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Sabrina Kay Smith Date

Performance of Screening Tools for Cervical Neoplasia Among Women in Low- and Middle-income Countries: A Systematic Review and Meta-Analysis

By

Sabrina Smith
MPH

The Hubert Department of Global Health

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Mohammed K. Ali, MD, MSc, MBA
Thesis Committee Chair

Performance of Screening Tools for Cervical Neoplasia Among Women in Low- and Middle-income Countries: A Systematic Review and Meta-Analysis

By

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Bachelor of Arts | Biology, Psychology
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2017

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An abstract of
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Abstract

Performance of Screening Tools for Cervical Neoplasia Among Women in Low- and Middle-income Countries: A Systematic Review and Meta-Analysis

By Sabrina Smith

Introduction: Cervical cancer continues to be a public health threat worldwide, disproportionately affecting low- and middle-income countries (LMICs). Screening for cervical cancer allows for early detection and treatment. Given the context of limited resources and challenges of traditional screening via Pap smears, the performance of different screening methods needs further evaluation.

Objective/Aim: This systematic review and meta-analysis evaluates the performance of visual inspection with acetic acid (VIA) testing, visual inspection with Lugol's iodine (VILI), primary HPV testing, and conventional Pap smear in detecting cervical intraepithelial neoplasia grade 2 or greater (CIN2+) among non-pregnant women aged 30-65 in LMICs between 1990 and 2020.

Methods: CENTRAL, CINAHL, Embase, Global Health, PubMed and Web of Science databases were systematically searched to identify studies. Diagnostic test accuracy meta-analysis evaluated the performance of screening methods in detecting CIN2+. Summary statistics for sensitivity, specificity, diagnostic odds ratios (DORs), and summary receiver operating characteristic (SROC) curves were determined for each method. Subgroup analyses were performed to examine whether there was variation in performance based on different reference standards, specifically: colposcopy-directed biopsy, biopsy alone, colposcopy alone, or liquid-based cytology.

Results: Eighteen studies were identified through systematic review. Six were narratively synthesized and excluded from meta-analysis given limitations in outcome values reported. Of the 12 studies included in meta-analysis, 11 were cross-sectional and 1 was a randomized controlled clinical trial. Summary estimates for sensitivity for VIA, VILI, HPV, and conventional Pap smear were 72.3%, 64.5%, 79.5%, and 60.2%, respectively; summary estimates for specificity were 74.5%, 68.5%, 72.6%, and 97.4%, respectively; the DORs were 7.31, 3.73, 10.42, 69.48, respectively; and the area under the SROC curves were 0.766, 0.647, 0.959, and 0.818, respectively. Performance of screening method varied based on reference standard used; summary estimates using colposcopy-directed biopsy or biopsy generally reported lower estimates; summary estimates using colposcopy or liquid-based cytology generally reported higher estimates.

Conclusion: This meta-analysis found primary HPV testing and VIA to be the highest performing cervical cancer screening methods in accurately identifying or excluding CIN2+. In resource-constrained countries, VIA may be the most feasible screening method. Further evaluation of performance at different CIN thresholds is warranted.

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This systematic review would not have been possible without the unwavering support and dedication from my study team. Dr. Mohammed Ali was a guiding light for this review, unparalleled in his methodological expertise. His mentorship helped to refine my research methods, ultimately enhancing the quality of the review, and for this I am extremely grateful. I would like to thank Kara Suvada for her resolute commitment and consistent enthusiasm in conducting this review amidst many competing priorities. Her passion and diligence are admirable and continue to inspire me in my own work. Thank you both for your expertise, as well as your friendship.

Thank you to Dr. Oguchi Nwosu for recognizing this gap in knowledge in the field and pursuing a study team to conduct this systematic review. Without your vision, this review would not have come to life. I would also like to express my gratefulness for your clinical expertise throughout this process and providing thoughtful feedback to all of our inquiries.

I would like to express my sincere gratitude to members of the Emory community who were willing to provide guidance during the development of review search terms, as well as the meta-analysis of study results. Mia White is an adept informationist and was able to quickly develop search terms based on exclusion and inclusion criteria, an essential component of the review. Thank you to Alex Edwards for providing data analyst expertise and contributing to the interpretation and visualization of study results. Both Mia and Alex's contributions to this project elevated the quality of the review. Their considerable contributions help to highlight the value of collaboration across disciplines to enhance the rigor and quality of research.

I am incredibly thankful to my Global Health peers as we navigated thesis writing amidst a global pandemic. The support and sense of solidarity in this community helped me to remain driven and focused in completing my review.

Finally, I would like to thank my friends and family for their words of advice and encouragement throughout this entire process. Thank you to all of my personal cheerleaders!

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Abbreviations

ASCUS	Atypical Squamous Cells of Undetermined Significance
CI	Confidence Interval
DC	Visual Inspection with Acetic Acid
DOR	Diagnostic Odds Ratio
DVI	Direct Visual Inspection
FN	False Negative
FP	False Positive
GNI	Gross National Income
IRB	Institutional Review Board
CIN	Cervical Intraepithelial Neoplasia
HIC	High-Income Country
HPV	Human Papillomavirus
HR	High-Risk
HSIL	High-Grade Squamous Intraepithelial Lesions
LBC	Liquid-Based Cytology
LR	Likelihood Ratio
LSIL	Low-Grade Squamous Intraepithelial Lesions
LMIC	Low- and Middle-income Country
MeSH	Medical Subject Heading
NPV	Negative Predictive Value
NLR	Negative Likelihood Ratio
OR	Odds Ratio
PICOTS	Patient population/Intervention/Comparison/Outcome/Timing/Setting
PLR	Positive Likelihood Ratio
PPV	Positive Predictive Value
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
SC	Suggesting Cancer
SCCx	Squamous Cell Carcinomas of the Cervix
SCJ	Squamocolumnar Junction
SROC	Summary Receiver Operating Characteristic

TN	True Negative
TP	True Positive
UVI	Unaided Visual Inspection
VI	Visual Inspection
VIA	Visual Inspection with Acetic Acid
VIAM	Visual Inspection with Acetic Acid with Magnification
VILI	Visual Inspection with Lugol's Iodine
WHO	World Health Organization

INTRODUCTION

Introduction & Rationale

Cervical cancer is the fourth most common cancer in women globally, despite being both preventable and treatable (Lemp, 2020). Approximately 570,000 women received a cervical cancer diagnosis in 2018, and an estimated 311,000 women died from the disease in the same year (WHO, 2021). Of these cases, over 85% were reported in low- and middle-income countries (LMICs), demonstrating the disproportionate burden of cervical cancer in these countries (Huy, 2018). The global reduction of cervical cancer incidence and mortality relies on vaccination, as well as the implementation of effective screening strategies to identify patients at risk for developing the disease and to select these patients for treatment (Gakidou, 2008). Given vaccine constraints in resource-limited countries, in order to adequately address the uneven distribution of cervical cancer cases in LMICs, it is necessary to focus on the barriers to effective cervical cancer screening in these countries.

Following the introduction of organized cervical cancer screening in the 1960s, high-income countries have experienced a steady decline in cervical cancer rates. In the United States, cervical cancer incidence and mortality have decreased by more than 70% since the 1950s (Safaeian, 2007), declining by 4.6% per year from 1973 through 1982 and by 1.6% per year from 1982 through 1997 (Wingo, 2003). The overall decline in death rates for cervical cancer is largely credited to these screening programs (Wingo, 2003). However, the burden of cervical cancer remains prevalent in many LMICs where screening programs are either unavailable or poorly implemented. According to the World Health Survey, the mean crude coverage (focusing solely on intervention use and

access) of cervical cancer screening in LMICs was reported to be 45%, and effective coverage (a measure that combines intervention need, use and quality) was reported at 19% (Gupta, 2017). These estimates point to potential deficiencies in infrastructure, resources and political will (Canfell, 2020). LMICs can be heavily resource-constrained and may lack access to standard cervical cancer screening methods including cervical cytology (Pap smear) and primary HPV testing. These recommended screening methods require adequately trained providers, ample screening tools and enhanced laboratory capacity. Additionally, these screening methods do not allow for a screen-and-treat approach, requiring patient follow-up after the initial appointment which proves to be a barrier for patients in LMIC settings where travel and transport may be an issue (Gallay, 2017).

In order to increase screening coverage in LMICs, it is essential to identify and implement feasible and cost-effective screening strategies appropriate for these settings. An alternate screening method with proven benefit in low-resource settings is visual inspection of the cervix. This visual screening can be done through unaided visual inspection (UVI), visual inspection with acetic acid (VIA), or visual inspection with Lugol's iodine (VILI). The most common practice in these settings is the utilization of VIA (Huy, 2018). During VIA testing, the provider swabs the patient's cervix with an acetic acid solution (vinegar) and allows the solution to highlight differences in cell structure and absorption, causing pre-cancerous lesions to turn white in color. The use of VIA as a screening method for cervical cancer represents a cost-effective alternative that requires less provider training and produces immediate results, reducing patient loss to follow-up and allowing for a screen-and-treat approach. While this strategy has proven

benefit in implementation, VIA lacks comprehensive evidence supporting the performance of the test as compared to more commonly used screening methods such as the Pap smear and primary HPV test (Sinha, 2018).

Problem Statement

Cervical cancer continues to disproportionately affect women in LMICs given limitations in the availability of standard screening modalities including the Pap smear and primary HPV test. Screening for cervical cancer allows for early detection and treatment of pre-cancerous lesions, ultimately reducing morbidities and mortalities of cervical cancer. Mass cytologic screening coverage is not feasible to implement in resource-constrained settings given the necessity of trained manpower, screening tools, and laboratory capacity (Gupta, 2017). Visual inspection methods like VIA or VILI offer a cost-effective solution and reduce patient loss to follow-up with a see-and-treat prevention strategy. While visual inspection methods have benefits to implementation in low-resourced settings, there lacks consensus on the proven efficacy of these methods in LMICs (Huchko, 2014). This systematic review aims to explore this gap in the literature by determining the performance of VIA compared to VILI, Pap test and primary HPV test in LMICs.

Purpose Statement

This thesis seeks to bridge the gap in knowledge regarding the performance of visual inspection with acetic acid (VIA) testing as a screening method for cervical cancer in low- and middle-income countries (LMICs) compared to the performance of other screening methods including visual inspection with Lugol's iodine (VILI), Pap test, and primary HPV testing.

Objectives and Aims

The objective of this thesis is to perform a systematic review and meta-analysis to determine the performance of visual inspection with acetic acid (VIA) testing compared to visual inspection with Lugol's iodine, primary HPV testing and Pap smear as a screening method for cervical cancer in non-pregnant women aged 30-65 in low- and middle-income countries (LMICs) between 1990 and 2020.

Underlying this objective are the following aims:

1. To collect and synthesize relevant literature based on predefined exclusion and inclusion criteria
2. To evaluate screening outcomes of sensitivity and specificity for each comparison group and determine diagnostic accuracy

Significance Statement

This study will contribute to the existing body of literature on the efficacy of screening methods for cervical cancer by providing a thorough review of the current literature and determining the performance of VIA compared to standard screening methods including the Pap smear and primary HPV test. This thesis will focus on VIA testing in LMICs, providing a unique and necessary perspective as the scientific community continues to work towards the advancement of organized cervical cancer screening globally. This systematic review will also help to identify any additional gaps in the literature, whether methodological or geographical. By determining the performance of VIA testing, the results of this thesis are expected to inform decision-making and potentially guidelines for the best and most optimal cervical cancer screening in LMICs and low-resourced communities.

Definition of Terms

The following key terms are used throughout this thesis, as well as in the corresponding literature referenced in this study.

Cervical Cancer

Cervical cancer occurs in the cells of the cervix (the tissue that connects the vagina to the uterus). The majority of cervical cancers are squamous cell carcinomas (SCCx), beginning in the thin, flat cells lining the outer portion of the cervix and comprising about 70% of all cervical cancer cases. Adenocarcinoma compromises about 25% of cases, and adenosquamous carcinoma is the least common with about 3-5% of cases (Brown, 2012). About 99% of all cervical cancer cases are caused by high-risk (HR) strains of human papillomavirus (HPV). Cervical cancer screening and treatment of pre-cancerous lesions has been shown to prevent most cervical cancer cases (WHO, 2021).

Cervical Intraepithelial Neoplasia/Dysplasia

Cervical intraepithelial neoplasia (CIN) are pre-cancerous cells occurring on the surface of the cervix, typically caused by HR strains of HPV. These cells are graded on a scale of 1 to 3, based on how abnormal the cell is and how much of the cervical tissue is affected. CIN1 is considered a low-grade lesion, and CIN 2 and 3 are considered high-grade lesions and more likely to progress to cervical cancer (WHO, 2014). The Bethesda system was introduced in 1988 and is an additional reporting system for cervical cytologic diagnoses, specifically used for reporting Pap smear results. This scale combines CIN2 and CIN3 into one group, termed high-grade squamous intraepithelial lesions (HSIL). CIN1 results are termed low-grade squamous intraepithelial lesions

(LSIL) (Chatterjee, 2000). On both the CIN scale and Bethesda scale, results that do not fit into either of these well-defined categories but appear different from the normal cervix are deemed ASCUS, or atypical cells of undetermined significance (Chatterjee, 2000; WHO, 2014). Given the ability of ASCUS and CIN1 lesions to resolve on their own and the higher risk for CIN2/3 to progress to cervical cancer, this review focuses on detecting for the CIN2+ threshold. Study results reported as HSIL have been translated to the CIN scale and deemed CIN2+ for purposes of analysis in this review.

Low- and Middle-Income Countries (LMIC)

This thesis classifies low- and middle-income countries (LMICs) utilizing the World Bank classification of economies. LMICs discussed in this thesis include three World Bank income groups: low, lower-middle, and upper-middle income groups. Groupings are developed using gross national income (GNI) per capita data in U.S. dollars. Estimates are updated annually and obtained from World Bank country unit economists based on official data published by each country (World Bank, 2021).

Human Papillomavirus (HPV)

Human papillomavirus (HPV) is a group of common viruses transmitted through sexual contact. Of more than 100 types of HPV, at least 14 are cancer causing and more specifically, two types of HPV (16 and 18) cause over 70% of cervical cancers and pre-cancerous cervical lesions (WHO, 2021).

Primary HPV Testing

An HPV test screens for high-risk HPV types likely to cause cervical cancer by detecting these HPV types of DNA in cells from the cervix. During an HPV test, a health provider examines the vagina and cervix and collects cell specimen for laboratory

examination and results. An HPV test requires a certified pathologist to interpret and typically takes 1-3 weeks for return of results. A *primary* HPV test is one that is done as a screening test without any other testing conducted. In contrast, an HPV *co-test* includes an HPV test and a Pap test conducted at the same time to screen for cervical cancer (American Cancer Society, 2021). This thesis focuses on primary HPV testing in order to isolate each method and more precisely determine screening efficacy.

