of anal precancer using study-specific definitions of histologic anal HSIL or anal epithelial neoplasia (AIN) grades 2 or 3 (hereafter referred to as "AIN2+"; anal cancers were rare and grouped with AIN2+) and non-cases as <HSIL or <AIN2. We estimated pooled AIN2+ prevalence (i.e., pre-test or baseline risk) and the risk of AIN2+ among test-positives (i.e., the positive predictive value), and among test-negatives (i.e., the complement of the negative predictive value) with 95% CI's using multilevel logistic-normal random effects models with *metaprop_one*.(10) Between-study variance was quantified using the τ^2 statistic.(11) We planned subgroup-specific analyses on the following populations where sufficient data were available: PLWH (including studies with ≥80% PLWH); MSM LWH; women LWH or those with history of gynecologic cancer/precancer (hereafter referred to collectively as "women"); and MSM without HIV. In studies that evaluated multiple populations, we analyzed these separately and referred to them as individual studies in subgroup analyses. Absolute risks for cytology, HPV, and co-testing were compared in studies that evaluated all three approaches within the same population, and post-test risks were plotted in relation to the baseline prevalence of AIN2+. To account for potential verification bias among studies in which only individuals with positive screening tests were referred to HRA, we stratified analyses by HRA referral algorithm (complete HRA vs. partial HRA referral), where possible.

Results

Characteristics of Included Studies

The systematic review process is summarized in the PRISMA diagram (Supplemental Figure1). Briefly, 4,717 articles were identified, after removal of duplicates (n=863), 3,748 were excluded based on title/abstract review. Of the remaining 106 articles, 39 were included after full-text review (Table 1; Supplemental Material)(12-50). Most studies reported data for PLWH (n=29)(12, 13, 15, 16, 18-34, 36-39, 41, 43, 47, 50), a majority were among MSM LWH (n=18)(12, 13, 15, 16, 21, 25-27, 29, 31-34, 36, 38,

39, 47, 50). Nine studies reported data exclusively among women(17, 28, 30, 41, 43, 45-47, 49), including six among women LWH and six were conducted among MSM without HIV(12, 15, 25, 31, 32, 47). Several studies reported data on multiple subgroups (n=10).(12, 15, 20, 25, 31, 32, 43, 46, 47, 51) Most studies were conducted in the United States (n=17), followed by Europe (n=11), and Thailand (n=3).

Prevalence of AIN2+

The prevalence of AIN2+ from 36 studies was 20% (range:1% to 58%; (Table 2; Supplemental Figure 2) and was lower among studies with partial HRA referral among screen-positives compared to studies with complete HRA referral (13% vs. 23%, p=0.07). Within subgroups, the prevalence of AIN2+ was highest among MSM LWH and PLWH (22%, respectively) and lowest among MSM without HIV (12%). Among women, AIN2+ prevalence was 13% overall, but was significantly higher in studies with complete HRA referral compared with partial HRA referral (19% vs. 6%, p=0.01; Supplemental Figures 3-6).

Anal Cytology

Overall, 33 studies evaluated anal cytology at an atypical squamous cells of undetermined significance or worse (ASC-US+) threshold for anal precancer detection (Table 3). ASC-US+ positivity was 42% (95% CI, 32-52%; τ^2=1.38) and the sensitivity and specificity were 81.0% (95% CI, 72-87%) and 62.4% (95% CI, 54-70%), respectively (Figure 1). Among ASC-US+, the risk of AIN2+ was 35% (95% CI, 29-43%, τ^2=0.75) and among those with negative for intraepithelial lesions or malignancy (NILM) cytology, the risk was 6% (95% CI, 4-10%, τ^2=2.20; Supplemental Figures 7-8). In 25 studies with complete HRA referral, ASC-US positivity was slightly higher (48%), and the sensitivity and specificity were slightly lower (76.1% and 57.7%, respectively, Supplemental Table 1). In MSM LWH (n=16 studies), ASC-US+ positivity was 51% (95% CI, 40-62%, τ^2=0.65) and the sensitivity and specificity were 85.2% (95% CI, 77-

91%) and 52.8% (95% CI, 43-62%), respectively (Figure 1). Among ASC-US+, the risk of AIN2+ was 32% (95% CI, 23-42%, τ^2=0.81) and 6% (95% CI, 3-11%, τ^2=1.38) among those with NILM (Table 3; Supplemental Figures 9-10). Among women (n=8), ASC-US+ positivity was 21% (95% CI, 9-41%, τ^2=1.89) and the sensitivity and specificity were 65.7% (95% CI, 38-86%) and 82.2% (95% CI, 64-92%), respectively (Figure 1). Among ASCUS+, the risk of AIN2+ was 33% (95% CI, 22-46%, τ^2=0.45), and 5% (95% CI, 2-11%, τ^2=1.27) in those with NILM (Table 3; Supplemental Figures 11-12). In MSM without HIV (n=4), ASC-US+ positivity was 39% (95% CI, 19-63%, τ^2=1.02) and the sensitivity and specificity were 56.6% (95% CI, 25-83%) and 66.5% (95% CI, 44-83%), respectively. Among ASC-US+, the risk of AIN2+ was 38% (95% CI, 5-48%, τ^2=0.00) and 14% (95% CI, 9-21%, τ^2=0.14) in those with NILM (Table 3; Supplemental Figures 13-15). In studies that evaluated both MSM with and without HIV, the risks among MSM LWH were higher than those without HIV (data not shown).

Data on cytologic high-grade squamous intraepithelial lesions (HSIL) were available in 26 studies. HSIL prevalence was 7% (6% in studies with complete HRA referral; Supplemental Table 1) and was similar across subgroups (Table 3). Among all studies, the sensitivity and specificity of HSIL were 21.1% and 96.4%, respectively. The risk of AIN2+ among those with HSIL was 64%; only two studies reported a risk of 90% or greater, which is a benchmark of the IANS HRA recommendations (Table 3; Supplemental Figures 16-18).(3)

HPV DNA Testing

Twenty-one studies evaluated high-risk HPV testing for anal precancer detection. HPV positivity was 67% (95% CI, 59-73%, τ^2=0.53) and the sensitivity and specificity were 91.9% (95% CI, 87-95%) and 41.8% (95% CI, 35-49%), respectively (Table 3, Figure 2). The risk of AIN2+ was 31% (95% CI, 24-40%, τ^2=0.62) among HPV positives and 4% (95% CI, 2-8%, τ^2=1.36) among HPV negatives (Supplemental Figures 19-20). In MSM LWH (n=12 studies), HPV positivity was 76% (95% CI, 69-81%, τ^2=0.30) and the

sensitivity and specificity were 96.1% (95% CI, 90-99%) and 29.9.% (95% CI, 22-39%), respectively (Figure 2). Among HPV positives, the risk of AIN2+ was 30% (95% CI, 21-41%, τ^2=0.71) and 4% (95% CI, 2-9%, τ^2=1.80) among HPV negatives (Table 3; Supplemental Figures 21-22). Among women (n=6), HPV positivity was 59% (95% CI, 45-71%, τ^2=0.40) and the sensitivity and specificity were 91.1% (95% CI, 76-97%) and 47.1% (95% CI, 37-58%), respectively (Table 3, Figure 2). Among HPV positives, the risk of AIN2+ was 27% (95% CI, 15-43%, τ^2=0.70) and among HPV negatives, the risk was 4% (95% CI, 2-11%, τ^2=0.70; Supplemental Figures 23-24). Among MSM without HIV (n=4), HPV positivity was 53% (95% CI, 30-74%, τ^2=0.90) and the sensitivity and specificity were 76.1% (95% CI, 43-93%) and 53.7% (95% CI, 32-74%), respectively. The risk of AIN2+ among HPV positives was 26% (95% CI, 12-47%, τ^2=0.69) and was 10% (95% CI, 7-16%, τ^2=0.00) among HPV negatives (Table 3; Supplemental Figures 25-27). In the four studies that evaluated both MSM with and without HIV, risks were higher among MSM LWH compared to those without HIV (data not shown).

