CERVICAL PATHOLOGY AND CONNECTIONS TO CLINICAL AND HEALTH FACTORS

Ph.D. Thesis

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"The works must be conceived with fire in the soul but executed with clinical coolness."

Joan Miro

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1. LIST OF ABBREVIATIONS

AGC: Atypical Glandular Cells

ASC-H: Atypical Squamous Cells, cannot rule out High-grade Squamous Intraepithelial Lesions

ASCUS: Atypical Squamous Cells of Undetermined Significance

ASCCP: American Society for Colposcopy and Cervical Pathology

CA: Cervical Cancer

CAP: College of American Pathologists

CENTRAL: Cochrane Central Register of Controlled Trials

CIN: Cervical Intraepithelial Neoplasia

CI: Confidence Interval

CoCoPop: Condition, Context, Population

CTC: Common Toxicity Criteria

CTCAE: Common Terminology Criteria for Adverse Events

DOI: Digital Object Identifier

FDA: US Food and Drug Administration

GRADE: Grades of Recommendation, Assessment, Development, and Evaluation

HCII: Hybrid Capture II

HIV: Human Immunodeficiency Virus

HPV: Human Papillomavirus

HR-HPV: High-Risk Human Papillomavirus

HSIL: High-Grade Squamous Intraepithelial Lesion

I2: Heterogeneity Index

IFN-α: Interferon-alpha

IL: Interleukin

ITT: Intention-to-Treat

JBI: Joanna Briggs Institute

JBI Critical Appraisal Checklists: A set of tools to appraise the quality of evidence in studies

LSIL: Low-Grade Squamous Intraepithelial Lesion

MOOSE: Meta-analysis Of Observational Studies in Epidemiology

MIP: Macrophage Inflammatory Protein

NA: Not Available

NIH: National Cancer Institute

OR: Odds Ratio

PCR: Polymerase Chain Reaction

PEO: Population, Exposure, Outcome

PICO: Population, Intervention, Comparator, Outcome

PP: Per Protocol

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PROSPERO: International Prospective Register of Systematic Reviews

QUIPS: Quality in Prognostic Studies

RCT: Randomized Controlled Trial

ROB2: Risk of Bias 2 Tool

ROBINS I: Risk of Bias in Non-Randomized Studies

RR: Risk Ratio

SD: Standard Deviation

STI: Sexually Transmitted Infection

TNF-α: Tumor Necrosis Factor-alpha

TV: Trichomonas Vaginalis

TV-HPV: Trichomonas Vaginalis - Human Papillomavirus

TV-HSIL: Trichomonas Vaginalis - High-Grade Squamous Intraepithelial Lesion

TV-LSIL: Trichomonas Vaginalis - Low-Grade Squamous Intraepithelial Lesion

USA: United States of America

VAS: Visual Analog Scale

WHO: World Health Organization

2. STUDENT PROFILE

2.1. Vision and mission statement, specific goals

My vision is that primary HPV-based cervical cancer screening will be implemented in Hungary in the above 30-year age, thus allowing prompt diagnosis of cervical precancer. Additionally, and more broadly, I hope to contribute to a better understanding of the factors influencing the development of cervical cancer as well as the aspects that can reduce the burden of cervical disease.

Relatedly, my mission is to effect change in the Hungarian guidelines on cervical cancer screening and treatment.

My specific goals are firstly, to investigate the effect of Trichomonas vaginalis on the development of cervical cancer and secondly, to investigate the utility of Imiquimod in the treatment of cervical intraepithelial neoplasia patients.

2.2. Scientometrics

Number of all publications:	4
Cumulative IF:	11.5
Av IF/publication:	2.88
Ranking (Sci Mago):	D1: 1, Q1: 2, Q4:1
Number of publications related to the subject of the thesis:	2
Cumulative IF:	7.1
Av IF/publication:	3.6
Ranking (Sci Mago):	Q1: 2
Number of citations on Google Scholar:	10
Number of citations on MTMT (independent):	8
H-index:	2

2.3. Future plans

I aim to continue researching cervical pathology and to establish a register in our clinic aimed at improving scientific recognition and patient care. Moreover, as a practicing physician, I wish to improve my skills, which are essential for high-quality care. My personal view on specialization is that it is necessitated by the continual widening of medical knowledge. By specializing in one area of medicine, the clinician can practice at an exceptional level and contribute to greater effect in that chosen area. As Obstetrics and Gynecology is a wide field, I hope to join the surgical division of gynecology.

3. SUMMARY OF THE PH.D.

Cervical cancer is a multivariate disease; even though HPV infection is a key element, other factors are also crucial. When it comes to therapeutic intervention in cervical precancer, the accepted method is surgical. To assess the development of cervical cancer and find possible conservative treatment options for cervical precancer we conducted two meta-analyses. These two analyses may seem tenuously connected at first sight, but their common goal is to better understand cervical disease and reduce the burden of HPV-induced illness.

Our clinical question was whether TV can be a risk factor for the development of cervical cancer. Our second question concerned the extent to which topical Imiquimod had a positive effect on cervical intraepithelial neoplasia.

The results showed an association between TV and HPV co-infection. Moreover, we found an association between TV and cytological aberrations (ASCUS, HSIL), and cervical cancer. Regarding our second question, we found evidence that topical Imiquimod is effective in reducing cervical intraepithelial neoplasia and enhances HPV clearance. However, Imiquimod was inferior compared to conization.

We concluded that in the case of TV detection, the odds are increased for a cervical lesion and cancer. In clinical practice, when TV is diagnosed, HPV screening is advisable, along with tests for other cervical diseases. Although it is less effective than conization, topical Imiquimod has potential as a valuable treatment option for high-grade CIN patients.

4. GRAPHICAL ABSTRACT

Cervical Pathology and Connections to Clinical and Health Factors

Context: To understand the development of cervical cancer and identify conservative therapeutic interventions for cervical precancer to reduce human papillomavirus (HPV)-induced

Trichomonas Vaginalis Infection is Associated with Increased Risk of Cervical Carcinogenesis: A Systematic Review and Meta-Analysis of 480.000 Patients.

Hamar et al., Int J Gynaecol Obstet, 2023

TV-HPV	OR: 1.79 (CI: 1.27-2.53; I^2: 95%)
TV-HSIL*	OR 2.34 (CI: 1.10-4.95; I^2: 75%)
TV-cervical cancer	OR: 5.23 (CI: 3.03-9.04; I^2: 3%)

*high grade squamous intraepithelial lesion

→ Association between Tv and cervical carcinogenesis.

↓ *In case of TV detection, it's essential to screen for HPV and cervical lesions*

Imiquimod is Effective in Reducing Cervical Intraepithelial Neoplasia: A Systematic Review and Meta-Analysis

Hamar et al., Cancers, 2024

Imiquimod CIN 2-3	regression rate: 0.63 (CI: 0.46–0.75)
Imiquimod-HPV	regression rate: 0.50 (CI: 0.31–0.81)

→ Imiquimod accelerates HPV clearance and promotes the regression of cervical intraepithelial neoplasia

Cervical cancer prevention requires comprehensive diagnostic and treatment strategies. Our findings may assist policymakers in creating guidelines for women with cervical lesions.

5. INTRODUCTION

5.1. Overview of the topic

5.1.1. What is the topic?

The topic is cervical diseases. The primary objective of our assessment was to investigate factors influencing cervical diseases, such as cervical dysplasia and cervical cancer, and their roles in modifying the progression of these diseases.

5.1.2. What is the problem that needs to be solved?

The problem is the prevalence of cervical cancer and the relatively low numbers of vaccination and screening among girls and women. The World Health Organization (WHO) has proposed an ambitious strategy with the goal of eliminating cervical cancer by 2030. In this protocol, the WHO wish to vaccinate 90% of girls under 15 of age. For cervical cancer screening, the objective is to screen 70% of all women by the age of 35 using a highly sensitive method and to conduct a rescreening at the age of 45. Women identified with cervical disease (cervical precancer and cervical cancer) should receive treatment, with a target of 90% undergoing the necessary interventions. (1)

5.1.3. What is the importance of the topic?

Among the cancers most commonly diagnosed in women, and likewise in terms of mortality rate, cervical cancer ranks fourth.(2) Cervical cancer can be combated effectively through immunization, screening, and oncologic treatment.(3) HPV vaccination offers strong protection against oncogenic HPV strains and can lessen the burden of cervical cancer. Meanwhile, Pap smear and HPV tests significantly improve the reliability of cervical cancer screening.(4) Regarding cervical cancer treatment, immunotherapy and target therapy show increasing potential alongside established chemotherapeutic regimens.(5) Notwithstanding these reasons for optimism, cervical cancer remains the most frequently diagnosed cancer in developing countries, and is responsible for the highest cancer-related mortality rate in emergent nations.(2, 3)

5.1.4. What would be the impact of our research results?

Our inquiry into disease-modifying factors in cervical diseases has the potential to enhance everyday patient care. Additionally, investigating these factors could aid in personalizing therapies and redirecting awareness efforts. The identification of new risk factors for cervical cancer can enhance the vigilance of healthcare policymakers and raise public awareness. There is a current need for alternative conservative therapies for cervical precancers, particularly for women of childbearing age, and our research results could aid clinicians in responding to this need.

5.2. Trichomonas vaginalis and cervical carcinogenesis

The main risk factor for cervical cancer is high-risk human papillomavirus (HPV) infection, responsible for various cancer types. In particular, HPV 16 and HPV 18 are responsible for 70% of cervical cancers internationally.(6) Once integrated into the host cell genome, the virus causes the overexpression of proto-oncogene proteins.(7, 8) The persistence of the HPV infection and the failure of the immune system to resolve the infection in the cervix are the principal factors of the carcinogenesis.(6) Persistent HPV infection is precipitated by the disruption of the vaginal microbiota, leading to vaginal dysbiosis, an increase in proinflammatory cytokines, and reduced immune clearance.(9) Additional risk factors for cervical cancer include smoking, multiple sexual partners, use of oral contraceptives, immunosuppressed state, and sexually transmitted infections (STIs), all of which are known to contribute to the development of cervical cancer.(8, 10-12)

Trichomonas vaginalis (TV), a common STI, accounts for 170-190 million infections each year(13). Once the genital tract is infected with these anaerobic protozoa, symptoms may include odorous discharge, dysuria, itching, and irritation of the vulva; however, up to 85% of trichomoniases produce no symptoms in women. In addition, between 5% and 35% of women may be reinfected.(14) TV increases the risk of cervical cancer development by causing inflammation and abruption of the cervical epithelium while prompting the immune system to eliminate HPV. There is currently conflicting evidence regarding the relationship between TV infection, cervical dysplasia, and cervical cancer. While some articles have indicated strong associations, others do not report TV as a risk factor for cervical carcinogenesis.(15-18) Two meta-analyses have been published on this subject. The first, published in 1994, included populations in which TV detection depended exclusively on cytology, which is associated with

underdetection of TV.(19-21) The second meta-analysis focused on cervical dysplasia and failed to distinguish the different states of cervical lesions; moreover, the relationship between TV and HPV was not investigated.(22)

5.2. Imiquimod for cervical precancer

Only a minority of cases lead to invasive cancer, following years of persistence, and in a majority of patients, CIN regresses to a normal condition.(23) In the case of histologic high-grade intraepithelial lesion (HSIL), excisional treatment is preferred, in line with the 2019 American Society for Colposcopy and Cervical Pathology (ASCCP) Risk-Based Management Consensus Guideline.(24) At the same time, these procedures; impact pregnancy outcomes, leading to preterm delivery, premature rupture of membranes, and low birth weight.(25) Furthermore, persistent HPV is associated with an increased recurrence rate following surgical intervention.(26) In the context of these considerations, it is necessary to consider alternative conservative therapies with the aim of reducing the occurrence of surgical interventions and associated complications.

Topical Imiquimod has gained approval from the US Food and Drug Administration (FDA) for the treatment of external genital and perianal warts, basal cell carcinoma, and actinic keratoses.(27) The compound is thought to activate immune cells in the role of a Toll-like receptor-7 agonist. Its antiviral effects are produced through the activation of dendritic cells and by inducing cytokines including tumor necrosis factor-alpha (TNF-α), interferon-alpha (IFN-α), and interleukins (ILs).(28) Numerous studies have identified Imiquimod as a potential conservative treatment for precursor cervical lesions, due to its role in accelerating viral clearance. (29-31) At the same time, other studies have found Imiquimod to be ineffective in reducing CIN.(32) No meta-analyses were available on this topic to resolve this conflict and thus answer this important question.

6. OBJECTIVES

6.1. Trichomonas vaginalis- cervical carcinogenesis

With reference to the available literature, this study set out to conduct a comprehensive investigation into the association between TV and HPV, cervical dysplasia, and carcinogenesis. Our hypothesis was that TV presented a risk factor for developing cervical cancer.

6.2. Imiquimod cervical precancer

With reference to the available literature, the aim of this study was to determine the efficacy and safety of topical Imiquimod therapy in reducing the incidence of cervical intraepithelial neoplasia (CIN) and its impact on HPV clearance.

7. METHODS

This systematic review and meta-analysis was conducted in accordance with the PRISMA 2020 and MOOSE guidelines (see Table S1-S2), and the recommendations of the Cochrane Handbook were adhered to throughout.(33-35) The pre-study protocol was registered in PROSPERO (Study I: CRD42021286097, Study II: CRD420222870), and was followed in full.

7.1.1 Literature search and eligibility criteria

The systematic search was carried out across five major study databases: 20 October 2021 (first study) and October 10, 2022 (second study): MEDLINE (via PubMed), Embase, Cochrane Central Register of Controlled Trials (CENTRAL), Scopus and Web of Science. Only peer-reviewed articles were accepted; accordingly, no search was performed on ClinicalTrials.gov., and a preliminary search did not identify any suitable studies. During the search, no filters or restrictions were applied.

7.1.1.1. Study I Trichomonas vaginalis-cervical carcinogenesis

Two population, exposure, and outcome (PEO) frameworks were used to define the eligibility criteria for the articles.(36) All studies reporting on sexually active (P_1) or HPV-positive women (P_2) screened for TV infection (E) were considered eligible. Outcomes of interest (O_1) were HPV positivity, cervical dysplasia, and cervical cancer. In HPV-positive women (P_2), the investigated outcomes (O_2) were cervical dysplasia and cervical cancer. Included articles were required to include a population of TV-negative women comprising the control group.

Articles in which TV was detected with cytology, wet mount, culture or PCR methods were considered for inclusion. Articles were excluded from the study if TV was diagnosed on the basis of clinical features or medical history. Studies in which HPV exposure was diagnosed with any nuclear amplification method were considered suitable. Articles in which HPV was detected only by cytology were excluded due to the low sensitivity of the method.(37) Cyto- and histopathological diagnoses were considered acceptable for confirmation of cervical intraepithelial neoplasia (CIN) and cancer. The following outcomes in the dysplasia group were

evaluated: atypical squamous cells of undetermined significance (ASC-US), atypical glandular cells (AGC), atypical squamous cells for which high-grade squamous intraepithelial lesions could not be ruled out (ASC-H), low-grade squamous intraepithelial lesions (LSIL), and high-grade squamous intraepithelial lesions (HSIL). The Bethesda classification was required for cytological samples. Where articles reported CIN1–3 diagnoses, these were categorized into LSIL (CIN1) and HSIL (CIN2–3) groups to facilitate a simpler interpretation.

Observational studies, including cross-sectional studies, case-control studies, and cohort analyses, were accepted. Abstracts were excluded from our review. Non-English language articles were translated before being considered for evaluation.

7.1.1.2. Imiquimod

Two frameworks were used to determine the eligibility criteria for the articles. For studies with no comparators available for assessment, the CoCoPop framework was used. Investigated were women with cervical intraepithelial neoplasia (**Pop**ulation) who applied topical Imiquimod (**Co**ntext). Cervical dysplasia regression, estimation of treatment success, assessment of HPV clearance, and adverse events (**Co**ndition) were determined. Thereafter, the PICO framework was used. Assessed were women (P) with cervical dysplasia or who were HPV positive. In the intervention group (I), women had to have received topical Imiquimod products for the treatment of their cervical disease. Patients in the comparator group (C) were given the standard treatment, mainly surgical solutions including conization, cryotherapy, laser therapy, or expectant management. The outcome (O) parameters included the assessment of cervical dysplasia regression, assessment of HPV clearance, and adverse events.(36) Cervical dysplasia regression was defined either by the absence of dysplasia or regression from CIN 2-3 to CIN 1. HPV clearance was considered effective when the original HPV types were not detectable following treatment. Cohorts, case-control studies, and randomized controlled trials (RCT) were accepted for evaluation. Patient follow-up was required for articles to be included. There were no language restrictions; any non-English articles were translated into English before being evaluated.

7.2 **Study selection and data collection**

Articles were selected using a reference management program (Endnote X9). Following the removal of duplicates, two independent reviewers (BH, EH) performed a title and abstract selection and then finally, full-text selection. Cohen's kappa coefficient (κ) was used to measure the degree of agreement.(38) Any disagreements were resolved by a third independent

investigator (ZSH). If an article could not be found, or data were found to be missing, the corresponding author was contacted.

Extracted variables from the eligible studies were collated into a pre-defined Microsoft Excel spreadsheet (Windows 11 Pro) by two independent reviewers (BH, EH). For the first study, the following variables were collected: first author, publication year, digital object identifier (DOI), study design, study type, demography (age, sample size), country, centers, and the detection methods for TV, HPV, and cytological/histological lesions. Where possible, outcome data were extracted into two-by-two tables. In all other cases, the unadjusted odds ratios (ORs) were collected. To manage confounding factors, adjusted ORs were collected where possible, and the variables for these results were adjusted. In any cases of disagreement, consensus was achieved with the involvement of a third investigator (ZSH).

For the second study, the following outcomes were investigated: first author, year of publication, digital object identifier, study type, study design, country, study period, centers, and follow-up duration. For both the intervention group and the control group the following were extracted: patient numbers, patient age, pregnancy status, smoking status, number of sexual partners, histological findings (cervical intraepithelial neoplasia 2-3), and HPV status. For the intervention group, the dose, duration, and application form were recorded. Outcomes were collected in two-by-two tables. Risk ratios (RRs) were extracted directly where possible. Intention-to-treat (ITT) and per-protocol (PP) data were collected from RCTs. Response rate data, if available, were recorded separately. Data on adverse events were collected using the Common Terminology Criteria for Adverse Event protocols, as published on the website of the National Cancer Institute (NIH).(39) Adverse events were graded on a scale of 1 to 4 for the following: fatigue, headache, myalgia, flu-like symptoms, fever, abdominal pain, vaginal pruritus, vaginal discharge, vaginal bleeding, and inflammation. Any disagreements were resolved by a third reviewer (ZSH).

7.3. Risk of bias and quality assessment of the included articles

For the purpose of critically assessing the outcome data in the first study, a risk of bias assessment was performed using the Quality in Prognostic Studies (QUIPS) tool.(40) The QUIPS tool comprises six domains: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis reporting. For each domain, there is a choice of four classifications: not applicable, low risk, moderate risk, and high risk of bias.

For the second study, RCTs were evaluated with the Risk of Bias II (ROB II) tool, while non-randomized interventions were evaluated using the Risk of Bias In Non-Randomized Studies (ROBINS I).(41, 42) Response rates lacking a control group were assessed using the Joanna Briggs Institute (JBI) Critical Appraisal Checklists.(43) To grade the level of evidence of our findings, the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) approach was used. The GradePro tool was used to prepare the Summary of Findings Tables.(44) QUIPS and GRADE were conducted by two independent reviewers (BH EH), with any cases of disagreement settled by a third investigator (ZSH).

7.4 Synthesis methods:

In the course of data synthesis, both qualitative and quantitative analyses were performed using the R statistical programming language (R version 4.2 for the first study and R version 4.3 for the second study). A minimum of three studies was required for quantitative analysis, the results of which were presented in forest plots. In the first study, subgroup analyses were based on the TV detection method along with the country of origin of the article, and sensitivity analyses were conducted for four outcomes. Subgroup analyses in the second study were based on article type and grade of cervical dysplasia, with ITT data from RCTs analyzed separately from other study types, and grouped as cohorts. Regarding cervical dysplasia, subgroups included studies without CIN, CIN 1-2-3, and CIN 2-3. Per-protocol data were analyzed only for RCTs with complete treatment. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were estimated in the first study using a random-effects model with the tel-Haenszel method and the Paule-Mandel method for between-study variance estimation. In the second study, risk ratios (RR) with 95% CIs were used to assess effect sizes, employing the Clopper-Pearson method to calculate CIs. Statistically significant results excluded the null value within pooled CIs for both studies, with a p-value threshold of <0.05 indicating significance. Heterogeneity was assessed using Higgins & Thompson's I^2 and Cochran Q tests, with τ^2 indicating variance in the second study. Heterogeneity levels were categorized as 0%–40% (possibly not important), 30%–60% (moderate), 50%–90% (substantial), and 75%–100% (considerable). Subgroups in the second study used a fixed-effects "plural" model, and the Cochrane Q test was used to evaluate subgroup differences, with the null hypothesis rejected at a 5% significance level. The first study also reported prediction intervals for pooled estimates provided the minimum study number was satisfied. In the first study, funnel plots were used to assess publication bias, and Egger's test was applied where a minimum of 10 articles were

available for one outcome. However, publication bias was not assessed in the second study due to the limited number of studies (<10).

8. RESULTS

8.1. Trichomonas vaginalis-cervical carcinogenesis

8.1.1. Search and selection

The comprehensive search identified 1,707 articles. Following duplicate removal, 1,259 publications were screened on the basis of title and abstract. In the following full-text selection, a total of 355 articles were screened, producing 29 studies eligible for the quantitative and qualitative data syntheses. Cohen's Kappa was 0.9 for title and abstract selection, and 0.85 for full-text selection. Regarding the articles that could not be retrieved, the authors were contacted but no response was received. (see Figure 1)

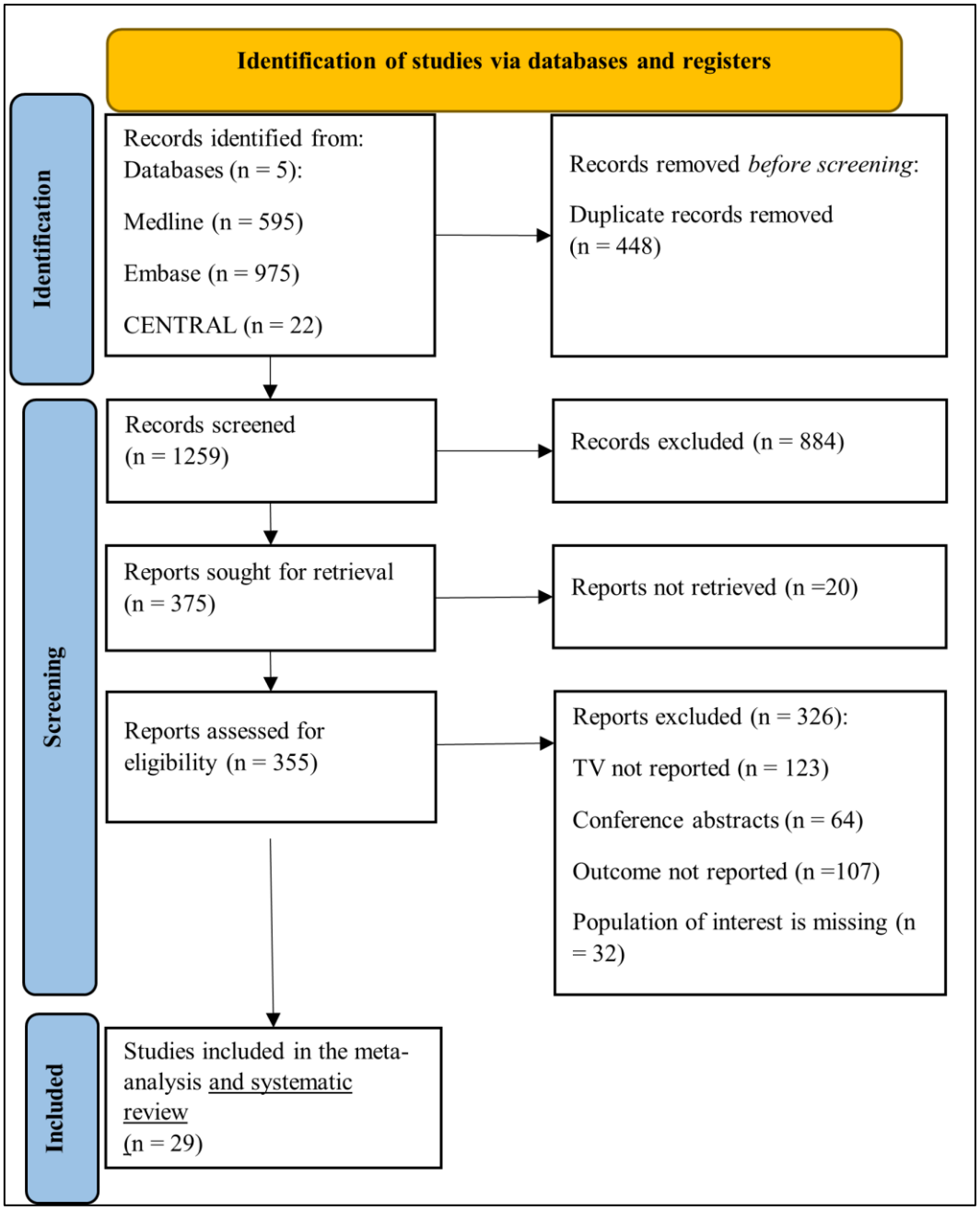

Figure 1. PRISMA 2020 flowchart representing the study selection process.