Papanicolaou test (Pap smear/test)

The Pap smear, or cervical cytology, is a cervical cancer screening method that detects pre-cancerous lesions, or cell changes on the cervix that may progress to cervical cancer if left untreated. During a Pap smear, a health provider examines the vagina and cervix and collects cell specimen for laboratory examination and results (NCI, 2021). A Pap smear requires a certified pathologist to interpret and typically takes 1-3 weeks for return of results.

Visual Inspection with Acetic Acid

Visual inspection of the cervix is a common screening tool for low-resource settings given the low-cost nature of the exam and ability to provide immediate results. There are three types of visual inspection of the cervix: unaided visual inspection (UVI), also known as direct visual inspection (DVI), or Visual Inspection with Acetic Acid (VIA) and Visual Inspection with Lugol's Iodine (VILI). VIA can also be conducted with magnification, called visual inspection with acetic acid under magnification (VIAM) (Sarian, 2005). This systematic review focuses on VIA testing to better understand the VIA test performance compared to VILI, primary HPV testing, and Pap smear.

LITERATURE REVIEW

Burden of Cervical Cancer

Cervical cancer continues to be an important public health problem in many low- and middle-income countries. Cervical cancer is the fourth most common female cancer and a major cause of cancer-related death globally (WHO, 2021). Of the 311,000 cervical cancer deaths that occurred in 2018, between 84-90% were in LMICs (Hull, 2020). This high burden of cervical cancer in LMICs is primarily due to lack of access to cervical cancer screening services (Wingo, 2003).

Screening prevents cervical cancer by finding abnormal cell changes, or pre-cancerous lesions, on the surface of the cervix. Once these abnormalities are identified, providers can treat patients before the lesions can progress to cervical cancer. The standard screening method for cervical cancer screening is cervical cytology by the conventional Papanicolaou test, commonly referred to as the Pap smear. The Pap smear is a routine procedure conducted by a provider that involves the scraping of the cervix with a swab to collect a cell sample. This sample is then examined in a laboratory to identify pre-cancerous or cancerous cells (NCI, 2021).

Effective cytology-based screening programs for early detection and treatment have been shown to reduce the risk of developing cervical cancer by 80% (Safaeian, 2007). However, only ~5% of eligible women in LMICs undergo screening (Catarino, 2015). It can be challenging to implement screening programs in LMICs due to infrastructural barriers, scarce resources, and lack of trained manpower (Wingo, 2003). Reproductive health funding is often limited and tends to be prioritized for urgent needs, as opposed to preventive care like screening programs. Additionally, cytology-based

screening programs generally occur in urban areas at teaching hospitals and private laboratories, making it difficult for rural populations to access these services and contributing to an increased risk for loss to follow-up (Gakidou, 2008).

Given these implementation challenges for cytology-based screening programs in LMIC settings, alternative screening methods should be considered. Studies have pointed to both visual inspection with acetic acid (VIA) and visual inspection with Lugol's Iodine (VILI) as alternate screening methods that are viable, accurate, inexpensive and effective in detecting cervical cancer (Huchko, 2014; Hull, 2020). These methods can be conducted in low-resourced, community-based settings and provide instant results, allowing providers to immediately treat pre-cancerous or cancerous lesions and reduce loss to follow-up (Gakidou, 2008). In order to develop effective screening programs and reduce the global burden of cervical cancer, the performance of these screening methods should be further evaluated for use in LMIC settings.

Risk Factors for Cervical Cancer

There are several risk factors that increase the likelihood of developing cervical cancer. The most important risk factor for cervical cancer is infection with human papillomavirus (HPV) (ACS, 2021). HPV is a common virus that affects both men and women and is spread through sexual intercourse. There are over 150 related viruses of HPV, but only some are high-risk for cervical cancer occurring in women. These most high-risk (HR) HPV strains include HPV 16 and 18, causing about 70% of cervical cancers. Additional high-risk strains include HPV 31, 33, 45, 52, and others (ACS, 2021).

HR-HPV can lead to cervical cancer by causing an abnormal growth of cells on the surface of the cervix, most commonly occurring at the squamocolumnar junction (SCJ). This is referred to as cervical intraepithelial neoplasia (CIN), or cervical dysplasia (WHO, 2014). It is important to note that many women with HPV infection do not develop CIN. CIN growth is graded on a 3-point scale, with CIN3 being the most abnormal. Low grade dysplasia, or CIN1, typically resolves on its own and does not require treatment. Moderate or high-grade dysplasia, including CIN2+ (CIN2 or CIN3) require treatment, which may include cryotherapy, laser therapy, loop electrosurgical procedures, or cone biopsy to remove the abnormal tissue. While CIN of any grade tends to either resolve on its own or respond to treatment, about 5% of CIN2 and 12% of CIN3 progress to cervical cancer (ACS, 2021).

Cervical cancer risk increases with risk of exposure to HPV. Sexual history plays a role in HPV risk, including age at sexual debut, number of sexual partners, and having high-risk sexual partners. Additional risk factors include having given birth to three or more children, having an immunodeficiency disorder, like HIV, and the use of birth control for a period longer than five years. Age is also a risk factor for women; while women aged 20-24 years old have the highest incidence for HPV infection, they are most likely to clear the infection and CIN without treatment or follow up. When women aged 30 and older have HPV infection, they are more likely to have persistent infection with CIN that is higher risk for progressing to cervical cancer (ACS, 2021).

Screening Methods

The conventional Pap smear was developed by George Papanicolaou in 1941 and became the first cancer screening test of the modern era, having a profound effect on

cervical cancer morbidity and mortality (Shaw, 2000). While the Pap smear is consistently recognized as the gold standard for cervical cancer screening due to its success in decreasing cervical cancer rates, there are limitations with the performance of the screening test. Studies have shown Pap smear to have low sensitivity for the detection of CIN2+ in women, and high rates of false negatives (Longatto, 2012). Additionally, Pap smears require adequate sample collection to yield results. About 1-8% of Pap smears are reported unsatisfactory, and even when samples are satisfactory, cytologic interpretation is up to the observer (Boone, 2012). Liquid-based cervical cytology (LBC) was introduced as a method to respond to this challenge and improve diagnostic reliability of the conventional Pap smear. LBC involves the rinsing of cervical cells in preservatives to remove obscurities and allows for additional HPV testing of the sample. However, recent systematic reviews report no convincing evidence of greater diagnostic accuracy for LBC over the conventional Pap smear (Koliopoulos, 2017). LBC also represents a less feasible and more expensive screening option in low-resource settings.

HPV primary screening is another method of testing for cervical cancer. This method is performed by a provider; a speculum opens the vagina, and a spatula or brush is used to scrape cells from the cervix. This cervical cell sample is tested for high-risk HPV types in a laboratory setting (ACS, 2021). Primary HPV screening is the preferred and recommended test, as opposed to co-testing, when an HPV test and Pap test are conducted at the same time. A primary HPV test has been shown to better prevent cancer than a Pap test done alone, given the higher sensitivity of the primary HPV test (Koliopoulos, 2017). In addition, a primary HPV test does not increase the burden of unnecessary testing, which can happen with a co-test. Both Pap smears and primary HPV

tests are the most commonly used screening methods in high-income countries (HICs). HPV testing has been more frequently used in LMICs, as this method is slightly less resource intensive than Pap smears (Koliopoulos, 2017). This method of testing also allows for innovative techniques such as HPV self-sampling, a method that empowers women by removing access barriers and allowing them to collect their own specimen at a time and place comfortable for the individual (Defo, 2020).

There are multiple visual inspection methods used to screen for cervical cancer. Visual inspection with acetic acid (VIA) is performed by a provider during a vaginal speculum exam. Acetic acid (vinegar) is applied on the cervix and the provider views the cervix to identify color changes; pre-cancerous and cancerous lesions undergo an acetowhite change in the presence of acetic acid due to differences in their proteins. The visual inspection can be done using the naked eye, also called cervicoscopy or direct visual inspection (DVI) (Sarian, 2005). It can also be done using magnification, referred to as gynoscopy, aided visual inspection (VI), or visual inspection with acetic acid under magnification (VIAM). Another type of visual inspection is done with Lugol's iodine (VILI), or Schiller's test, applying Lugol's iodine instead of acetic acid. VIA has been validated as a stand-alone test and is more widely used than VILI, though VILI shows promise as an easier and more specific screening test (Huchko, 2015). Both of these methods represent practical solutions to the challenges that LMICs face in screening implementation, as visual inspection requires minimal infrastructure and immediate return of results.

The results of visual inspection are categorized as test-negative, test-positive and suspicious for or suggesting cancer (SC). Negative results indicate that no acetowhite

lesions are present, while positive results indicate that there are sharp, distinct, well-defined and dense acetowhite areas. SC lesions have clinically visible ulcerative, cauliflower-like growths, or oozing and/or bleeding on touch (ACS, 2021).

As previously detailed, the most notable strengths of visual inspection methods include the immediate availability of results, screen-and-treat approach and minimal reliance on infrastructure (Gakidou, 2008). Additional strengths are the low start-up and sustaining costs, simple training for providers, and potential to integrate these screening methods into primary care services. Visual inspection is limited in that the specificity of the test tends to be low, leading to overtreatment and associated increase in cost and morbidities (Gakidou, 2008). Previous and ongoing studies report test positivity rates varying from 10-35% following VIA testing, indicating overtreatment as a challenge (Cagle, 2010; Longatto, 2012). Similar to screening methods like the Pap smear, the test is rater dependent and varies based on the ability of the provider performing the test. Standardized criteria and quality assurance measures have been implemented to mitigate this risk.

Cervical cancer screening tests should strive for a balance between high sensitivity and acceptable specificity in order to correctly identify positive cases and minimize overtreatment. Cervical cancer persists as a public health threat in LMICs, with considerable variation in the type of screening methods employed. The most commonly used screening methods in these settings include the conventional Pap smear, primary HPV test and VIA. Therefore, the performance of these most standardized methods should be further evaluated for use in detecting pre-cancerous and cancerous lesions in LMIC settings.

VIA as an Alternative Approach to Cytology Screening in LMICs

Cervical cancer is the most widely screened cancer in both LMICs and HICs, given the nature of the cancer and the prolonged preclinical detection phase (WHO, 2021). While cytology-based screening programs have reduced cancer incidence and mortality by up to 80% in HICs (Safaeian, 2007), these programs continue to perform sub-optimally in LMICs because of the associated implementation challenges. Alternatively, VIA-based screening programs have been adopted to increase the uptake of cervical cancer screening in these settings. VIA has been widely investigated in various settings and by different providers for performance characteristics in detecting cervical neoplasia. To evaluate the performance of any screening test, it is essential to determine test characteristics in terms of sensitivity, specificity, positive predictive value, and negative predictive value. Current literature reports a broad range of test characteristic outcomes for VIA testing, depending on the context of the study. A systematic review of 11 cross-sectional studies conducted in India by Bobdey et al. demonstrated a pooled VIA sensitivity of 67.65% and VIA pooled specificity of 84.32% (Bobdey, 2016). In a different systematic review and meta-analysis occurring in China, Chen et al. estimated a VIA combined sensitivity and specificity of 77% and 87%, respectively (Chen, 2012). Additional meta-analyses report pooled outcomes that are both similar to these studies and varying significantly from these studies. The current body of evidence typically investigates VIA as a screening method in a singular country, rather than evaluating VIA performance in multiple countries and meta-analyzing these results.

Knowledge Gap

While many studies have reported on cervical cancer screening methods, there remains a gap in knowledge concerning the performance of these screening methods in low- and middle-income countries. Current systematic reviews and meta-analyses tend to explore VIA performance in one country or region, rather than evaluation across continents to include any LMIC. There is also a gap in knowledge concerning the performance of VIA in patient populations above the age of 30, when women less easily eradicate HPV and are more likely to progress to cervical cancer.

Conclusion

There is an abundance of literature exploring VIA performance as a screening method for cervical cancer. Many of these studies aim to determine the accuracy or performance of VIA compared to other cervical screening methods including the Pap smear, HPV test and VILI. Current literature fails to evaluate test characteristics in LMICs, and among an older patient population. This study intends to evaluate the performance of VIA, VILI, conventional Pap smear, and primary HPV testing in detecting CIN2+ among women ages 30-65 in LMICs.

STUDENT CONTRIBUTION

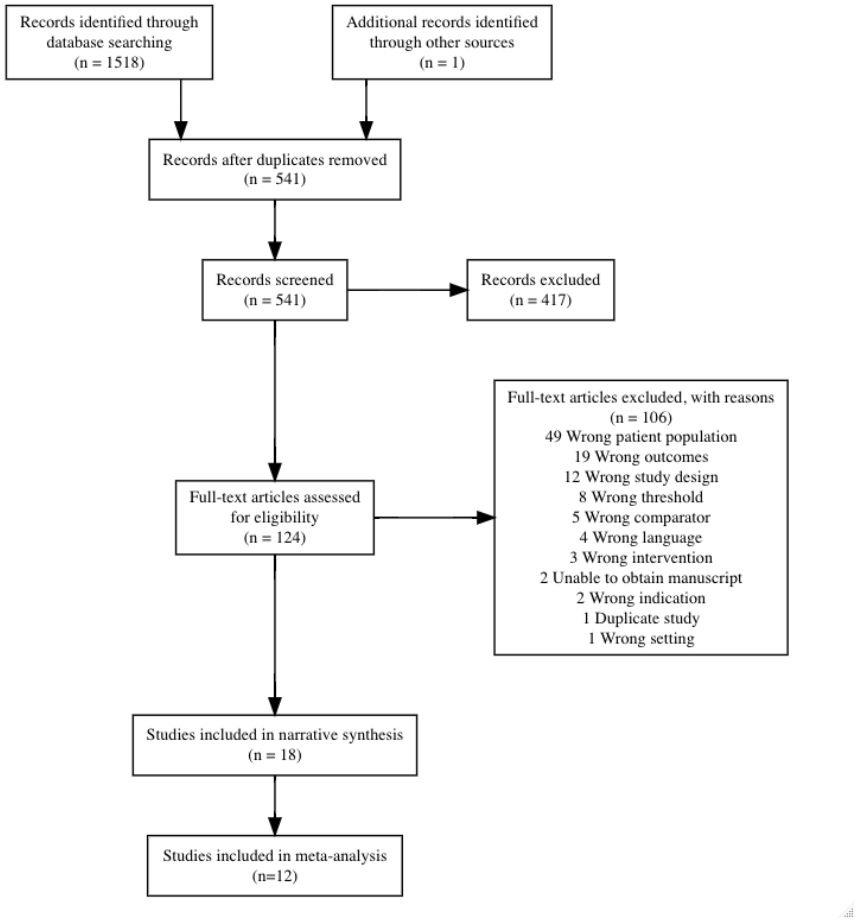
As the primary investigator of this study, I led the study team and contributed to all aspects of the review and meta-analysis. The current study was proposed by Oguchi Nwosu, MD, a practicing physician in Atlanta, GA, who noticed a gap in knowledge related to the performance of visual inspection with acetic acid (VIA) as a screening method for cervical cancer in low- and middle income countries. As a first step, I conducted formative research to review the literature and confirm this gap in knowledge. Following this informal review, I developed a PICOTS framework to narrow down the scope of the study and determine inclusion and exclusion criteria. Based on the PICOTS framework, I worked with an informationist to develop search terms and specify databases to run these searches. I then registered the review in PROSPERO (CRD42020206154).