HPV and Cytology Co-Testing

In 12 studies that evaluated HPV and cytology co-testing (HPV and cytology performed on all samples), the positivity was 74% (95% CI, 63-82%, τ^2=0.78) and the sensitivity and specificity were 93.0% (95% CI, 86-97%) and 33.4% (95% CI, 25-43%), respectively. The risk of AIN2+ was 27.0% (95% CI, 20-35%, τ^2=0.44), and 5.0% (95% CI, 3-7%, τ^2=0.40) among those with a positive and negative co-test, respectively (Table 3; Supplemental Figures 28-30).

Limited HPV Genotyping

In 10 studies that evaluated HPV16 genotyping, HPV16 positivity was 23% (95% CI, 20-26%, τ^2=0.05) and the sensitivity and specificity were 45.5% (95% CI, 34-57%) and 83.4% (95% CI, 79-87%), respectively. Among HPV16 positives, the risk of AIN2+ was 39% (95% CI, 25-56%, τ^2=0.92) and was 13% (95% CI, 8-20%, τ^2=0.58) among HPV16 negatives. Among MSM with HIV (n=5 studies), HPV16 positivity

was 24% (95% CI, 20-28%, τ^2=0.03) and the sensitivity and specificity were 42.4% (95% CI, 27-59%) and 80.4% (95% CI, 74-85%), respectively. Among HPV16 positives, the risk of AIN2+ was 29% (95% CI, 13-54%, τ^2=1.18) and was 12% (95% CI, 6-25%, τ^2=0.80) among HPV16 negatives. In the 8 studies evaluating HPV16/18 genotyping, the positivity was slightly higher (30%) and while sensitivity was nearly equivalent (44.1%), the specificity was lower compared to HPV16 alone (77.4%; Table 3; Supplemental Figures 31-39).

HPV mRNA

Six studies evaluated HPV E6/E7 mRNA testing for anal precancer detection including the NucliSENS easyQ HPV v1 assay (Biomérieux, Marcy l'Etoile, France) the HPV OncoTectTM E6, E7 mRNA Kit (IncellDx, Menlo Park, CA, USA) and Aptima (Hologic) (Table 1). Importantly, the NucliSens assay restricts to 5 high-risk HPV genotypes whereas the other two assays test for 14 types. HPV E6/E7 mRNA positivity was 47% (95% CI, 45-50%, τ^2=0.0) and the sensitivity and specificity were 74.2% (95% CI, 69-79%) and 64.3% (95% CI, 58-70%), respectively. Among E6/E7 mRNA positives, the risk of AIN2+ was 49% (95% CI, 35-64%, τ^2=0.45) was 16% (95% CI, 10-25%, τ^2=0.32) among mRNA negatives (Table 3; Supplemental Figures 40-42)

p16 or p16/Ki-67

Seven studies evaluated the performance of p16 or p16/Ki-67 dual stain (DS, CINtec PLUS, Roche, Tucson, Arizona, USA) testing for anal precancer detection. Among four studies of DS, the positivity was 41% (95% CI, 28-56%; τ^2=0.23) and the sensitivity and specificity were 65.8% (95% CI, 39-85%) and 70.3% (95% CI, 5-82%), respectively. Among DS-positives, the risk of AIN2+ was 44% (95% CI, 23-68%, τ^2=0.93) and was 15% (95% CI, 8-28%, τ^2=0.51) in DS-negatives (Table 3; Supplemental Figures 43-45)

Quality Assessment of Studies Evaluating Cytology and HPV Testing

Risks of bias for study population, index test, reference standard, and study flow and timing were deemed low for 80%, 72%, 74%, and 72% of studies, respectively. Concerns regarding applicability were deemed low for patient selection, index test, and reference standard in 56%, 77% and 82% of studies, respectively (Supplemental Tables 2-3). Sensitivity analyses evaluating the prevalence of AIN2+ and performance of cytology and HPV testing among high-quality studies are shown in Supplemental Table 4.

Comparative Evaluation of Cytology and HPV

Absolute risks for cytology (ASC-US+), HPV, and co-testing were compared in 12 studies that evaluated all three approaches (Figure 3). HPV testing provided slightly more reassurance of a low AIN2+ risk among HPV negatives (7%, 95% CI, 4-11%) compared with NILM cytology (9%, 95% CI, 6-15%); conversely, more individuals tested positive for HPV compared with cytology (64% vs. 43%). Risks were lowest among co-test negatives (5%, 95% CI, 3-7%) but co-testing had the highest positivity of all strategies (74%). Evaluation of the clinical implications of risk stratification achieved by different tests requires specific clinical risk action thresholds, which are currently not defined.

Discussion

Recent findings from the ANCHOR trial demonstrate that detecting and treating anal precancers can reduce anal cancer risk. To identify optimal approaches for detecting anal precancers, we performed a systematic review and meta-analysis of tests for anal cancer screening in different populations. Overall, the prevalence of AIN2+ was 20% and varied across different populations, ranging from 22% in MSM LWH to 13% and 12% in women and MSM without HIV, respectively. Anal cytology and high-risk HPV testing were the most commonly evaluated screening tests, with summary sensitivity and specificity estimates of 81% and 63% for cytology (ASC-US+) and 92% and 39% for HPV across all studies. Cytology and HPV testing were more sensitive, but less specific among MSM LWH compared to women

and MSM without HIV, although data were more limited for these two groups, especially MSM without HIV. In studies with HPV genotyping, the sensitivity and specificity of HPV16 were 46% and 83%, respectively; performance did not seem to improve with the addition of HPV18 in studies evaluating HPV16/18 genotyping, although direct comparisons are needed. Fewer studies evaluated other biomarkers including E6/E7 mRNA and p16 or DS. In general, we found higher specificity, but lower sensitivity of these biomarkers compared to anal cytology and HPV, with high variability across studies.

Our findings highlight important complexities that need to be considered when evaluating the literature on anal cancer screening tests. These include heterogeneity in anal precancer prevalence (within and across subgroups), limited data on populations other than MSM LWH, differences in assays evaluated, and variability in study quality and reporting. The underlying disease prevalence in a population is an important determinant of diagnostic test performance, so understanding the differences in reported anal precancer prevalence is critical. Among studies of MSM LWH, AIN2+ prevalence ranged from 3% to 50%, which may in part be due to temporal factors, particularly between the pre- and post-HAART eras,(52) but variability in clinical practice, HRA expertise, and study procedures are also important determinants.(3, 38) While the IANS HRA guidelines suggest that histologic HSIL should be identified in ≥90% of cases with cytologic HSIL, only two studies in our review achieved this metric, whereas most were below 75%. This is similar to what has been observed in studies of cervical cancer screening, where the positive predictive value of HSIL cytology for cervical precancer (CIN3) is highly variable across different studies and populations.(53) Our findings also demonstrate how differences in HRA referral algorithms can impact anal precancer prevalence and clinical performance estimates, particularly when HRA referral is based on abnormal cytology. Finally, differences in diagnostic criteria and pathology practice (e.g., use of AIN terminology vs. LAST criteria)(54) may also introduce variability. With respect to the screening tests themselves, we observed greater variability in the performance of cytology compared to HPV testing, which may be due to the

lower reproducibility of cytology.(55) For HPV testing, a wide range of assays were used, including some that are currently not approved for clinical use in cervical screening. The definitions of "high-risk" HPV varied across studies and type coverage was particularly variable for HPV E6/E7 mRNA studies. Like anal cytology, performance varied for anal p16 and p16/Ki-67 DS.