8.1.2. Basic characteristics of included studies

Publication dates of the eligible articles ranged between 2009 and 2021. Regarding country of origin, 11 publications were from Asia, 7 from South America, 5 from Europe, 5 from Africa, and 1 from North America. Regarding study type, 22 were cross-sectional studies, five were case-control studies, and one was a prospective cohort study.

Regarding demographics, the mean age of the female patients was 37.57 years. In 15 articles, TV was detected with PCR, in 8 articles, TV was detected with wet mount, in 4 articles, TV was detected with cytology, and in 2 articles, TV was detected with cultures and wet mount. All the studies assessed the exposure and the outcome simultaneously.

A total of 473,740 women were included in our meta-analysis. Of these, 8,518 women had TV infection in the exposure group. The baseline characteristics of the eligible studies are shown in Table 1.

Author, year	Study type	Region	Number of patients	Age (mean)	Diagnosis of Trichomonas vaginalis	Diagnosis of HPV	Diagnosis of cervical lesion	Cervical lesion related outcomes
Verteramo et al, 2009(45)	cross-sectional	Europe	860	32.7	culture &wet-mount	PCR	NA	NA
Noel et al, 2010(46)	case-control	Europe	507	<30–50[a]	cytology	HCII	NA	NA
Depuydt et al, 2010(47)	cross-sectional	Europe	62,944	42	PCR	PCR	NA	NA
Caiyan et al, 2012(48)	cross-sectional	Asia	6,339	39.2	wet-mount	HCII	histology	LSIL, HSIL
Donders et al, 2013(17)	cross-sectional	Europe	63,251	NA	PCR	PCR	cytology	ASCUS, LSIL, HSIL
Mendoza et al, 2013(49)	cross-sectional	South-America	181	30[b]	culture &wet-mount	PCR	NA	NA
Paesi et al, 2013(50)	cross-sectional	South America	208	13–69[a]	cytology	PCR	NA	NA
Lazenby et al, 2014(15)	cross-sectional	Africa	324	38	PCR	HCII	cytology/histology	LSIL, HSIL
Liu et al, 2015(51)	cross-sectional	Asia	429	39	wet-mount	PCR	NA	NA
Casillas-Vega et al., 2016(52)	cross-sectional	South America	662	31	PCR	PCR	NA	NA
Camporiondo et al, 2016(53)	cross-sectional	Europe	309	49[b]	PCR	PCR	NA	NA
Dey et al, 2016(54)	cross-sectional	Asia	7,962	NA	cytology	NA	cytology	ASCUS, LSIL, HSIL
de Abreau et al, 2016(55)	cross-sectional	South America	685	40.3	PCR	NA	cytology/histology	HSIL
Kim et al, 2016(56)	case-control	Asia	1,000	NA	PCR	PCR	cytology	ASCUS, ASC-H LSIL, HSIL,
Amorim et al, 2017(57)	case-control	South America	132	38.2	PCR	NA	cytology/histology	LSIL, HSIL
Costa-Lira et al, 2017(58)	cross-sectional	South America	180	16–50[a]	PCR	PCR	-	NA

Ghosh et al, 2017(18)	case-control	Asia	483	30–60[a]	wet-mount	HCII	histology	LSIL, HSIL, CA
Al-Awadhi et al, 2018(16)	cross-sectional	Asia	8,836	NA	cytology	NA	cytology	ASCUS, LSIL, HSIL
Lockhart et al, 2019(59)	prospective-cohort	Africa	344	18–49[a]	PCR	PCR	NA	NA
Ferre et al, 2019(60)	cross-sectional	Africa	320	25	PCR	PCR	NA	NA
Lv et al, 2019(61)	cross-sectional	Asia	826	38.5	wet-mount	PCR	NA	NA
Cunha et al, 2020(62)	cross-sectional	South America	353	39.7	PCR	PCR	NA	NA
Wang et al, 2020(63)	cross-sectional	Asia	4,449	43.6	wet-mount	PCR	NA	NA
Yang et al, 2020(64)	cross-sectional	Asia	310,545	>30	wet-mount	PCR	NA	NA
Zheng et al, 2020(65)	case-control	Asia	532	42.2	wet-mount	PCR	histology	LSIL, HSIL, CA
Gupta et al, 2020(66)	case-control	Asia	168	21–65[a]	wet-mount	NA	histology	CA
Taku et al, 2021(67)	cross-sectional	Africa	205	45[b]	PCR	PCR	NA	NA
Jary et al, 2021(68)	cross-sectional	Africa	144	37	PCR	PCR	NA	NA
Belfort, 2021(69)	cross-sectional	South America	562	30–49[c]	PCR	PCR	NA	NA

Table 1. Basic characteristics of included studies

[a]minimum-maximum age values, [b]median age value, [c]in this age range 48.40% of patients were included.

Abbreviations: PCR: polymerase chain reaction, HCII: Hybrid capture II, NA: not available, ASCUS: atypical squamous cells of undetermined significance, ASC-H: atypical squamous cells for which one cannot rule out high-grade squamous intraepithelial lesions, LSIL: low-grade squamous intraepithelial lesions, HSIL: high-grade squamous intraepithelial lesions .CA: cervical cancer

8.1.3. Quantitative and qualitative analysis

8.1.3.1. The association between TV and HPV infections

Twenty-four studies, representing 7,291 women in the TV infected group and 452,161 in the control group, reported an association between TV and HPV infections.(15, 17, 45-53, 56),(18, 58-65, 67-69) Based on our results, TV-positive women were shown to be 1.79 times more likely to receive a HPV co-infection diagnosis (CI: 1.27–2.53; I^2: 95%; Figure 2) than TV-negative women.

Where a TV infection was confirmed via the wet-mount method, slightly higher odds of detecting a co-infection with HPV were observed, by the odds of 2.29 (CI: 1.23–4.28; I^2: 97%;). Based on regional subgroup results, it was observed that TV-positive women from Asia were most likely to experience HPV co-infection (OR: 2.05, CI: 1.08–3.88; I^2: 97%; (see the Supplementary Material of the original publication, Figure S1). A sensitivity analysis (leave-one-out method) did not indicate any influential study. (see Figure S2)

In one article(69) a multivariate analysis resulted in 2.29 odds (CI:1.46–3.60) for the diagnosis of HPV where TV was detected. A second study(15) showed higher chances still for HPV-co-infection. (OR: 4.10, CI: 1.70–9.80, see Table S3)

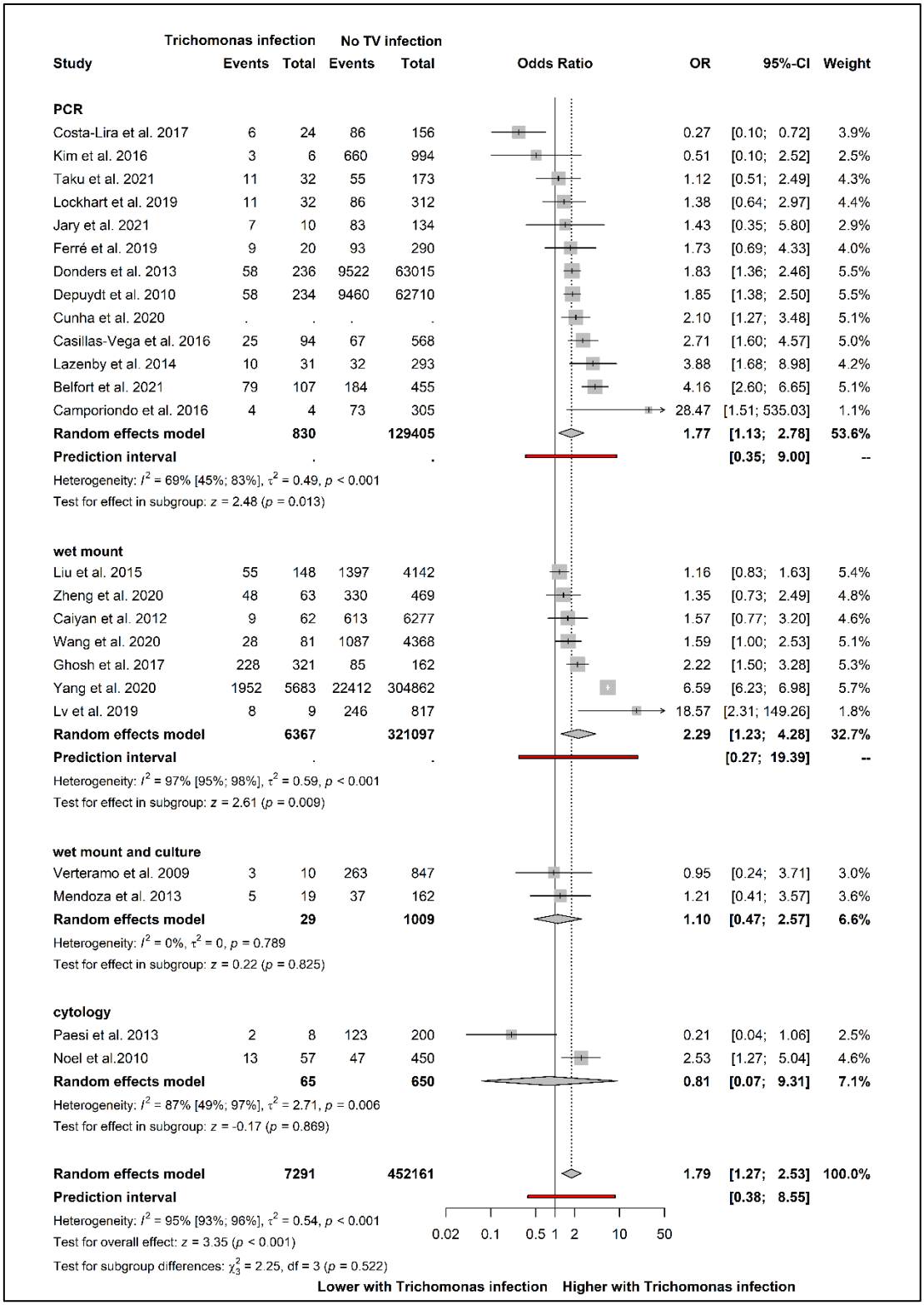

Figure 2. Forest plot of studies representing an association between **TV** and **HPV** infection.

Abbreviations: PCR: polymerase chain reaction, TV: Trichomonas vaginalis, HPV: human papillomavirus.

8.1.3.2. The association between TV and cervical dysplasia

8.1.3.2.1. Atypical Squamous Cells of Undetermined Significance (ASCUS)

Regarding the ASCUS outcome, five studies reported on 1,493 women in the exposure group and 75,135 women in the control group. (16, 17, 54, 56, 69) TV positive women were 2.3 times more likely to receive an ASCUS diagnosis (CI: 1.63–3.26; I^2: 52%, see Figure S3) than non-TV infected women. The results of a subgroup analysis based on the screening method showed an even stronger association when TV was detected with PCR (OR: 2.91; CI: 1.95–4.35; I^2: 0%;). Studies from South America and Europe showed nearly triple the odds for the diagnosis of ASCUS (Belfort et al.(69); OR: 2.99; CI: 1.06–8.43; Donders et al.(17); OR: 2.94, CI: 1.88–4.57, respectively; see Figure S4). In conducting the leave-one-out analysis, the exclusion of Al-Awadhi et al. (16) from ASCUS led to a higher association (OR: 2.79; CI: 2.21–3.53; I^2: 0%; see Figure S5).

Only one article(47) reported a multivariate analysis, finding an OR of 2.65 (CI: 0.87–8.05) for the diagnosis of ASCUS.

8.1.3.2.2. Atypical Glandular Cells

Two studies investigated AGC and TV infection in sexually active women. No association was found between TV positivity and AGC in either study (Donders et al.(17): OR: 1.33; CI: 0.08–21.40; Al-Awadhi et al.(16): OR: 1.55, CI: 0.46–5.41).

8.1.3.2.3. Low-Grade Squamous Intraepithelial Lesion

In total, there were 10 eligible studies reporting on 1,740 women in the TV group and 82,362 in the control group.(15-18, 48, 54, 56, 57, 65, 69)

In investigating the association between TV and LSIL, it was observed that TV-infected women were nearly twice as likely to have LSIL (OR: 1.92; CI: 0.78–4.77; I^2: 91%; see Figure S6) than women who were not TV infected, although the findings were not statistically significant. In cases where TV was detected with PCR, women were found to have higher odds of receiving a diagnosis for LSIL (OR: 3.66; CI: 1.51–8.86; I^2: 69%). In analyzing regional differences, it was found that TV-infected women from South America were nine times more likely to have LSIL (OR: 9.36; CI: 2.34–37.36; I^2: 63%; Figures S7). A leave-one-out analysis excluding the study by Al-Awadhi et al. (16) led to a higher association between TV and LSIL detection (OR: 2.79; CI: 1.61–4.82; I^2: 65%). Moreover, excluding the article by Amorim et al.(70) resulted in an OR of 1.51 (CI: 0.65–3.55; I^2: 95%; see Figure S8). One study(69) performed multivariate analysis and found 3.17 odds (CI: 1.09–9.02) for the diagnosis of LSIL where TV infection was present (see Table S3).

8.1.3.2.4. Atypical Squamous Cells, cannot rule out High-grade Squamous Intraepithelial Lesions

No association was found between TV and ASC-H, (OR: 1.78; CI: 0.21–15.12; see Figure S9).(17, 56, 69) Only one study(69) performed a multivariate analysis and showed a positive association between TV and ASC-H. (OR:12.04; CI: 1.98–73.06) (see Table S3)

8.1.3.2.5. High-Grade Squamous Intraepithelial Lesion

Regarding the association between TV infection and HSIL, eleven studies reported on 1,796 women in the exposure group and 80,276 women in the control group.(15-18, 48, 54-57, 65, 69). TV- infected women were 2.34 times more likely to receive an HSIL diagnosis (CI: 1.10–4.95; I^2: 75% Figure 3) compared to non-TV-infected women. Regarding the TV detection method, it was observed that women diagnosed using PCR had higher odds of receiving an HSIL diagnosis (OR: 3.81; CI: 1.23–11.78; I^2: 81%). Regional subgroup analysis of the articles showed six times higher odds in South America (OR: 6.52; CI: 0.74–57.75; I^2: 92%;); however, the findings are not statistically significant. In one European study, high odds were shown for HSIL in the presence of TV (Donders et al(17).; OR: 3.14; CI: 1.496.78; see Figure S10). A leave-one-out analysis excluding the article by Al-Awadhi et al. (16) from HSIL resulted in an OR: 2.87; CI:1.43–5.75; I^2: 67%. Furthermore, the exclusion of the article by Amorim et al.(57) resulted in the OR changing to 1.72 (CI: 1.01–2.91; I^2: 42%; see Figure S11).

One paper conducted a multivariate analysis and found(69) 8.16 odds (CI: 0,8181.87) for HSIL diagnosis in the presence of TV (see Table S3).

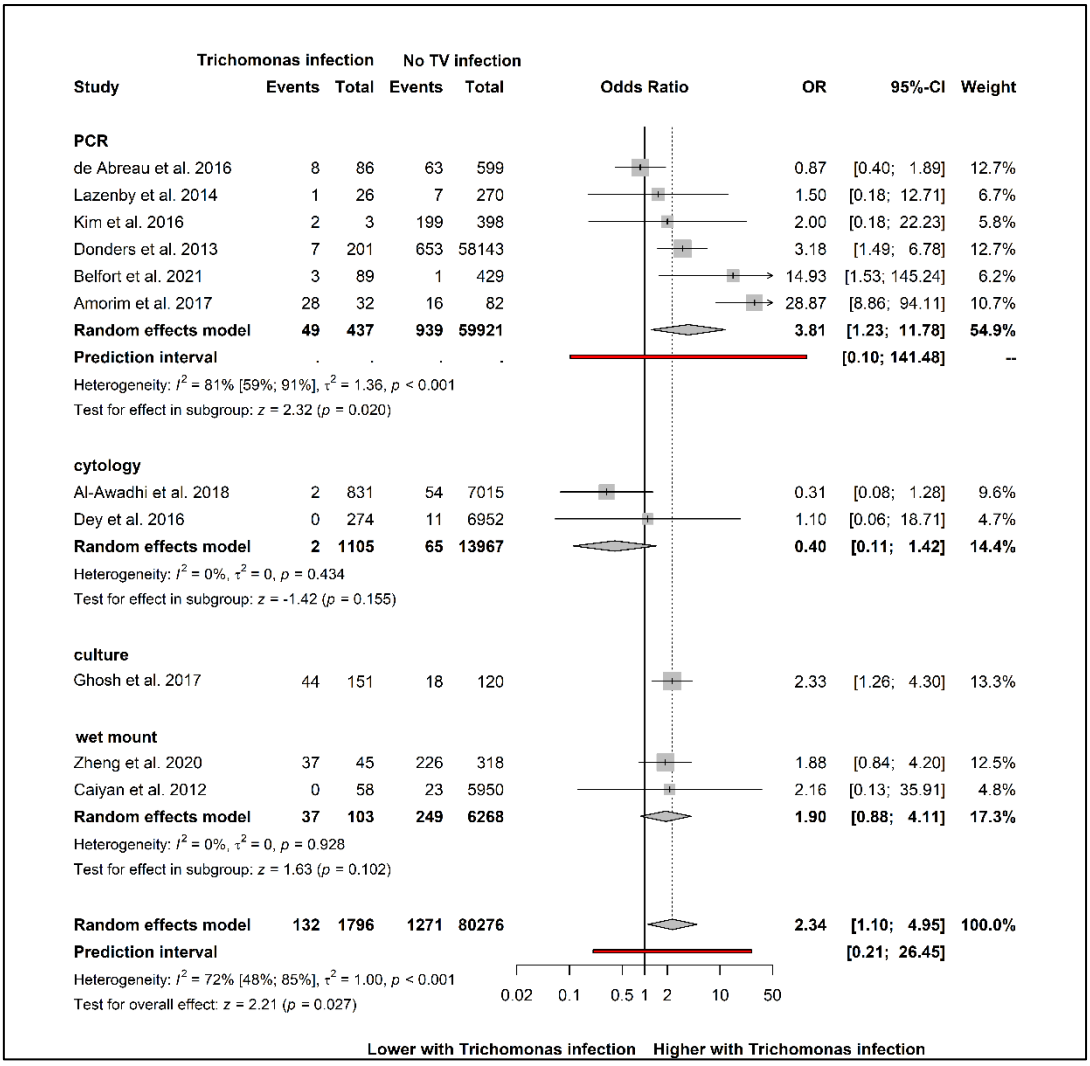

Figure 3. Forest plot of studies showing the association between **TV** infection and **HSIL**.

Abbreviations: PCR: polymerase chain reaction, TV: Trichomonas vaginalis: HSIL: high-grade squamous intraepithelial lesions

8.1.3.3. The association between TV and cervical cancer

Three articles were subjected to quantitative analysis, with 219 women in the TV positive group and 397 women in the control group.(18, 65, 66) TV-positive women were 5.24 times more likely to have cervical cancer (OR: 5.23; CI: 3.03–9.04; I^2: 3% Figure 4) than TV-negative women.

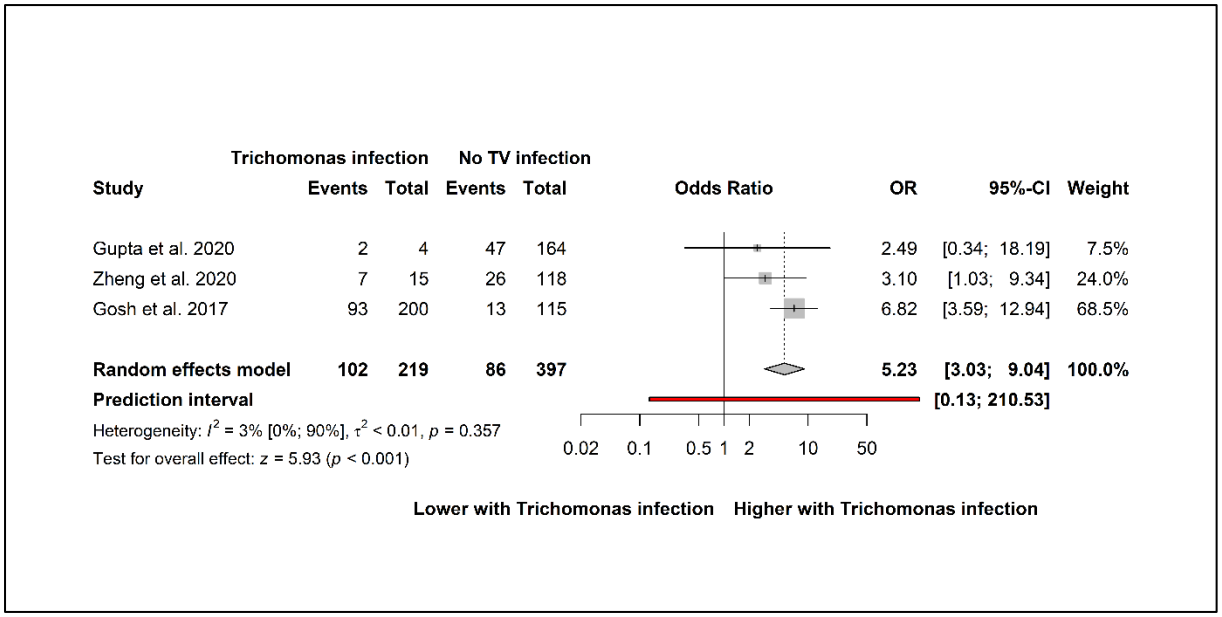

Figure 4. Forest plot of studies showing the association between **TV** infection and **cervical cancer**.

Abbreviation: TV: Trichomonas vaginalis

8.1.3.4. The association between TV, cervical lesions, and cervical cancer in the HPV-positive population

Four articles were identified for quantitative synthesis in respect of the association between TV infection and cervical lesions in the HPV-positive population.(18, 55, 64, 65)

Regarding LSIL, three articles were analyzed, in which 1,932 women in the exposure group and 20,033 women in the control group were assessed.(18, 64, 65) TV-positive women had 2.81 higher odds for LSIL diagnosis compared to non-TV-infected women (CI: 2.37–3.33; I^2: 0%; see Figure 6).

For HSIL, there was a total 1,921 women in the exposure group, with 20,750 women in the control group.(18, 55, 64, 65) TV-positive women were twice as likely to have HSIL than TV-negative women (OR: 2.36; CI: 1.79–3.11; I^2: 10%; see Figure 5).