CENTRAL (Cochrane Library), CINAHL, Embase, Global Health, PubMed and Web of Science databases returned 1518 results, and an additional study was found through an organic search. Author KS and I reviewed all study abstracts and full-texts for inclusion in the review, with disagreements adjucated by author MA. Following full-text review, I conducted all data extraction, with KS performing 10% data extraction to check for variability. Eighteen final studies were included in narrative synthesis and 12 final studies were included in meta-analysis, following PRISMA guidance (Figure 1). Covidence software was used for data management purposes.

To begin meta-analysis, I initially conducted descriptive analyses to examine test performance by screening method. I used Excel to develop a table outlining characteristics and demographics of studies included in narrative synthesis and meta-

analysis. I used RStudio (version 1.2.5042) to conduct a diagnostic test accuracy (DTA) meta-analysis, generating summary statistics and summary lines for each comparison group (RStudio Team, 2020). Specifically, I generated total effect sizes for sensitivity, specificity, and a diagnostic odds ratio. These descriptives were visualized in RStudio as forest plots. I also generated summary receiver operating characteristic curves as a summary line to help further visualize the data. I assessed heterogeneity based on these findings. After conducting the DTA meta-analysis, I worked with my study team to interpret findings and make conclusions.

Figure 1. PRISMA Diagram



*6 studies excluded from meta-analysis based on inability to extract true positive, false positive, true negative, and false negative values necessary for analysis.

Subsequently, I drafted the initial manuscript of the study for submission to either Lancet Global Health or JAMA Internal Medicine. Given time constraints associated with thesis deadlines, this thesis focuses on the analysis of test performance based on outcomes of sensitivity and specificity. For the manuscript submission, the meta-analysis will also evaluate positive predictive values, negative predictive values, and likelihood ratios. The manuscript will broaden the scope of the paper to include multiple CIN thresholds. Additionally, studies that were included solely in narrative synthesis will be considered for inclusion in meta-analysis, pending data received by study authors. My study team will continue to assist with editing and revising the manuscript prior to submission.

JOURNAL ARTICLE

Title Page

Title: Performance of Screening Tools for Cervical Neoplasia Among Women in Low- and Middle-income Countries: A Systematic Review and Meta-Analysis

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Abstract

Objective: To evaluate the performance of visual inspection with acetic acid (VIA) testing, visual inspection with Lugol's iodine (VILI), primary HPV testing, and conventional Pap smear in detecting CIN2+ among non-pregnant women aged 30-65 in LMICs between 1990 and 2020.

Design: Systematic review, narrative synthesis, and meta-analysis.

Setting and Participants: Low- and middle-income countries, non-pregnant women aged 30-65.

Methods: CENTRAL (Cochrane Library), CINAHL, Embase, Global Health, PubMed and Web of Science databases were systematically searched to identify studies evaluating the performance of cervical cancer screening methods in LMICs. A diagnostic test accuracy meta-analysis was conducted to evaluate the performance of 4 screening methods in detecting CIN2+. Summary statistics and summary lines for sensitivity, specificity, diagnostic odds ratios, and summary receiver operating characteristic curves were determined for each method. Subgroup analyses were performed to examine whether there was variation in performance based on different reference standards, specifically: colposcopy-directed biopsy, biopsy alone, colposcopy alone, or liquid-based cytology.

Results: Eighteen studies were identified through systematic review. Six were narratively synthesized and excluded from meta-analysis due to limitations in outcome values reported. Of the 12 studies included in meta-analysis, 11 were cross-sectional and 1 was a randomized controlled clinical trial. Summary estimates for sensitivity for VIA, VILI, HPV, and conventional Pap smear were 72.3%, 64.5%, 79.5%, and 60.2%, respectively;

summary estimates for specificity were 74.5%, 68.5%, 72.6%, and 97.4%, respectively; the diagnostic odds ratios were 7.31, 3.73, 10.42, 69.48, respectively; and the area under the summary receiver operating characteristic curves were 0.766, 0.647, 0.959, and 0.818, respectively. Performance of the screening method varied based on the reference standard used; summary estimates using either colposcopy-directed biopsy or biopsy alone as the reference standard generally reported lower estimates; summary estimates using either colposcopy alone or liquid-based cytology reported higher estimates.

Conclusions and Implications: This meta-analysis found primary HPV testing and VIA to be the highest performing cervical cancer screening methods in accurately identifying or excluding CIN2+. In resource-constrained countries, VIA may be the most feasible screening method and further evaluation of performance at different CIN thresholds is warranted.

Introduction

Cervical cancer is the fourth most common cancer in women globally, despite being both preventable and treatable. Approximately 570,000 women received a cervical cancer diagnosis in 2018, and an estimated 311,000 women died from the disease in the same year[1]. Of these cases, over 85% were reported in low- and middle-income countries (LMICs), demonstrating the disproportionate burden of cervical cancer in these countries[2]. The global reduction of cervical cancer incidence and mortality relies on vaccination, as well as the implementation of effective screening strategies to identify patients at risk for developing the disease and to select these patients for treatment[3]. Given vaccine constraints in resource-limited countries, in order to adequately address the uneven distribution of cervical cancer cases in LMICs, it is necessary to focus on the barriers to effective cervical cancer screening in these countries.

Following the introduction of organized cervical cancer screening in the 1960s, high-income countries have experienced a steady decline in cervical cancer rates. In the United States, cervical cancer incidence and mortality have decreased by more than 70% since the 1950s[4], declining by 4.6% per year from 1973 through 1982 and by 1.6% per year from 1982 through 1997[5]. The overall decline in death rates for cervical cancer is largely credited to these screening programs[5]. However, the burden of cervical cancer remains prevalent in many LMICs where screening programs are either unavailable or poorly implemented. According to the World Health Survey, the mean crude coverage (focusing solely on intervention use and access) of cervical cancer screening in LMICs was reported to be 45%, and effective coverage (a measure that combines intervention need, use and quality) was reported at 19%[6]. These estimates point to potential

deficiencies in infrastructure, resources and political will. LMICs can be heavily resource-constrained and may lack access to standard cervical cancer screening methods including cervical cytology (Pap smear) and primary HPV testing[7]. These recommended screening methods require adequately trained providers, ample screening tools and enhanced laboratory capacity. Additionally, these screening methods do not allow for a screen-and-treat approach, requiring patient follow-up after the initial appointment which proves to be a barrier for patients in LMIC settings where travel and transport may be an issue[8].

In order to increase screening coverage in LMICs, it is essential to identify and implement feasible and cost-effective screening strategies appropriate for these settings. An alternate screening method with proven benefit in low-resource settings is visual inspection of the cervix. This visual screening can be done through unaided visual inspection (UVI), visual inspection with acetic acid (VIA), or visual inspection with Lugol's iodine (VILI). The most common practice in these settings is the utilization of VIA[2]. During VIA testing, the provider swabs the patient's cervix with an acetic acid solution (vinegar) and allows the solution to highlight differences in cell structure and absorption, causing pre-cancerous lesions to turn white in color. The use of VIA as a screening method for cervical cancer represents a cost-effective alternative that requires less provider training and produces immediate results, reducing patient loss to follow-up and allowing for a screen-and-treat approach. While this strategy has proven benefit in implementation, VIA lacks comprehensive evidence supporting the performance of the test as compared to more commonly used screening methods such as the Pap smear and primary HPV test[9].

Methods

Study Search and Selection

CENTRAL (Cochrane Library), CINAHL, Embase, Global Health, PubMed and Web of Science databases were systematically searched to identify studies assessing test performance of VIA, VILI, Pap smear and primary HPV testing, published from January 1, 1990 to December 31, 2020. These databases were searched using Medical Subject Heading (MeSH) terms such as “cervical cancer”, “visual inspection”, “pap smear”, “developing country”, “mass screening”, and “clinical outcome”. Publications were restricted to the English language. The review protocol was registered in PROSPERO (CRD42020206154) and the report adheres to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for reporting systematic reviews[10].

The study population of interest included non-pregnant women aged 30-65 in low- and middle-income countries (LMICs) who received VIA testing and either VILI, Pap smear, or primary HPV test between 1990-2020. The decision to exclude studies on women aged 29 and younger aimed to decrease the false positive rates of high-risk HPV. Women under 30 have a low prevalence of underlying high-grade lesions and a high prevalence of transient HPV infection, meaning that they contract and eradicate HPV more quickly than women 30 years and older[11]. Studies that included participants who were pregnant or had a history of hysterectomy were excluded from this review. Studies with an inclusion criterion specifying that participants be symptomatic were also excluded from this review. Additionally, studies that utilized co-testing, as opposed to primary HPV testing, were excluded. HPV co-testing includes both a Pap test and HPV test performed at the same time, while primary HPV tests involve a singular HPV test.

Only primary HPV test results were collected in order to isolate the test results and determine performance of the singular test. This review evaluated diagnostic accuracy at the CIN2+ threshold, given the higher likelihood of CIN2+ progression to cervical cancer[12]. Studies included in this review consisted of randomized controlled clinical trials, cross-sectional studies and cohort studies. Case reports were excluded from this review because these reports are individual cases and associated with a higher risk of bias. Systematic reviews and meta-analyses were excluded from this review due to differing inclusion criteria and to avoid duplication of studies included in this review. Good and fair quality studies were assessed for inclusion in this review, as defined by the NIH Quality Assessment Tool[13]. Data that was available only in abstract form or grey literature were not eligible.

Covidence software was used for data management throughout this review[14]. Two reviewers independently screened all study titles and abstracts identified through MeSH database searches and selected studies for full-text review. Reviewers then individually screened all full texts to select studies for data extraction. Disagreements between independent reviewers were adjudicated by a third reviewer for title and abstract screening, full-text review, and data extraction.

Data Extraction

A standardized data extraction sheet was designed to extract data relevant to the review. Data extraction was primarily conducted by author SS and validated by authors KS and MA using a decision tree approach. In addition, author KS extracted a subsample of 10% of studies to check for variation in extraction. The primary outcome included extraction of the sensitivity and specificity of VIA, Pap tests, primary HPV test and VILI

at the CIN2+ threshold. When raw data was available, including true positives, true negatives, false positives and false negatives, these results were extracted. When only computed data for sensitivity and specificity was available, individual raw data were calculated based on identified proportions. When studies reported CIN2 and CIN3 as separate thresholds, these measures were weighted and combined to determine sensitivity and specificity of the combined CIN2+ threshold. Measures that were reported as HSIL on the Bethesda scale were converted to the CIN reporting method and denoted as CIN2+[12]. When studies reported outcomes for aggregates by age group, outcomes were either extracted or backed into using raw TP, TN, FP, FN for the desired age group ($\geq$ 30 years). Secondary outcomes included the extraction of positive predictive value, negative predictive value, adverse effects and likelihood ratios. Information was also extracted regarding the study-level characteristics and participant-level characteristics.

Data Synthesis and Analyses

This study utilizes the diagnostic test accuracy (DTA) approach to quantitatively synthesize extracted data[15,16]. Through DTA, representative summary statistics of sensitivity and specificity are combined into one effect size. Additional representative summary statistics generated include the diagnostic odds ratio (DOR) and forest plot, as well as the summary receiver operating characteristic (SROC) curve. These representative summary statistics and summary lines are derived from raw data including the true positive (TP), false positive (FP), false negative (FN), and true negative (TN) values that make up a 2 x 2 table[15].

To perform the DTA approach, raw data (TP, FP, FN, TN) was coded in RStudio (version 1.2.5042) to generate summary statistics of sensitivity, specificity and DOR, as

well as a summary line (SROC curve) [17]. The R "mada" package reitsma model was used to calculate these summary statistics using the bivariate model[16], estimating pooled measures of sensitivity and specificity separately for each comparison group while accounting for the potential correlation between sensitivity and specificity[18]. The bivariate model is similar to the random effects meta-analysis model of a pair-wise comparison, and is able to estimate heterogeneity, or the within-study and between-study variation of studies. This model assumes a binomial distribution to model representative summary statistics for within-study variation, and a bivariate normal distribution for between-study variation[16]. The bivariate approach produces unbiased estimates of sensitivity, specificity, and their correlation, and does not rely on ad hoc continuity correction for zero marginal counts[18]. A subgroup analysis was also performed, generating summary estimates for each comparison group by reference standard. Summary receiver-operating characteristics curves (SROC) were obtained along with 95% confidence regions for the bivariate estimates of sensitivity and 1-specificity. This curve indicates how discriminating a model is, and how well one is able to discern an individual study from another study included in the review[15].

Following the generation of summary statistics and summary lines, heterogeneity was verified and reported. Heterogeneity could be due to chance, difference in cut-off value, difference in study design, prevalence, research environment, and demographic factors of the sample population[15]. To assess statistical heterogeneity, the Higgins' I^2 measure was quantified, indicating the percentage of total variation across studies due to heterogeneity rather than chance[19]. Additionally, the symmetry and scattering of the

SROC curve was assessed, and the correlation coefficient of sensitivity and specificity was calculated using the R "mada" package.

The R "meta" package was used to calculate the total effect sizes of summary statistics through univariate analysis, such as the combined sensitivity for all studies included in the VIA comparison group[20]. This data was then plotted via "mada" on an SROC curve utilizing bivariate analysis[16]. This was a necessary step given the limitations of the "mada" package in calculating total effect sizes and allowed for each summary statistic value to be verified through univariate analysis in addition to bivariate analysis. For the interested reader, the R "meta" and "mada" packages have been described in depth at https://cran.r-project.org/web/packages/meta/meta.pdf and https://CRAN.R-project.org/package=mada[16,20].