Management guidelines for cervical cancer screening have recently shifted to a risk-based approach, where management is determined by risk of cervical precancer based on test results in relation to consensus clinical action risk thresholds.(56) Compared to the decades-long history of established clinical practice in cervical cancer screening, experience with anal cancer screening is limited, and we need to accept more uncertainty when developing clinical guidelines. There are two important components that are needed for clinical guidelines development: One is the underlying clinical evidence, including test performance and absolute risk data, which we present here. The other is specific clinical action thresholds, which are influenced by clinical standards and considerations, the risk tolerance of a society, and other factors. These are value-driven decisions based on consensus stakeholder efforts. Despite many gaps in the current literature, our systematic review and meta-analysis provide important clinical evidence and test performance data for developing evidence-based guidelines for screening and management of anal cancer precursors. Anal cytology is used in many settings for clinical management and reflects a standard of care that can support setting clinical action thresholds. The performance of anal cytology in MSM LWH is similar to the cytology performance observed in the triage of HPV-positive women and can be a standalone screening test with frequent re-testing. While the specificity of anal cytology at an HSIL threshold was high, the sensitivity was too low to be considered as a standalone screening test, with high residual AIN2+ risks (e.g., from 15% to 20%) among individuals testing HSIL negative. Like cervical screening, anal HPV testing is more sensitive compared to anal cytology and provides better reassurance of not having anal precancer. However, the high HPV prevalence in some populations, particularly MSM LWH, results in a low specificity and only

reassures a minority of the population who test HPV negative of a low anal cancer risk. However, this HPV-negative population could be referred to longer screening intervals, which could make this approach worthwhile. Additional triage is still needed for the majority of individuals who test HPV positive. Triage of HPV with cytology (e.g., at an ASC-US+ threshold), which is a preferred strategy for cervical cancer screening(56), can improve specificity and reduce HRA referral at the cost of somewhat reduced sensitivity; however, observational data were lacking to directly evaluate this approach in the current review. Summary estimates based on co-testing do not suggest that adding cytology to HPV testing provides additional benefit. Biomarkers like p16/Ki-67 DS have shown promise for HPV triage in cervical cancer screening (57) but have not been sufficiently studied for anal cancer screening. Decisions on specific strategies ultimately depend on how the value-driven risk thresholds are determined and may differ between target populations and in different healthcare settings.

Strengths of our study include a comprehensive systematic review of the current literature on studies evaluating the performance of diagnostic tests for anal cancer screening in different populations. We estimated several measures of diagnostic accuracy (i.e., sensitivity, specificity, and predictive values) that inform clinical-decision making and the development of risk-based anal cancer screening guidelines. We also performed a comprehensive quality assessment of all included studies. Some limitations are worth noting. Due to the timeliness of this review for ongoing guideline development on anal cancer screening, we relied on published data and did not reach out to study authors for additional information. Because most studies did not separate analyses by AIN2 and AIN3 endpoints, we were only able to assess risk of AIN2+ as a combined outcome. Similar to CIN2 in cervical cancer screening, AIN2 is a poorly reproducible, heterogenous disease category, whereas AIN3 is likely a better surrogate of anal cancer risk. There is accumulating evidence that some biomarkers, like HPV16, could stratify the heterogeneous group of AIN2/3 into those that are more or less likely to progress to cancer.(2, 58)

Similarly, other biomarkers such as p16/Ki-67 and methylation could be considered to improve anal disease classification in future analyses. A second limitation of this study is that due to the urgency of generating evidence for ongoing guidelines efforts, we did not obtain individual-level data from studies which would allow to evaluate more test combinations such as anal cytology and HPV16/18 genotyping.

Conclusions

Our systematic review and meta-analysis summarize the best available evidence, providing a foundation for the development of anal cancer screening guidelines, while also highlighting several gaps in the literature that need to be addressed in future clinical studies. Clinical data from settings that conduct anal cancer screening can provide important information about clinical standards to inform development of risk action thresholds. Longitudinal studies evaluating the cumulative risks of anal precancer and cancer are needed to understand how long negative tests provide reassurance, a requirement to determine screening intervals. These data are also needed for mathematical modeling of long-term harms versus benefits of anal cancer screening and to develop cost-effective algorithms. Ultimately, the tradeoff between disease detection and resource utilization (i.e., HRA capacity) needs to be considered for each target population and test strategy.

Data Availability Statement:
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: MC: Conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft; AD: Conceptualization, data curation, investigation, writing – review & editing; RS: Data curation, investigation, writing – review & editing; JR: Conceptualization, data curation, investigation, writing – review & editing; RG: Conceptualization, data curation, investigation, writing – review & editing; NJ: Conceptualization, data curation, investigation, writing – review & editing; ES: Conceptualization, data curation, investigation, writing – review & editing; NW: Conceptualization, data curation, investigation, methodology, writing – review & editing. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

Conflict of Interest:

Drs. Clarke, Deshmukh, Gilson, Suk and Wentzensen: No conflicts

Dr. Naomi Jay has received consulting fees from Merck Pharmaceuticals, and has received support for conference travel and attendance from ASCCP. Dr. Jay served in an unpaid role as the past president and is a current unpaid Board Member and Anal Cancer Screening Guidelines' Task Force Leader of the International Anal Neoplasia Society.

Dr. Elizabeth Stier has received honorariums from the Physicians' Research Network and the British Association for Sexual Health and HIV. Dr. Steir has received reimbursement for travel from the British Association for Sexual Health and HIV, Eurogin Congress, and ASCCP. Dr. Steir serves as an unpaid leader of the International Anal Neoplasia Society's Anal Cancer Screening Guidelines' Task Force, and has received in-kind support for HPV testing from Hologic, LLC and Qiagen.

Dr. Jennifer Roberts has received payment from Sonic Healthcare, and consumables and antibodies donated from Hologic Australia and Roche Australia, respectively.

Funding: This work was supported by the Intramural Research Program of the National Institutes of Health and the National Cancer Institute (#Z01 CP010124-21).

Role of the funder: No sponsor had any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

References

1. Clifford GM, Georges D, Shiels MS, Engels EA, Albuquerque A, Poynten IM, et al. A meta-analysis of anal cancer incidence by risk group: Toward a unified anal cancer risk scale. Int J Cancer. 2021;148(1):38-47.
2. Lin C, Franceschi S, Clifford GM. Human papillomavirus types from infection to cancer in the anus, according to sex and HIV status: a systematic review and meta-analysis. Lancet Infect Dis. 2017.
3. Hillman RJ, Cuming T, Darragh T, Nathan M, Berry-Lawthorn M, Goldstone S, et al. 2016 IANS International Guidelines for Practice Standards in the Detection of Anal Cancer Precursors. J Low Genit Tract Dis. 2016;20(4):283-91.
4. Joel Palefsky JL, Teresa Darragh, Stephen Goldstone, Naomi Jay, Hillary Dunlevy, Timothy Wilkin, Isabella Rosa-Cunha, Abigail Arons, Julia Pugliese, Gary Bucher, Lisa Flowers, Rebecca Levine, Michael Berry-Lawhorn, editor Treatment of Anal High-Grade Squamous Intraepithelial Lesions to Prevent Anal Cancer. Conference on Retroviruses and Opportunistic Infections; 2022; Virtual.
5. Brown G. Screening for Anal Dysplasia and Cancer in Patients With HIV. New York State Department of Health AIDS Institute Clinical Guidelines. Baltimore (MD)2020.
6. Plotzker RE, Barnell GM, Wiley DJ, Stier EA, Jay N. Provider Preferences for Anal Cancer Prevention Screening: Results of the International Anal Neoplasia Society Survey. Tumour Virus Res. 2022:200235.
7. Clarke MA, Wentzensen N. Strategies for screening and early detection of anal cancers: A narrative and systematic review and meta-analysis of cytology, HPV testing, and other biomarkers. Cancer Cytopathol. 2018.
8. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529-36.
9. Harbord RM, Whiting P. metandi: Meta-analysis of diagnostic accuracy using hierarchical logistic regression. The Stata Journal. 2009;9(2):211-29.
10. Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. Arch Public Health. 2014;72(1):39.
11. Higgins JP, Thompson SG, Spiegelhalter DJ. A re-evaluation of random-effects meta-analysis. J R Stat Soc Ser A Stat Soc. 2009;172(1):137-59.
12. Palefsky JM, Holly EA, Hogeboom CJ, Berry JM, Jay N, Darragh TM. Anal cytology as a screening tool for anal squamous intraepithelial lesions. J Acquir Immune Defic Syndr Hum Retrovirol. 1997;14(5):415-22.
13. Palefsky JM, Holly EA, Efirdc JT, Da Costa M, Jay N, Berry JM, et al. Anal intraepithelial neoplasia in the highly active antiretroviral therapy era among HIV-positive men who have sex with men. Aids. 2005;19(13):1407-14.
14. Panther LA, Wagner K, Proper J, Fugelso DK, Chatis PA, Weeden W, et al. High resolution anoscopy findings for men who have sex with men: inaccuracy of anal cytology as a predictor of histologic high-grade anal intraepithelial neoplasia and the impact of HIV serostatus. Clin Infect Dis. 2004;38(10):1490-2.
15. 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(2):239-47.
16. Salit IE, Lytwyn A, Raboud J, Sano M, Chong S, Diong C, et al. The role of cytology (Pap tests) and human papillomavirus testing in anal cancer screening. Aids. 2010;24(9):1307-13.