Three studies investigated cervical cancer, with 1,811 women included in the exposure group and 19,331 women in the control group.(18, 64, 65) It was observed that TV-positive women had increased odds of receiving a cervical cancer diagnosis compared to TV-negative women (OR: 3.09; CI: 1.66–5.77; I^2: 45%; see Figure 5).

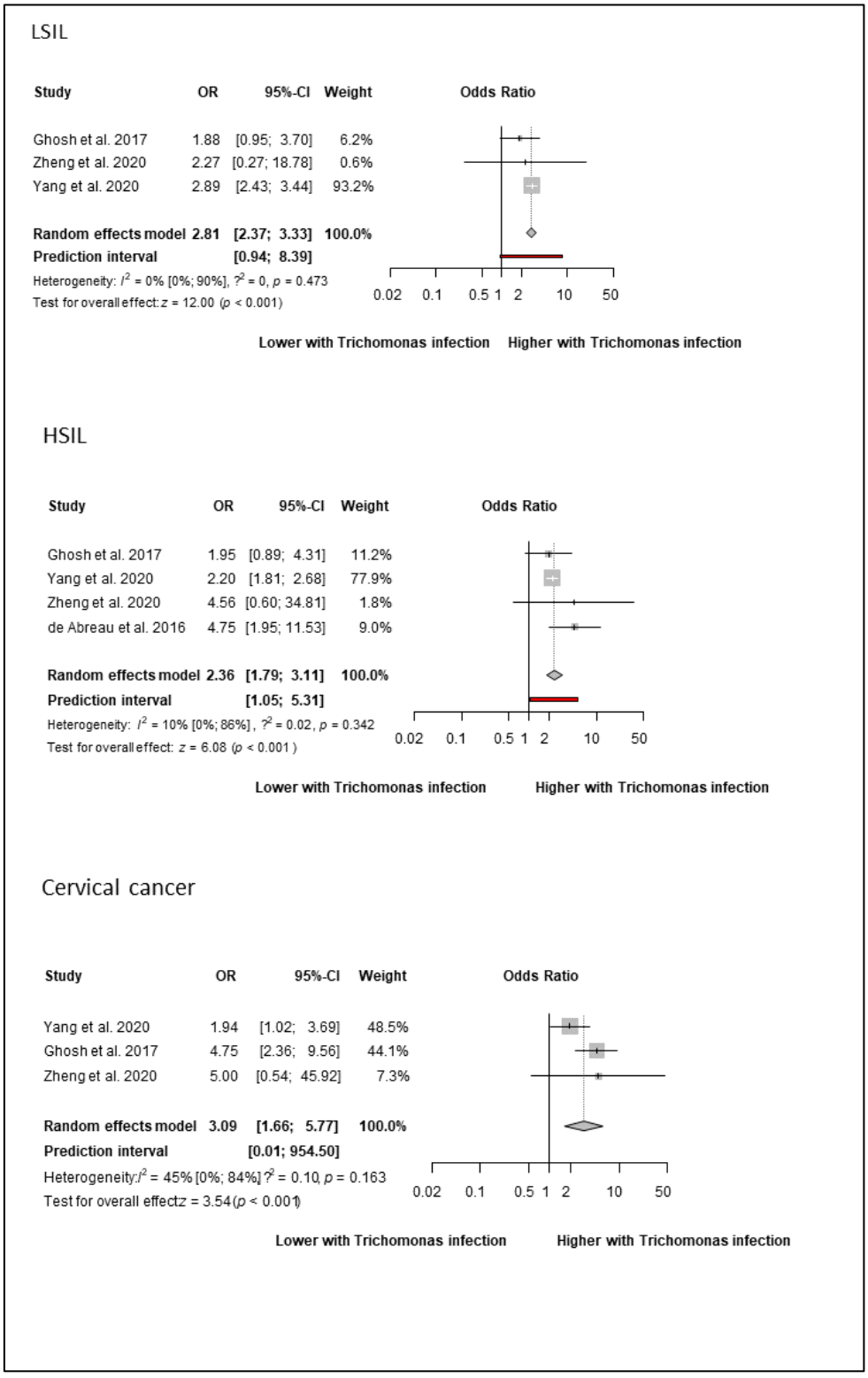

Figure 5: Forest plot of studies showing the association between **TV** and **LSIL, HSIL,** and **cervical cancer** in the **HPV-positive population**.

Abbreviations: LSIL: low-grade squamous intraepithelial lesions, HSIL: high-grade squamous intraepithelial lesions, HPV: human papillomavirus

8.1.4. Risk of bias assessment and quality of evidence

Risk of bias assessment results are shown for all outcomes. Regarding the TV-HPV co-infection outcome, seven articles presented a moderate risk for study confounding bias, while three articles presented a high risk of bias. A high risk of bias in the study participation domain was determined in three articles. There was a low risk of bias among the cervical dysplasia groups. In the domain of study confounding there was one article deemed as presenting a high risk of bias in ASCUS, with two articles presenting a high risk of bias in the LSIL and HSIL groups. Within the cervical cancer group, and in the study confounding domain, there was one article found to present a high risk of bias. (see Figure S14–22.) Included in the Summary of Findings were six outcomes for the first PEO and three for the second PEO. (see Table S3–4.) Regarding quality of evidence, this was determined as 'low' for six outcomes and 'very low' for three outcomes.

8.1.5. Publication bias and heterogeneity

Egger's test was conducted and a funnel plot created for the purpose of assessing publication bias in the TV-HPV co-infection, TV-LSIL, and the TV HSIL groups. The funnel plots presented a degree of asymmetry in all three groups. No significant publication bias was found, as the p values were greater than 0.1. (see Figures S23–25.).

8.2. Imiquimod cervical precancer

8.2.1. Search and Selection

The systematic search identified 3,141 articles from five databases. Following removal of duplicates, 2,218 articles were analyzed during title and abstract selection. For the full-text selection, 13 eligible articles were screened. In the final selection, eight articles were considered eligible for quantitative and qualitative synthesis (see Figure 6).

Author, years	Study type	Region	Folow up time (months)	Number of patients in intervention	Age (mean) intervention, SD	Number of patients in control	Age (mean) control, SD	CIN[B]	CIN2/CIN3 ratio	HPV[D] type	Dose of Imiquimod/ patient	Intervention of control group	Adverse event reporting.	Dropout of patients
Grimm et al 2012(30)	RCT[A]	Austria	5	30	29.2±6.1	29	31.8±7.8	CIN 2-3	1.73	HPV 16/18, other HR[E] HPV	243.75 mg	observation	CTCAE[F] 3.0	6.70%
Hendriks et al 2022(31)	Non-randomized interventional	Netherland	6	61	33.3±9.1	62	35.2±7	CIN 2-3	0.69	HPV 16/18, other HR HPV	300 mg	conization	VAS[G]	22.90%
Cokan et al 2021(71)	RCT	Slovenia	6	52	28.3±4.2	52	26±4.6	CIN 2-3	0.79	NA	600 mg	conization	CTCAE 5.0	17.30%
Lin et al 2012(72)	Retrospective cohort analysis	Taiwan	33.4	72	51.75 [B]	20	50[B]	NA[C]	NA	persistent HR-HPV	150 mg	observation	NA	NA
Fonseca et al(29) 2021	RCT	Brazil	24	45	32[B]	45	36[B]	CIN 2-3	0.4	NA	150 mg	observation	CTCAE 4.0	15.60%
Pachman et al(32) 2012	RCT	USA	37.2	28	30±8.9	28	29±9.7	CIN 1-2-3	1.42	HR-HPV	12.5 mg	conization, laser, cryotherapy	CTCH[H] 2.0	7.14%
Polterauer et al(73) 2022	RCT	Austria	24	51	31.4[B]	42	30.1[B]	CIN 2-3	0.28	HPV 16/18, other HR HPV	243,75 mg	conization	CTCAE 3.0	9.80%
Kim et al 2019(74)	retrospective cohort analysis	South-Korea	13.4	55	30[B]	NA	NA	CIN 2-3	0.74	HPV 16/18, other HR HPV	100 mg	NA	NA	1.80 %

Table 2: Basic characteristics of included studies

A - randomized control trial; B - median cervical intraepithelial neoplasia; C - Not applicable; D - Human papillomavirus; E - High-risk; F - Common Terminology Criteria for Adverse Events; G - Visual Analog Scale; H - Common Toxicity Criteria

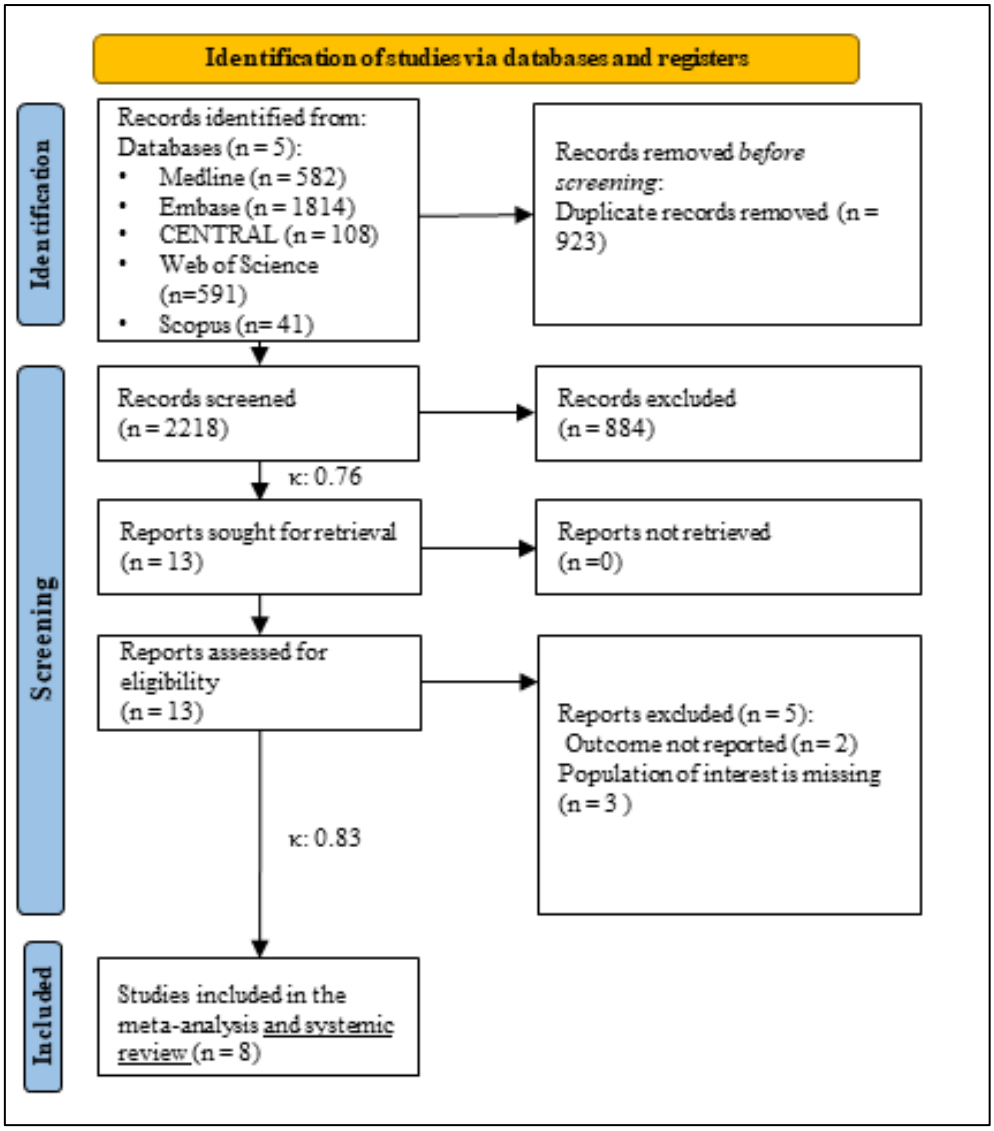

Figure 6. PRISMA flowchart of selection n: number of studies, κ: cohen's kappa coeficient

8.2.2. Basic Characteristics of Included Studies

Publication year of the eligible articles ranged from 2012 to 2022. In terms of demographics, the women included in the studies had a mean age of 30.41 years (± 2.15). Studies presented a mean follow-up time of 18.62 (± 12.00) months. In six of the studies, the women had histologically proven CIN 2-3, while in one of the remaining studies, both cytology and histology were used.(74) It was possible to conduct quantitative synthesis only for a subpopulation with HPV status. HPV tests were conducted in seven studies. Information concerning the doses and the application of Imiquimod are presented in Table S2.

In total, the eight eligible studies included 672 patients,(29-32, 71-74) of whom 398 received Imiquimod treatment. Detailed baseline characteristics are presented in (Table 2)

8.2.3. CIN 2-3 regression

Altogether, 294 women were treated with topical Imiquimod for CIN 2-3.(29, 31, 71, 73, 74) A regression rate of 61% (CI: 0.46–0.75; I^2: 77%) to CIN 1 or no disease was observed following topical Imiquimod therapy (see Figure 2). A subgroup analysis was conducted based on study type, and this showed a 59% histologic regression rate (CI: 0.47–0.70) in the ITT-RCTs, and a response rate of 64% in the cohort studies (CI: 0–1.00 I^2: 94) (see Figure 7). Among the PP population, comprising 155 patients, there was a regression rate of 67% (CI: 0.54–0.78; I^2: 0%) (see the Supplementary Material of the original publication- Figure S1).

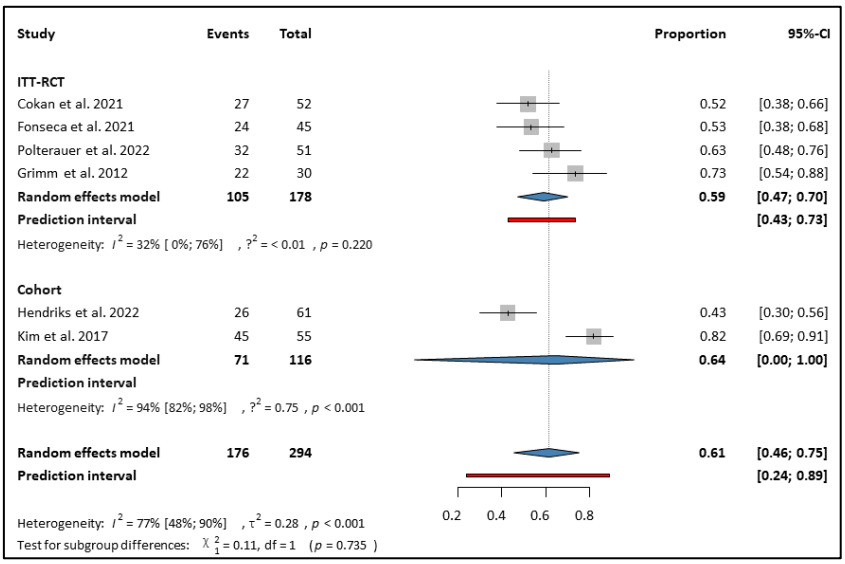

Figure 7. Forest plot of studies showing **Imiquimod** and **CIN 2-3 regression based on study type.** ITT: intention to treat, RCT: randomized control trial

Two articles examined the efficacy of topical Imiquimod,(29, 30) with both studies showing the RR for CIN regression to be higher when comparing Imiquimod to no treatment (RR: 1.87; CI: 1.12–3.10 and RR: 2.37; CI: 1.25–4.48, respectively) (see Figure S2).

For the experimental group, 196 women were treated with Imiquimod, with 196 women in the control group treated with conization.(29, 31, 71, 73) For women in the conization group, there was a 38% decrease in the risk for persistence or progression in CIN in comparison to women who had received Imiquimod (RR: 0.62; CI: 0.42–0.92; I^2: 64%) (see Figure 8). Results from the subgroup analysis included those of a randomized clinical trial in which conization was shown to be superior to Imiquimod and to have a 44% decrease in the risk of unsuccessful

treatment (RR: 0.56; CI: 0.43–0.74) (see Figure 3).(71) Similarly in the PP analysis, Imiquimod did not outperform conization (RR: 0.78; CI: 0.56–1.07; I^2: 0%) (see Figure S3).

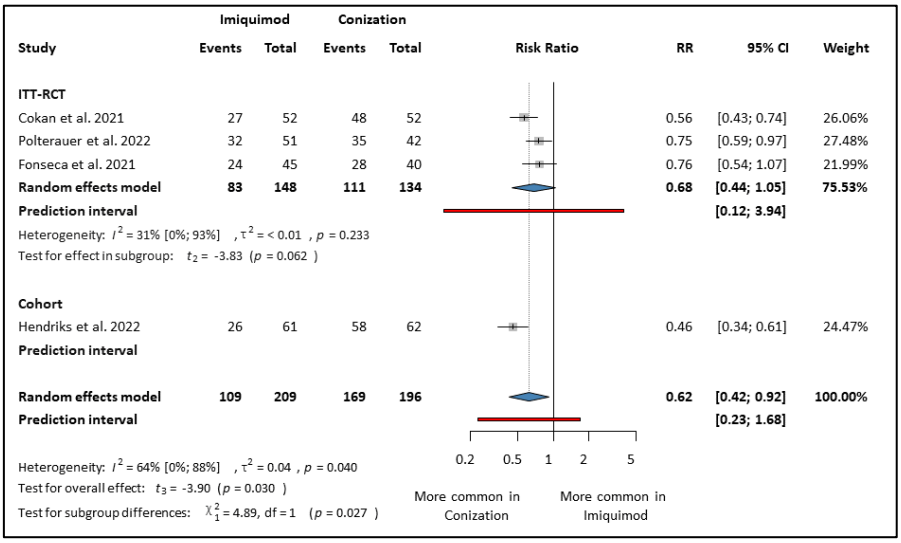

Figure 8. Forest plot of studies showing the **Imiquimod group** compared to **conization** on **CIN 2-3 regression based on study type.** ITT: intention to treat, RCT: randomized control trial

8.2.4. Imiquimod on HPV clearance

Out of the 254 women treated with Imiquimod, 50% (CI: 0.35–0.64; I^2: 64) experienced HPV clearance. (see Figure 9).(29, 32, 72-74) A subgroup analysis was conducted, based on the grade of cervical dysplasia (see Figure 4). For diagnosed CIN 2-3, there was a HPV clearance rate of 42% (CI: 0.29–0.56; I^2:49%); for diagnosed CIN 1-3, there was a HPV clearance rate of 68% (CI: 0.48–0.84). Finally, for HPV positivity with no CIN, there was a HPV clearance rate of 65% (CI: 0.44–0.83). It must be noted however, that only one study was available for each outcome. The subgroup analysis based on the study types showed a 56% (CI: 0.28–0.80; I^2: 59%) HPV clearance in the ITT-RCTs, while a 44% (CI: 0.17–0.75; I^2:73%) HPV clearance was observed in the cohort studies (see figure S4). Furthermore, in the PP population of 100 women, a higher HPV clearance rate of 60% (CI: 0.35–0.84; I^2: 57%) (see Figure S5) was observed. The subgroup analysis in the PP for CIN 2-3 showed a HPV clearance of 54% (CI: 0.06–0.96; I^2: 47%), while for CIN 1-3, the HPV clearance was 73% (CI: 0.52–0.88) (see

Figure S6). The HPV tests were performed a mean of 2.33 (SD: ± 1.91) months following completion of Imiquimod treatment.

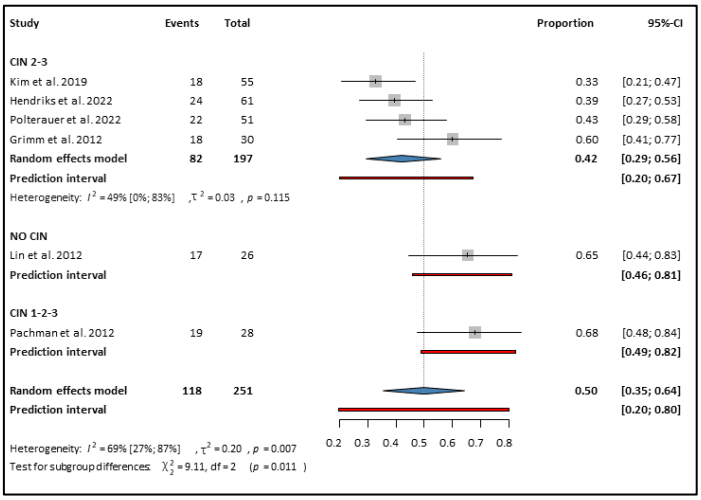

Figure 9. Forest plot of studies representing **Imiquimod on HPV clearance based on the CIN status** CIN: cervical intraepithelial neoplasia

The Imiquimod group comprised 196 women, and the control arm comprised 180 women. HPV clearance as a result of Imiquimod treatment was not better than that observed in the control group (RR: 1.29; CI: 0.52–3.21; I^2: 80%) (see Figure 10). Within the control group, treatment varied according to the studies. Conization proved more effective than Imiquimod in those studies in which control group patients received conization (RR: 0.67; CI: 0.46–0.99).(73) On the other hand, Imiquimod proved more effective in those studies where no intervention took place in the control group, and where HPV infection was persistent (RR: 4.20; CI: 1.62–10.89).(30) Imiquimod also proved more effective in one study in which only persistent HPV positivity was diagnosed, with no cervical dysplasia, and with no intervention in the control arm (RR: 2.18; CI: 1.06–4.05).(72) In another study, in which surgical interventions (conization, cryotherapy, laser) were implemented in the control arm,(32), Imiquimod treatment was not more effective than the control group in terms of HPV clearance (RR: 1.19; CI: 0.79–1.79).(32) Examination of the PP group showed that Imiquimod did not produce a higher HPV clearance rate than that observed in the control group (see Figure S7).

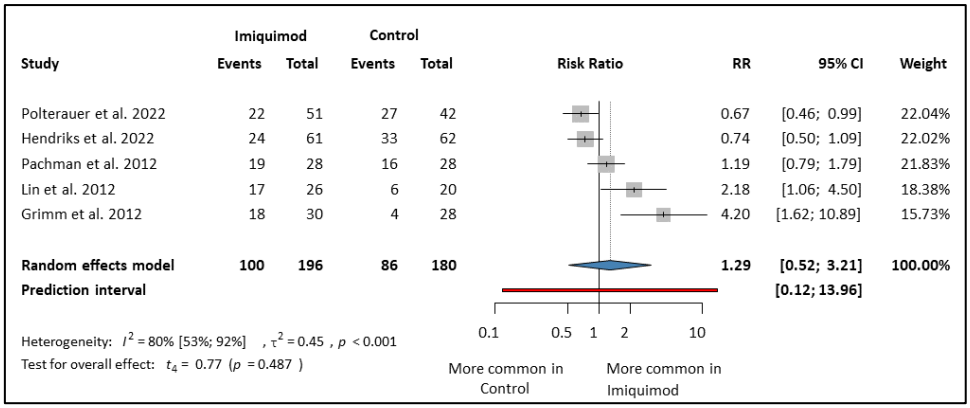

Figure 10. Forest plot of studies showing the **Imiquimod group** compared to the **control group** in terms of **HPV clearance**

For 186 patients treated with Imiquimod, HPV 16/18 clearance was compared to clearance of other high-risk HPV (HR-HPV) types.(30, 31, 73, 74) Our findings show no significant difference between HPV 16/18 clearance and clearance of other HR-HPV types (RR: 0.89; CI: 0.58–1.37; I^2:0) (see Figure S8).

8.2.5 Adverse events

For five studies, it was possible to quantitatively synthesize the adverse events in patients treated with Imiquimod, due to the similar grading system employed by these studies (see Table 2).(29, 30, 32, 71, 73) Side effects were graded on a scale ranging from one to five, with grades defined as mild, moderate, serious, life-threatening, and death (see Figure 11, Figure S9-S18).