Results

Characteristics of All Included Studies

This search strategy generated 1518 citations, with 1 additional study added for consideration for inclusion in the review. Following abstract screening and full-text review, 18 studies were included in the narrative synthesis and 12 studies were pooled for meta-analysis. Figure 1 shows the flowchart of study selection according to PRISMA[10]. The 6 studies excluded from meta-analysis but included in narrative synthesis fit the inclusion criteria and scope of the review but did not explicitly state the raw data needed to conduct meta-analysis, including true positive, false positive, true negative, and false negative values per group. For the purpose of this thesis, these studies were narratively synthesized to provide relevant context to the review.

The 12 studies included in meta-analysis were published between 2004 and 2020. Participant sample size from these studies totaled 110,657 and ranged from 100 to 54,981 women. Participant age ranged from 18 years to 65 years, with all studies reporting either a mean or median $\geq$ 35 years. These studies were geographically diverse, representing the African Region (36.9%), South-East Asian Region (38.1%), and Western Pacific Region (25%). Of these studies, 3 (25%) were comprised solely of HIV-positive populations. All women included in this review had enrolled in cervical cancer screening and were apparently healthy upon enrollment. The quality of all studies was high, as determined by the NIH Quality Assessment Tool[13]. Eleven (91.7%) studies were cross sectional and 1 (8.33%) was a randomized control trial. Table 1 lists the main characteristics of all integrated studies.

These 12 studies employed 4 different comparison groups to detect for pre-cancerous lesions of the cervix at the CIN2+ threshold: visual inspection with acetic acid (VIA), visual inspection with Lugol's iodine (VILI), primary HPV testing, and Pap smear (Table 2). Each of the studies included in this review conducted VIA as a screening method; 3 (25%) of the studies conducted VILI; 5 (41.7%) of the studies conducted HPV testing; and 5 (41.7%) of the studies conducted Pap smears. Studies used a variety of reference standards to determine the diagnostic accuracy of each of these methods. Reference standards included colposcopy-directed biopsy (50% of studies), biopsy alone (33.3% of studies), colposcopy alone (8.3% of studies), and liquid-based cytology (LBC) (8.3% of studies). Test characteristics for the CIN2+ threshold were aggregated based on screening method and reference group, as displayed in Figures 3-18. All studies assessed the sensitivity and specificity of one or more groups for detecting CIN2+.

Subgroup Analysis: VIA Comparison Group

From a total of 12 studies, the VIA screening approach had a combined sensitivity of 0.723 [0.641; 0.792], with lower sensitivity noted when the reference standard was LBC. Specificity of VIA was, on average, 0.745 [0.569; 0.866], and did not vary significantly by reference standard. The studies reporting on VIA were generally heterogenous, with an I^2 of 95.7% [94.0%; 97.0%], $p < 0.0001$ and 99.7% [99.6%; 99.7%], $p = 0$ for sensitivity and specificity, respectively.

The combined DOR for the VIA comparison group was 7.3078 [3.6547; 14.6122], with a higher DOR reported when the reference group was colposcopy (37.5711 [15.4299; 91.4842]). The combined DOR I^2 was calculated at 95.7% [94.0%; 97.0%], $p < 0.0001$, indicating considerable heterogeneity. The VIA SROC curve fell around 0.75-0.80, with an AUC of 0.766. The curve was determined to be relatively symmetrical with moderate scattering across the X axis, causing the curve to be stretched across the X axis. The correlation coefficient was -0.528.

Subgroup Analysis: HPV Comparison Group

Of 5 total studies, the primary HPV screening approach had a combined sensitivity of 0.795 [0.604; 0.908] and a higher sensitivity observed when the reference standard was LBC. The combined specificity of HPV was 0.726 [0.340; 0.932], but varied substantially for the colposcopy-directed biopsy reference group, with a specificity of 0.376 [0.213; 0.572]. The studies reporting on HPV were generally heterogenous, with an I^2 of 88.3% [77.1%; 94.0%], $p = 0.2127$ and 99.5% [99.3%; 99.6%], $p < 0.0001$ for sensitivity and specificity, respectively.

The combined DOR for the HPV comparison group was 10.4183 [1.7443; 62.2257], with a higher DOR reported when the reference group was colposcopy (101.9792 [30.1424; 345.0202]). The combined DOR I^2 was calculated at 96.9% [95.1%; 98.0%], $p < 0.0001$, indicating considerable heterogeneity. The HPV SROC curve fell around 0.75-0.85, with an AUC of 0.959. The curve was determined to be relatively symmetrical with moderate scattering across the X axis, causing the curve to be stretched across the X axis. The correlation coefficient was -0.558.

Subgroup Analysis: Pap smear Comparison Group

From a total of 5 studies, the Pap smear screening approach had a combined sensitivity of 0.602 [0.361; 0.803], with higher sensitivity noted when the reference standard was colposcopy alone. Specificity of Pap smear was, on average, 0.974 [0.955; 0.985], and did not vary by reference standard. The studies reporting on Pap smear were generally heterogenous, with an I^2 of 84.8% [66.1%; 93.2%], $p < 0.0001$ and 88.0% [74.6%; 94.4%], $p < 0.0001$ for sensitivity and specificity, respectively.

The combined DOR for the Pap smear comparison group was 69.4863 [25.6440; 188.2837], with a higher DOR reported when the reference group was colposcopy (499.4741 [165.5978; 1506.5078]). The combined DOR I^2 was calculated at 80.4% [53.9%; 91.6%], $p < 0.0004$, indicating considerable heterogeneity. The Pap smear SROC curve fell around 0.70, with an AUC of 0.818. The curve was determined to be symmetrical with moderate scattering across the Y axis, causing the curve to be stretched across the Y axis. The correlation coefficient could not be determined given limitations with the dataset.

Subgroup Analysis: VILI Comparison Group

From 2 total studies, the VILI screening approach had a combined sensitivity of 0.645 [0.571; 0.713] and did not vary by reference standard. Specificity of VILI was, on average, 0.685 [0.460; 0.847]; reported at 0.563 [0.391; 0.722] and 0.856 [0.846; 0.865] for colposcopy-directed biopsy and biopsy, respectively. The studies reporting on VILI were somewhat heterogenous, with an I^2 of 0% [0.0%; 46.8%], $p = 0.9934$ and 99.6% [99.4%; 99.7%], $p < 0.0001$ for sensitivity and specificity, respectively.

The combined DOR for the VILI comparison group was 3.7331 [0.8797; 15.8418], with a higher DOR reported when the reference group was biopsy (10.7737 [7.4001; 15.6853]). The combined DOR I^2 was calculated at 91.9% [79.5%; 96.8%], $p < 0.0001$. The VILI SROC curve fell around 0.65-0.70, with an AUC of 0.647. The curve was determined to be symmetrical, and the correlation coefficient was -0.190.

Narrative Synthesis

Six studies fit the inclusion and exclusion criteria for the systematic review but were not included in meta-analysis because of limitations in data reported in the manuscript. While not included in meta-analysis, these studies generally tended to demonstrate acceptable VIA performance and recommended the use of VIA when Pap is not feasible[21]. These 6 studies included data from India[21,22], China[23, 24], Iran[25], Brazil[26], and Argentina[26]. Among these studies, sensitivities for VIA ranged from 43% to 94.6%; specificity for VIA demonstrated less variation, ranging from 81.6% to 96.7%. These variations in diagnostic accuracy of VIA reflect the considerable heterogeneity observed in the meta-analysis of findings.

Discussion

To our knowledge, this was the first systematic review and meta-analysis evaluating the diagnostic performance of cervical cancer screening tests in non-pregnant women living in LMICs. Disease status was confirmed with either colposcopy-directed biopsy, biopsy alone, colposcopy alone, or liquid-based cytology. Results indicated that VIA had a sensitivity of 72.3%, a specificity of 74.5% and a DOR of 7.31, achieving a high efficiency in accurately identifying or excluding CIN2+. Primary HPV testing had a slightly higher diagnostic performance than VIA, with a sensitivity of 79.5% and DOR of 10.42, but a lower specificity of 72.6%. Conventional Pap smear demonstrated the highest specificity of the screening methods at 97.4% and a DOR of 69.48. However, Pap smear sensitivity ranked lowest at 60.2%, losing efficacy in accurately identifying those with CIN2+ and decreasing the favorability of this particular screening method. Of the 4 methods evaluated, VILI was the lowest performing with a sensitivity of 64.5%, specificity of 68.5%, and DOR of 3.73.

Conventional Pap smear continues to be the most widely used cervical cancer screening method worldwide, despite constraints with the sensitivity of the test. This study demonstrated results corresponding with similar meta-analyses that evaluated performance and test characteristics of Pap smear[27]. There is an abundance of literature evaluating test characteristics of Pap smear to screen for cervical cancer in a variety of settings and populations, with results for sensitivity remaining consistently low across studies. A meta-analysis by Fahey et al. that estimated accuracy of the Pap smear using a SROC curve suggested that the Pap smear may be unable to achieve concurrently high sensitivity and specificity[28]. A Bayesian analysis of Fahey et al.'s dataset estimated

similar results for the accuracy of the test[29]. The low sensitivity associated with Pap smear appears to be attributable to the nature of the test and necessitates the use of co-testing with HPV testing or rescreening for cervical cancer annually or bi-annually.

Given these limitations of Pap smear, this study determined the highest performing diagnostic test to be primary HPV testing. This screening test was less specific than Pap smear but was generally more efficacious when considering both the sensitivity and specificity of the test. However, cost-effectiveness, loss to follow-up and inadequate testing conditions continue to be a barrier for HPV testing in LMIC settings[3, 30]. Visual inspection methods of VIA and VILI are more desirable in these settings, proving to be advantageous in minimizing cost and reducing loss to follow-up[3]. In this meta-analysis, VIA improved diagnostic performance with sensitivity and specificity values rivaling those of primary HPV testing. VILI had a worse diagnostic performance than VIA, appearing to be the most undesirable screening method of the 4 methods evaluated. These results suggest that VIA has acceptable diagnostic performance and should be more widely used in LMIC settings in the future.

Another benefit of visual inspection is that these methods tend to be less invasive than Pap smear or HPV testing. While all methods are relatively safe, added discomfort during the procedure may discourage women to continue screening throughout their adult life, particularly in settings where services are less accessible. It is also important to note the burden of misdiagnosis and over-screening. False-negative reports, or failing to identify CIN, contributes to increased rates of cervical cancer[31]. Alternatively, false-positive reports, or inaccurately characterizing cells as abnormal, are shown to cause both physical and psychological burden in patients, including anxiety, unnecessary invasive

investigative procedures and treatment[31]. In both cases, inaccurate results contribute to patient harm and over-screening, leading to increased cost and patient mistrust in screening practices[31]. These outcomes stress the importance of a high-performing screening test to minimize harm in patient populations[32].

With the exception of the VILI group, results indicated considerable heterogeneity for VIA, Pap, and HPV comparison groups when analyzing SROC curves. This finding suggests that there is very little similarity between studies in each comparison group. Studies were all high quality as determined by the NIH Quality Assessment Tool[13], and comparisons were performed in similar patient populations with a mean $\geq$ 35 years of age living in LMICs. Differences in heterogeneity may be a function of the science, the dataset, the sample that we took, or a combination of these issues. Further analysis should be done to confirm whether certain variables contribute to increased heterogeneity in outcomes, such as the study region, study size of the population, or capacity of test providers[21].

One of the major strengths of this systematic review and meta-analysis is the ability to examine test characteristics in the population of patients aged 30-65 years. Focusing on this patient population addresses CIN findings that are more likely to progress to cervical cancer and less likely to resolve without treatment[11]. The scope of this review allowed for any low- or middle-income country to be included, contributing to the breadth of the dataset and evaluating performance of these screening tests on a global stage. This review helped to identify gaps in the literature; the majority of studies evaluated screening methods in peri-urban and urban areas of middle-income countries. Future studies should explore test performance of screening methods in expanded

geographies, to include rural populations and low-income countries. Additionally, most studies did not report outcomes beyond sensitivity, specificity, positive predictive value, and negative predictive value. Studies should expand reporting to include values such as likelihood ratios and odds ratios to aid in the assessment of clinical utility of the results.

This study has several limitations that should be taken into account when interpreting these results. By evaluating performance of test characteristics solely at the CIN2+ threshold, performance of screening methods at ASCUS and CIN1 thresholds were missed. Evaluating performance at multiple thresholds would help to provide context for results at the CIN2+ threshold, as well as contribute to the body of evidence supporting the use of certain screening methods. Another limitation related to threshold was the standardization of the Bethesda system under the CIN reporting system umbrella. While this crosswalk allowed for standardized comparisons groups, studies using the Bethesda system to report CIN may have had slight variations in CIN diagnosis by clinicians that could not be accounted for. The studies included in this review did not always stratify data by pregnancy status as outlined in the exclusion criteria, including data from the LAMS study in the narrative synthesis, which had a 3.5% pregnant population at the time of the screening[26, 33]. Additionally, when backing into desired outcomes for aggregates by age group, manual calculations and rounding may have contributed to slight discrepancies in extracted outcomes[32]. The majority of studies included in this study employed a cross-sectional design, which limits the ability to derive causal relationships and assumes a representative sample. Finally, this review was influenced by publication bias, and limited to articles published in English. The review

and meta-analysis would have benefited from the inclusion of additional languages, particularly given its' global scope.

Conclusions and Implications

Based on these findings, primary HPV testing and VIA testing demonstrated the highest diagnostic accuracy for early detection of CIN2+ in women aged 30-65 years low- and middle-income settings. In resource-constrained settings, VIA may be the most optimal screening method given the cost-effectiveness of the test. However, the heterogeneity demonstrated in this study suggests disparities across studies and highlights the need for reproducible research in this topic area to better understand the performance of screening methods in low- and middle-income countries. Additionally, further exploration should be done to evaluate performance of these screening methods in detecting a range of CIN thresholds.

Conflicts of Interest: The authors have no conflicts of interest to report.