17. Santoso JT, Long M, Crigger M, Wan JY, Haefner HK. Anal intraepithelial neoplasia in women with genital intraepithelial neoplasia. Obstet Gynecol. 2010;116(3):578-82.
18. Weis SE, Vecino I, Pogoda JM, Susa JS, Nevoit J, Radaford D, et al. Prevalence of anal intraepithelial neoplasia defined by anal cytology screening and high-resolution anoscopy in a primary care population of HIV-infected men and women. Dis Colon Rectum. 2011;54(4):433-41.
19. Tramujas da Costa ESI, Coelho Ribeiro M, Santos Gimenez F, Dutra Ferreira JR, Galvao RS, Vasco Hargreaves PE, et al. Performance of p16INK4a immunocytochemistry as a marker of anal squamous intraepithelial lesions. Cancer Cytopathol. 2011;119(3):167-76.
20. Goldstone SE, Lowe B, Rothmann T, Nazarenko I. Evaluation of the hybrid capture 2 assay for detecting anal high-grade dysplasia. Int J Cancer. 2012;131(7):1641-8.
21. 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(17):2185-92.
22. Mallari AO, Schwartz TM, Luque AE, Polashenski PS, Rauh SM, Corales RB. Anal cancer screening in HIV-infected patients: is it time to screen them all? Dis Colon Rectum. 2012;55(12):1244-50.
23. Wilkin T, Lee JY, Lensing SY, Stier EA, Goldstone SE, Berry MJ, et al. High-grade anal intraepithelial neoplasia among HIV-1-infected men screening for a multicenter clinical trial of a human papillomavirus vaccine. HIV Clin Trials. 2013;14(2):75-9.
24. Darwich L, Videla S, Canadas MP, Pinol M, Garcia-Cuyas F, Vela S, et al. Distribution of human papillomavirus genotypes in anal cytological and histological specimens from HIV-infected men who have sex with men and men who have sex with women. Dis Colon Rectum. 2013;56(9):1043-52.
25. Phanuphak N, Teeratakulpisarn N, Keelawat S, Pankam T, Barisri J, Triratanachat S, et al. Use of human papillomavirus DNA, E6/E7 mRNA, and p16 immunocytochemistry to detect and predict anal high-grade squamous intraepithelial lesions in HIV-positive and HIV-negative men who have sex with men. PLoS One. 2013;8(11):e78291.
26. Sendagorta E, Herranz P, Guadalajara H, Bernardino JI, Viguer JM, Beato MJ, et al. Prevalence of abnormal anal cytology and high-grade squamous intraepithelial lesions among a cohort of HIV-infected men who have sex with men. Dis Colon Rectum. 2014;57(4):475-81.
27. Sendagorta E, Romero MP, Bernardino JI, Beato MJ, Alvarez-Gallego M, Herranz P. Human papillomavirus mRNA testing for the detection of anal high-grade squamous intraepithelial lesions in men who have sex with men infected with HIV. J Med Virol. 2015;87(8):1397-403.
28. Heard I, Etienney I, Potard V, Poizot-Martin I, Moore C, Lesage AC, et al. High Prevalence of Anal Human Papillomavirus-Associated Cancer Precursors in a Contemporary Cohort of Asymptomatic HIV-Infected Women. Clin Infect Dis. 2015;60(10):1559-68.
29. Cheng SH, Wang CC, Chang SL, Chu FY, Hsueh YM. Oncogenic human papillomavirus is not helpful for cytology screening of the precursor lesions of anal cancers in Taiwanese men who are infected with human immunodeficiency virus. Int J Clin Oncol. 2015;20(5):943-51.
30. Sananpanichkul P, Pittyanont S, Yuthavisuthi P, Thawonwong N, Techapornroong M, Bhamarapravatana K, et al. Anal papanicolaou smear in women with abnormal cytology: a thai hospital experience. Asian Pac J Cancer Prev. 2015;16(3):1289-93.
31. Jin F, Grulich AE, Poynten IM, Hillman RJ, Templeton DJ, Law CL, et al. The performance of anal cytology as a screening test for anal HSILs in homosexual men. Cancer Cytopathol. 2016;124(6):415-24.
32. Pankam T, Kerr SJ, Teeratakulpisan N, Rodbamrung P, Wongkanya R, Keelawat S, et al. Human papillomavirus in anal biopsy tissues and liquid-based cytology samples of HIV-positive and HIV-negative Thai men who have sex with men. Papillomavirus Res. 2017;3:149-54.
33. Burgos J, Hernandez-Losa J, Landolfi S, Guelar A, Dinares M, Villar J, et al. The role of oncogenic human papillomavirus determination for diagnosis of high-grade anal intraepithelial neoplasia in HIV-infected MSM. Aids. 2017;31(16):2227-33.

34. Hidalgo-Tenorio C, Gil-Anguita C, Ramirez-Taboada J, Esquivias J, Lopez-Ruz MA, Balgahata OM, et al. Risk factors for infection by oncogenic human papillomaviruses in HIV-positive MSM patients in the ART era (2010-2016). Medicine (Baltimore). 2017;96(39):e8109.
35. Jin F, Roberts JM, Grulich AE, Poynten IM, Machalek DA, Cornall A, et al. The performance of human papillomavirus biomarkers in predicting anal high-grade squamous intraepithelial lesions in gay and bisexual men. Aids. 2017;31(9):1303-11.
36. Serrano-Villar S, Hernandez-Novoa B, de Benito A, Del Romero J, Ocampo A, Blanco JR, et al. Screening for precancerous anal lesions with P16/Ki67 immunostaining in HIV-infected MSM. PLoS One. 2017;12(11):e0188851.
37. Frank M, Lahiri CD, Nguyen ML, Mehta CC, Mosunjac M, Flowers L. Factors Associated with High-Grade Anal Intraepithelial Lesion in HIV-Positive Men in a Southern U.S. City. AIDS Res Hum Retroviruses. 2018;34(7):598-602.
38. Clifford GM, Siproudhis L, Piroth L, Poizot-Martin I, Radenne S, Reynes J, et al. Determinants of high-grade anal intraepithelial lesions in HIV-positive MSM. AIDS. 2018;32(16):2363-71.
39. Pernot S, Boucheron P, Pere H, Lucas ML, Veyer D, Fathallah N, et al. Comparison of anal cancer screening strategies including standard anoscopy, anal cytology, and HPV genotyping in HIV-positive men who have sex with men. Br J Cancer. 2018;119(3):381-6.
40. Sambursky JA, Terlizzi JP, Goldstone SE. Testing for Human Papillomavirus Strains 16 and 18 Helps Predict the Presence of Anal High-Grade Squamous Intraepithelial Lesions. Dis Colon Rectum. 2018;61(12):1364-71.
41. Stier EA, Lensing SY, Darragh TM, Deshmukh AA, Einstein MH, Palefsky JM, et al. Prevalence of and Risk Factors for Anal High-grade Squamous Intraepithelial Lesions in Women Living with Human Immunodeficiency Virus. Clin Infect Dis. 2020;70(8):1701-7.
42. Wiley DJ, Hsu HK, Ganser MA, Brook J, Elashoff DA, Moran MG, et al. Comparison of nylon-flocked swab and Dacron swab cytology for anal HSIL detection in transgender women and gay, bisexual, and other men who have sex with men. Cancer Cytopathol. 2019;127(4):247-57.
43. Ramos-Cartagena JM, Perez CM, Guiot HM, Amaya-Ardilla CP, Tirado-Gomez M, Ortiz AP. Assessment of Anal Cancer Screening Tools in Detecting High-Grade Anal Squamous Intraepithelial Lesions in Women. J Low Genit Tract Dis. 2020;24(1):75-81.
44. Chiao EY, Lensing SY, Wiley DJ, Deshmukh AA, Lee J, Darragh TM, et al. Screening strategies for the detection of anal high-grade squamous intraepithelial lesions in women living with HIV. AIDS. 2020;34(15):2249-58.
45. Wohlmuth C, Ghorab Z, Shier M, Tinmouth J, Salit IE, Covens A, et al. Cytology-based screening for anal intraepithelial neoplasia in women with a history of cervical intraepithelial neoplasia or cancer. Cancer Cytopathol. 2021;129(2):140-7.
46. Kimura CMS, Nahas CSR, Silva-Filho EV, Ribeiro VL, Segurado AC, Alcantara FFP, et al. High-risk human papillomavirus test in anal smears: can it optimize the screening for anal cancer? AIDS. 2021;35(5):737-45.
47. Gaisa MM, Sigel KM, Deshmukh AA, Lenskaya V, Chan CA, Silvera R, et al. Comparing Anal Cancer Screening Algorithms Using Cytology and Human Papillomavirus DNA Testing in 3 High-Risk Populations. J Infect Dis. 2021;224(5):881-8.
48. Swanson AA, Hartley C, Long ME, Chantigian PDM, Casey PM, Jenkins SM, et al. Evaluation of high-risk human papillomavirus testing and anal cytology to detect high-grade anal intraepithelial neoplasia. J Am Soc Cytopathol. 2021;10(4):406-13.
49. Larsen HK, Haedersdal M, Thomsen LT, Hertzum-Larsen R, Lok TT, Bonde J, et al. Risk of Anal High-grade Squamous Intraepithelial Lesions Among Renal Transplant Recipients Compared With Immunocompetent Controls. Clin Infect Dis. 2021;73(1):21-9.