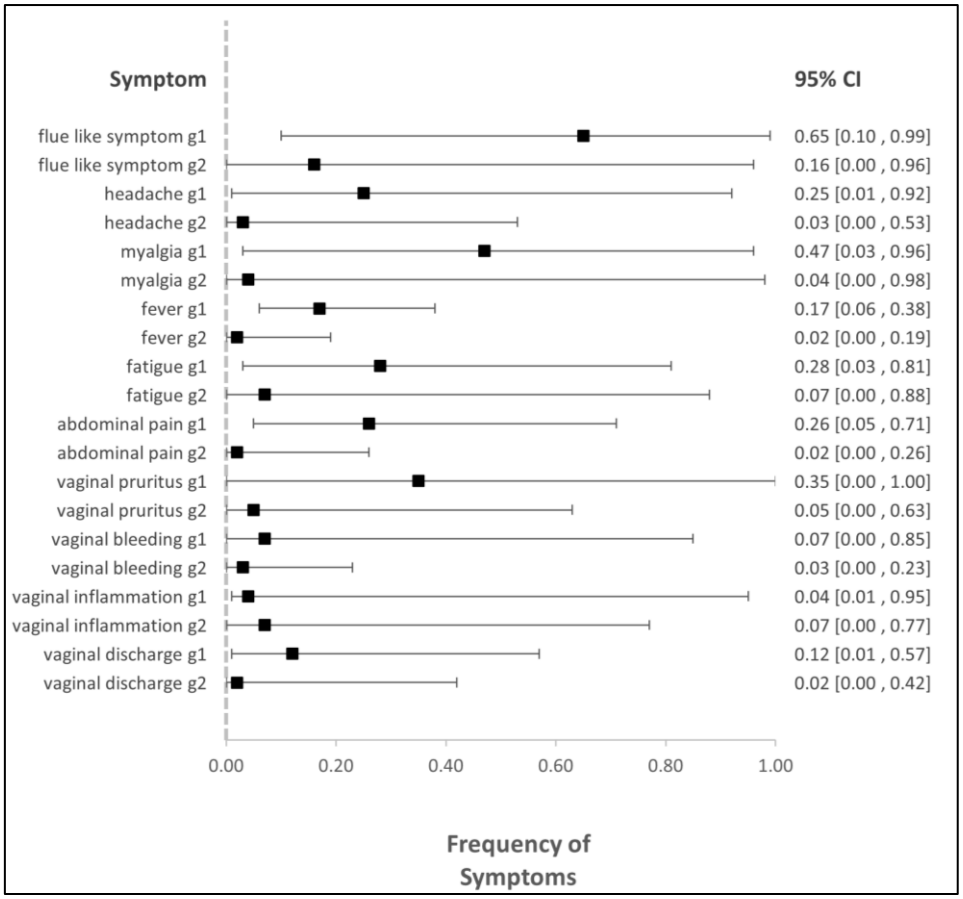

Figure 11. Forest plot of studies showing the frequency of all symptoms g1: grade1, g2: grade2, CI: confidence interval.

The most frequent systemic side effects were flu-like symptoms and myalgia; regarding local side effects, the most common was vaginal pruritus .

Side effects at grade 3 were reported 8 times. Two articles(29, 71) reported abdominal pain, and two articles(71, 73) reported headache. The other four instances of grade 3 side effects comprised 1 report of flu-like symptoms(30), 1 report of fever(73), 1 report of myalgia(73), and 1 report of vaginal inflammation(71).

8.2.6. Risk of bias assessment and GRADE

Among randomized controlled studies, the ROB2 indicated some concern for risk of bias in five outcomes, along with a low risk of bias in two outcomes. In non-randomized clinical trials meanwhile, the ROBINSON tool indicated a moderate risk of bias in one outcome and a serious risk of bias in another. The latter study was a retrospective cohort analysis utilizing a historical control group for comparison, and presenting methodological issues. Following analysis of the

response rates in line with the JBI critical appraisal checklist, sample size was identified as the most frequent issue (see Figures S20-S23).

Our summary of findings comprised five outcomes with the inclusion of a control group. Quality of evidence was rated high for two outcomes, and low for the three other outcomes (see Table S4-S6).

9. DISCUSSION

9.1. Summary of findings, international comparisons (including all studies)

Our study, including almost 500,000 women from population-based studies, identified a positive association between TV and cervical carcinogenesis. First, we examined the association between TV and HPV. We observed that women with a TV infection had greater odds of being diagnosed with a concomitant HPV infection compared to TV-negative women. Regarding TV and cervical dysplasia, a significant association was observed. On evaluating the relationship between TV and cervical cancer, we similarly observed a statistically significant association, such that TV-infected women had higher odds of developing cervical cancer. Concerning our second clinical question related to the HPV-positive population, we observed a positive association between TV, LSIL, HSIL, and cervical cancer.

Looking into the association between TV and HPV, it may be observed that STIs often coexist alongside similar behavioral risk factors including young age, multiple sexual partners, and unprotected sexual intercourse.(17, 75) Accordingly, it cannot be determined that TV infection affects HPV acquisition, given that both infections can be present concomitantly. Cervical cancer and most cervical intraepithelial neoplasias are attributable to high-risk HPV viruses;(6) therefore, HPV might be a confounding factor in the outcomes in our study concerning the cervix. However, focusing exclusively on HPV-positive population, an even greater association between TV cervical dysplasia and cancer could be observed.

On the contrary, not all HPV types are associated with the same degree of cancer risk as HPV 16 and 18, which together cause around 70% of all cervical cancers worldwide.(6) Accordingly, HPV-positive women are not a homogeneous population in terms of cancer risk. Some prospective studies indicate that persistent HPV infection is more likely in the presence of concomitant TV infection.(59, 76) This observation is predicated on a certain mechanism by which TV can affect HPV clearance. In this mechanism, TV may produce micro-lesions to the cervical epithelium, reduce the protective mucus layer of the vagina, and prompt proinflammatory cytokines through the immune response, potentially facilitating the spread of

an HPV infection into the basal layer of the cervical epithelium, thereby causing persistent HPV infection.(8, 77, 78). With persistent HPV infection comes an increased probability of cervical dysplasia, in turn promoting cervical carcinogenesis.(79) Meanwhile, the coexistence of various genital infections, Chlamydia trachomatis, and bacterial vaginosis may also induce persistent HPV infection, leading to cervical dysplasia progression.(80, 81)

In the assessment of ASCUS, we observed a significant association with TV infection. A Belgian study similarly found that women diagnosed with ASCUS had, in some cases, been TV-positive, indicating TV as a potential precursor to ASCUS.(17) Among the cervical dysplasia group, we observed the highest odds for cervical lesions in those cases where TV was detected using PCR, most likely due to PCR being the most sensitive method for TV detection.(20) Among the TV and cervical dysplasia group, the South American ASCUS and LSIL groups showed higher odds; however, there was only one article in the ASCUS group. TV prevalence is strongly linked to socioeconomic variables, sexual behaviors, and access to healthcare. In the absence of surveillance programs, there is no accurate picture of the epidemiological state of TV. In general, countries with higher incomes tend towards lower TV prevalence of TV, and vice versa.(14) The sensitivity analysis highlighted two articles with the potential to alter our results. One of the outliers(16) resulted in a lower association between TV and cervical dysplasia. However, in this study, TV diagnosis was via cytology, considered to be an inferior detection method for TV(20). The second article(57) originated in a part of Brazil notable for its high poverty rate, and where cervical cancer was the second most common cancer. [3] These contexts might explain the high odds ratios presented in the TV and cervical dysplasia group.

Lipophophoglycan (LPG) is a virulence factor found on the surface of TV which can induce immunological reactions according to the LPG type. As a reaction to these LPG particles, the host epithelial cells can produce the proinflammatory cytokines, IL-8 and MIP 3α, which in turn induce inflammation of the cervix and the vagina. However, other LPGs found on TV may reduce the level of proinflammatory cytokines and evade immune response. This is in agreement with the clinical finding that TV can be asymptomatic or the cause of persistent infection.(78)

In one study, inflammation of the cervix was linked to an increased risk of CIN.(8) In another study, elevated levels of IL-6, and IL-8 were found in cases of CIN and cervical cancer.(82) In general, inflammation is considered a risk factor for the development of many types of cancer.(83) One study examined the microbial aspect of the vagina in cervical cancer patients

and non-cervical cancer patients, hypothesizing that cervical cancer disrupts the vaginal microbiota in such a way as to make it more susceptible to infectious diseases. Accordingly, it might be speculated that TV could be less a cofactor than a consequence of cervical cancer.(84) Certainly, a healthy vaginal microbiome colonized with Lactobacillus species is essential for protection against STIs. Disruption of this complex balance reduces the natural defensive barriers and increases the likelihood of genital infections.(85) One study evidenced the proinflammatory synergy between vaginal dysbiosis and TV, with the authors suggesting a surface biofilm that might make bacterial vaginosis more resistant to antibiotic treatment.(86) Generally, STIs and vaginal infections are considered potential cofactors in the development of cervical intraepithelial neoplasia and cervical cancer, and this is supported by other meta-analyses. *Chlamydia trachomatis* was found to be associated with cervical cancer in one meta-analysis, and bacterial vaginosis was found to be associated with cervical lesions in another meta-analysis.(75, 81) Similarly, our findings provide further evidence that STIs and vaginal infections might act as cofactors in the development of cervical cancer.

Following Imiquimod treatment in CIN 2-3 patients, a regression rate of 61% was observed. In evaluating efficacy, we analyzed the biopsies of CIN 2-3 patients, with the conclusion that women who were treated with Imiquimod had a higher rate of CIN regression compared to those women who were not treated with Imiquimod. We observed that conization is more effective than Imiquimod in the treatment of CIN 2-3 patients, with a 38% increase in treatment success in the conization arm.

The HPV clearance rate in HPV-positive women treated with Imiquimod was 50%, rising to 60% in women who completed the course of treatment. Overall, Imiquimod treatment did not produce HPV clearance superior to that of the control group. In one study comparing Imiquimod to conization, HPV clearance proved higher in the conization group.(73) However, in another study where Imiquimod was compared to placebo, HPV clearance was higher in the Imiquimod arm.(30)

While side effects were often reported, most were mild, with hospitalization required only in a minority of cases.

Increased Imiquimod dosage did not lead to a higher rate of CIN 2-3 regression. Women who completed the Imiquimod treatment had a similar rate of dysplasia regression, regardless of dosage. In two studies, the remission rates were notably higher than in other studies.(30, 74) In the first, this was explained by the higher CIN 2/CIN 3 ratio. CIN 2 is characterized by a higher

rate of spontaneous regression than CIN 3 and is considered to be a milder lesion. (87, 88) Furthermore, the College of American Pathologists and ASCCP have suggested p16 immunostaining in CIN 2 cases to allocate them to LSIL category if p16 negative, or HSIL category if p16 positive.(89) The second study employed cytology for confirmation of CIN regression.(74) While cytology is not a reproducible method of detecting cervical dysplasia,(90) Imiquimod has been demonstrated as effective in reducing vulvar intraepithelial neoplasia and vaginal intraepithelial neoplasia.(91, 92) The American College of Obstetricians and Gynecologists recognizes the off-label use of Imiquimod for treating vulvar intraepithelial neoplasia. (93) Our findings on Imiquimod in cervical dysplasia regression are in line with the findings of previous studies on other lower genital intraepithelial neoplasia. (91, 92) Imiquimod is effective in reducing cervical CIN.

In comparing Imiquimod with established surgical therapy for CIN 2-3, we found Imiquimod to be inferior. However, when the topical immunomodulator was used prior to conization, positive margins of the resected tissue were found to be lower than the average recorded in the literature. (29, 94, 95) It might be inferred that Imiquimod reduced the depth and width of the lesion, thus making it more amenable to surgical excision.(29) For selected patients, Imiquimod may represent a beneficial treatment choice. For women planning to have children in the future, for example, a more conservative treatment is preferable(96) to conization, which increases the risk of miscarriage and preterm birth through cervical incompetence.(25) One study suggested low-grade CIN patients experience more anxiety than high-grade CIN patients, on the basis that the surgical interventions received by the latter are more reassuring than the observation and follow-up typically implemented in the former.(97) Accordingly, women with low-grade CIN who are very anxious might be considered for Imiquimod treatment. The ASCCP guideline recommends a six-month diagnostic evaluation of the cervix in the case of positive surgical excision margins.(24) Imiquimod treatment could reduce the need for additional surgical excision in positive margin cases. Again, this is a potentially desirable option for women considering future pregnancy, as repeated surgical intervention in the cervix increases the risk of preterm birth.(98) A recent study showed how patients could be preselected for Imiquimod treatment using an immunohistochemical method, that predicts whether the patient will respond to Imiquimod treatment.(99) This would help clinicians to personalize treatment and prescribe Imiquimod more efficaciously and cost-effectively, as not all patients respond to it.

Following examination of the HPV clearance for HPV 16 and 18 and other HR-HPV types, we observed that the clearance rate was not inferior for HPV 16 and 18. This is notable given that HPV 16 and 18 are known to be more aggressive and responsible for 70% of all cervical cancer cases.(100) We investigated the top three HPV clearance rates from among the studies. In the first study, we found there were only persistent HPV infections with no CIN lesions.(72) In the second study, the CIN 2/CIN 3 ratio was the highest among all publications.(30) In the third study, the CIN 2/CIN 3 ratio was high, and CIN 1 also featured;(32) furthermore, the HPV tests were performed 6 months after Imiquimod discontinuation, potentially long enough to overlap with natural clearance of HPV.(101) As is widely observed, patients with CIN 2+ lesions represent an extremely heterogeneous population when considering the molecular level; with progression, more extensive cellular changes are observed, and the spontaneous regression of CIN and HPV is reduced.(102) Our findings support this observation: in the case of more CIN 3 lesions, HPV clearance rates are lower with Imiquimod. Higher Imiquimod doses did not lead to a higher HPV clearance rate. Imiquimod did not show a better HPV clearance rate compared to the control group. However, it is important to note that the control differed between studies: for some studies, the control was surgical excision of the HPV-infected area(73), while for other studies the control was expectant management and no intervention.(30) In the case of the former, the surgical solution was comparable or more effective than Imiquimod; in the case of the latter, Imiquimod was more effective than expectant management.

Systemic and local side effects were frequent but mainly mild, and symptoms could be reduced with non-steroidal anti-inflammatory drugs.(30) The variability observed can be attributed to the systemic side effects associated with Imiquimod, also potentially influenced by other common infections and health-related conditions. In addition, local side effects of Imiquimod are commonly seen in general gynecological practice. This variability in respect of side effects also contributes to and helps to explain the differences observed between the studies.

Dropout rate is affected by various factors (travel requirements, financial considerations, patient dissatisfaction, etc.); however, it is notable that the two highest rates of dropout were observed in the two studies in which the highest doses of Imiquimod were implemented.(31, 71) Severe side effects were reported in only 8 cases, seven of which involved high doses of Imiquimod. Two studies involved lower doses of Imiquimod applied by doctors. Accordingly, direct application might be linked to less frequent and milder side effects.(29, 74)

9.2. Strengths

Regarding the topic of TV and cervical carcinogenesis, our study is the first meta-analysis to investigate the relationship between TV, HPV, and cervical lesions in detail. Due to the large sample size in the assessed studies, we were able to include nearly half a million patients in the analysis. An additional strength is the low risk of bias in the majority of the included studies.

Regarding Imiquimod and high-grade CIN, our meta-analysis is the first to synthesize the findings on Imiquimod use in cervical dysplasia and HPV-positive patients.

9.3. Limitations

The results of our study must be interpreted alongside the limitations. First, is the absence of follow-up in the TV-infected population, which together with the simultaneous screening of all participants for TV, HPV, and cervical carcinogenesis, meant that we were not able to determine how TV contributed to the development of the various outcomes. Second, many of the studies lacked multivariate analyses, meaning we could not calculate pooled adjusted ORs. The inadequate control of confounders may lead to an under- or overestimation of the analyzed associations. Third, it is unclear whether TV infection causes the cervical environment to be more susceptible to HPV infection and to the subsequent cervical intraepithelial neoplasia, or whether cervical dysplasia makes the environment more susceptible to TV infection.(103) Fourth, in diagnosing cervical lesions, some studies used cytology – a subjective method, and one that is difficult to replicate.(90) Fifth, the GRADE assessment identified the quality of evidence as low in six outcomes, and very low in three outcomes. Sixth, while not all HPVs are oncogenic, in the TV HPV association 10 studies included non-oncogenic HPV strains in their investigation.

In addition, several other limitations should be taken into account. First, many studies were affected by poor patient recruitment, with efforts made to enroll more women. Second, the patients were for the most part selected using specific criteria, thus limiting the generalizability of the study and its implications to all cervical dysplasia patients. Third, in some cases, methods differed among control groups, affecting comparisons. Fourth, many studies did not include longer follow-up intervals, calling into question the longevity of dysplasia remission. Fifth, there was often inconsistency in the endpoint and timing of different outcome measures, which

is a particular issue considering the tendency of these lesions to spontaneously regress. Sixth, a large clinical and statistical heterogeneity was notable in several cases.

10. CONCLUSIONS

Our results show that TV infection may increase the odds of cervical lesions and cancer development in sexually active women. In cases of TV diagnosis, clinicians should evaluate HPV and cervical dysplasia.

Our findings show Imiquimod to be safe and effective in reducing CIN and facilitating HPV clearance. In conclusion, while Imiquimod is not a substitute for cone biopsy, it can be a valuable option for the treatment of high-grade cervical dysplasia. Additionally, Imiquimod could also be considered for the management of low-grade cervical dysplasia.

11. IMPLICATIONS FOR PRACTICE

Based on our findings, we recommend that clinicians always consider HPV infection and cervical lesions when diagnosing TV infection. We cannot confirm a causative relationship between TV and cervical carcinogenesis; however, TV is associated with HPV infection, cervical lesions, and cervical cancer. Accordingly, follow-up after TV diagnosis is advised. Many countries have implemented HPV-based cervical cancer screening programs, which promise greater detection of HPV strains. (104) The results of our study show an association between TV and HPV; accordingly, TV screening and treatment are advisable following diagnosis of HPV, due to its potential carcinogenic effect on the cervix.

Although Imiquimod is less effective than conization, clinicians may consider it for use in selected patients, and in particular, to avoid subsequent surgical excision of the cervix following positive margins of conization.

12. IMPLICATIONS FOR RESEARCH

While translating scientific knowledge into patient benefits is the ultimate objective, more studies on this topic are required in order to control the confounding factors. Consequently, the true effect of TV on cervical carcinogenesis can be evaluated more reliably.

Similarly, further interventional studies on this topic are required to aid understanding of how Imiquimod can reduce the burden of cervical dysplasia.

13. Implications for Policymakers

It is important to emphasize that integrated care is key to decreasing cervical cancer and precancer prevalence. Besides regular screening for cervical cancer, the detection of concomitant STIs, such as Trichomonas, is crucial as they can contribute to the progression of cervical cancer. Policymakers should provide accessible and cost-reduced tests for STIs in cases of HPV detection or cervical lesions. Furthermore, Imiquimod should be adopted in treatment guidelines due to its potential as a conservative treatment option for selected patients. However, the cost of this drug is high, so insurance coverage is necessary. Additionally, organizing public information campaigns is required to disseminate information on cervical cancer prevention and treatment.

14. Future Perspectives

The proposition of the WHO for reducing the burden of cervical cancer is very ambitious and potentially difficult to achieve, given that the developing world accounts for the majority cervical cancer cases.(1) The cost of a cervical cancer screening program and treatment is very high, raising doubts about financial coverage, as many countries lack sanitation, suffer famine, and are afflicted by other diseases such as HIV.(105-107) Personalized treatment with Imiquimod is promising, as prior immunohistological examination can predict therapeutic response, given that not all patients respond to Imiquimod.

15. REFERENCES

1. WHO;. Global strategy to accelerate the elimination of cervical cancer as a public health problem 2020.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-49.
3. Simms KT, Steinberg J, Caruana M, Smith MA, Lew JB, Soerjomataram I, et al. Impact of scaled up human papillomavirus vaccination and cervical screening and the potential for global elimination of cervical cancer in 181 countries, 2020-99: a modelling study. Lancet Oncol. 2019;20(3):394-407.
4. Giannini A, Bogani G, Vizza E, Chiantera V, Laganà AS, Muzii L, et al. Advances on Prevention and Screening of Gynecologic Tumors: Are We Stepping Forward? Healthcare (Basel). 2022;10(9).
5. D'Oria O, Corrado G, Laganà AS, Chiantera V, Vizza E, Giannini A. New Advances in Cervical Cancer: From Bench to Bedside. Int J Environ Res Public Health. 2022;19(12).
6. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer. 2002;2(5):342-50.
7. Mercer F, Johnson PJ. Trichomonas vaginalis: Pathogenesis, Symbiont Interactions, and Host Cell Immune Responses. Trends Parasitol. 2018;34(8):683-93.
8. Castle PE, Hillier SL, Rabe LK, Hildesheim A, Herrero R, Bratti MC, et al. An association of cervical inflammation with high-grade cervical neoplasia in women infected with oncogenic human papillomavirus (HPV). Cancer Epidemiol Biomarkers Prev. 2001;10(10):1021-7.
9. Brusselaers N, Shrestha S, van de Wijgert J, Verstraelen H. Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. Am J Obstet Gynecol. 2019;221(1):9-18.e8.
10. Slattery ML, Robison LM, Schuman KL, French TK, Abbott TM, Overall JC, Jr., et al. Cigarette smoking and exposure to passive smoke are risk factors for cervical cancer. Jama. 1989;261(11):1593-8.
11. Huh WK. Human papillomavirus infection: a concise review of natural history. Obstet Gynecol. 2009;114(1):139-43.
12. Hawes SE, Kiviat NB. Are genital infections and inflammation cofactors in the pathogenesis of invasive cervical cancer? J Natl Cancer Inst. 2002;94(21):1592-3.
13. Van der Pol B. Trichomonas vaginalis infection: the most prevalent nonviral sexually transmitted infection receives the least public health attention. Clin Infect Dis. 2007;44(1):23-5.
14. Kissinger P. Trichomonas vaginalis: a review of epidemiologic, clinical and treatment issues. BMC Infect Dis. 2015;15:307.
15. Lazenby GB, Taylor PT, Badman BS, McHaki E, Korte JE, Soper DE, et al. An association between trichomonas vaginalis and high-risk human papillomavirus in rural tanzanian women undergoing cervical cancer screening. Clinical Therapeutics. 2014;36(1):38-45.

16. Al-Awadhi R, Al-Shaheen A, Al-Juwaiser A, George SS, Sharma P, Kapila K. Prevalence of Infectious Organisms Observed in Cervical Smears Between 1997-2014 at Mubarak Al-Kabeer Hospital, Kuwait. Sultan Qaboos University medical journal. 2018;18(3):e324-e8.
17. Donders GG, Depuydt CE, Bogers JP, Vereecken AJ. Association of Trichomonas vaginalis and cytological abnormalities of the cervix in low risk women. PLoS One. 2013;8(12):e86266.
18. Ghosh I, Muwonge R, Mittal S, Banerjee D, Kundu P, Mandal R, et al. Association between high risk human papillomavirus infection and co-infection with Candida spp. and Trichomonas vaginalis in women with cervical premalignant and malignant lesions. Journal of Clinical Virology. 2017;87:43-8.
19. Zhang ZF, Begg CB. Is Trichomonas vaginalis a cause of cervical neoplasia? Results from a combined analysis of 24 studies. Int J Epidemiol. 1994;23(4):682-90.
20. Asmah RH, Agyeman RO, Obeng-Nkrumah N, Blankson H, Awuah-Mensah G, Cham M, et al. Trichomonas vaginalis infection and the diagnostic significance of detection tests among Ghanaian outpatients. BMC Womens Health. 2018;18(1):206.
21. Aslan DL, Gulbahce HE, Stelow EB, Setty S, Brown CA, McGlennen RC, et al. The diagnosis of Trichomonas vaginalis in liquid-based Pap tests: correlation with PCR. Diagn Cytopathol. 2005;32(6):341-4.
22. Yang S, Zhao W, Wang H, Wang Y, Li J, Wu X. Trichomonas vaginalis infection-associated risk of cervical cancer: A meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2018;228:166-73.
23. Loopik DL, Bentley HA, Eijgenraam MN, IntHout J, Bekkers RLM, Bentley JR. The Natural History of Cervical Intraepithelial Neoplasia Grades 1, 2, and 3: A Systematic Review and Meta-analysis. J Low Genit Tract Dis. 2021;25(3):221-31.
24. 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.
25. Weinmann S, Naleway A, Swamy G, Krishnarajah G, Arondekar B, Fernandez J, et al. Pregnancy Outcomes after Treatment for Cervical Cancer Precursor Lesions: An Observational Study. PLoS One. 2017;12(1):e0165276.
26. Bogani G, V DID, Sopracordevole F, Ciavattini A, Ghelardi A, Lopez S, et al. Recurrence rate after loop electrosurgical excision procedure (LEEP) and laser Conization: A 5-year follow-up study. Gynecol Oncol. 2020;159(3):636-41.
27. Food and Drug Administration (FDA).
28. Stary G, Bangert C, Tauber M, Strohal R, Kopp T, Stingl G. Tumoricidal activity of TLR7/8-activated inflammatory dendritic cells. J Exp Med. 2007;204(6):1441-51.
29. Fonseca BO, Possati-Resende JC, Salcedo MP, Schmeler KM, Accorsi GS, Fregnani JHTG, et al. Topical Imiquimod for the Treatment of High-Grade Squamous Intraepithelial Lesions of the Cervix: A Randomized Controlled Trial. Obstetrics and gynecology. 2021;137(6):1043-53.
30. Grimm C, Polterauer S, Natter C, Rahhal J, Hefler L, Tempfer CB, et al. Treatment of cervical intraepithelial neoplasia with topical imiquimod: a randomized controlled trial. Obstetrics and gynecology. 2012;120(1):152-9.
31. Hendriks N, Koeneman MM, Van De Sande AJM, Penders CGJ, Piek JMJ, Kooreman LFS, et al. Topical Imiquimod Treatment of High-grade Cervical Intraepithelial Neoplasia (TOPIC-3): A Nonrandomized Multicenter Study. Journal of Immunotherapy. 2022;45(3):180-6.