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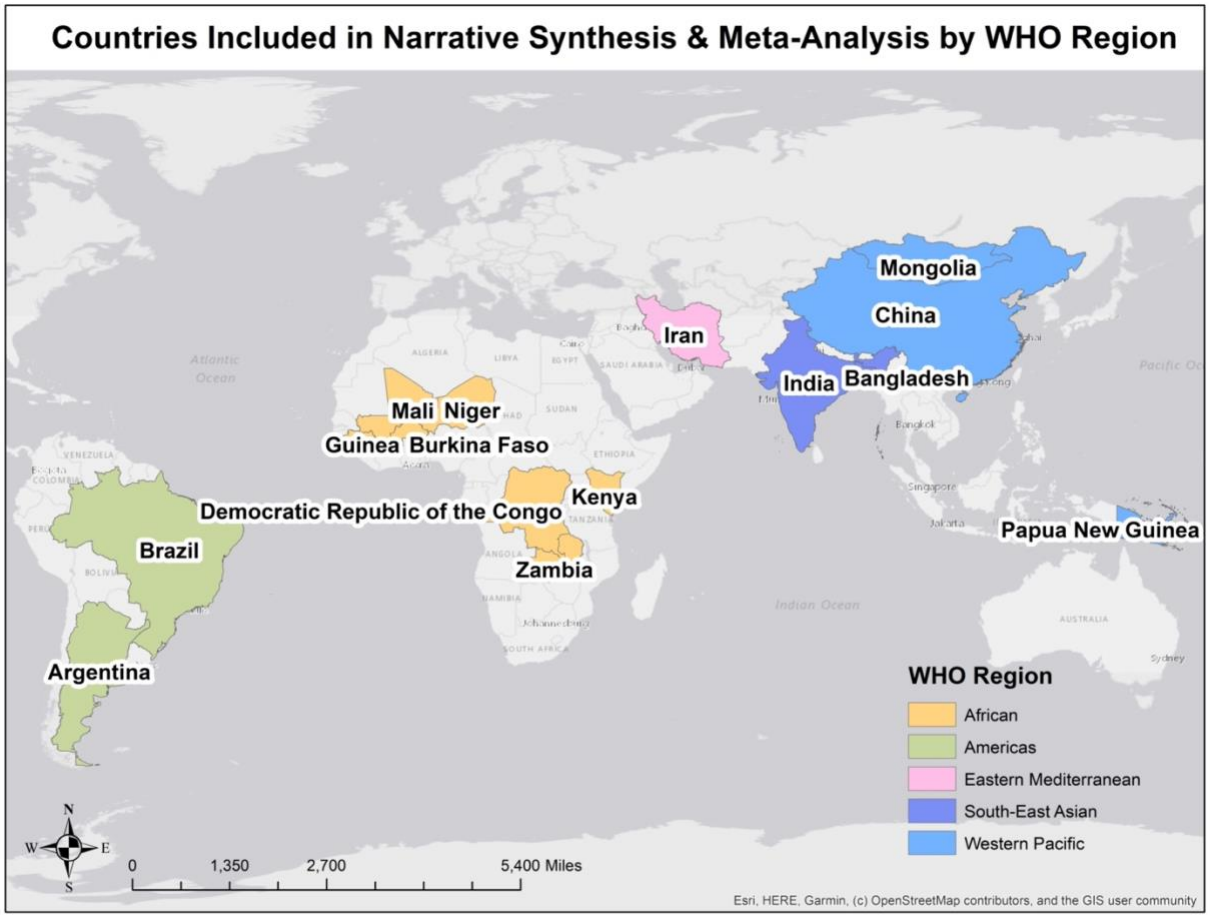
Tables and Figures

Figure 2. Map of Countries Included in Narrative Synthesis and Meta-Analysis by WHO Region



*All countries displayed are included in narrative synthesis. Countries also included in meta-analysis are Argentina, Bangladesh, Brazil, China, Democratic Republic of the Congo, India, Iran, Kenya, Mongolia, Papua New Guinea, Zambia.

Table 1. Characteristics of 12 Studies on Cervical Cancer Screening

Study	Study Design	Country	Region	Study Period	Study Population (n)	Age Mean (SD)/Median	Age Range (Years)	Education (%)	Marital Status (%)	Parity (%)	Symptomatic (%)	HIV Positive (%)
Basu (2015)	Cross-sectional	India	Southeast Asian	2010-2014	39740	–	30-60	< Secondary School: 78% ≥ Secondary School: 22%	Married: 92.8% Unmarried: 7.2%	None: 1.8% Some: 98.2%	No	No
Chibwesha (2016)	Cross-sectional	Zambia	African	2015	200	42.00	–	< Secondary School: 25% > Secondary School: 75%"	Married: 47% Unmarried: 53%	–	No	Yes (100%)
Chung (2013)	Cross-sectional	Kenya	African	2009	500	38.00	18-50	< Secondary School: 21% > Secondary School: 79%"	Married: 43% Unmarried: 57%	–	No	Yes (100%)
Deodhar (2012)	Cross-sectional	India	Southeast Asian	2006-2007	5519	–	30-49	< Secondary School: 57% > Secondary School: 43%"	–	None: 0% Some: 100%	No	No
Elit (2006)	Cross-sectional	Mongolia	Western Pacific	2002-2004	2009	43.70	31.5-65.8	–	Married: 86.2% Unmarried: 13.8%	–	Symptomatic: 8.3% Asymptomatic: 91.7%	No
Huchko (2015)	Cross-sectional	Kenya	African	2010-2012	1439	–	30-39	–	Married: 54% Unmarried: 46%	–	No	Yes (100%)
Khodakarami (2011)	Cross-sectional	Iran	Southeast Asian	2011	100	36 (7.90)	20-60	< Secondary School: 64% > Secondary School: 36%"	Married: 88% Unmarried: 12%"	None: 21% Some: 79%	No	No
Naizhaer (2020)	RCT	China	Western Pacific	2015	1993	44.95 (6.66)	35-64	< Secondary School: 93.6% > Secondary School: 6.4%"	–	–	No	No
Nessa (2013)	Cross-sectional	Bangladesh	Southeast Asian	2008-2010	650	–	30-45	< Secondary School: 51.1% ≥ Secondary School: 48.9%	Married: 100% Unmarried: 0%	None: 3.9% Some: 96.1%	No	No
Sangwa-Lugoma (2006)	Cross-sectional	DRC	African	2003-2004	1528	–	30-50	< Secondary School: 66% ≥ Secondary School: 34%	Married: 75% Unmarried: 25%	–	Symptomatic: 45.7% Asymptomatic: 54.3%	No
Sankaranarayanan (2004)	Cross-sectional	Multiple	African, Southeast Asian	1998-2003	54981	–	25-65	< Secondary School: 100% > Secondary School: 0%"	Married: 89.2% Unmarried: 10.8%"	None: 3.6% Some: 96.4%	No	No
Toliman (2018)	Cross-sectional	Papau New Guinea	Western Pacific	2014-2015	991	–	30-59	< Secondary School: 79.7% > Secondary School: 20.3%"	Married: 89.6% Unmarried: 10.4%	–	Symptomatic: 61% Asymptomatic: 39%	No

Table 2. Screening Methods and Reference Standards

Study	Screening Method	Reference Standard	HPV Assay	Study Population (n)
Basu (2015)	VIA	Colposcopy-directed biopsy	HC2	39740
	HPV	Colposcopy-directed biopsy		39740
Chibwesha (2016)	VIA	Biopsy	OncoE6, Xpert HPV	200
	HPV	Biopsy		200
				200
Chung (2013)	VIA	Colposcopy-directed biopsy	EIA	500
	Pap	Colposcopy-directed biopsy		500
	HPV	Colposcopy-directed biopsy		500
Deodhar (2012)	VIA	Biopsy	–	5519
	VILI	Biopsy		5519
	Pap	Biopsy		5519
Elit (2006)	VIA	Colposcopy	–	2009
	Pap	Colposcopy		2009
Huchko (2015)	VIA	Biopsy	–	1439
Khodakarami (2011)	VIA	Colposcopy-directed biopsy	–	100
	Pap	Colposcopy-directed biopsy		100
Naizhaer (2020)	VIA	Colposcopy-directed biopsy	careHPV	1007
	VILI	Colposcopy-directed biopsy		1007
	HPV	Colposcopy-directed biopsy		1993
Nessa (2013)	VIA	Biopsy	–	650
	Pap	Biopsy		650
Sangwa-Lugoma (2006)	VIA	Colposcopy-directed biopsy	–	1528
	VILI	Colposcopy-directed biopsy		1528
Sankaranarayanan (2004)	VIA	Colposcopy-directed biopsy	–	54981
Toliman (2018)	VIA	Liquid-based Cytology	HPV Xpert	462
	HPV			529