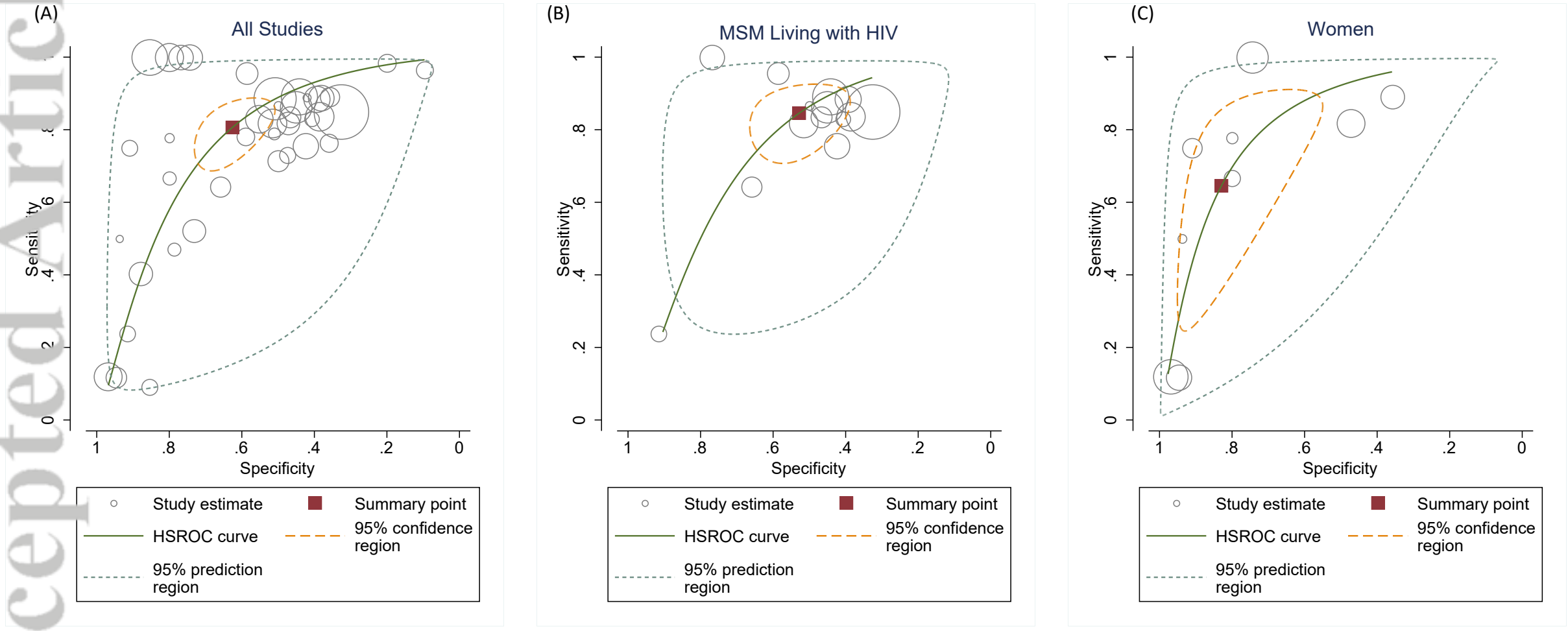
50. Silva-Klug AC, Saumoy M, Baixeras N, Trenti L, Catala I, Vidal A, et al. Comparison of two sample collection devices for anal cytology in HIV-positive men who have sex with men: Cytology brush and Dacron swab. Cytopathology. 2021;32(5):646-53.
51. Jin FR, J. M.; Grulich, A. E.; Poynten, I. M.; Machalek, D. A.; Cornall, A.; Phillips, S.; Ekman, D.; McDonald, R. L.; Hillman, R. J.; Templeton, D. J.; Farnsworth, A.; Garland, S. M.; Fairley, C. K.; Tabrizi, S. N. The performance of human papillomavirus biomarkers in predicting anal high-grade squamous intraepithelial lesions in gay and bisexual men. Aids. 2017;31(9):1303-11.
52. Colon-Lopez V, Shiels MS, Machin M, Ortiz AP, Strickler H, Castle PE, et al. Anal Cancer Risk Among People With HIV Infection in the United States. J Clin Oncol. 2018;36(1):68-75.
53. Silver MI, Andrews J, Cooper CK, Gage JC, Gold MA, Khan MJ, et al. Risk of Cervical Intraepithelial Neoplasia 2 or Worse by Cytology, Human Papillomavirus 16/18, and Colposcopy Impression: A Systematic Review and Meta-analysis. Obstet Gynecol. 2018;132(3):725-35.
54. 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(3):205-42.
55. Darragh TM, Tokugawa D, Castle PE, Follansbee S, Borgonovo S, LaMere BJ, et al. Interrater agreement of anal cytology. Cancer Cytopathol. 2013;121(2):72-8.
56. Perkins RB, Guido RS, Castle PE, Chelmow D, Einstein MH, Garcia F, et al. 2019 ASCCP Risk-Based Management Consensus Guidelines for Abnormal Cervical Cancer Screening Tests and Cancer Precursors. J Low Genit Tract Dis. 2020;24(2):102-31.
57. Wentzensen N, Clarke MA, Bremer R, Poitras N, Tokugawa D, Goldhoff PE, et al. Clinical Evaluation of Human Papillomavirus Screening With p16/Ki-67 Dual Stain Triage in a Large Organized Cervical Cancer Screening Program. JAMA Intern Med. 2019;179(7):881-8.
58. Wei F, Xia N, Ocampo R, Goodman MT, Hessol NA, Grinsztejn B, et al. Age-specific prevalence of anal and cervical HPV infection and high-grade lesions in 11 177 women by HIV status: a collaborative pooled analysis of 26 studies. J Infect Dis. 2022.