32. Pachman DR, Barton DL, Clayton AC, McGovern RM, Jefferies JA, Novotny PJ, et al. Randomized clinical trial of imiquimod: An adjunct to treating cervical dysplasia. American Journal of Obstetrics and Gynecology. 2012;206(1):42.e1-.e7.
33. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Bmj. 2021;372:n71.
34. Cumpston M, Li T, Page MJ, Chandler J, Welch VA, Higgins JP, et al. Updated guidance for trusted systematic reviews: a new edition of the Cochrane Handbook for Systematic Reviews of Interventions. Cochrane Database Syst Rev. 2019;10:Ed000142.
35. van Zuuren EJ, Fedorowicz Z. Moose on the loose: checklist for meta-analyses of observational studies. Br J Dermatol. 2016;175(5):853-4.
36. Munn Z, Stern C, Aromataris E, Lockwood C, Jordan Z. What kind of systematic review should I conduct? A proposed typology and guidance for systematic reviewers in the medical and health sciences. BMC Med Res Methodol. 2018;18(1):5.
37. Roteli-Martins CM, Alves VA, Santos RT, Martinez EZ, Syrjänen KJ, Derchain SF. Value of morphological criteria in diagnosing cervical HPV lesions confirmed by in situ hybridization and hybrid capture assay. Pathol Res Pract. 2001;197(10):677-82.
38. McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb). 2012;22(3):276-82.
39. National Cancer Institute (NIH).
40. Hayden JA, van der Windt DA, Cartwright JL, Côté P, Bombardier C. Assessing bias in studies of prognostic factors. Ann Intern Med. 2013;158(4):280-6.
41. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. Bmj. 2019;366:l4898.
42. Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. Bmj. 2016;355:i4919.
43. Joanna Briggs Institute (JBI) critical appraisal checklist. 2017.
44. GradePro. [Available from: https://www.gradepro.org.
45. Verteramo R, Pierangeli A, Mancini E, Calzolari E, Bucci M, Osborn J, et al. Human Papillomaviruses and genital co-infections in gynaecological outpatients. BMC Infectious Diseases. 2009;9.
46. Noël JC, Fayt I, Romero Munoz MR, Simon P, Engohan-Aloghe C. High prevalence of high-risk human papillomavirus infection among women with Trichomonas vaginalis infection on monolayer cytology. Archives of Gynecology and Obstetrics. 2010;282(5):503-5.
47. Depuydt CE, Leuridan E, Van Damme P, Bogers J, Vereecken AJ, Donders GG. Epidemiology of Trichomonas vaginalis and human papillomavirus infection detected by real-time PCR in flanders. Gynecol Obstet Invest. 2010;70(4):273-80.
48. Caiyan X, Weiyuan Z, Minghui W, Songwen Z. Prevalence and risk factors of lower genital tract infections among women in Beijing, China. Journal of Obstetrics and Gynaecology Research. 2012;38(1):310-5.
49. Mendoza L, Mongelos P, Paez M, Castro A, Rodriguez-Riveros I, Gimenez G, et al. Human papillomavirus and other genital infections in indigenous women from Paraguay: A cross-sectional analytical study. BMC Infectious Diseases. 2013;13(1).
50. Paesi SO, Aver L, Barea F, Vanni A, Roesch-Ely M. Human papillomavirus and infections of the lower genital tract in women with abnormal cervical cytological examination. Scientia Medica. 2013;23(1):41-6.

51. Liu J, Liu W, Liu Y, Zhou X, Zhang Z, Sun Z. Prevalence of microorganisms co-infections in human papillomaviruses infected women in Northern China. Archives of Gynecology and Obstetrics. 2016;293(3):595-602.
52. Casillas-Vega N, Morfín-Otero R, García S, Llaca-Díaz J, Rodríguez-Noriega E, Camacho-Ortiz A, et al. Sexually transmitted pathogens, coinfections and risk factors in patients attending obstetrics and gynecology clinics in Jalisco, Mexico. Salud Publica Mex. 2016;58(4):437-45.
53. Camporiondo MP, Farchi F, Ciccozzi M, Denaro A, Gallone D, Maracchioni F, et al. Detection of HPV and co-infecting pathogens in healthy Italian women by multiplex real-time PCR. Infezioni in Medicina. 2016;24(1):12-7.
54. Dey S, Pahwa P, Mishra A, Govil J, Dhillon PK. Reproductive Tract infections and Premalignant Lesions of Cervix: Evidence from Women Presenting at the Cancer Detection Centre of the Indian Cancer Society, Delhi, 2000–2012. Journal of Obstetrics and Gynecology of India. 2016;66:441-51.
55. de Abreu ALP, Malaguti N, Souza RP, Uchimura NS, Ferreira ÉC, Pereira MW, et al. Association of human papillomavirus, Neisseria gonorrhoeae and Chlamydia trachomatis co-infections on the risk of high-grade squamous intraepithelial cervical lesion. American Journal of Cancer Research. 2016;6(6):1371-83.
56. Kim HS, Kim TJ, Lee IH, Hong SR. Associations between sexually transmitted infections, high-risk human papillomavirus infection, and abnormal cervical Pap smear results in OB/GYN outpatients. Journal of Gynecologic Oncology. 2016;27(5).
57. Amorim AT, Marques LM, Campos GB, Lobão TN, de Souza Lino V, Cintra RC, et al. Co-infection of sexually transmitted pathogens and Human Papillomavirus in cervical samples of women of Brazil. BMC Infectious Diseases. 2017;17(1).
58. Costa-Lira E, Jacinto AHVL, Silva LM, Napoleão PFR, Barbosa-Filho RAA, Cruz GJS, et al. Prevalence of human papillomavirus, Chlamydia trachomatis, and Trichomonas vaginalis infections in Amazonian women with normal and abnormal cytology. Genetics and Molecular Research. 2017;16(2).
59. Lockhart A, Senkomago V, Ting J, Chitwa M, Kimani J, Gakure H, et al. Prevalence and risk factors of trichomonas vaginalis among female sexual workers in Nairobi, Kenya. Sexually Transmitted Diseases. 2019;46(7):458-64.
60. Ferré VM, Ekouevi DK, Gbeasor-Komlanvi FA, Collin G, Le Hingrat Q, Tchounga B, et al. Prevalence of human papillomavirus, human immunodeficiency virus and other sexually transmitted infections among female sex workers in Togo: a national cross-sectional survey. Clinical Microbiology and Infection. 2019;25(12):1560.e1-.e7.
61. Lv P, Zhao F, Xu X, Xu J, Wang Q, Zhao Z. Correlation between Common Lower Genital Tract Microbes and High-Risk Human Papillomavirus Infection. Canadian Journal of Infectious Diseases and Medical Microbiology. 2019;2019.
62. Cunha APA, Belfort IKP, Mendes FPB, dos Santos GRB, de Lima Costa LH, de Matos Monteiro P, et al. Human papillomavirus and Its Association with Other Sexually Transmitted Coinfection among Sexually Active Women from the Northeast of Brazil. Interdisciplinary Perspectives on Infectious Diseases. 2020;2020.
63. Wang W, Zhang XH, Li M, Hao CH, Liang HP. Association between Vaginal Infections and the Types and Viral Loads of Human Papillomavirus: A Clinical Study Based on 4,449 Cases of Gynecologic Outpatients. Canadian Journal of Infectious Diseases and Medical Microbiology. 2020;2020.
64. Yang M, Li L, Jiang C, Qin X, Zhou M, Mao X, et al. Co-infection with trichomonas vaginalis increases the risk of cervical intraepithelial neoplasia grade 2-3 among HPV16 positive female: A large population-based study. BMC Infectious Diseases. 2020;20(1).

65. Zheng JJ, Miao JR, Wu Q, Yu CX, Mu L, Song JH. Correlation between HPV-negative cervical lesions and cervical microenvironment. Taiwanese Journal of Obstetrics and Gynecology. 2020;59(6):855-61.
66. Gupta R, Singh N, Kalyan RK, Agrawal S. Case-Control Study to Find Association of Common RTIs with CIN and Cervical Cancer. Indian Journal of Gynecologic Oncology. 2020;18(4).
67. Taku O, Brink A, Meiring TL, Phohlo K, Businge CB, Mbulawa ZZA, et al. Detection of sexually transmitted pathogens and co-infection with human papillomavirus in women residing in rural Eastern Cape, South Africa. PeerJ. 2021;9.
68. Jary A, Teguete I, Sidibé Y, Kodio A, Dolo O, Burrel S, et al. Prevalence of cervical HPV infection, sexually transmitted infections and associated antimicrobial resistance in women attending cervical cancer screening in Mali. International Journal of Infectious Diseases. 2021;108:610-6.
69. Belfort IKP, Cunha APA, Mendes FPB, Galvao-Moreira LV, Lemos RG, Costa LHD, et al. Trichomonas vaginalis as a risk factor for human papillomavirus: a study with women undergoing cervical cancer screening in a northeast region of Brazil. Bmc Womens Health. 2021;21(1).
70. IntHout J, Ioannidis JP, Rovers MM, Goeman JJ. Plea for routinely presenting prediction intervals in meta-analysis. BMJ Open. 2016;6(7):e010247.
71. Cokan A, Pakiž M, Serdinšek T, Dovnik A, Kodrič T, Fokter AR, et al. Comparison of conservative treatment of cervical intraepithelial lesions with imiquimod with standard excisional technique using lletz: A randomized controlled trial. Journal of Clinical Medicine. 2021;10(24).
72. Lin CT, Qiu JT, Wang CJ, Chang SD, Tang YH, Wu PJ, et al. Topical imiquimod treatment for human papillomavirus infection in patients with and without cervical/vaginal intraepithelial neoplasia. Taiwanese Journal of Obstetrics and Gynecology. 2012;51(4):533-8.
73. Polterauer S, Reich O, Widschwendter A, Hadjari L, Bogner G, Reinthaller A, et al. Topical imiquimod compared with conization to treat cervical high-grade squamous intraepithelial lesions: multicenter, randomized controlled trial. Gynecologic oncology. 2022;165(1):23-9.
74. Kim JH, Kim DY. Imiquimod as an alternative option for young women with high-grade cervical intraepithelial neoplasia. European Journal of Gynaecological Oncology. 2019;40(6):943-7.
75. Zhu H, Shen Z, Luo H, Zhang W, Zhu X. Chlamydia Trachomatis Infection-Associated Risk of Cervical Cancer: A Meta-Analysis. Medicine (Baltimore). 2016;95(13):e3077.
76. Shew ML, Fortenberry JD, Tu W, Juliar BE, Batteiger BE, Qadadri B, et al. Association of condom use, sexual behaviors, and sexually transmitted infections with the duration of genital human papillomavirus infection among adolescent women. Archives of Pediatrics and Adolescent Medicine. 2006;160(2):151-6.
77. Madeiro Da Costa RF, De Souza W, Benchimol M, Alderete JF, Morgado-Díaz JA. Trichomonas vaginalis perturbs the junctional complex in epithelial cells. Cell Research. 2005;15(9):704-16.
78. Fichorova RN, Yamamoto HS, Fashemi T, Foley E, Ryan S, Beatty N, et al. Trichomonas vaginalis Lipophosphoglycan Exploits Binding to Galectin-1 and -3 to Modulate Epithelial Immunity. J Biol Chem. 2016;291(2):998-1013.
79. Bogani G, Taverna F, Lombardo C, Borghi C, Martinelli F, Signorelli M, et al. Retrospective study of the influence of HPV persistence on outcomes among women with high-risk HPV infections and negative cytology. Int J Gynaecol Obstet. 2017;138(1):62-8.

80. Naldini G, Grisci C, Chiavarini M, Fabiani R. Association between human papillomavirus and chlamydia trachomatis infection risk in women: a systematic review and meta-analysis. Int J Public Health. 2019;64(6):943-55.
81. Gillet E, Meys JF, Verstraelen H, Verhelst R, De Sutter P, Temmerman M, et al. Association between bacterial vaginosis and cervical intraepithelial neoplasia: systematic review and meta-analysis. PLoS One. 2012;7(10):e45201.
82. Tjiong MY, van der Vange N, ten Kate FJ, Tjong AHSP, ter Schegget J, Burger MP, et al. Increased IL-6 and IL-8 levels in cervicovaginal secretions of patients with cervical cancer. Gynecol Oncol. 1999;73(2):285-91.
83. Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. Proc Natl Acad Sci U S A. 1995;92(12):5258-65.
84. Kovachev SM. Cervical cancer and vaginal microbiota changes. Archives of Microbiology. 2020;202(2):323-7.
85. Alimena S, Davis J, Fichorova RN, Feldman S. The vaginal microbiome: A complex milieu affecting risk of human papillomavirus persistence and cervical cancer. Curr Probl Cancer. 2022;46(4):100877.
86. Fichorova RN, Buck OR, Yamamoto HS, Fashemi T, Dawood HY, Fashemi B, et al. The villain team-up or how Trichomonas vaginalis and bacterial vaginosis alter innate immunity in concert. Sex Transm Infect. 2013;89(6):460-6.
87. Chan JK, Monk BJ, Brewer C, Keefe KA, Osann K, McMeekin S, et al. HPV infection and number of lifetime sexual partners are strong predictors for 'natural' regression of CIN 2 and 3. Br J Cancer. 2003;89(6):1062-6.
88. Tainio K, Athanasiou A, Tikkinen KAO, Aaltonen R, Cárdenas J, Hernándes, et al. Clinical course of untreated cervical intraepithelial neoplasia grade 2 under active surveillance: systematic review and meta-analysis. Bmj. 2018;360:k499.
89. Waxman AG, Chelmow D, Darragh TM, Lawson H, Moscicki AB. Revised terminology for cervical histopathology and its implications for management of high-grade squamous intraepithelial lesions of the cervix. Obstet Gynecol. 2012;120(6):1465-71.
90. Kitchener HC, Castle PE, Cox JT. Chapter 7: Achievements and limitations of cervical cytology screening. Vaccine. 2006;24 Suppl 3:S3/63-70.
91. Terlou A, van Seters M, Ewing PC, Aaronson NK, Gundy CM, Heijmans-Antonissen C, et al. Treatment of vulvar intraepithelial neoplasia with topical imiquimod: seven years median follow-up of a randomized clinical trial. Gynecol Oncol. 2011;121(1):157-62.
92. Tranoulis A, Laios A, Mitsopoulos V, Lutchman-Singh K, Thomakos N. Efficacy of 5% imiquimod for the treatment of Vaginal intraepithelial neoplasia-A systematic review of the literature and a meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2017;218:129-36.
93. Committee Opinion No. 675 Summary: Management of Vulvar Intraepithelial Neoplasia. Obstet Gynecol. 2016;128(4):937-8.
94. Panna S, Luanratanakorn S. Positive margin prevalence and risk factors with cervical specimens obtained from loop electrosurgical excision procedures and cold knife conization. Asian Pac J Cancer Prev. 2009;10(4):637-40.
95. O'Shea ASS, C. K. The impact of LEEP margin status on subsequent abnormal cervical cytology. Proceedings in Obstetrics and Gynecology.4(2):1-8.
96. Koeneman MM, Essers BA, Gerestein CG, van de Sande AJM, Litjens R, Boskamp D, et al. Treatment of Cervical Intraepithelial Neoplasia: Patients Preferences for Surgery or Immunotherapy with Imiquimod. J Immunother. 2017;40(4):148-53.
97. Lee Mortensen G, Adeler AL. Qualitative study of women's anxiety and information needs after a diagnosis of cervical dysplasia. Z Gesundh Wiss. 2010;18(5):473-82.

98. Kyrgiou M, Athanasiou A, Kalliala IEJ, Paraskevaidi M, Mitra A, Martin-Hirsch PP, et al. Obstetric outcomes after conservative treatment for cervical intraepithelial lesions and early invasive disease. Cochrane Database Syst Rev. 2017;11(11):Cd012847.
99. Abdulrahman Z, Hendriks N, A JK, Somarakis A, A JMvdS, H JvB, et al. Immune-based biomarker accurately predicts response to imiquimod immunotherapy in cervical high-grade squamous intraepithelial lesions. J Immunother Cancer. 2022;10(11).
100. da Silva RL, da Silva Batista Z, Bastos GR, Cunha APA, Figueiredo FV, de Castro LO, et al. Role of HPV 16 variants among cervical carcinoma samples from Northeastern Brazil. BMC Womens Health. 2020;20(1):162.
101. Gravitt PE, Winer RL. Natural History of HPV Infection across the Lifespan: Role of Viral Latency. Viruses. 2017;9(10).
102. Steenbergen RD, Snijders PJ, Heideman DA, Meijer CJ. Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. Nat Rev Cancer. 2014;14(6):395-405.
103. Lin S, Zhang B, Lin Y, Lin Y, Zuo X. Dysbiosis of Cervical and Vaginal Microbiota Associated With Cervical Intraepithelial Neoplasia. Front Cell Infect Microbiol. 2022;12:767693.
104. Smith MA, Sherrah M, Sultana F, Castle PE, Arbyn M, Gertig D, et al. National experience in the first two years of primary human papillomavirus (HPV) cervical screening in an HPV vaccinated population in Australia: observational study. Bmj. 2022;376:e068582.
105. Popkin BM, Corvalan C, Grummer-Strawn LM. Dynamics of the double burden of malnutrition and the changing nutrition reality. Lancet. 2020;395(10217):65-74.
106. Wolf J, Johnston RB, Ambelu A, Arnold BF, Bain R, Brauer M, et al. Burden of disease attributable to unsafe drinking water, sanitation, and hygiene in domestic settings: a global analysis for selected adverse health outcomes. Lancet. 2023;401(10393):2060-71.
107. McBain RK, Jordan M, Kapologwe NA, Kagaayi J, Kiracho EE, Nandakumar AK. Costing of HIV services, Uganda and United Republic of Tanzania. Bull World Health Organ. 2023;101(10):626-36.

16. BIBLIOGRAPHY

16.1. Publications related to the thesis

Q1

Balázs Hamar, Brigitta Teutsch, Eszter Hoffmann, Péter Hegyi, Alex Váradi, Péter Nyirády, Zsombor Hunka, Nándor Ács, Balázs Lintner, Réka Juhász Hermánné, Zsolt Melczer. (2023) Trichomonas vaginalis infection is associated with increased risk of cervical carcinogenesis: A systematic review and meta-analysis of 470,000 patients. **Int J Gynecol Obstet,** 163(1)31-43

Q1

Balázs Hamar, Brigitta Teutsch, Eszter Hoffmann, Péter Hegyi, Andrea Harnos, Péter Nyirády, Zsombor Hunka, Nándor Ács, Ferenc Bánhidy, Zsolt Melczer. (2024) Imiquimod is Effective in Reducing Cervical Intraepithelial Neoplasia: A Systematic Review and Meta-Analysis. **Cancers,** 22;16(8):1610

16.2. Publications not related to the thesis

D1

Eszter Hoffmann, Szilárd Váncsa, Alex Váradi, Péter Hegyi, Rita Nagy, **Balázs Hamar**, Vanda Futács, Begüm Kepep, Péter Nyirády, Csaba Demendi, Nándor Ács. (2023) Routine screening of vaginal flora during pregnancy reduces the odds of preterm births: a systematic review and meta-analysis. **Sci. Rep**, 25;13(1):13897

Q4

Balázs Hamar, Balázs Börzsönyi, Nándor Ács, Tibor Glasz, Zsolt Melczer. (2022) Rare abdominal bleeding from the branches of the splenic vein during pregnancy **Orv. Hetil.** 20;163(8):328-332

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REVIEW ARTICLE

Gynecology

Trichomonas vaginalis infection is associated with increased risk of cervical carcinogenesis: A systematic review and meta-analysis of 470 000 patients

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Abstract

Background: *Trichomonas vaginalis* infection is the most prevalent non-viral sexually transmitted infection (STI) in women and has been suggested as a risk factor for developing cervical cancer.

Objective: We aimed to investigate the associations between *T. vaginalis* infection and cervical carcinogenesis.

Search Strategy: A comprehensive systematic search was conducted in five databases on 21 October 2021.

Selection Criteria: Studies assessing the relationship between *T. vaginalis* infection, HPV co-infections, cervical dysplasia, and cervical cancer were found eligible.

Data Collection and Analysis: Summary estimates for pooled odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated with a random-effects model. Statistical heterogeneity was measured with I^2 and Cochran's Q tests.

Main Results: The 29 articles included 473 740 women, of whom 8518 were *T. vaginalis*-positive. Our results showed that *T. vaginalis*-infected women had 1.79 times higher odds of being diagnosed with HPV co-infection (95% CI 1.27–2.53; I^2 95%). We also found that *T. vaginalis* infection was associated with high-grade squamous intraepithelial lesion diagnosis (OR 2.34, 95% CI 1.10–4.95; I^2 75%) and cervical cancer (OR 5.23, 95% CI 3.03–9.04; I^2 3%).

Conclusions: Our results showed an association between *T. vaginalis* and cervical carcinogenesis in sexually active women.