Figure 3. Forest Plot for VIA Sensitivity Summary Statistics

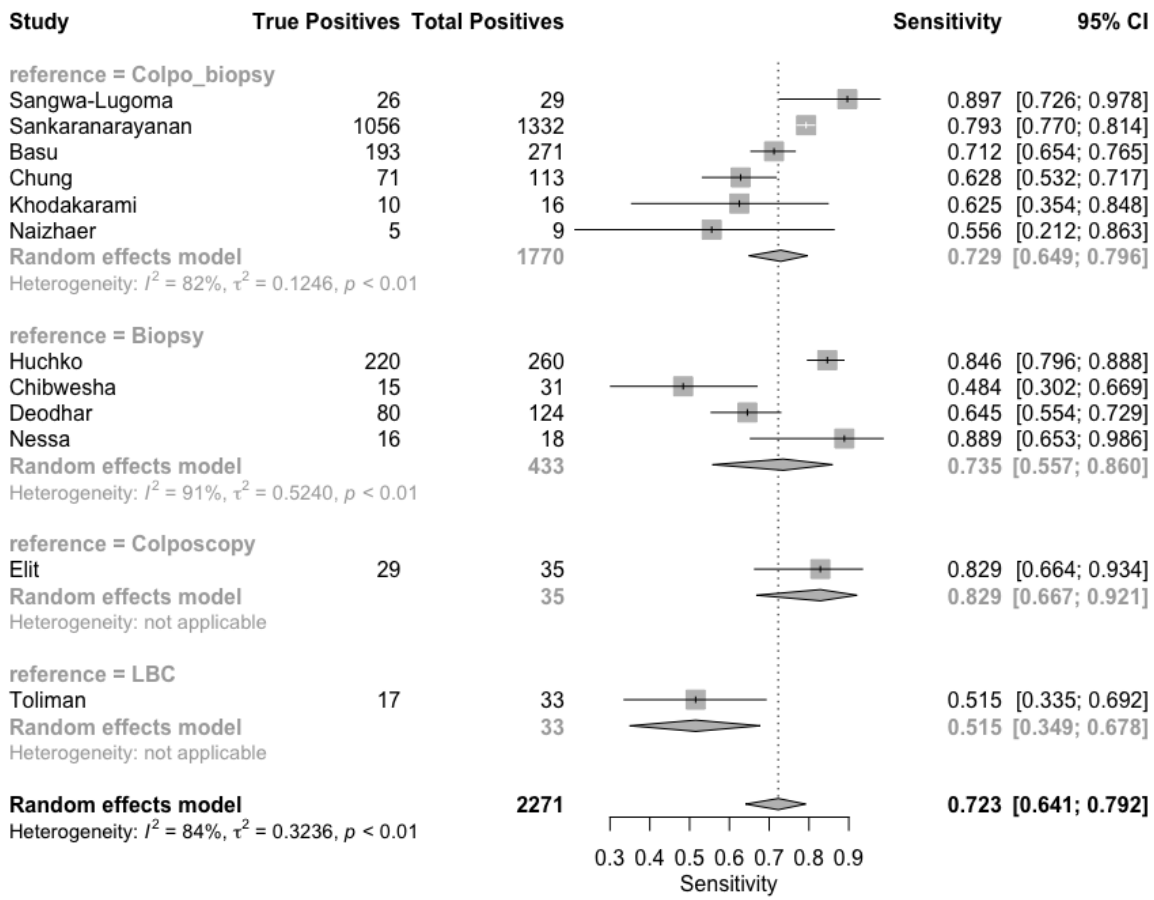


Figure 4. Forest Plot for VIA Specificity Summary Statistics

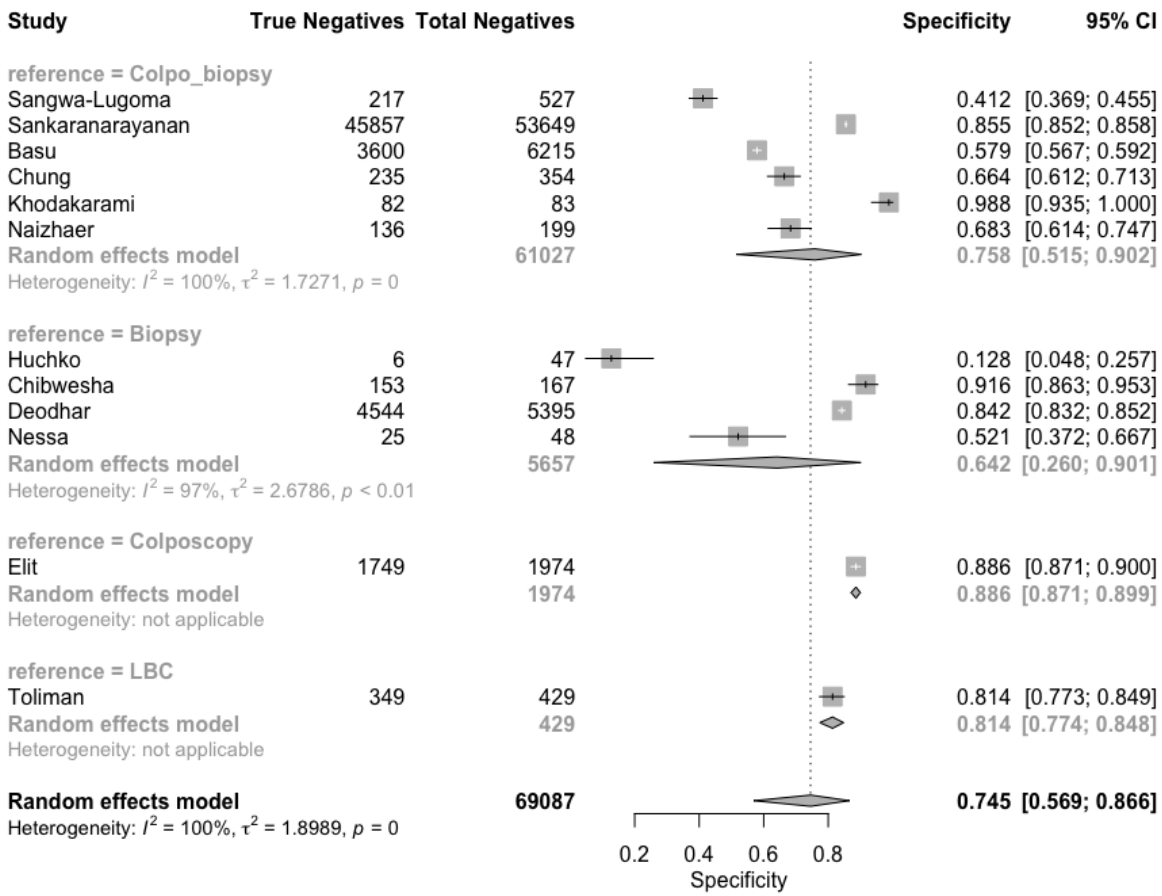


Figure 5. Forest Plot for VIA Diagnostic Odds Ratios

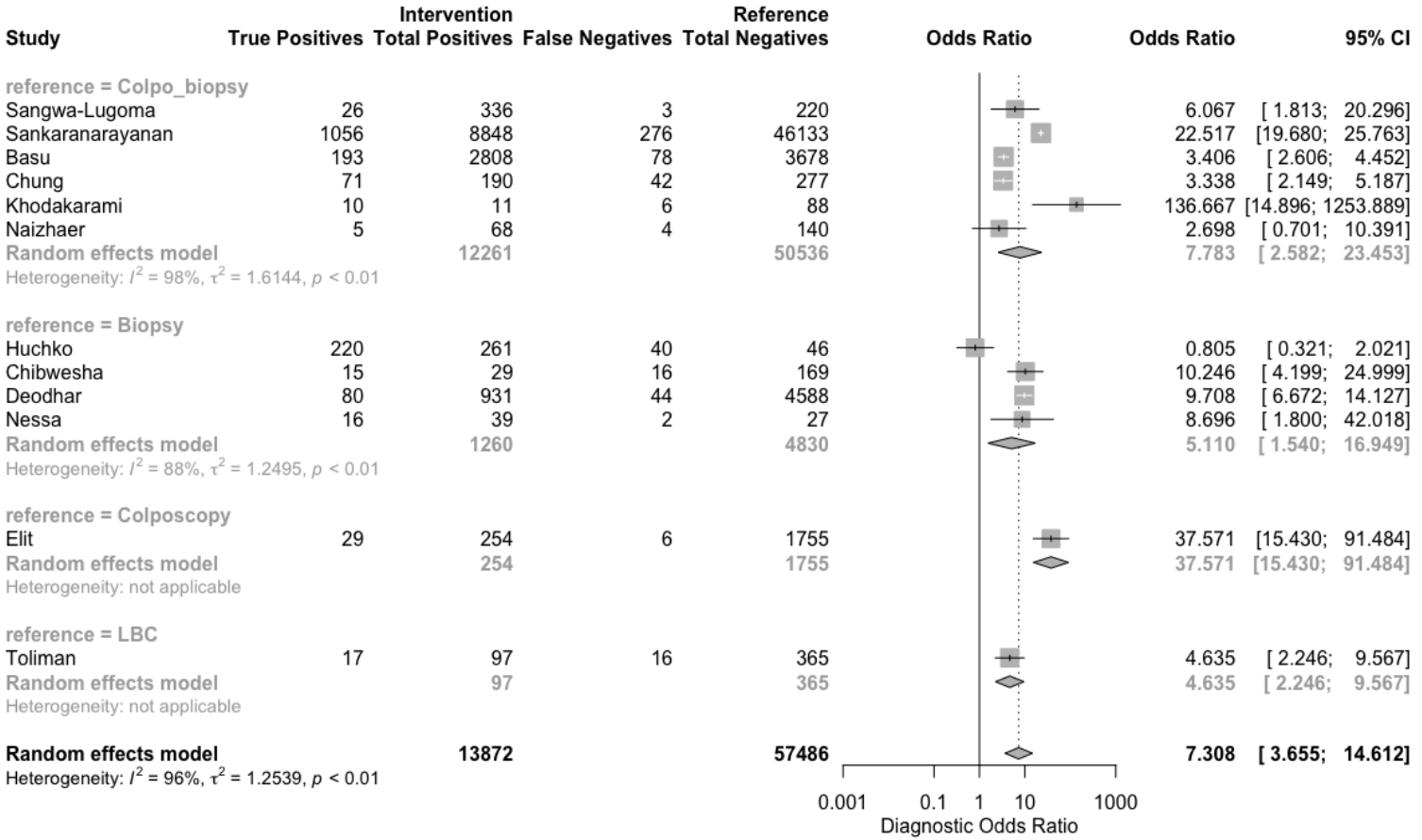


Figure 6. VIA SROC Curve

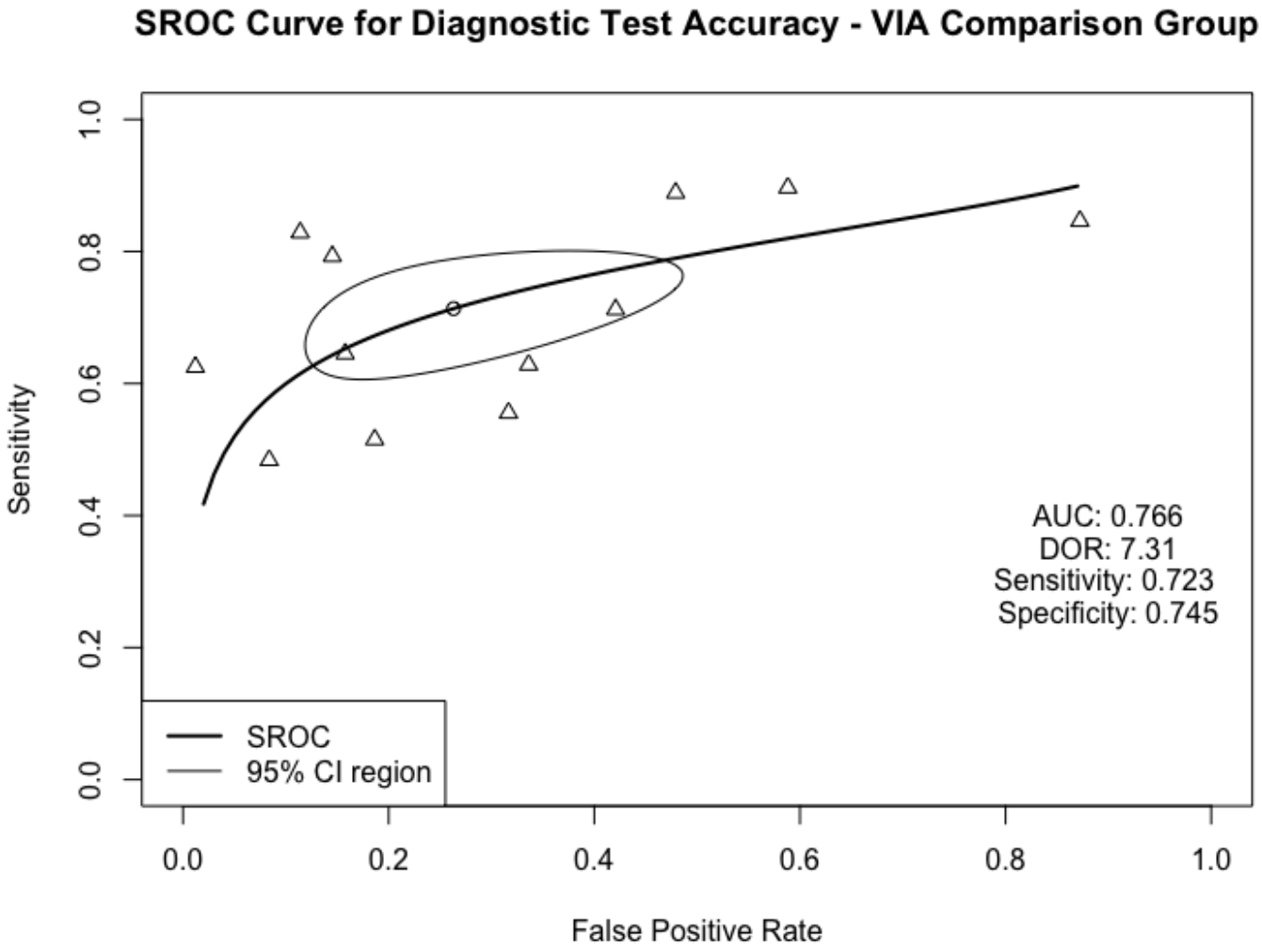


Figure 7. Forest Plot for VILI Sensitivity Summary Statistics

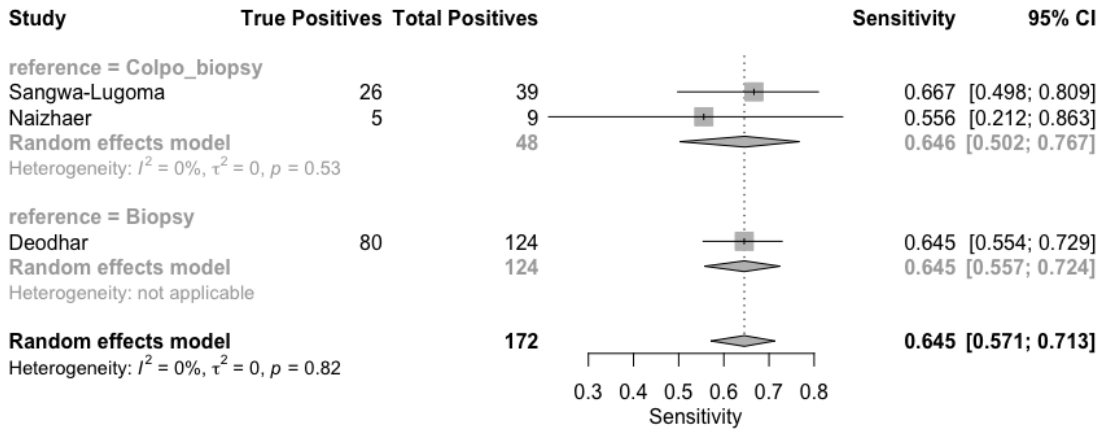


Figure 8. Forest Plot for VILI Specificity Summary Statistics

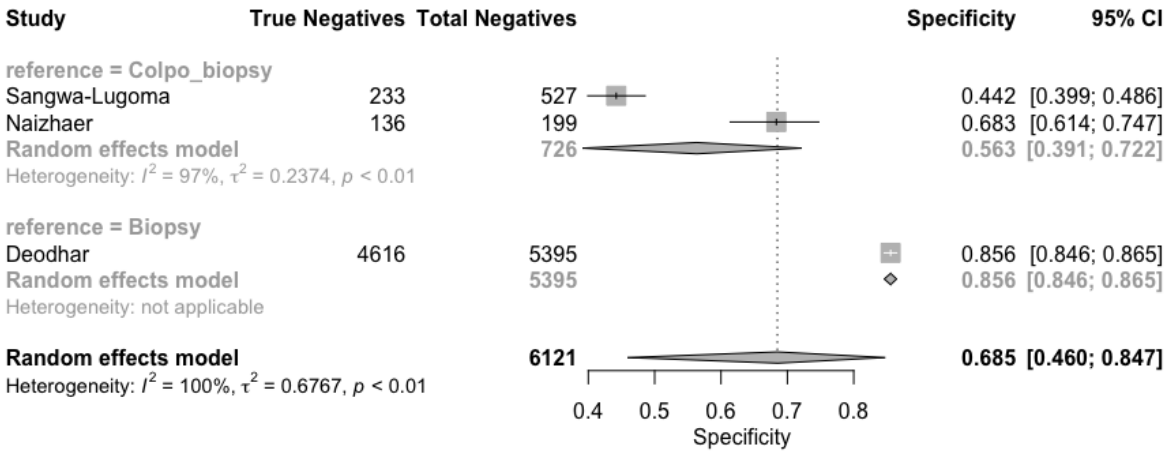


Figure 9. Forest Plot for VILI Diagnostic Odds Ratios

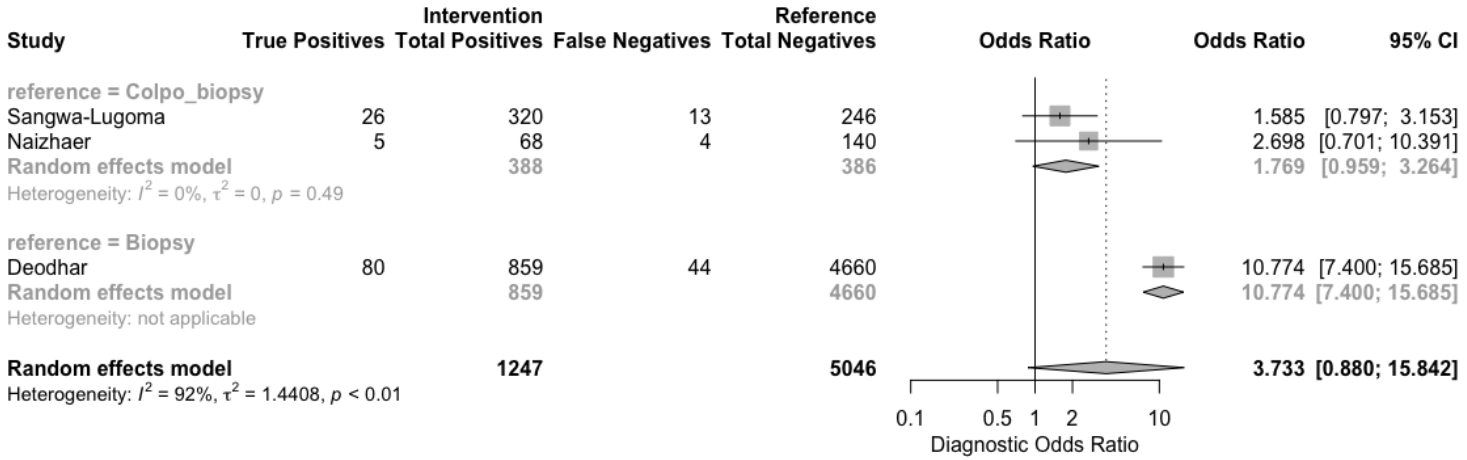


Figure 10. VILI SROC Curve

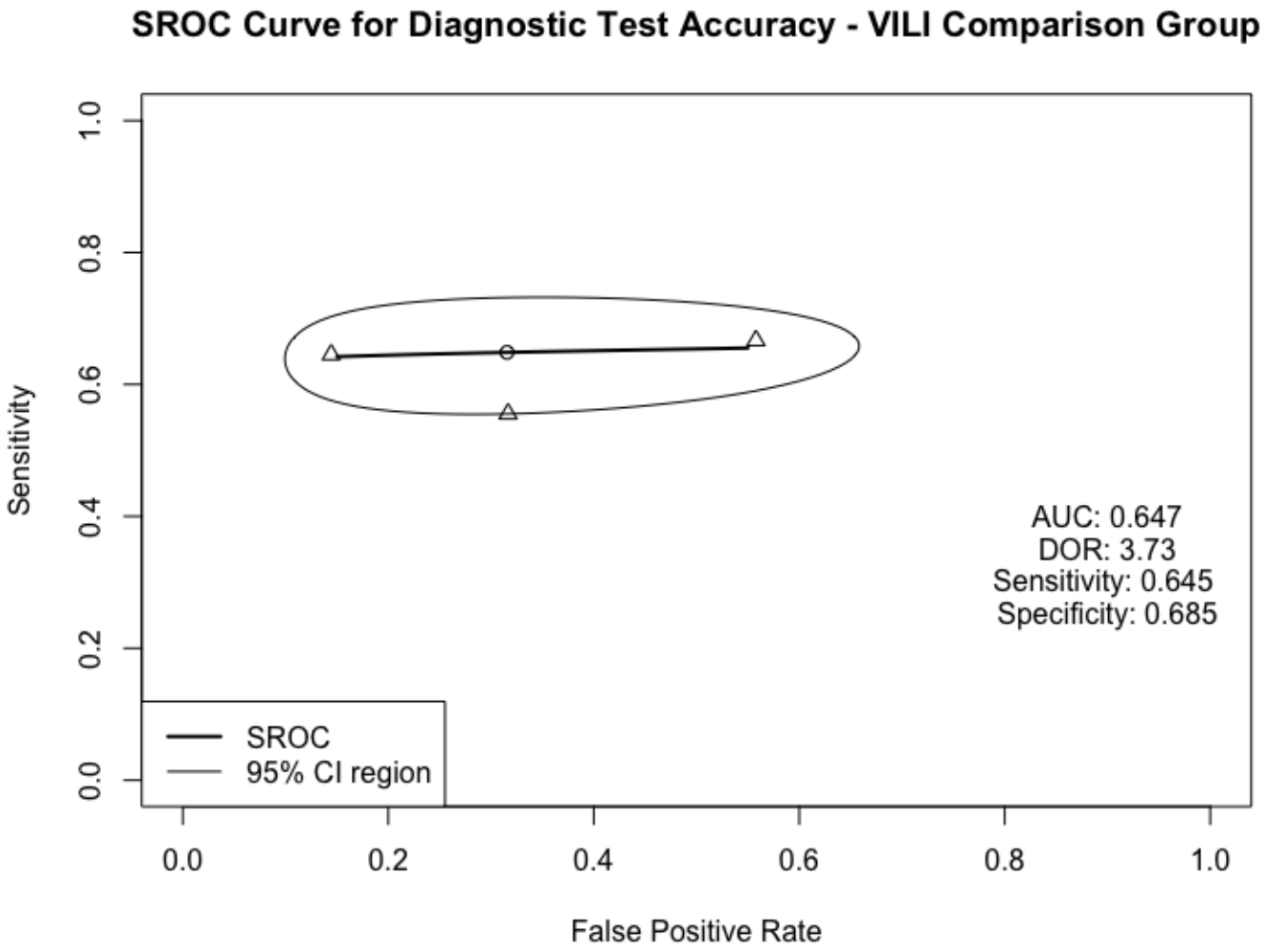


Figure 11. Forest Plot for Pap Sensitivity Summary Statistics

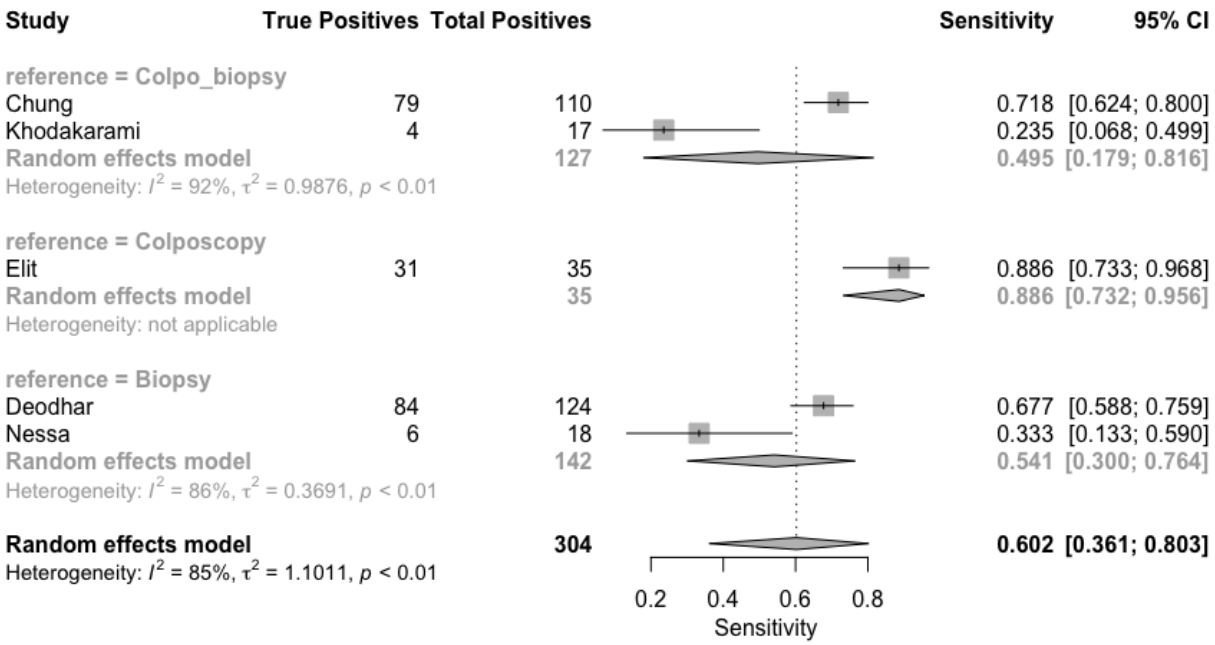


Figure 12. Forest Plot for Pap Specificity Summary Statistics

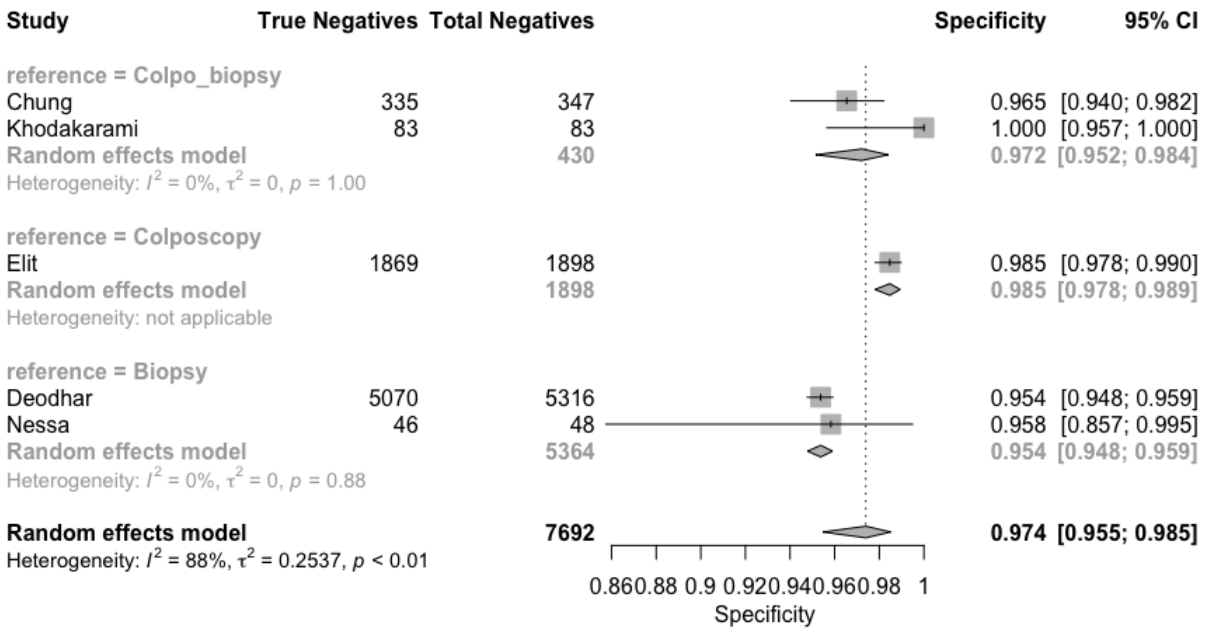


Figure 13. Forest Plot for Pap Diagnostic Odds Ratios

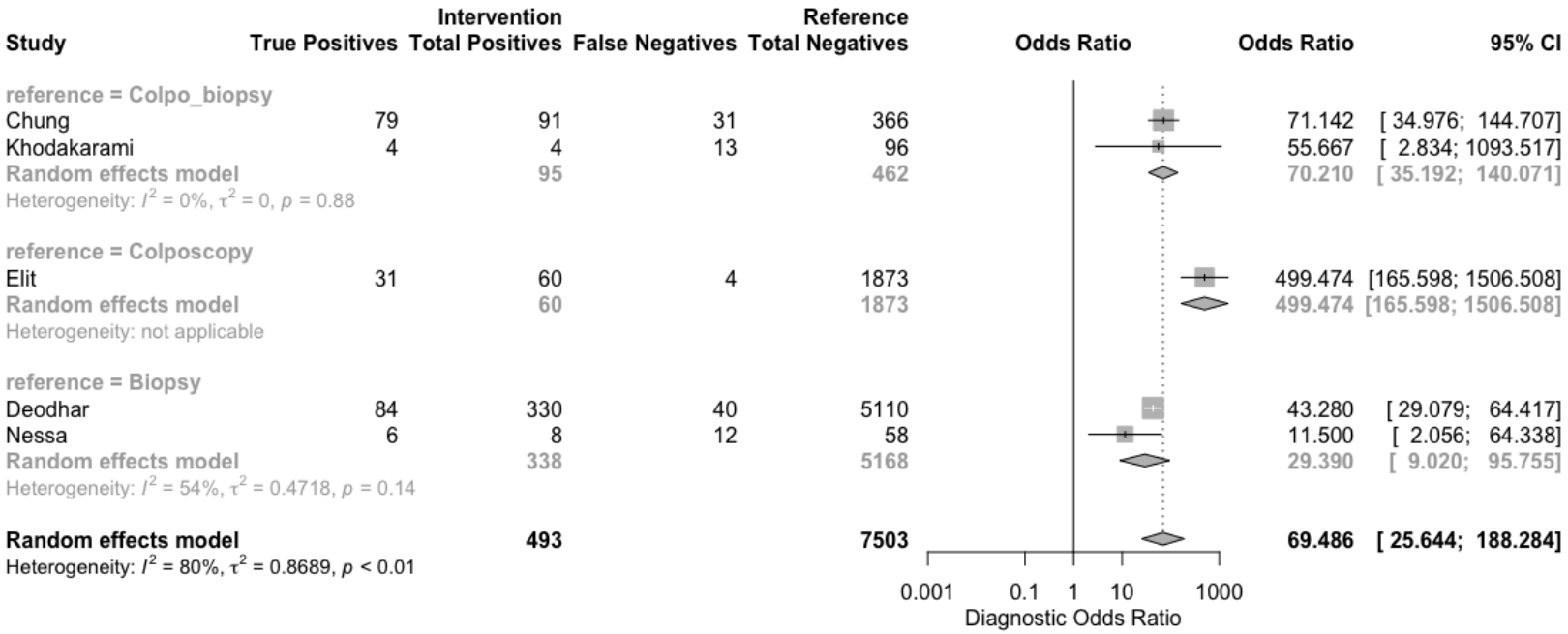


Figure 14. Pap SROC Curve

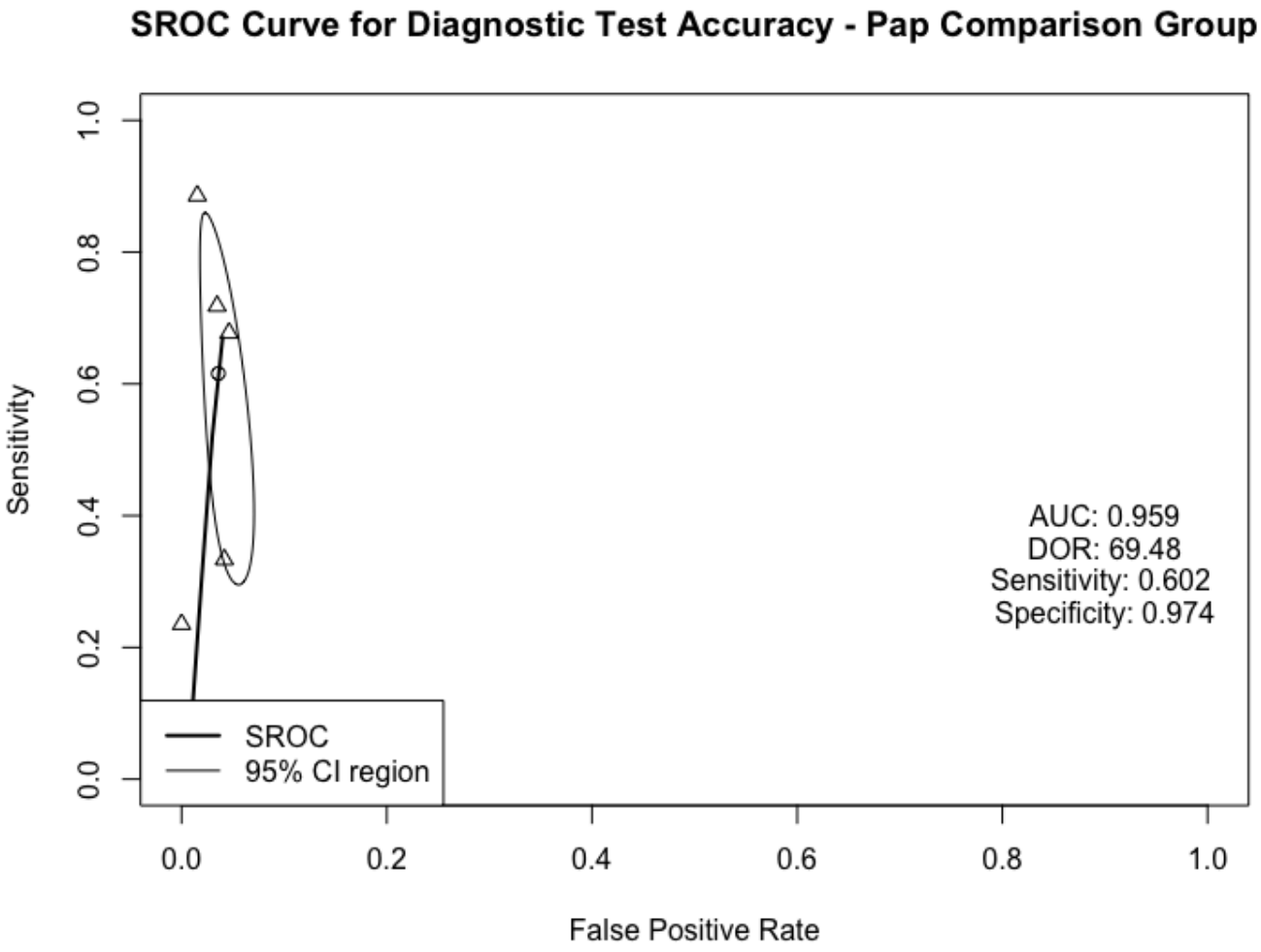


Figure 15. Forest Plot for HPV Sensitivity Summary Statistics

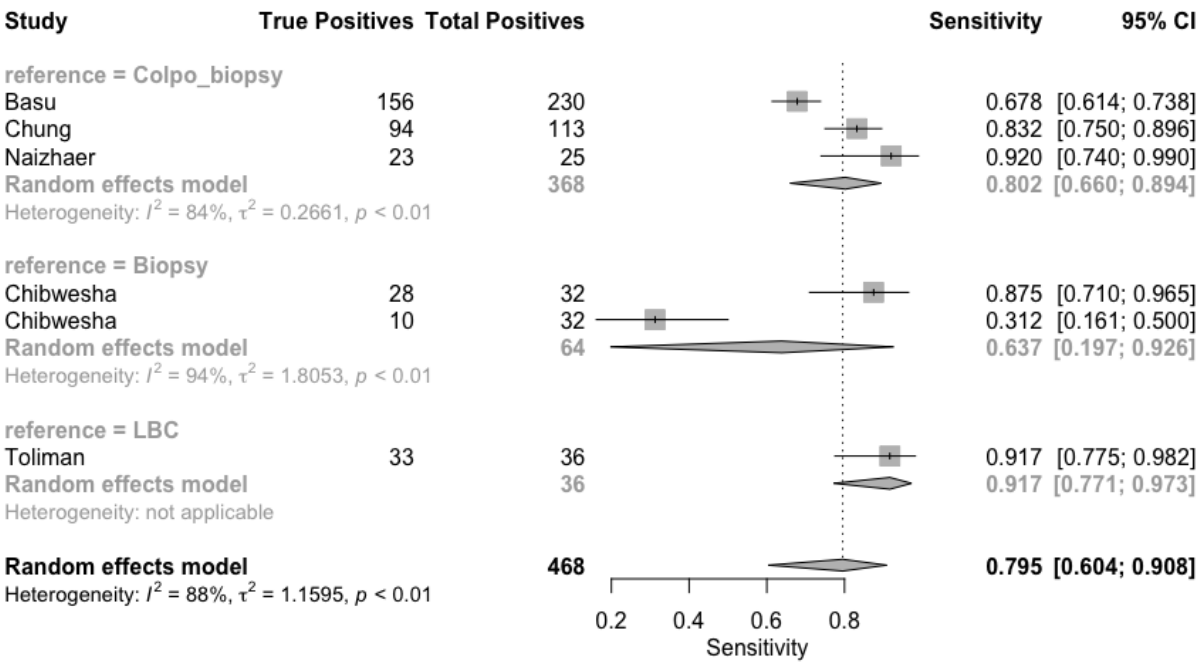


Figure 16. Forest Plot for HPV Specificity Summary Statistics

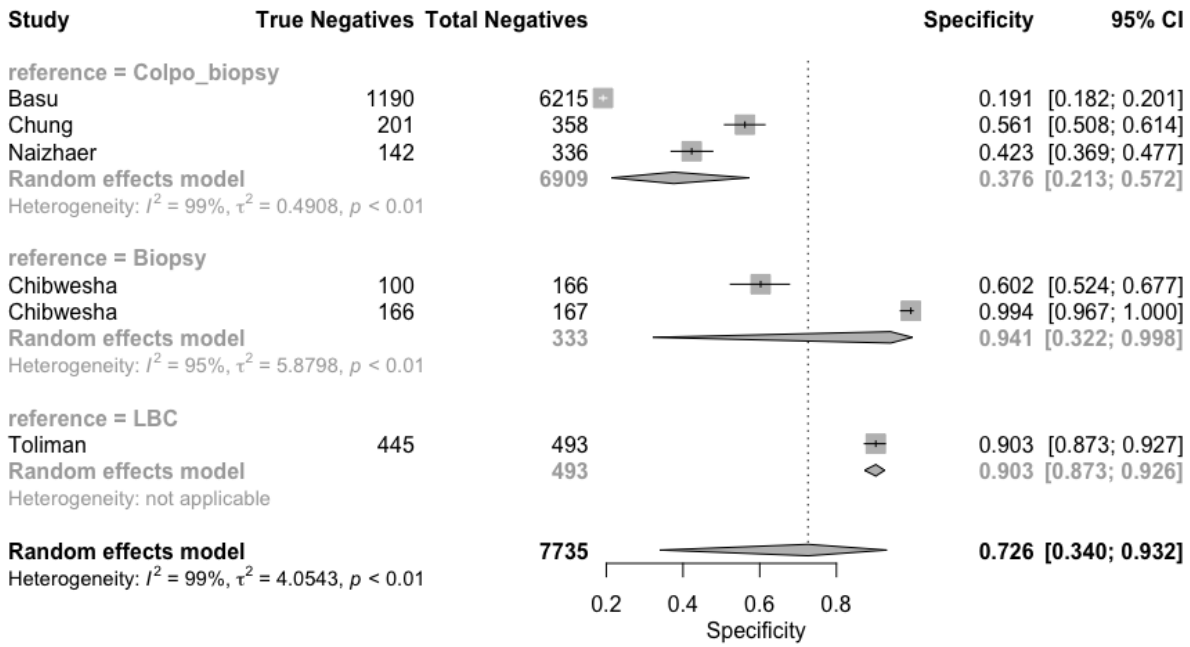


Figure 17. Forest Plot for HPV Diagnostic Odds Ratios

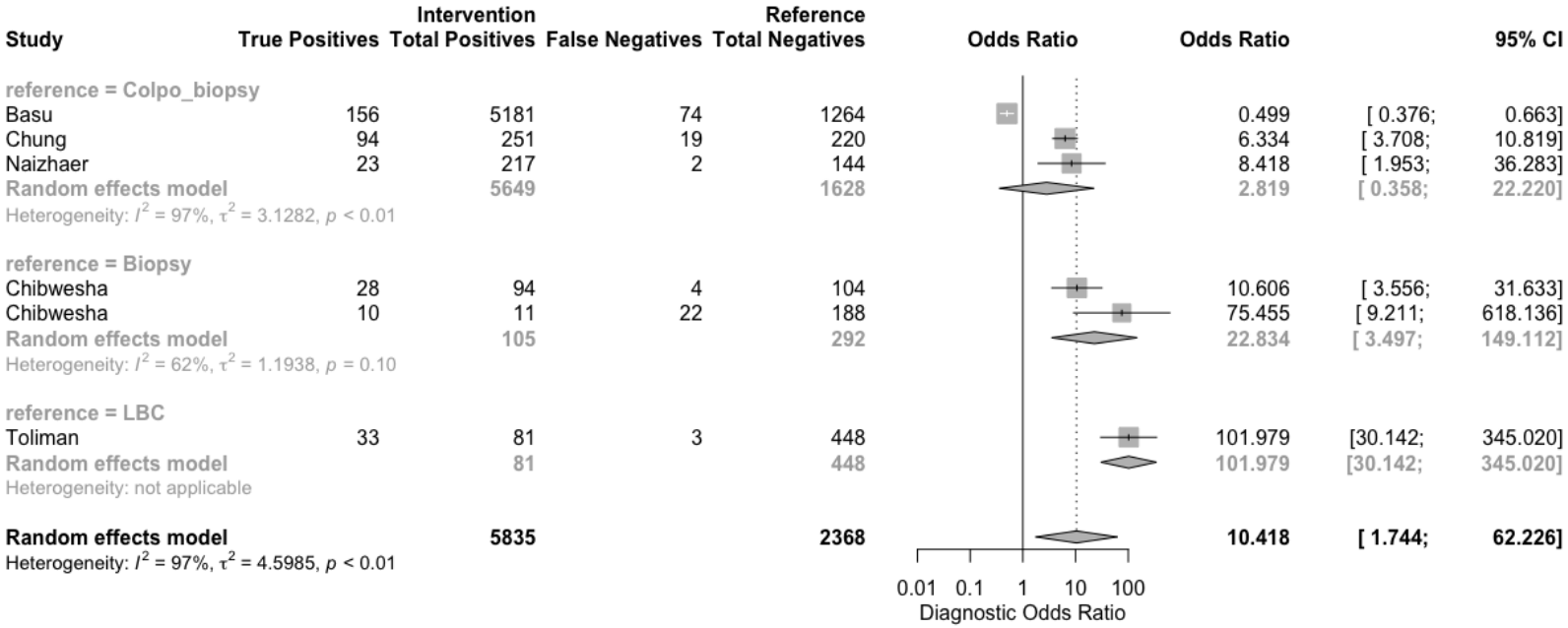
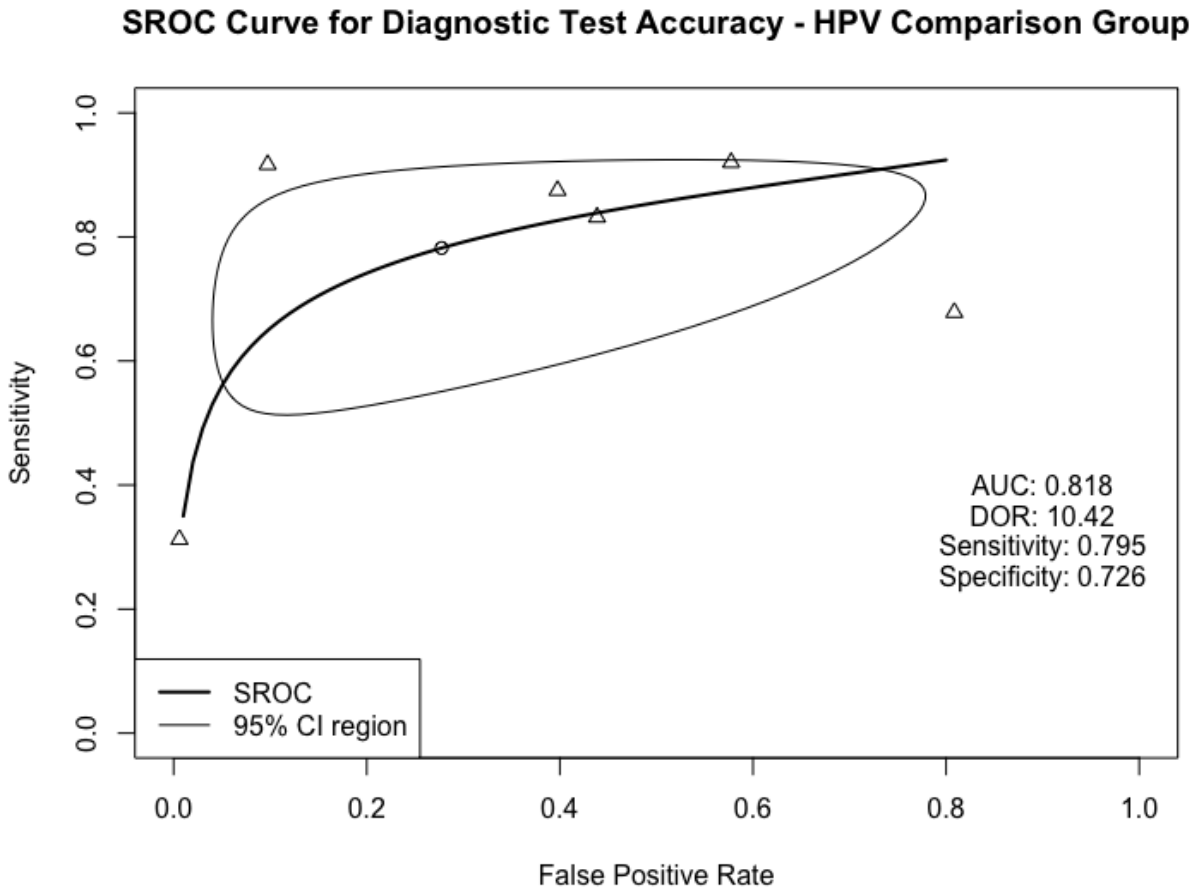


Figure 18. HPV SROC Curve



PUBLIC HEALTH IMPLICATIONS