Figure Legends

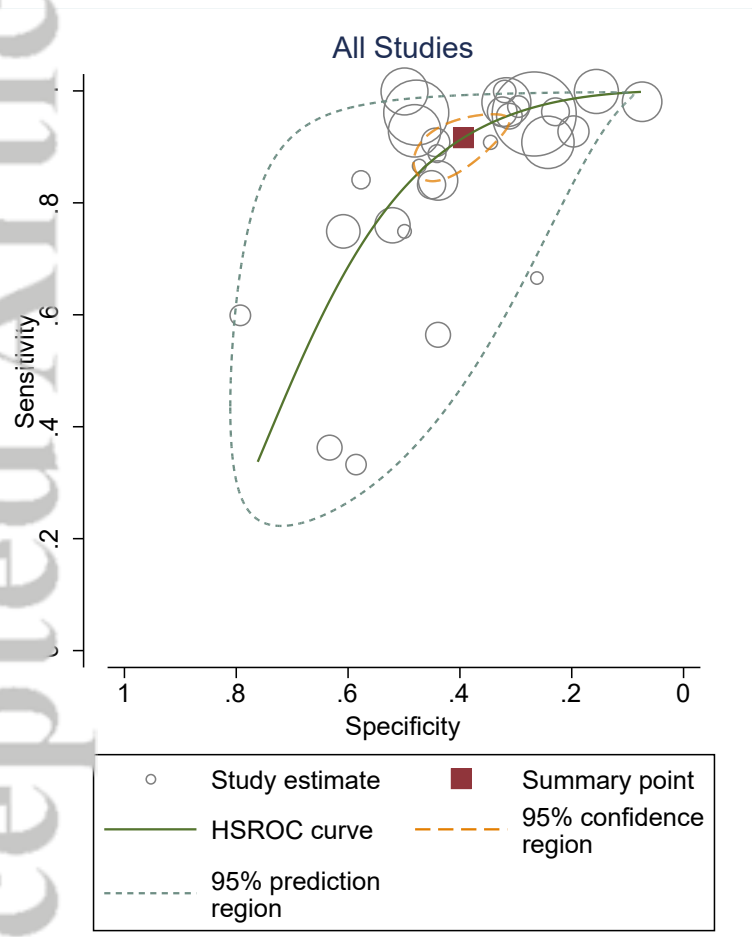
Figure 1. Summary ROC curves for the Performance of Anal Cytology at an ASC-US+ Threshold for AIN2+ Detection. Summary ROC curves are plotted for all studies (A), for studies of MSM living with HIV (B), and for studies of women (C). Individual estimates for the sensitivity and specificity of cytology at an ASC-US+ threshold for AIN2+ are shown as hollow circles, with their size determined by the total number of study participants, and the summary estimate is indicated by a solid red square with a 95% confidence region (orange dashed line). The summary ROC curve is plotted (solid green line) and the 95% prediction region (green dashed line) represents potential values of sensitivity and specificity that might be observed in a future study by describing the full extent of uncertainty of the summary points. Abbreviations: ROC, receiver operating characteristic curve; ASC-US+, atypical squamous cells of undetermined significance or worse; AIN2+, anal intraepithelial neoplasia grade 2 or worse; HSROC, hierarchical summary ROC curve

Figure 2. Summary ROC curves for the Performance of High-Risk HPV Testing for AIN2+ Detection. Summary ROC curves are plotted for all studies (A), for studies of MSM living with HIV (B), and for studies of women (C). Individual estimates for the sensitivity and specificity of high-risk HPV testing for AIN2+ are shown as hollow circles, with their size determined by the total number of study participants, and the summary estimate is indicated by a solid red square with a 95% confidence region (orange dashed line). The summary ROC curve is plotted (solid green line) and the 95% prediction region (green dashed line) represents potential values of sensitivity and specificity that might be observed in a future study by describing the full extent of uncertainty of the summary points. Abbreviations: ROC, receiver operating characteristic curve; HPV, human papillomavirus; AIN2+, anal intraepithelial neoplasia grade 2 or worse; HSROC, hierarchical summary ROC curve

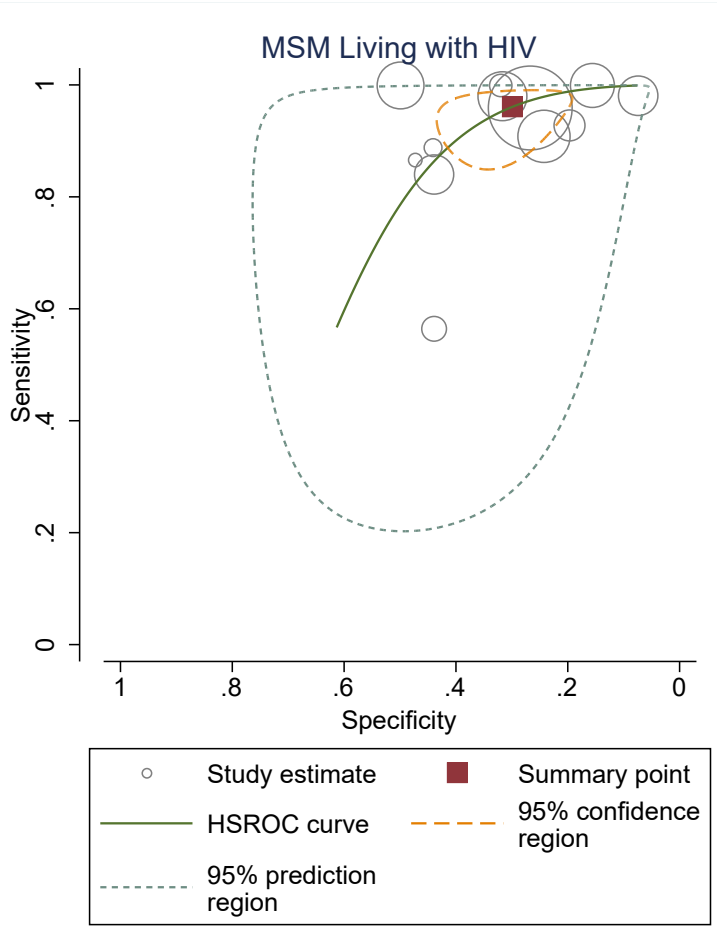
Figure 3. The Absolute Risk of AIN2+ for Cytology, High-Risk HPV, and Co-testing. The pre-and post-test risks of AIN2+ with 95% confidence intervals are plotted for anal cytology (ASC-US threshold), high-risk HPV testing, and HPV and cytology co-testing using data from 11 studies that evaluated all three strategies in the same study. The percentage with a given test result are shown in parentheses next to each result. Baseline risk corresponds to the prevalence of AIN2+ (i.e., pre-test risk). Abbreviations: AIN2+, anal intraepithelial neoplasia grade 2 or worse; HPV, human papillomavirus; ASC-US+, atypical squamous cells of undetermined significance or worse; NILM, negative for intraepithelial neoplasia or malignancy; AIN2+, anal intraepithelial neoplasia grade 2 or worse



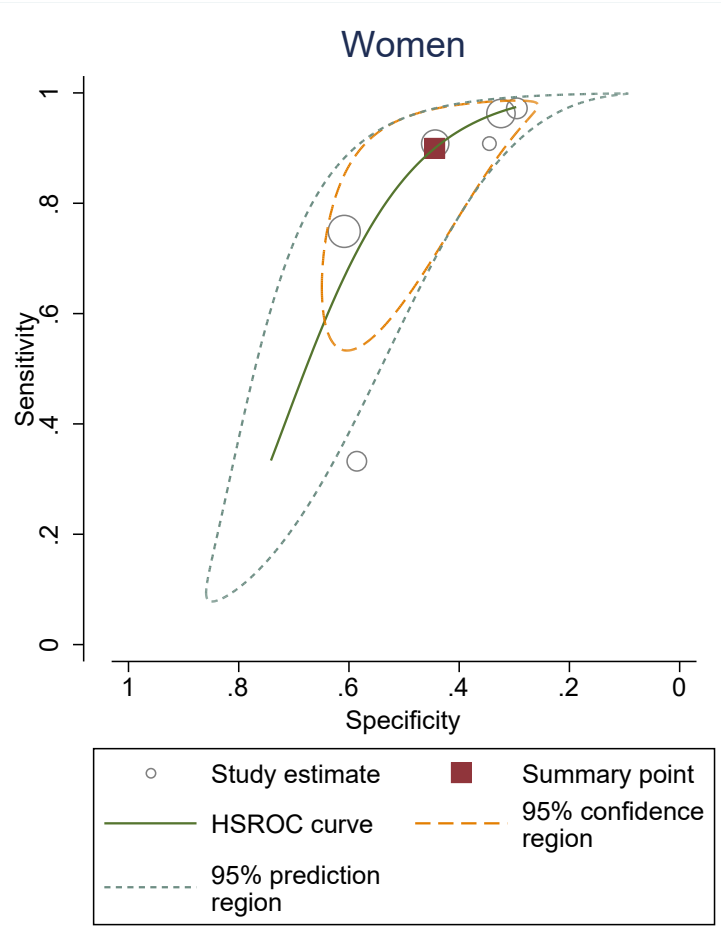
(A)