KEYWORDS
cervical intraepithelial neoplasia, cervical lesion, cervical precancer, protozoal infection

1 | INTRODUCTION

Cervical cancer is the fourth most commonly diagnosed cancer and the fourth leading cause of cancer-related death in women.[1] Currently, there are effective ways of fighting cervical cancer through immunization, screening, and oncologic treatment.[2] HPV vaccination provides a high level of protection against oncogenic HPV strains and can reduce the burden of cervical cancer. Moreover, cervical smear and HPV tests improve the reliability of cervical cancer screening significantly.[3] In the treatment line of cervical cancer, immunotherapy and target therapy can have an increasing role besides the classical chemotherapeutic regimens.[4] Despite all of these, cervical cancer is still the most frequently diagnosed cancer in developing countries, and the leading cause of cancer-related mortality in emergent nations.[1,2]

The main risk factor of cervical cancer is infection with high-risk HPV types, responsible for various cancer types. Most notably, HPV 16 and HPV 18 types are accountable for 70% of cervical cancers worldwide.[5] After being incorporated into the host cell genome, the virus leads to overexpression of proto-oncogene proteins.[6,7] A persistent HPV infection and the inability of the immune system to clear out the infection in the cervix are key elements of the carcinogenesis.[5] The disruption of the vaginal microbiota performs an essential role in persistent HPV infection, as vaginal dysbiosis occurs, proinflammatory cytokines are increased and immunclearance is reduced.[8] It is well known that other risk factors, including smoking, promiscuity, using oral contraceptive drugs, immunosuppressed state, and sexually transmitted infections (STIs), can also contribute to developing cervical cancer.[7,9–11]

Trichomonas vaginalis, a common cause of STI, causes around 170–190 million infections annually.[12] Infection of the genital tract with these anaerobic protozoa can lead to discomfort by causing odorous discharge, dysuria, itching, and vulvar irritation. However, up to 85% of trichomoniases can be symptomless in women. Moreover, 5%–35% of women can also be reinfected.[13] *Trichomonas vaginalis* can contribute to the development of cervical cancer by causing inflammation, abruption of the cervical epithelium, and influencing the immune system to eliminate HPV. Current evidence on the relationship between *T. vaginalis* infection, cervical dysplasia, and cervical cancer is conflicting. Although several articles report strong associations, other publications do not find *T. vaginalis* to be a risk factor for cervical carcinogenesis.[14–17] Two meta-analyses have been conducted on this topic. The first article, published in 1994, included populations where *T. vaginalis* detection was based only on cytology, which can often underdetect *T. vaginalis*.[18–20] The other meta-analysis focused on cervical dysplasia without differentiating between the different states of cervical lesions and did not investigate the relationship between *T. vaginalis* and HPV.[21]

Hence, on the basis of the available literature, this study aimed to investigate the association between *T. vaginalis* and HPV, cervical dysplasia, and carcinogenesis. We hypothesized that *T. vaginalis* was a risk factor for developing cervical cancer.

2 | MATERIALS AND METHODS

We conducted our systematic review and meta-analysis according to the PRISMA 2020 and MOOSE guidelines (see Figure 1; Tables S1 and S2) while we followed the recommendations of the Cochrane Handbook.[22–24] The pre-study protocol was registered in PROSPERO (CRD42021286097), and we fully adhered to it.

2.1 | Literature search and eligibility criteria

The systematic search was conducted using five major databases on October 20, 2021: MEDLINE (via PubMed), Embase, Cochrane Central Register of Controlled Trials (CENTRAL), Scopus, and Web of Science. We accepted only peer-reviewed articles; therefore, we did not search on ClinicalTrials.gov, nor did our preliminary search find any suitable studies. No filters or restrictions were applied during the search. We used two population, exposure, and outcome (PEO) frameworks to define the eligibility criteria for the articles.[25] All studies reporting sexually active (P_1) or HPV-positive (P_2) women who were screened for *T. vaginalis* infection (E) were deemed eligible. The outcomes of interest (O_1) were HPV positivity, cervical dysplasia, and cervical cancer. In HPV-positive women (P_2), the investigated outcomes (O_2) were cervical dysplasia and cervical cancer. The articles had to include a population of *T. vaginalis*-negative women forming the control group.

Articles were considered where *T. vaginalis* was detected with cytology, wet-mount, culture, or polymerase chain reaction (PCR) methods. Articles were excluded in which *T. vaginalis* was diagnosed on the basis of clinical features or medical history. Studies were suitable if HPV exposure was diagnosed with any nuclear amplification method. Articles where HPV was detected only by cytology were excluded because of the low sensitivity of the method.[26] Cytologic and histopathologic diagnoses were acceptable for cervical intraepithelial neoplasia (CIN) and cancer confirmation. We evaluated the following outcomes in the dysplasia group: atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells, atypical squamous cells for which one could not rule out high-grade squamous intraepithelial lesions (ASC-H), low-grade squamous intraepithelial lesions (LSIL), and high-grade squamous intraepithelial lesions (HSIL). For cytologic samples, the Bethesda classification was required. Articles that proclaimed CIN1–3 diagnoses were divided into LSIL (CIN1) and HSIL (CIN2–3) groups for a more straightforward interpretation.

Observational studies, such as cross-sectional, case–control, and cohort analyses, were accepted. Abstracts were excluded in our review. Non-English language articles were translated for possible evaluation.

2.2 | Search strategy

During the systematic search, we used the following main concepts: "trichomonas", "human papillomavirus", "cervical intraepithelial

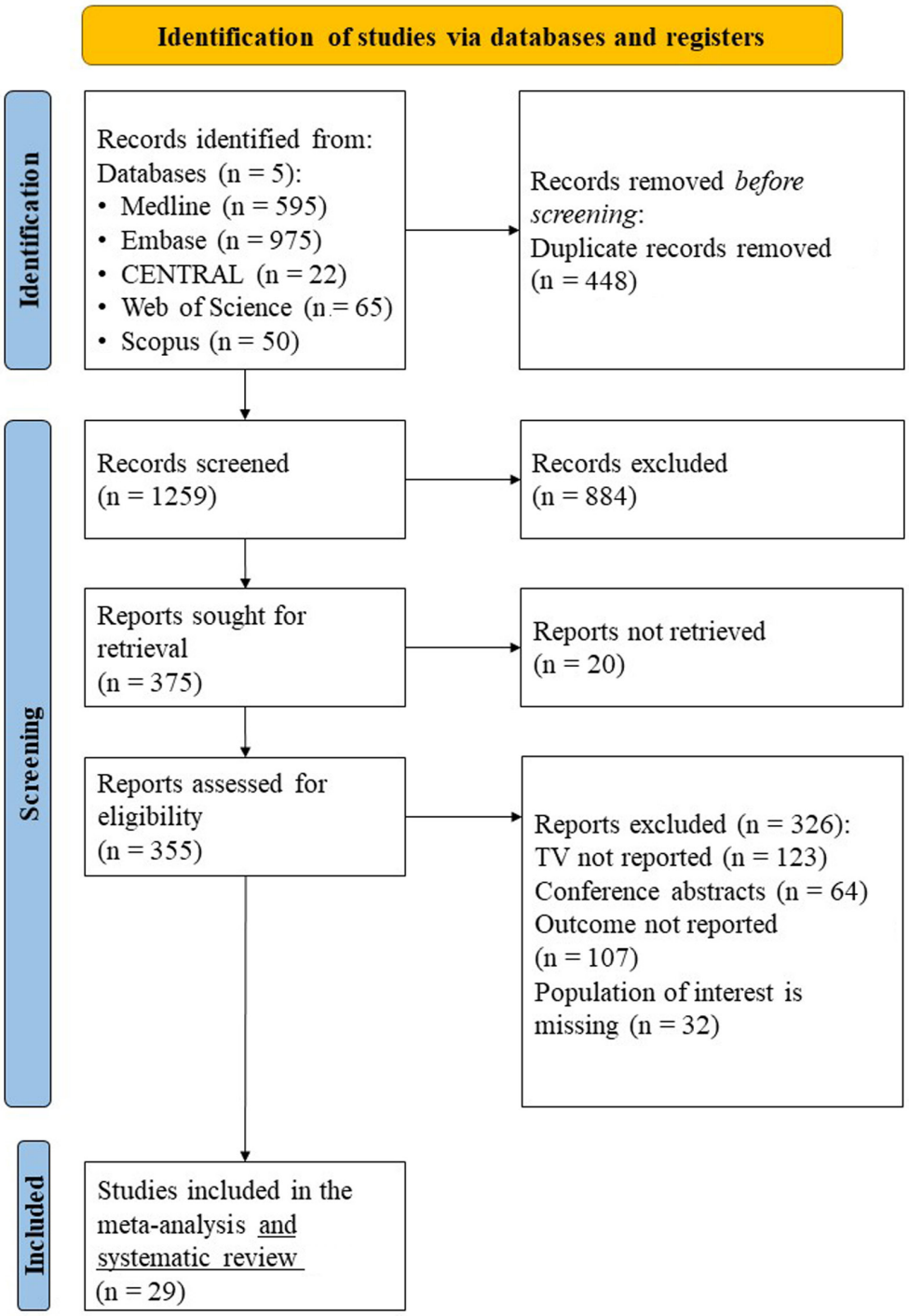

FIGURE 1 PRISMA 2020 flowchart representing the study selection process.

neoplasia", and "cervical cancer". The whole search key can be found in Supporting Information.

2.3 | Study selection and data collection

A reference management program (ENDNOTE X9) was used to select the articles. First, a duplicate removal was performed, then, two independent reviewers (BH, EH) carried out a title and abstract selection and then full-text selection. Cohen's κ coefficient measured the degree of agreement.[27] A third independent investigator (ZSH) agreed on debated articles. If we could not find an article, or data were missing, we contacted the authors.

Two independent reviewers (BH, EH) extracted variables from the eligible studies into a pre-defined Microsoft EXCEL spreadsheet (Windows 11 Pro). The following variables were collected from each article: first author, publication year, digital object identifier, study design, study type, demography (age, sample size), country,

centers, the detection method of *T. vaginalis*, HPV, and cytologic/histologic lesions. Where possible, data regarding the outcomes were extracted in two-by-two tables. Otherwise, we collected the unadjusted odds ratios (ORs). In order to handle confounding factors, when possible, we collected adjusted ORs, and the variables for these results were adjusted. In case of any disagreement, a consensus was reached involving a third investigator (ZSH).

2.4 | Risk of bias and quality assessment of the included articles

To critically assess the outcome data, we performed a risk of bias assessment with the help of the Quality in Prognostic Studies (QUIPS) tool.[28] The QUIPS tool includes six domains: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis reporting. In each domain, four classifications can be given: not applicable, low risk, moderate risk, and high risk of bias. To grade the level of evidence of our findings, we implemented the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) approach. The Summary of Findings table was prepared by using the GradePro tool.[29] Both QUIPS and GRADE were performed by two independent reviewers (BH, EH), and in case of disagreement, a third investigator resolved the dispute (ZSH).

2.5 | Synthesis methods

During data synthesis, both qualitative and quantitative assessments were carried out. The R programming language was used for statistical data analysis (R Core Team, 2022; R version 4.2). The minimum number of studies for performing the quantitative synthesis was three. Forest plots were used to visualize individual studies and overall results. Subgroup analyses were performed based on the detection method of *T. vaginalis* and the country of origin of the article. Sensitivity analyses were carried out for four outcomes.

Where possible, we estimated pooled ORs with 95% confidence intervals (CIs) with a random-effects model, using the tel-Haenszel method with the metabin function from meta v5.50 package, and we applied the Paule-Mandel method to estimate the between-study variance.[30–32] Statistical significance was substantiated for a result when $P < 0.05$. I^2 and Cochran's Q tests were used to measure statistical heterogeneity, where $P < 0.1$ indicated significant heterogeneity.[28] A general interpretation of the heterogeneity values is as follows: 0%–40% possibly not important heterogeneity; 30%–60% moderate heterogeneity; 50%–90% substantial heterogeneity; and 75%–100% considerable heterogeneity. Beside I^2, we also reported the prediction intervals (i.e. the expected range of effects of future studies) of the pooled estimates if the minimum study number was reached.[33]

The inspection of funnel plots and an Egger's test were used to assess publication bias when a minimum of 10 articles were available for one outcome.

3 | RESULTS

3.1 | Search and selection

Our comprehensive search identified 1707 articles. After duplicate removal, 1259 publications were screened based on title and abstract. During the full-text selection, 355 articles were screened, resulting in 29 eligible studies for the quantitative and qualitative data syntheses. Cohen's κ was 0.9 for title and abstract selection; and 0.85 for full-text selection. Despite contacting authors for non-retriveble articles, we received only a few responses.

3.2 | Basic characteristics of included studies

The eligible articles were published between 2009 and 2021, with 11 publications from Asia, five from Europe, seven from South America, five from Africa, and one from North America. According to study type, we found 22 cross-sectional, five case–control, and one prospective cohort study.

As for demographics, the mean age of women was 37.57 years. In 15 articles, *T. vaginalis* was detected with PCR, in eight with wet-mount, in four with cytology and in two with cultures and wet-mount. All the studies assessed the exposure and the outcome at the same time.

Altogether 473 740 women were included in our meta-analysis. Of them, 8518 patients had *T. vaginalis* infection in the exposure group. Baseline characteristics of the eligible studies are detailed in Table 1.

3.3 | Quantitative and qualitative analysis

The association between *T. vaginalis* and HPV infections

Twenty-four studies including 7291 women in the *T. vaginalis*-infected group and 452 161 in the control group reported an association between *T. vaginalis* and HPV infections.[14,16,17,34–54] Our results showed that *T. vaginalis*-positive women were 1.79 times more likely to be diagnosed with an HPV co-infection (95% CI 1.27–2.53; I^2 95%; Figure 2) compared with *T. vaginalis*-negative women.

When a *T. vaginalis* infection was confirmed with the wet-mount method, the odds of detecting a co-infection with HPV were slightly higher, by the odds of 2.29 (95% CI 1.23–4.28; I^2 97%). The results from the subgroups based on region showed that *T. vaginalis*-positive women from Asia had the highest chance for HPV co-infection (OR 2.05, 95% CI 1.08–3.88; I^2 97%; see Figure S1). A sensitivity analysis (leave-one-out method) did not recognize any influential study (see Figure S2).

In one article,[54] a multivariate analysis showed 2.29 odds (95% CI1.46–3.60) for the diagnosis of HPV in the case of *T. vaginalis*

TABLE 1 Basic characteristics of included studies.

Author, year	Study type	Region	Number of patients	Mean age, y	Diagnosis of *Trichomonas vaginalis*	Diagnosis of HPV	Diagnosis of cervical lesion	Cervical lesion-related outcomes
Verteramo et al., 2009[34]	Cross-sectional	Europe	860	32.7	Culture and wet-mount	PCR	NA	NA
Noel et al., 2010[35]	Case–control	Europe	507	<30–50[a]	Cytology	HCII	NA	NA
Depuydt et al., 2010[36]	Cross-sectional	Europe	62 944	42	PCR	PCR	NA	NA
Caiyan et al., 2012[37]	Cross-sectional	Asia	6339	39.2	Wet-mount	HCII	Histology	LSIL, HSIL
Donders et al., 2013[16]	Cross-sectional	Europe	63 251	NA	PCR	PCR	Cytology	ASCUS, LSIL, HSIL
Mendoza et al., 2013[38]	Cross-sectional	South America	181	30[b]	Culture and wet-mount	PCR	NA	NA
Paesi et al., 2013[39]	Cross-sectional	South America	208	13–69[a]	Cytology	PCR	NA	NA
Lazenby et al., 2014[14]	Cross-sectional	Africa	324	38	PCR	HCII	Cytology/histology	LSIL, HSIL
Liu et al., 2015[40]	Cross-sectional	Asia	429	39	Wet-mount	PCR	NA	NA
Casillas-Vega et al., 2016[41]	Cross-sectional	South America	662	31	PCR	PCR	NA	NA
Camporiondo et al., 2016[42]	Cross-sectional	Europe	309	49[b]	PCR	PCR	NA	NA
Dey et al., 2016[55]	Cross-sectional	Asia	7962	NA	Cytology	NA	Cytology	ASCUS, LSIL, HSIL
de Abreau et al., 2016[57]	Cross-sectional	South America	685	40.3	PCR	NA	Cytology/histology	HSIL
Kim et al., 2016[43]	Case–control	Asia	1000	NA	PCR	PCR	Cytology	ASCUS, ASC-H LSIL, HSIL
Amorim et al., 2017[56]	Case–control	South America	132	38.2	PCR	NA	Cytology/histology	LSIL, HSIL
Costa-Lira et al., 2017[44]	Cross-sectional	South America	180	16–50[a]	PCR	PCR	—	NA
Ghosh et al., 2017[17]	Case–control	Asia	483	30–60[a]	Wet-mount	HCII	Histology	LSIL, HSIL, CA
Al-Awadhi et al., 2018[15]	Cross-sectional	Asia	8836	NA	Cytology	NA	Cytology	ASCUS, LSIL, HSIL
Lockhart et al., 2019[45]	Prospective cohort	Africa	344	18–49[a]	PCR	PCR	NA	NA
Ferre et al., 2019[46]	Cross-sectional	Africa	320	25	PCR	PCR	NA	NA
Lv et al., 2019[47]	Cross-sectional	Asia	826	38.5	Wet-mount	PCR	NA	NA
Cunha et al., 2020[48]	Cross-sectional	South America	353	39.7	PCR	PCR	NA	NA
Wang et al., 2020[49]	Cross-sectional	Asia	4449	43.6	Wet-mount	PCR	NA	NA
Yang et al., 2020[50]	Cross-sectional	Asia	310 545	>30	Wet-mount	PCR	NA	NA
Zheng et al., 2020[51]	Case–control	Asia	532	42.2	Wet-mount	PCR	Histology	LSIL, HSIL, CA
Gupta et al., 2020[58]	Case–control	Asia	168	21–65[a]	Wet-mount	NA	Histology	CA
Taku et al., 2021[52]	Cross-sectional	Africa	205	45[b]	PCR	PCR	NA	NA
Jary et al., 2021[53]	Cross-sectional	Africa	144	37	PCR	PCR	NA	NA
Belfort, 2021[54]	Cross-sectional	South America	562	30–49[c]	PCR	PCR	NA	NA

Abbreviations: ASC-H, atypical squamous cells for which one cannot rule out high-grade squamous intraepithelial lesions; ASCUS, atypical squamous cells of undetermined significance; CA, cervical cancer; HCII, Hybrid capture II; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions; NA, not available; PCR, polymerase chain reaction.

[a]Minimum–maximum age values.

[b]Median age value.

[c]In this age range, 48.40% of patients were included.

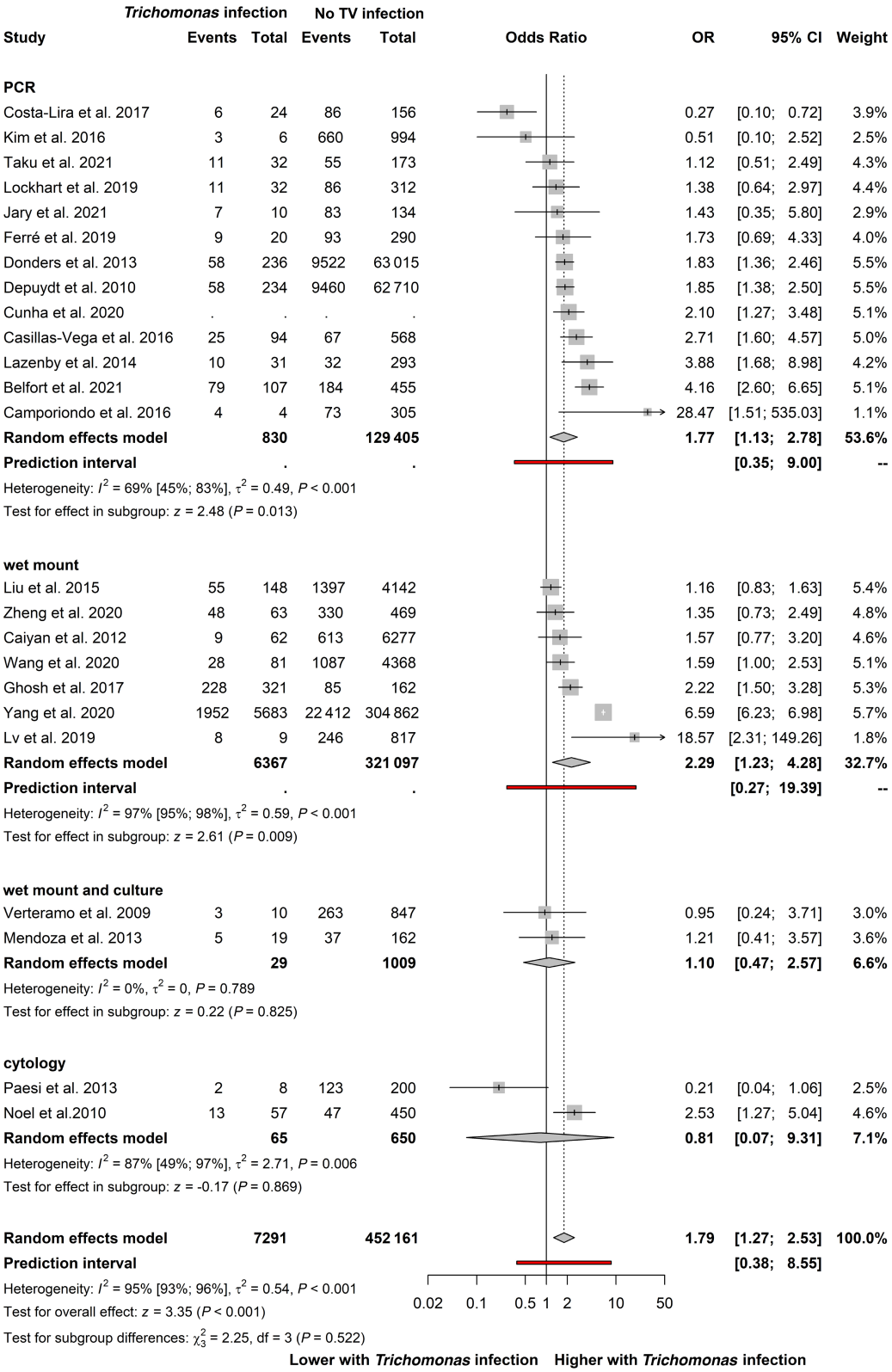

FIGURE 2 Forest plot of studies representing that *Trichomonas vaginalis* infection was associated with HPV co-infection. CI, confidence interval; OR, odds ratio; PCR, polymerase chain reaction; TV, *Trichomonas vaginalis*.

detection. A second study[14] found even higher chances for HPV-co-infection (OR 4.10, 95% CI 1.70–9.80, see Table S3).

The association between *T. vaginalis* and cervical dysplasia

For the ASCUS outcome, five studies evaluated 1493 women in the exposure group and 75 135 women in the control group.[15,16,43,54,55] Women who were *T. vaginalis*-positive had a 2.3 times higher chance for ASCUS diagnosis (95% CI 1.63–3.26; I^2 52%, see Figure S3) compared with women who did not have a *T. vaginalis* infection. A subgroup analysis based on the screening method showed that when *T. vaginalis* was detected with PCR, this association was even stronger (OR 2.91; 95% CI 1.95–4.35; I^2 0%;). Articles from South America and Europe found almost threefold increased odds for the diagnosis of ASCUS (Belfort et al.[54]: OR 2.99, 95% CI 1.06–8.43; Donders et al.[16]: OR 2.94, 95% CI 1.88–4.57, respectively; see Figure S4). When the leave-one-out analysis was carried out, the exclusion of Al-Awadhi et al.[15] from ASCUS resulted in a higher association (OR 2.79, 95% CI 2.21–3.53; I^2 0%; see Figure S5).

Two studies investigated atypical glandular cells and *T. vaginalis* infection in sexually active women. Neither found an association between *T. vaginalis* positivity and atypical glandular cells (Donders et al.[16]: OR 1.33, 95% CI 0.08–21.40; Al-Awadhi et al.[15]: OR 1.55, 95% CI 0.46–5.41).

Altogether there were 10 eligible studies concerning LSIL, investigating 1740 women in the *T. vaginalis* group and 82 362 in the control group.[14–17,37,43,51,54–56]

When examining the association between *T. vaginalis* and LSIL, we found that women who were infected with *T. vaginalis* had almost twofold odds of having LSIL (OR 1.92, 95% CI 0.78–4.77; I^2 91%; see Figure S6), compared with women who were not *T. vaginalis*-infected. However, the findings were statistically not significant. When *T. vaginalis* was detected with PCR, women had higher odds of having an LSIL diagnosis (OR 3.66, 95% CI 1.51–8.86; I^2 69%). Regarding the analysis of regional differences, we detected a ninefold chance for LSIL when *T. vaginalis* was present in women from South America (OR 9.36, 95% CI 2.34–37.36; I^2 63%; Figure S7). When the leave-one-out analysis was carried out, the exclusion of the study by Al-Awadhi et al.[15] resulted in a higher association between *T. vaginalis* and LSIL detection (OR 2.79; 95% CI 1.61–4.82; I^2 65%). Furthermore, when the article by Amorim et al.[56] was excluded, we found an OR of 1.51 (95% CI 0.65–3.55; I^2 95%; see Figure S8).