Cervical cancer screening continues to be an important public health initiative, particularly in low- and middle-income settings where populations experience a disproportionate rate of cervical cancer compared to high-income countries. Particularly in the context of low vaccination, screening programs are essential for cervical cancer prevention. Even when vaccination is readily available, hundreds of millions of women living in LMICs have already been exposed to HPV and require continuous monitoring for cervical cancer and CIN throughout their lifetimes (Huchko, 2015). For these reasons, it is essential to scale up and improve cervical cancer screening on a global scale, determining the most feasible options for screening in LMIC contexts. Cervical cancer screening methods should be evaluated to better understand test characteristics and performance in these settings, so that the most efficacious method can be recommended for scale up in implementation.

There is a wide body of research on the performance of visual inspection with acetic acid (VIA) testing, but very limited research that focuses on a patient population over 30 years of age in LMICs. Therefore, this study aimed to evaluate the performance of VIA, VILI, conventional Pap smear and primary HPV testing in non-pregnant women aged 30-65 in LMICs. This systematic review and meta-analysis found that primary HPV testing and VIA were the highest performing screening methods in diagnostic accuracy. These findings will contribute to the development of global protocol and program planning for cervical cancer screening (Huchko, 2015), suggesting that if the feasibility of implementing HPV screening is low in certain settings, VIA is an acceptable standard for organized cervical cancer screening. These findings validate the performance of both

HPV testing and VIA testing for use in clinical settings by licensed providers. While this study demonstrates superior diagnostic performance of HPV and VIA screening methods, it is important to note that patients should partake in whichever cervical cancer screening method is readily available and accessible to them.

Based on the findings of this systematic review and meta-analysis, the research community should focus on conducting studies in a broader range of geographies, specifically to include rural populations in low-income countries. In terms of methodology, this study demonstrated significant disparities across studies and the need for scientific consensus on the evaluation of performance characteristics of these screening methods. Studies should expand on the analysis of performance to include likelihood ratios and odds ratios, aiding in the assessment of clinical utility of the results. Future research should also explore performance of these screening methods at different CIN thresholds. Finally, new innovations in cervical cancer screening have become more widely used in LMIC settings, including self-sampling HPV test kits, given their enhanced feasibility and cost-effectiveness. Further analysis should be conducted to determine the performance of these new technologies in detecting cervical neoplasia compared to standard methods evaluated in this review.

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