(B)



(C)



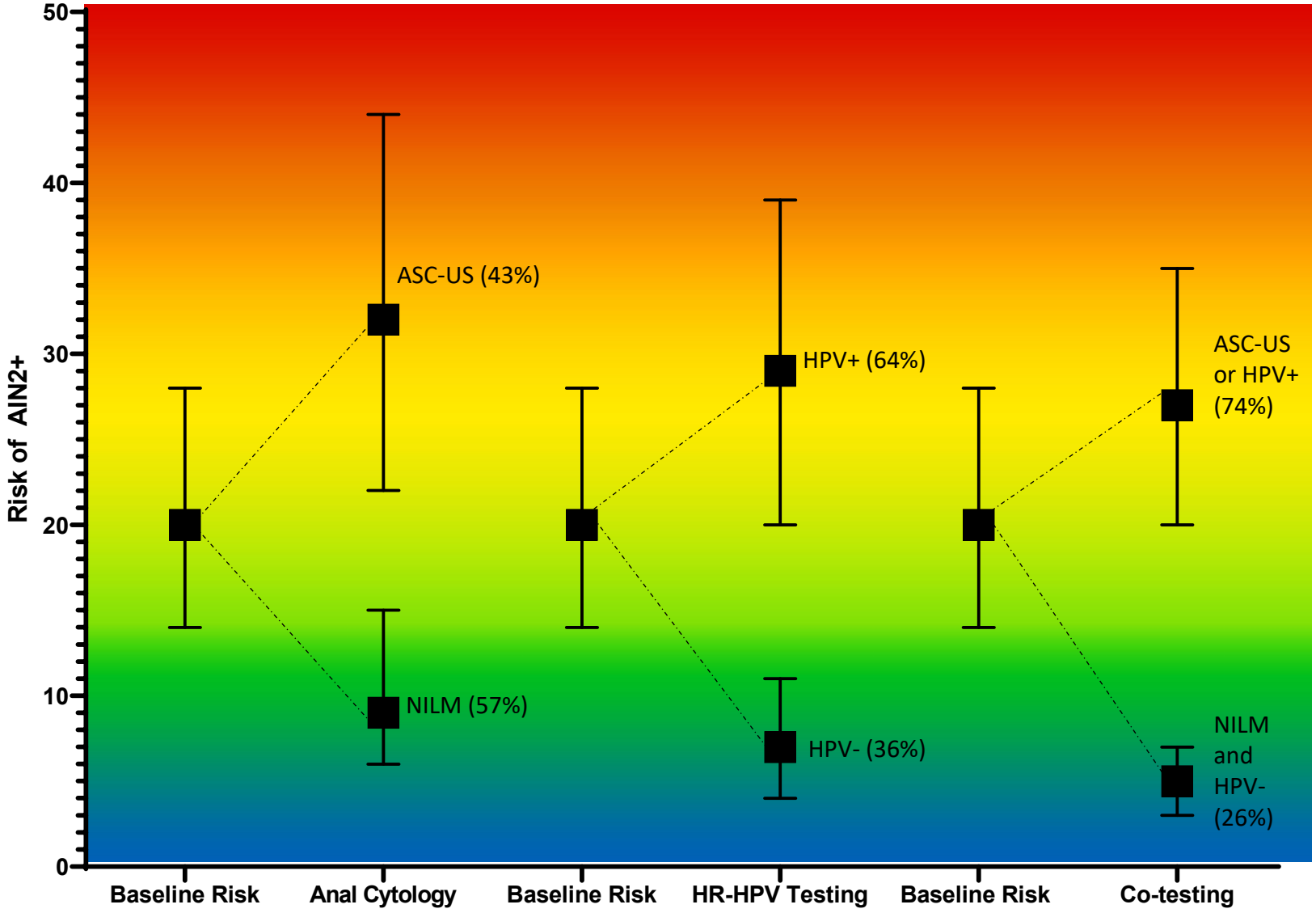


Table 1. Characteristics of Included Studies

First Author	Year	Location	Total N	AIN2+ N	Populations	Index Tests[1]					HRA on All[2]
						Cytology	High-Risk HPV	HPV Genotyping	Co-testing[3]	Biomarkers	
Palefsky	1997	USA	658	12	MSM LWH; MSM, no HIV	Conventional					Yes
[illegible]ther	2004	USA	153	63	MSM	Conventional					Yes
Palefsky	2005	USA	357	180	MSM LWH		MY09/MY11				Yes
Berry	2009	USA	125	38	MSM LWH; MSM, no HIV	LBC	MY09/MY11	HPV16	Yes		Yes
Sa[illegible]	2010	Canada	401	98	MSM LWH	LBC	HC2				Yes
Santoso	2010	USA	205	17	Women with LGTD	LBC					Yes
[illegible]	2011	USA	692	173	PLWH	LBC					No
Tramujas da [illegible]a E Silva	2011	Brazil	169	32	PLWH					p16 (clone 6H12)	Yes
Goldstone	2012	USA	298	104	PLWH; Men (majority), no HIV	LBC	HC2		Yes		Yes
Wentzensen	2012	USA	363	109	MSM LWH	LBC	cobas4800	HPV16/18		E6/E7 mRNA (PreTect HPVProofer); p16/Ki-67 dual stain (CINtec PLUS)	Yes
Mallari	2012	USA	329	125	PLWH	LBC					Yes
Wilkin	2013	USA	235	63	PLWH	LBC		HPV16			Yes
Darwich	2013	Spain	483	20	PLWH						No
Phanuphak	2013	Thailand	246	34	MSM LWH; MSM no HIV	LBC	Linear Array	HPV16/18	Yes	E6/E7 mRNA (OncoTect); p16 (p16INK4a antibody)	Yes
Sendagorta	2014	Spain	298	66	MSM LWH	LBC					No
[illegible]ard	2015	France	171	11	Women LWH	LBC	Linear Array	HPV16			No
Cheng	2015	Taiwan	196	14	MSM LWH	LBC	Linear Array	HPV16/18; HPV16	Yes		Yes
Sahanpanichkul	2015	Thailand	393	25	Women LWH	Conventional					No
Sendagorta	2015	Spain	101	47	MSM LWH	LBC	CLART Genomica HPV2			E6/E7 mRNA (NUcliSENS EasyQ HPV)	Yes
[illegible]	2016	Australia	617	191	MSM LWH; MSM, no HIV	LBC					Yes
[illegible]	2017	Thailand	95	22	MSM LWH; MSM, no HIV		SPF10-LiPA25	HPV16			Yes
Burr[illegible]os	2017	Spain	692	83	MSM LWH	LBC	CLART Genomica HPV2	HPV16/18	Yes		Yes
Hidalgo-Tenorio	2017	Spain	319	44	MSM LWH	LBC	Linear Array				Yes
Se[illegible]no-Villar	2017	Spain	328	74	MSM LWH	LBC				p16/Ki-67 dual stain (CINtec PLUS)	Yes
Jin	2017	Australia	617	231	MSM LWH; MSM, no HIV		cobas4800			p16/Ki-67 dual stain (CINtec PLUS); E6/E7 mRNA (NucliSENS easyQ HPV v1)	Yes
[illegible]k	2018	USA	147	85	Men LWH	LBC					No