Regarding a relationship between *T. vaginalis* and ASC-H, we could not find any association (OR 1.78, 95% CI 0.21–15.12; see Figure S9).[16,43,54]

Eleven studies assessed 1796 women in the exposure group and 80 276 women in the control group for the association between *T. vaginalis* infection and HSIL.[14–17,37,43,51,54–57] Patients diagnosed with *T. vaginalis* infection had 2.34 times higher odds of having an HSIL diagnosis (95% CI 1.10–4.95; I^2 75%; Figure 3) than women who were not diagnosed with *T. vaginalis*. According to the *T. vaginalis* detection method, women diagnosed with PCR had higher odds of receiving an HSIL result (OR 3.81, 95% CI 1.23–11.78; I^2 81%). The subgroup analysis of the origins of the articles displayed sixfold odds in South America (OR 6.52, 95% CI 0.74–57.75; I^2 92%), although the findings were statistically not significant. One study from Europe found high odds for HSIL when *T. vaginalis* was present (Donders et al.[16]: OR 3.14, 95% CI 1.49–6.78; see Figure S10). When the leave-one-out analysis was carried out, the exclusion of the article by Al-Awadhi et al.[15] from HSIL resulted in an OR of 2.87 (95% CI 1.43–5.75; I^2 67%). In addition, when the article by Amorim et al.[56] was excluded, the OR changed to 1.72 (95% CI 1.01–2.91; I^2 42%; see Figure S11).

One paper[54] performed a multivariate analysis (see Table S3).

The association between *T. vaginalis* and cervical cancer

Three articles were quantitatively analyzed, with 219 women in the *T. vaginalis*-positive group and 397 women in the control group.[17,51,58] Women who were *T. vaginalis*-positive had 5.24 times higher odds of having cervical cancer (OR 5.23, 95% CI 3.03–9.04; I^2 3%; Figure 4) compared with *T. vaginalis*-negative women.

Association between *T. vaginalis*, cervical lesions, and cervical cancer in the HPV-positive population

We found four articles for the quantitative synthesis regarding the HPV-positive population when evaluating the association between *T. vaginalis* infection and cervical lesions.[17,50,51,57]

For LSIL, three articles were analyzed, assessing 1932 women in the exposure group and 20 033 in the control group.[17,50,51] *Trichomonas vaginalis*-positive women had 2.81 higher odds for LSIL diagnosis than women who were not *T. vaginalis* infected (95% CI 2.37–3.33; I^2 0%; see Figure 5).

In total, there were 1921 women in the exposure group and 20 750 women in the control group for HSIL.[17,50,51,57] Patients who were diagnosed with *T. vaginalis* had more than twofold odds of having HSIL compared with women who were not diagnosed with *T. vaginalis* (OR 2.36, 95% CI 1.79–3.11; I^2 10%; see Figure 5).

Three studies examined cervical cancer, with 1811 women in the exposure group and 19 331 in the control group.[17,50,51] We found that women who were *T. vaginalis*-positive had increased odds of being diagnosed with cervical cancer compared with women who were not *T. vaginalis*-positive (OR 3.09, 95% CI 1.66–5.77; I^2 45%; see Figure 5).

3.4 | Risk of bias assessment and quality of evidence

The results of the risk of bias assessment are presented for every outcome. For the *T. vaginalis*-HPV co-infection outcome, seven articles demonstrated "a moderate risk for study confounding bias",

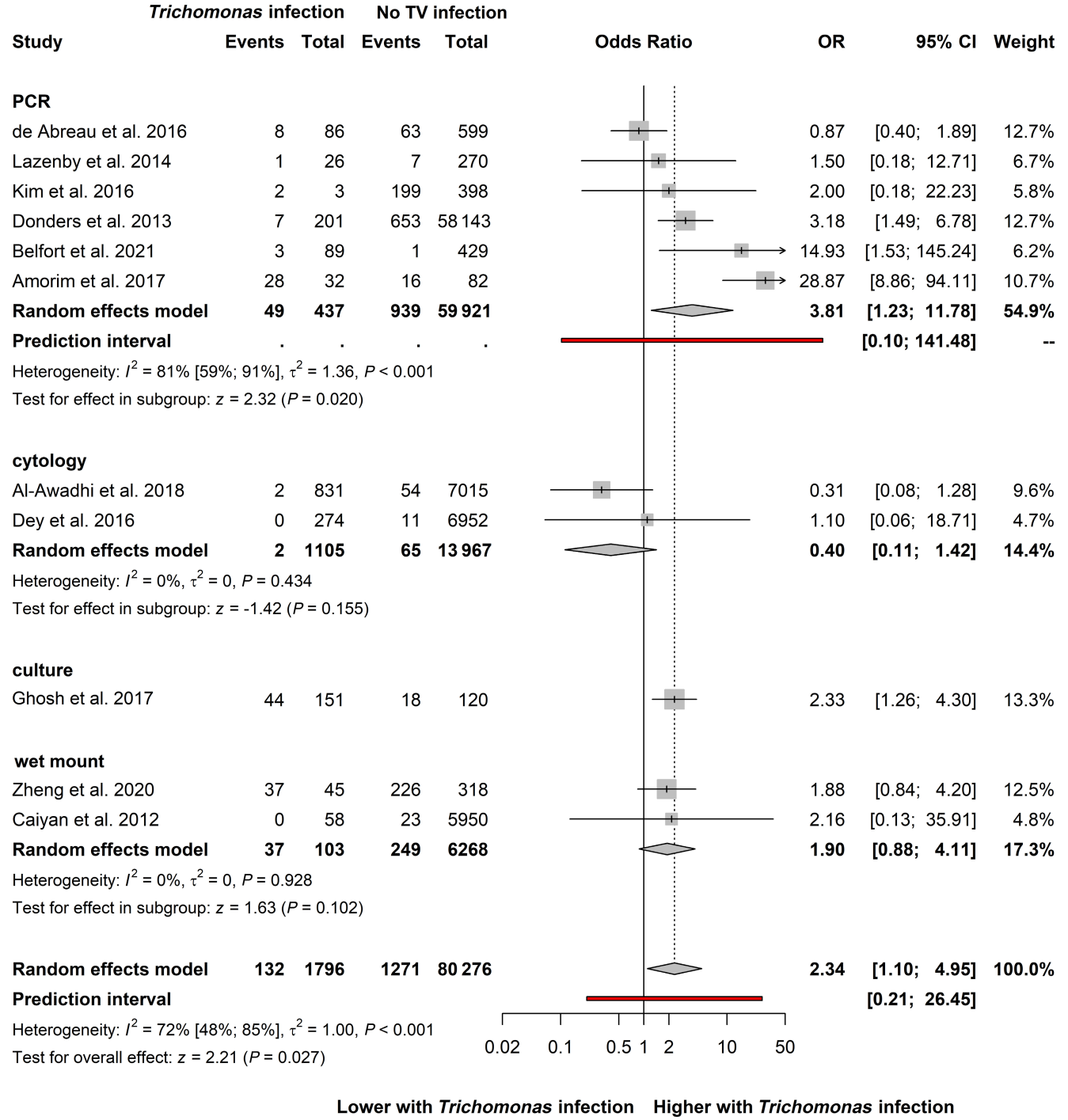

FIGURE 3 Forest plot of studies representing that *Trichomonas vaginalis* infection was associated with high-grade squamous intraepithelial lesions. CI, confidence interval; HSIL, high-grade squamous intraepithelial lesions; OR, odds ratio; PCR, polymerase chain reaction; TV, *Trichomonas vaginalis*.

and three articles demonstrated "a high risk of bias". In the study participation domain we detected a high risk of bias in three articles. In the cervical dysplasia groups, the risk of bias was low. In the study confounding domain, we found one article to be at a high risk of bias in ASCUS and two articles of a high risk of bias in the LSIL and HSIL groups. In the cervical cancer group, we found one article in the confounding domain of the study at a high risk of bias. (see Figures S12–S22).

Our Summary of Findings included six outcomes for the first PEO and three for the second PEO (see Tables S3 and S4). The quality of evidence was "low" for six outcomes and "very low" for three outcomes.

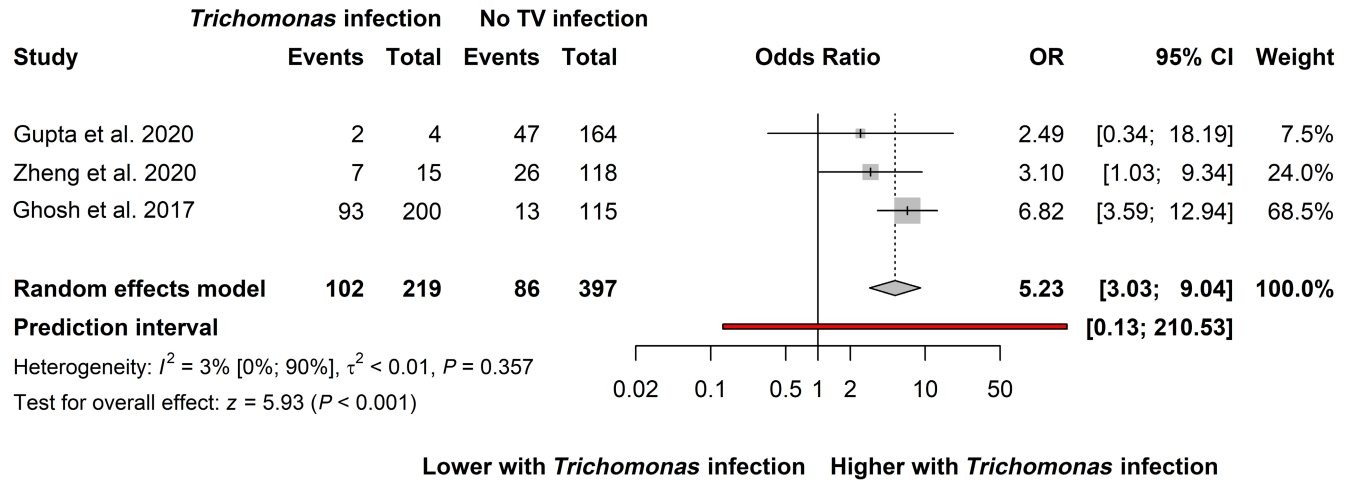

FIGURE 4 Forest plot of studies representing that *Trichomonas vaginalis* infection was associated with cervical cancer. CI, confidence interval; OR, odds ratio; TV, *Trichomonas vaginalis*.

3.5 | Publication bias and heterogeneity

We performed the Egger test and a funnel plot to assess publication bias in the *T. vaginalis*-HPV co-infection, *T. vaginalis*-LSIL, and *T. vaginalis*-HSIL groups. In all three cases, the funnel plots showed some asymmetry. Even on the basis of Egger test, we did not find a significant publication bias as the *P* values were greater than 0.1 (see Figures S23–S25).

4 | DISCUSSION

Our study, which included nearly half a million women from population-based studies, showed a positive association between *T. vaginalis* and cervical carcinogenesis. First, we investigated the association between *T. vaginalis* and HPV. We found that women with a *T. vaginalis* infection had higher odds of being diagnosed with a concomitant HPV infection than women who were *T. vaginalis*-negative. In the relation between *T. vaginalis* and cervical dysplasia, a significant association was found. When we evaluated the relationship between *T. vaginalis* and cervical cancer, we also found a statistically significant association resulting in higher odds of developing cervical cancer among women infected with *T. vaginalis*. Regarding our second clinical question in the HPV-positive population, we found a positive association between *T. vaginalis*, LSIL, HSIL, and cervical cancer.

As for strengths, our study is the first meta-analysis to investigate the relationship between *T. vaginalis*, HPV, and cervical lesions in detail. As a result of the large sample size in the assessed articles, we could include nearly half a million patients in the analysis. Moreover, most of the studies carried a low risk of bias.

However, the results need to be interpreted together with the limitations. First, as none of the studies followed up the *T. vaginalis*-infected population, and all participants were screened for *T. vaginalis*, HPV, and cervical carcinogenesis simultaneously, we do not know how *T. vaginalis* can contribute to the development of the outcomes. Second, as many studies did not perform multivariate analyses, we could not calculate pooled adjusted ORs. The inadequate control of confounders may lead to an underestimation or overestimation of the analyzed associations. Third, it is not clear whether *T. vaginalis* infection causes the cervical environment to be more susceptible to HPV infection and to the subsequent CIN, or whether cervical dysplasia makes the environment more attractive to *T. vaginalis* infection.[59] Fourth, in the diagnosis of cervical lesions, some studies used cytology, which is subjective, and it is a diagnostic method that is difficult to replicate.[60] Fifth, according to the GRADE assessment, the quality of evidence was low in six and very low in three outcomes. Sixth, not all HPVs are oncogenic though in the *T. vaginalis*-HPV association, 10 studies included non-oncogenic HPV strains in their investigation too.

The association between *T. vaginalis* and HPV showed that STIs often coexist because of similar behavioral risk factors such as young age, a high number of sexual partners, and unprotected intercourse.[16,61] Therefore, we cannot conclude that *T. vaginalis* infection affects HPV acquisition because both infections can be concomitantly present. The etiology of cervical cancer and most CIN are attributable to high-risk HPV types.[5] Therefore, HPV could be a confounding factor for our cervix-related outcomes. However, if we only investigate the HPV-positive population, we could observe an even more increased association between *T. vaginalis* cervical dysplasia and cancer.

In contrast, not all HPV types carry the same oncogenic risk as HPV 16 and HPV 18, which cause around 70% of all cervical cancers worldwide.[5] Therefore, HPV positivity in women does not represent a homogeneous population from an oncogenic point of view. Some prospective studies suggest that the likelihood of a persistent HPV infection increases in the presence of concomitant *T. vaginalis* infection.[45,62] Behind this observation there is a presumption of how *T. vaginalis* can alter HPV clearance. *Trichomonas vaginalis* can cause micro-lesions in the cervical epithelium,

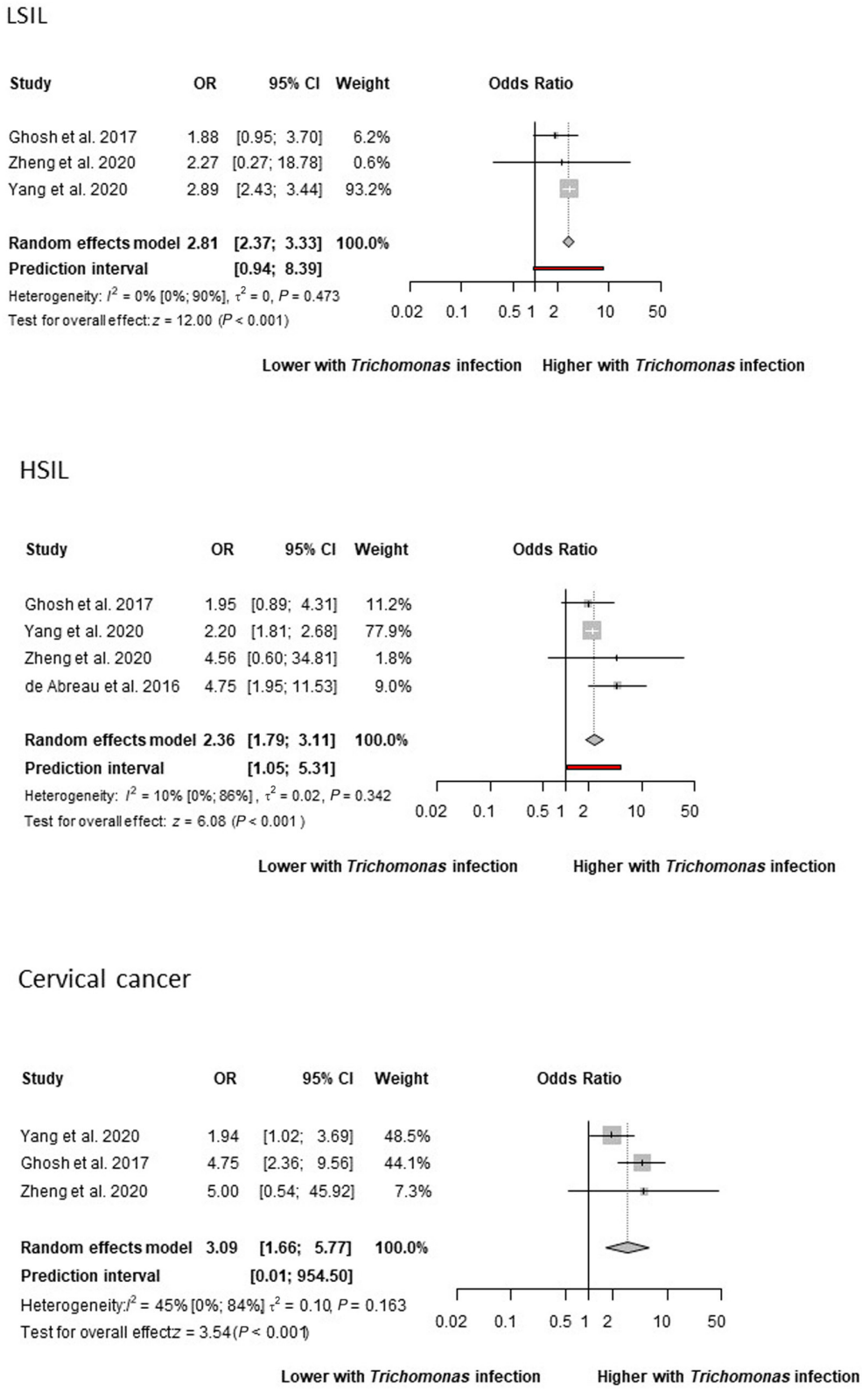

FIGURE 5 Forest plot of studies representing that *Trichomonas vaginalis* was associated with low-grade squamous intraepithelial lesions, high-grade squamous intraepithelial lesions, and cervical cancer in the HPV-positive population. CI, confidence interval; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions.

decrease the protective mucus layer of the vagina, and induce proinflammatory cytokines through immune response, which can facilitate the spread of an HPV infection into the basal layer of the cervical epithelium and induce persistent HPV infection.[7,63,64] As persistent HPV infection occurs, the probability of cervical dysplasia increases, promoting cervical carcinogenesis.[65] Coexistence

of different genital infections, *Chlamydia trachomatis*, and bacterial vaginosis can also induce persistent HPV infection, resulting in cervical dysplasia progression.[66,67]

When assessing ASCUS, we found a significant association with *T. vaginalis* infection. A Belgian study also found that women diagnosed with ASCUS had been HPV-negative but *T. vaginalis*-positive in a few cases, suggesting that *T. vaginalis* could also lead to ASCUS.[16] In the cervical dysplasia group, we found the highest odds for cervical lesions when *T. vaginalis* was detected with PCR, probably because this method was the most sensitive for *T. vaginalis* detection.[19] In the *T. vaginalis* and cervical dysplasia group, we found higher odds in the ASCUS and LSIL groups in South America, although we had only one article in the ASCUS group. The prevalence of *T. vaginalis* is deeply connected to socioeconomic variables, sexual behaviors, and access to health care. Without surveillance programs, the actual epidemiologic state of *T. vaginalis* is unknown. However, countries where the populations have higher incomes generally have a lower prevalence of *T. vaginalis*, and countries where the populations have lower incomes generally have a higher prevalence.[13] In the sensitivity analysis, two articles could have altered our results. One of the outliers[15] led to a lower association between *T. vaginalis* and cervical dysplasia. In this study, *T. vaginalis* was diagnosed with cytology, which is not a reference standard detection of *T. vaginalis*.[19] The other article[56] came from an area of Brazil where poverty rate was high, and cervical cancer was the second most common cancer.[3] These findings can explain the high ORs we experienced in the *T. vaginalis* and cervical dysplasia group.

Lipophosphoglycan (LPG), a virulence factor found on the surface of *T. vaginalis*, can induce immunologic reactions depending on the type of LPG. In reaction to these LPG particles, the host epithelial cells can secrete proinflammatory cytokines, interleukin-8 and macrophage inflammatory protein 3α, which induce the inflammation of the cervix and the vagina. At the same time, other LPGs found on *T. vaginalis* can decrease the level of proinflammatory cytokines and evade immune reactions. This is in line with the clinical finding that *T. vaginalis* can often be asymptomatic or can cause persistent infection.[64]

Inflammation of the cervix has been associated with an increased risk of CIN in one study.[7] Another article found elevated levels of interleukin-6 and interleukin-8 in CIN and cervical cancer.[68] Generally, inflammation is considered a risk factor for developing many cancer types.[69] One study investigated the microbial component of the vagina in cervical cancer patients and non-cervical cancer patients, assuming that cervical cancer disrupts the vaginal microbiota and makes it attractive to infectious diseases. *Trichomonas vaginalis* is possibly less of a cofactor than a consequence of cervical cancer.[70] The intact state of the vaginal microbiome with *Lactobacillus* species is essential for protection against STIs. The abruption of this complex microbiome increases the probability of genital infections due to decreased defensive barriers.[71] One study proved the proinflammatory synergism between vaginal dysbiosis and *T. vaginalis*; moreover, it suggested a surface biofilm that makes them more resistant to antibiotic treatment.[72] Overall, STIs and vaginal infections have been considered possible cofactors in the development of CIN and cervical cancer. In one meta-analysis, *Chlamydia trachomatis* was found to be associated with cervical cancer, whereas another meta-analysis also found an association between bacterial vaginosis and cervical lesions.[61,67] Our findings also support the idea that STIs and vaginal infections might act as cofactors in the development of cervical cancer.

We believe that more studies are needed to control the confounding factors; therefore, the true effect of *T. vaginalis* on cervical carcinogenesis could be estimated in a more reliable way. Second, we recommend that clinicians who treat women in their practice always consider HPV infection and cervical lesions when diagnosing *T. vaginalis* infection. Even though we cannot conclude a causative relationship between *T. vaginalis* and cervical carcinogenesis, *T. vaginalis* is associated with HPV infection, cervical lesions, and cervical cancer, so a follow up of patients after the *T. vaginalis* diagnosis might be beneficial. Many countries have implemented HPV-based cervical cancer screening programs, which means a greater detection rate in HPV strains.[73] According to our study, *T. vaginalis* and HPV are associated; therefore, in the case of an HPV diagnosis, the screening and treatment of *T. vaginalis* are advisable because of its potential carcinogenic effect on the cervix.

In conclusion, our results showed that *T. vaginalis* infection might increase the odds of cervical lesions and cancer development in sexually active women. We advise clinicians to evaluate HPV and cervical dysplasia in the case of a *T. vaginalis* diagnosis.