Clifford	2018	France	513	52	MSM LWH	LBC	cobas4800	HPV16	Yes	p16/Ki-67 dual stain (CINtec PLUS)	Yes
Pernot	2018	France	212	27	MSM LWH			HPV16			No
Sambursky	2018	USA	894	132	MSM (majority)	LBC	HC2 and cobas4800	HPV16/18	Yes		Yes
Wiley	2019	USA	326	149	MSM	LBC					Yes
Stier	2019	USA	256	69	Women LWH	LBC					Yes
Ramos-Cartagena	2020	Puerto Rico	128	48	Women LWH; Women, no HIV	LBC	cobas4800	HPV16	Yes		Yes
Chiao	2020	USA	256	60	Women LWH	LBC[4]	HC2		Yes	Aptima E6/E7 mRNA	Yes
Wohlmuth	2020	Canada	317	20	Women with history of CIN2+	LBC					No
Kimura	2021	Brazil	366	61	Women with elevated risk; Men with elevated risk; Immunocompromised men and women	LBC	Abbott RealTime High-Risk HPV assay	HPV16/18^	Yes		Yes
Gaisa	2021	USA	1,837	756	MSM LWH; MSM, no HIV; Women LWH	LBC	cobas4800	HPV16/18^	Yes		Yes
Swanson	2021	USA	64	19	People with elevated risk	LBC	cobas4800	HPV16	Yes		Yes
Larsen	2021	Denmark	250	27	Renal transplant recipients					INNO-LiPA HPV Genotyping Extra II	Yes
Silva-Klug	2021	Spain	239	37	MSM LWH	LBC					Yes

[1]Index tests are indicated for the tests with available data for meta-analyses; [2]A response of "yes" indicates HRA was performed on all individuals in the study, whereas a response of "no" indicates that HRA was only performed among individuals with positive screening test results; [3]Co-testing refers to cytology with high-risk HPV testing; [4]Cytology data for Chiao et al. not assessed in meta-analysis due to overlapping data

Abbreviations: AIN2+, anal intraepithelial neoplasia grade 2 or worse; HRA, high resolution anoscopy; HPV, human papillomavirus; LBC, liquid based cytology; MSM, men who have sex with men; LWH, living with human immunodeficiency virus; PLWH, people living with HIV; LGTD, lower genital tract disease; CIN2+, cervical intraepithelial neoplasia grade 2 or worse

Table 2. Prevalence of AIN2+ in Different Subgroups and by HRA Referral Algorithm						
	N Studies	Total N	N AIN2+	Summarized AIN2+ Prevalence (95% CI)	Range of AIN2+ Prevalence	τ^2
Population						
All[1]	36	13,247	3,131	20% (17-29%)	1-58%	0.90
HRA Referral^						
All	28	10,544	2,704	23% (17-29%)	1-51%	0.72
Screen positives	8	2,713	427	13% (7-24%)	4-58%	1.16
PLWH	29	9,804	2,457	22% (17-29%)	3-58%	0.95
HRA Referral						
All	22	7,408	2,050	25% (19-33)	3-51%	0.75
Screen positives	7	2,396	407	14% (7-28%)	4-58%	1.22
MSM LWH	18	6,359	1,674	22% (16-30%)	3-47%	0.81
HRA Referral						
All	16	5,849	1,581	23% (16-32%)	3-47%	0.88
Screen positives	2	510	93	18% (15-22%)	13-22%	0.00
Women	9	1,854	270	13% (8-21%)	6%-41%	0.70
HRA Referral*						
All	5	973	214	19% (11-30%)	7%-41%	0.54
Screen positives	3	881	56	6% (5-8%)	6%	0.00
MSM, no HIV	6	1,019	191	12% (4-31%)	0%-35%	2.11

[1]Two studies (Chiao et al., 2020 and Jin et al., 2017) not included in the prevalence estimates due to overlap in populations with other studies, one study (Tramujas da Costa E Silva et al., 2011) not included because the data were not available to accurately assess anal precancer prevalence

*p heterogeneity <0.05; ^p heterogeneity <0.1

Abbreviations: AIN2+, anal intraepithelial neoplasia grade 2 or worse; HRA, high resolution anoscopy; MSM, men who have sex with men; LWH, living with human immunodeficiency virus; PLWH, people living with HIV

Table 3. Summary of the Performance Estimates of Tests for Anal Precancer Detection among Different Populations							
						Immediate AIN2+ Risk	
	Screening Test	N Studies	Positivity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	Test Positives % (95% CI)	Test Negatives % (95% CI)
Population							
All	Cytology (ASC-US+)	33	42.0 (32-52)	81.0 (72-87)	62.4 (54-70)	35 (29-43)	6 (4-10)
	Cytology (HSIL)	26	7 (5-10)	21.1 (16-27)	96.4 (95-98)	64 (56-72)	19 (13-26)
	HPV Testing	21	67 (59-73)	91.9 (87-95)	41.8 (35-49)	31 (24-39)	4 (2-8)
	Cytology and HPV Co-testing	12	74 (63-82)	93.0 (86-97)	33.4 (25-43)	27 (20-35)	5 (3-7)
	HPV16 Genotyping	10	23 (20-26)	45.5 (34-57)	83.4 (79-87)	39 (25-56)	13 (8-20)
	HPV16/18 Genotyping	8	30 (23-38)	44.1 (33-56)	77.4 (70-83)	32 (17-53)	16 (11-21)
	HPV E6/E7 mRNA	6	47 (45-50)	74.2 (69-79)	64.3 (58-70)	49 (35-64)	16 (10-25)
	p16 or p16/Ki-67 Dual Stain	6	41 (32-51)	54.0 (34-73)	65.2 (55-75)	34 (17-55)	16 (10-24)
	p16/Ki-67 Dual Stain	4	41 (28-56)	65.8 (39-85)	70.3 (55-82)	44 (23-68)	15 (8-28)
PLWH	Cytology (ASC-US+)	27	45 (35-56)	84.1 (75-90)	60.0 (49-70)	37 (29-46)	6 (4-11)
	Cytology (HSIL)	18	8 (6-12)	22.6 (17-29)	95.6 (94-97)	64 (52-74)	20 (12-32)
	HPV Testing	17	73 (66-78)	93.8 (88-97)	34.9 (28-43)	31 (23-41)	5 (3-9)
	Cytology and HPV Co-testing	9	76 (63-86)	91.9 (82-97)	31.5 (21-44)	27 (18-39)	6 (4-9)
	HPV16 Genotyping	8	23 (20-26)	43.6 (32-56)	82.1 (77-86)	36 (22-53)	14 (8-25)
	HPV16/18 Genotyping	5	38 (35-40)	46.2 (31-62)	68.7 (63-74)	24 (9-52)	16 (11-24)

MSM LWH	Cytology (ASC-US+)	16	51 (41-61)	85.2 (77-91)	52.8 (43-62)	32 (23-42)	6 (3-11)
	Cytology (HSIL)	10	6 (3-12)	24.6 (19-31)	96.0 (93-98)	56 (44-67)	15 (8-27)
	HPV Testing	12	76 (69-81)	96.1 (90-99)	29.9 (22-39)	30 (21-41)	4 (2-9)
	HPV16 Genotyping	5	24 (20-28)	42.4 (27-59)	80.4 (74-85)	29 (13-54)	12 (6-25)
	HPV16/18 Genotyping	4	38 (35-41)	42.9 (24-64)	66.5 (61-72)	18 (6-43)	13 (10-18)
Women	Cytology (ASC-US+)	8	21 (9-41)	65.7 (36-86)	82.2 (64-92)	33 (22-46)	5 (2-11)
	Cytology (HSIL)	5	6 (2-19)	36.2 (26-48)	95.6 (93-97)	70 (51-85)	13 (7-24)
	HPV Testing	6	59 (45-71)	91.1 (76-97)	47.1 (37-58)	27 (15-43)	4 (2-11)
MSM, no HIV	Cytology (ASC-US+)	4	39 (19-63)	56.6 (25-83)	66.5 (44-83)	38 (5-48)	14 (9-21)
	HPV Testing	4	53 (30-74)	76.1 (43-93)	53.7 (32-74)	26 (12-47)	10 (7-16)

Abbreviations: AIN2+, anal intraepithelial neoplasia grade 2 or worse; CI, confidence interval; ASC-US+, atypical squamous cells of undetermined significance or worse; HRA, high resolution anoscopy; HSIL, high grade squamous intraepithelial lesion; MSM, men who have sex with men; LWH, living with human immunodeficiency virus; PLWH, people living with HIV