AUTHOR CONTRIBUTIONS

Balázs Hamar contributed to conceptualization, project administration, and writing the original draft; Brigitta Teutsch and Péter Hegyi contributed to conceptualization, project administration, methodology, and writing—review & editing; Eszter Hoffmann and Zsombor Hunka contributed to data curation and writing–review & editing; Alex Váradi contributed to data curation, formal analysis, visualization, and writing–review & editing; Nándor Ács and Zsolt Melczer contributed to conceptualization, supervision, and writing—review & editing; and Péter Nyirády, Balázs Lintner, and Réka Juhász Hermánné contributed to conceptualization, and writing—review & editing. All authors certify that they have participated sufficiently in the work, including the concept, design, analysis, writing, and/or revision of the manuscript, to take public responsibility for the content of the work.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data sets used in this study can be found in the full-text articles included in the systematic review and meta-analysis.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209-249.
2. Simms KT, Steinberg J, Caruana M, et al. Impact of scaled up human papillomavirus vaccination and cervical screening and the potential for global elimination of cervical cancer in 181 countries, 2020-99: a modelling study. *Lancet Oncol*. 2019;20(3):394-407.
3. Giannini A, Bogani G, Vizza E, et al. Advances on prevention and screening of gynecologic tumors: are we stepping forward? *Healthcare*. 2022;10(9):1.
4. D'Oria O, Corrado G, Laganà AS, Chiantera V, Vizza E, Giannini A. New advances in cervical cancer: from bench to bedside. *Int J Environ Res Public Health*. 2022;19(12):2-3.
5. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer*. 2002;2(5):342-350.
6. Mercer F, Johnson PJ. *Trichomonas vaginalis*: pathogenesis, symbiont interactions, and host cell immune responses. *Trends Parasitol*. 2018;34(8):683-693.
7. Castle PE, Hillier SL, Rabe LK, et al. An association of cervical inflammation with high-grade cervical neoplasia in women infected with oncogenic human papillomavirus (HPV). *Cancer Epidemiol Biomarkers Prev*. 2001;10(10):1021-1027.
8. Brusselaers N, Shrestha S, van de Wijgert J, Verstraelen H. Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. *Am J Obstet Gynecol*. 2019;221(1):9-18.e8.
9. Slattery ML, Robison LM, Schuman KL, et al. Cigarette smoking and exposure to passive smoke are risk factors for cervical cancer. *JAMA*. 1989;261(11):1593-1598.
10. Huh WK. Human papillomavirus infection: a concise review of natural history. *Obstet Gynecol*. 2009;114(1):139-143.
11. Hawes SE, Kiviat NB. Are genital infections and inflammation cofactors in the pathogenesis of invasive cervical cancer? *J Natl Cancer Inst*. 2002;94(21):1592-1593.
12. Van der Pol B. *Trichomonas vaginalis* infection: the most prevalent nonviral sexually transmitted infection receives the least public health attention. *Clin Infect Dis*. 2007;44(1):23-25.
13. Kissinger P. *Trichomonas vaginalis*: a review of epidemiologic, clinical and treatment issues. *BMC Infect Dis*. 2015;15:307.
14. Lazenby GB, Taylor PT, Badman BS, et al. An association between *Trichomonas vaginalis* and high-risk human papillomavirus in rural tanzanian women undergoing cervical cancer screening. *Clin Ther*. 2014;36(1):38-45.
15. Al-Awadhi R, Al-Shaheen A, Al-Juwaiser A, George SS, Sharma P, Kapila K. Prevalence of infectious organisms observed in cervical smears between 1997–2014 at Mubarak Al-Kabeer Hospital, Kuwait. *Sultan Qaboos Univ Med J*. 2018;18(3):e324-e328.
16. Donders GG, Depuydt CE, Bogers JP, Vereecken AJ. Association of *Trichomonas vaginalis* and cytological abnormalities of the cervix in low risk women. *PLoS One*. 2013;8(12):e86266.
17. Ghosh I, Muwonge R, Mittal S, et al. Association between high risk human papillomavirus infection and co-infection with Candida spp. and *Trichomonas vaginalis* in women with cervical premalignant and malignant lesions. *J Clin Virol*. 2017;87:43-48.
18. Zhang ZF, Begg CB. Is *Trichomonas vaginalis* a cause of cervical neoplasia? Results from a combined analysis of 24 studies. *Int J Epidemiol*. 1994;23(4):682-690.
19. Asmah RH, Agyeman RO, Obeng-Nkrumah N, et al. *Trichomonas vaginalis* infection and the diagnostic significance of detection tests among Ghanaian outpatients. *BMC Womens Health*. 2018;18(1):206.
20. Aslan DL, Gulbahce HE, Stelow EB, et al. The diagnosis of *Trichomonas vaginalis* in liquid-based pap tests: correlation with PCR. *Diagn Cytopathol*. 2005;32(6):341-344.
21. Yang S, Zhao W, Wang H, Wang Y, Li J, Wu X. *Trichomonas vaginalis* infection-associated risk of cervical cancer: a meta-analysis. *Eur J Obstet Gynecol Reprod Biol*. 2018;228:166-173.
22. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71.
23. Cumpston M, Li T, Page MJ, et al. Updated guidance for trusted systematic reviews: a new edition of the Cochrane handbook for systematic reviews of interventions. *Cochrane Database Syst Rev*. 2019;10:ed000142.
24. van Zuuren EJ, Fedorowicz Z. Moose on the loose: checklist for meta-analyses of observational studies. *Br J Dermatol*. 2016;175(5):853-854.
25. Munn Z, Stern C, Aromataris E, Lockwood C, Jordan Z. What kind of systematic review should I conduct? A proposed typology and guidance for systematic reviewers in the medical and health sciences. *BMC Med Res Methodol*. 2018;18(1):5.
26. Roteli-Martins CM, Alves VA, Santos RT, Martinez EZ, Syrjänen KJ, Derchain SF. Value of morphological criteria in diagnosing cervical HPV lesions confirmed by in situ hybridization and hybrid capture assay. *Pathol Res Pract*. 2001;197(10):677-682.
27. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med*. 2012;22(3):276-282.
28. Hayden JA, van der Windt DA, Cartwright JL, Côté P, Bombardier C. Assessing bias in studies of prognostic factors. *Ann Intern Med*. 2013;158(4):280-286.
29. GradePro. https://www.gradepro.org
30. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst*. 1959;22(4):719-748.
31. Thompson SG, Turner RM, Warn DE. Multilevel models for meta-analysis, and their application to absolute risk differences. *Stat Methods Med Res*. 2001;10(6):375-392.
32. Guido Schwarzer JRC. *Gerta Rücker. Meta-Analysis with R*. Springer International Publishing Switzerland; 2015.
33. IntHout J, Ioannidis JP, Rovers MM, Goeman JJ. Plea for routinely presenting prediction intervals in meta-analysis. *BMJ Open*. 2016;6(7):e010247.
34. Verteramo R, Pierangeli A, Mancini E, et al. Human papillomaviruses and genital co-infections in gynaecological outpatients. *BMC Infect Dis*. 2009;9:2-5.
35. Noël JC, Fayt I, Romero Munoz MR, Simon P, Engohan-Aloghe C. High prevalence of high-risk human papillomavirus infection among women with *Trichomonas vaginalis* infection on monolayer cytology. *Arch Gynecol Obstet*. 2010;282(5):503-505.
36. Depuydt CE, Leuridan E, Van Damme P, Bogers J, Vereecken AJ, Donders GG. Epidemiology of *Trichomonas vaginalis* and human papillomavirus infection detected by real-time PCR in flanders. *Gynecol Obstet Invest*. 2010;70(4):273-280.
37. Caiyan X, Weiyuan Z, Minghui W, Songwen Z. Prevalence and risk factors of lower genital tract infections among women in Beijing, China. *J Obstet Gynaecol Res*. 2012;38(1):310-315.
38. Mendoza L, Mongelos P, Paez M, et al. Human papillomavirus and other genital infections in indigenous women from Paraguay: a cross-sectional analytical study. *BMC Infect Dis*. 2013;13(1):2-6.
39. Paesi SO, Aver L, Barea F, Vanni A, Roesch-Ely M. Human papillomavirus and infections of the lower genital tract in women with abnormal cervical cytological examination. *Sci Med*. 2013;23(1):41-46.
40. Liu J, Liu W, Liu Y, Zhou X, Zhang Z, Sun Z. Prevalence of microorganisms co-infections in human papillomaviruses infected women in northern China. *Arch Gynecol Obstet*. 2016;293(3):595-602.
41. Casillas-Vega N, Morfín-Otero R, García S, et al. Sexually transmitted pathogens, coinfections and risk factors in patients attending obstetrics and gynecology clinics in Jalisco, Mexico. *Salud Publica Mex*. 2016;58(4):437-445.

42. Camporiondo MP, Farchi F, Ciccozzi M, et al. Detection of HPV and co-infecting pathogens in healthy Italian women by multiplex real-time PCR. *Inf Med*. 2016;24(1):12-17.
43. Kim HS, Kim TJ, Lee IH, Hong SR. Associations between sexually transmitted infections, high-risk human papillomavirus infection, and abnormal cervical pap smear results in OB/GYN outpatients. Journal of. *Gynecol Oncol*. 2016;27(5):2-6.
44. Costa-Lira E, Jacinto AHVL, Silva LM, et al. Prevalence of human papillomavirus, chlamydia trachomatis, and *Trichomonas vaginalis* infections in Amazonian women with normal and abnormal cytology. *Genet Mol Res*. 2017;16(2):2-5.
45. Lockhart A, Senkomago V, Ting J, et al. Prevalence and risk factors of *Trichomonas vaginalis* among female sexual workers in Nairobi, Kenya. *Sex Transm Dis*. 2019;46(7):458-464.
46. Ferré VM, Ekouevi DK, Gbeasor-Komlanvi FA, et al. Prevalence of human papillomavirus, human immunodeficiency virus and other sexually transmitted infections among female sex workers in Togo: a national cross-sectional survey. *Clin Microbiol Infect*. 2019;25(12):1560.e1-1560.e7.
47. Lv P, Zhao F, Xu X, Xu J, Wang Q, Zhao Z. Correlation between common lower genital tract microbes and high-risk human papillomavirus infection. *Can J Infect Dis Med Microbiol*. 2019;2019:1-6.
48. Cunha APA, Belfort IKP, Mendes FPB, et al. Human papillomavirus and its association with other sexually transmitted coinfection among sexually active women from the northeast of Brazil. *Interdiscip Perspect Infect Dis*. 2020;2020:1-8.
49. Wang W, Zhang XH, Li M, Hao CH, Liang HP. Association between vaginal infections and the types and viral loads of human papillomavirus: a clinical study based on 4,449 cases of gynecologic outpatients. *Can J Infect Dis Med Microbiol*. 2020;2020:1-6.
50. Yang M, Li L, Jiang C, et al. Co-infection with *Trichomonas vaginalis* increases the risk of cervical intraepithelial neoplasia grade 2–3 among HPV16 positive female: a large population-based study. *BMC Infect Dis*. 2020;20(1):642.
51. Zheng JJ, Miao JR, Wu Q, Yu CX, Mu L, Song JH. Correlation between HPV-negative cervical lesions and cervical microenvironment. *Taiwan J Obstet Gynecol*. 2020;59(6):855-861.
52. Taku O, Brink A, Meiring TL, et al. Detection of sexually transmitted pathogens and co-infection with human papillomavirus in women residing in rural eastern cape, South Africa. *PeerJ*. 2021;9:9.
53. Jary A, Teguete I, Sidibé Y, et al. Prevalence of cervical HPV infection, sexually transmitted infections and associated antimicrobial resistance in women attending cervical cancer screening in Mali. *Int J Infect Dis*. 2021;108:610-616.
54. Belfort IKP, Cunha APA, Mendes FPB, et al. *Trichomonas vaginalis* as a risk factor for human papillomavirus: a study with women undergoing cervical cancer screening in a northeast region of Brazil. *BMC Womens Health*. 2021;21(1):2-4.
55. Dey S, Pahwa P, Mishra A, Govil J, Dhillon PK. Reproductive tract infections and premalignant lesions of cervix: evidence from women presenting at the cancer detection Centre of the Indian Cancer Society, Delhi, 2000–2012. *J Obstet Gynaecol India*. 2016;66:441-451.
56. Amorim AT, Marques LM, Campos GB, et al. Co-infection of sexually transmitted pathogens and human papillomavirus in cervical samples of women of Brazil. *BMC Infect Dis*. 2017;17(1):769.
57. de Abreu ALP, Malaguti N, Souza RP, et al. Association of human papillomavirus, Neisseria gonorrhoeae and chlamydia trachomatis co-infections on the risk of high-grade squamous intraepithelial cervical lesion. *Am J Cancer Res*. 2016;6(6):1371-1383.
58. Gupta R, Singh N, Kalyan RK, Agrawal S. Case-control study to find Association of Common RTIs with CIN and cervical cancer. *Indian J Gynecol Oncol*. 2020;18(4):2-6.
59. Lin S, Zhang B, Lin Y, Lin Y, Zuo X. Dysbiosis of cervical and vaginal microbiota associated with cervical intraepithelial neoplasia. *Front Cell Infect Microbiol*. 2022;12:767693.
60. Kitchener HC, Castle PE, Cox JT. Chapter 7: achievements and limitations of cervical cytology screening. *Vaccine*. 2006;24(Suppl 3):S3/63-S3/70.
61. Zhu H, Shen Z, Luo H, Zhang W, Zhu X. Chlamydia trachomatis infection-associated risk of cervical cancer: a meta-analysis. *Medicine*. 2016;95(13):e3077.
62. Shew ML, Fortenberry JD, Tu W, et al. Association of condom use, sexual behaviors, and sexually transmitted infections with the duration of genital human papillomavirus infection among adolescent women. *Arch Pediatr Adolesc Med*. 2006;160(2):151-156.
63. Madeiro Da Costa RF, De Souza W, Benchimol M, Alderete JF, Morgado-Díaz JA. *Trichomonas vaginalis* perturbs the junctional complex in epithelial cells. *Cell Res*. 2005;15(9):704-716.
64. Fichorova RN, Yamamoto HS, Fashemi T, et al. *Trichomonas vaginalis* Lipophosphoglycan exploits binding to Galectin-1 and -3 to modulate epithelial immunity. *J Biol Chem*. 2016;291(2):998-1013.
65. Bogani G, Taverna F, Lombardo C, et al. Retrospective study of the influence of HPV persistence on outcomes among women with high-risk HPV infections and negative cytology. *Int J Gynaecol Obstet*. 2017;138(1):62-68.
66. Naldini G, Grisci C, Chiavarini M, Fabiani R. Association between human papillomavirus and chlamydia trachomatis infection risk in women: a systematic review and meta-analysis. *Int J Public Health*. 2019;64(6):943-955.
67. Gillet E, Meys JF, Verstraelen H, et al. Association between bacterial vaginosis and cervical intraepithelial neoplasia: systematic review and meta-analysis. *PLoS One*. 2012;7(10):e45201.
68. Tjiong MY, van der Vange N, ten Kate FJ, et al. Increased IL-6 and IL-8 levels in cervicovaginal secretions of patients with cervical cancer. *Gynecol Oncol*. 1999;73(2):285-291.
69. Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. *Proc Natl Acad Sci U S A*. 1995;92(12):5258-5265.
70. Kovachev SM. Cervical cancer and vaginal microbiota changes. *Arch Microbiol*. 2020;202(2):323-327.
71. Alimena S, Davis J, Fichorova RN, Feldman S. The vaginal microbiome: a complex milieu affecting risk of human papillomavirus persistence and cervical cancer. *Curr Probl Cancer*. 2022;46(4):100877.
72. Fichorova RN, Buck OR, Yamamoto HS, et al. The villain team-up or how *Trichomonas vaginalis* and bacterial vaginosis alter innate immunity in concert. *Sex Transm Infect*. 2013;89(6):460-466.
73. Smith MA, Sherrah M, Sultana F, et al. National experience in the first two years of primary human papillomavirus (HPV) cervical screening in an HPV vaccinated population in Australia: observational study. *BMJ*. 2022;376:e068582.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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cancers

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Systematic Review

Imiquimod Is Effective in Reducing Cervical Intraepithelial Neoplasia: A Systematic Review and Meta-Analysis

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Simple Summary: There are publications on the use of Imiquimod in cervical intraepithelial neoplasia (CIN) and HPV clearance; however, the literature is not consistent about its efficacy. Moreover, in cervical precancers, surgical solutions are widely accepted therapies, despite their association with increased obstetrical complications, such as miscarriage and preterm birth. Therefore, a conservative solution is needed. Topical Imiquimod reduced CIN and enhanced HPV clearance, though surgical intervention conization was found to be more effective than Imiquimod treatment. Side effects were common, though mostly mild. Topical Imiquimod could be a valuable therapeutic option for CIN patients, especially for women who have future pregnancy desires. Imiquimod should be incorporated into guidelines as evidence shows it is effective and safe.

Abstract: Introduction: Topical Imiquimod is an immune response modifier approved for the off-label use of vulvar intraepithelial neoplasia. We conducted this systematic review and meta-analysis to investigate the efficacy and safety of Imiquimod in treating cervical intraepithelial neoplasia (CIN) and human papillomavirus (HPV)-positive patients. Methods: The study was prospectively registered (CRD420222870) and involved a comprehensive systematic search of five medical databases on 10 October 2022. We included articles that assessed the use of Imiquimod in cervical dysplasia and HPV-positive patients. Pooled proportions, risk ratios (RRs), and corresponding 95% confidence intervals (CIs) were calculated using a random effects model to generate summary estimates. Statistical heterogeneity was assessed using I^2 tested by the Cochran Q tests. Results: Eight articles reported on 398 patients who received Imiquimod out of 672 patients. Among CIN-2–3 patients, we observed a pooled regression rate of 61% (CI: 0.46–0.75; I^2: 77%). When compared, Imiquimod was inferior to conization (RR: 0.62; CI: 0.42–0.92; I^2: 64%). The HPV clearance rate in women who completed Imiquimod treatment was 60% (CI: 0.31–0.81; I^2: 57%). The majority of side effects reported were mild to moderate in severity. Conclusions: Our findings indicate that topical Imiquimod is safe and effective in reducing cervical intraepithelial neoplasia and promoting HPV clearance. However, it was found to be inferior compared to conization. Imiquimod could be considered a potential medication for high-grade CIN patients and should be incorporated into guidelines for treating cervical dysplasia.

Keywords: Imiquimod; gynecologic cancer risk reduction; HPV; cervical cancer

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1. Introduction

Cervical intraepithelial neoplasia (CIN) 2–3 is the precursor lesion of cervical cancer, one of the leading causes of cancer-related death in women. In 2020, there were 604,000 new cases and 342,000 deaths worldwide, according to GLOBOCAN [1]. High-risk human papillomavirus (HPV) is the predominant factor (99.7%) responsible for cervical cancer [2].

While only a minority of cases progress to invasive cancer after years of persistence, most patients experience regression of CIN to a normal condition [3]. For histologic high-grade intraepithelial lesions (HSILs), excisional treatment is preferred according to the 2019 American Society for Colposcopy and Cervical Pathology (ASCCP) Risk-Based Management Consensus Guideline [4]. However, these procedures may impact pregnancy outcomes, such as preterm delivery, premature rupture of membranes, and low birth weight [5,6]. Moreover, the persistence of HPV has been linked to an increased recurrence rate following surgical intervention [7]. Consequently, alternative conservative therapies are necessary to reduce the frequency of surgical interventions and associated complications.

Topical Imiquimod has been approved by the US Food and Drug Administration (FDA) for treating external genital and perianal warts, basal cell carcinoma, and actinic keratoses [8]. This compound is believed to activate immune cells as a Toll-like receptor-7 agonist. It exerts its antiviral effects by activating dendritic cells and inducing cytokines such as tumor necrosis factor-alpha (TNF-α), interferon-alpha (IFN-α), and interleukins (ILs) [9]. Multiple studies have shown that Imiquimod could be a potential conservative treatment for precursor cervical lesions by accelerating viral clearance [10–12]. However, some other publications have found that it is ineffective in reducing CIN [13]. There were no meta-analyses on the subject to answer this important question.

In this present study, based on the available literature, we aimed to determine the efficacy and safety of topical Imiquimod therapy in reducing the incidence of cervical intraepithelial neoplasia (CIN) and its impact on HPV clearance.

2. Materials and Methods

We followed the PRISMA 2020 (Table S1) for conducting a systematic review and meta-analysis, as recommended by the Cochrane Handbook for Systematic Reviews of Interventions [14,15] (see Figure S1). The study design and protocol were registered in PROSPERO (CRD420222870), and we adhered to them completely.

2.1. Search Strategy

The complete search key is provided in the Supplementary Materials. During the systematic search, the following search strategy was used: 'Imiquimod', 'cervical intraepithelial neoplasia', 'cervical dysplasia', 'human papillomavirus'.

2.2. Literature Search and Eligibility Criteria

A systematic search was conducted using five major databases: MEDLINE (through PubMed), Embase, Cochrane Central Register of Controlled Trials (CENTRAL), Scopus, and Web of Science until 10 October 2022. No restrictions or filters were applied during the search. We used two frameworks to describe the eligibility criteria in the articles. First, the CoCoPop framework was used in studies with no comparators to assess. We investigated women with cervical intraepithelial neoplasia (Population) who applied topical Imiquimod (Context). We determined cervical dysplasia regression, estimation of treatment success, assessment of HPV clearance, and adverse events (Condition). Afterwards, the PICO framework was used. We assessed women (P) with cervical dysplasia or who were HPV positive. In the intervention group (I), women had to receive topical Imiquimod products for their cervical disease. Patients in the comparator group (C) received the standard treatment, predominantly surgical solutions: conization, cryotherapy, laser therapy or expectant management. Outcome (O) parameters included the assessment of cervical dysplasia regression, assessment of HPV clearance, and adverse events [16]. Cervical dysplasia regression was defined as the absence of dysplasia or regression from CIN 2–3 to

CIN 1. HPV clearance was effective when the original HPV types could not be detected after treatment. Cohorts, case-control studies, and randomized controlled trials (RCT) were accepted. Only studies with patient follow-up were included. We imposed no language restrictions; non-English articles were translated into English and evaluated afterwards.

2.3. Selection Process and Data Collection

Article selection was performed using the reference management program EndNote X9. Duplicate removal was conducted by two independent reviewers (B.H., H.E.) at each stage: after title and abstract selection and during full-text selection. Cohen's kappa coefficient (κ) was used to measure the level of agreement [17]. Disputed articles were resolved by a third independent reviewer (H.Z.S.).

Pre-defined variables were described in a Microsoft Excel spreadsheet (Windows 11 Pro 10) by two independent reviewers (B.H., E.H.). The following variables were extracted: first author, year of publication, digital object identifier, study type, study design, country, study period, centers, and duration of follow-up. The following were extracted for both the intervention group and the control group: patient numbers, patient age, pregnancy status, smoking status, number of sexual partners, histological findings (cervical intraepithelial neoplasia 2–3), and HPV status. The dose, duration, and application form were recorded in the intervention group. Outcomes were collected in two-by-two tables. Whenever possible, risk ratios (RRs) were extracted directly. Intention-to-treat (ITT) and per-protocol (PP) data were collected from RCTs. Response rate data were recorded separately when available. Adverse events were collected using the National Cancer Institute (NIH) website's Common Terminology Criteria for Adverse Event protocols [18]. Adverse events were graded from 1 to 4 for the following: fatigue, headache, myalgia, flu-like symptoms, fever, abdominal pain, vaginal pruritus, vaginal discharge, vaginal bleeding, and inflammation. A third reviewer (Z.S.H.) resolved the conflict in case of disagreements.

2.4. Risk of Bias and Quality Assessment of Included Articles

Assessing bias and quality risk of bias and quality assessment depended on study type. RCTs were evaluated using the Risk of Bias II (ROB II) tool, while non-randomized interventions used the Risk of Bias in Non-Randomized Studies (ROBINS I) [19,20]. Response rates without a control group were assessed with the Joanna Briggs Institute (JBI) Critical Appraisal Checklists [21]. GRADE was applied to grade evidence, and a Summary of Findings Table was formulated using GradePro [22]. Two reviewers (B.H., E.H.) conducted assessments, with disputes resolved by a third reviewer (Z.S.H.).

2.5. Synthesis Methods

In data synthesis, both qualitative and quantitative analyses utilized R statistical programming language (R version 4.3). Quantitative synthesis required a minimum of three studies, presented in forest plots. Subgroup analyses were conducted based on article type and cervical dysplasia grade. RCTs' ITT data were analyzed, while other study types were grouped as cohorts. For cervical dysplasia, subgroups included studies without CIN, CIN 1–2–3, and CIN 2–3. Per-protocol data were analyzed for RCTs with complete treatment. Risk ratios (RR) with 95% confidence intervals (CI) assessed effect sizes. The Clopper-Pearson method calculated CIs. Statistically significant results excluded the null value within pooled CI [23]. Forest plots summarized meta-analysis findings. Higgins & Thompson's I^2 assessed heterogeneity, with τ^2 indicating variance [24].

Heterogeneity levels were categorized: 0–40% possibly not important, 30–60% moderate, 50–90% substantial, 75–100% considerable. Subgroups used a fixed effects "plural" model. Cochrane Q test evaluated subgroup differences [25]. To assess the difference between the subgroups, a "Cochrane Q" test was used between subgroups (Harrer et al. 2021). The null hypothesis was rejected on a 5% significance level [26].

Publication bias was not assessed due to limited studies (<10).

3. Results

3.1. Search and Selection

Our systematic search identified 3141 articles from five databases. After removing duplicates, 2218 articles were analyzed for title and abstract selection. In the full-text selection, 13 eligible articles were screened. Finally, we found eight eligible articles for quantitative and qualitative synthesis (see Figure 1).

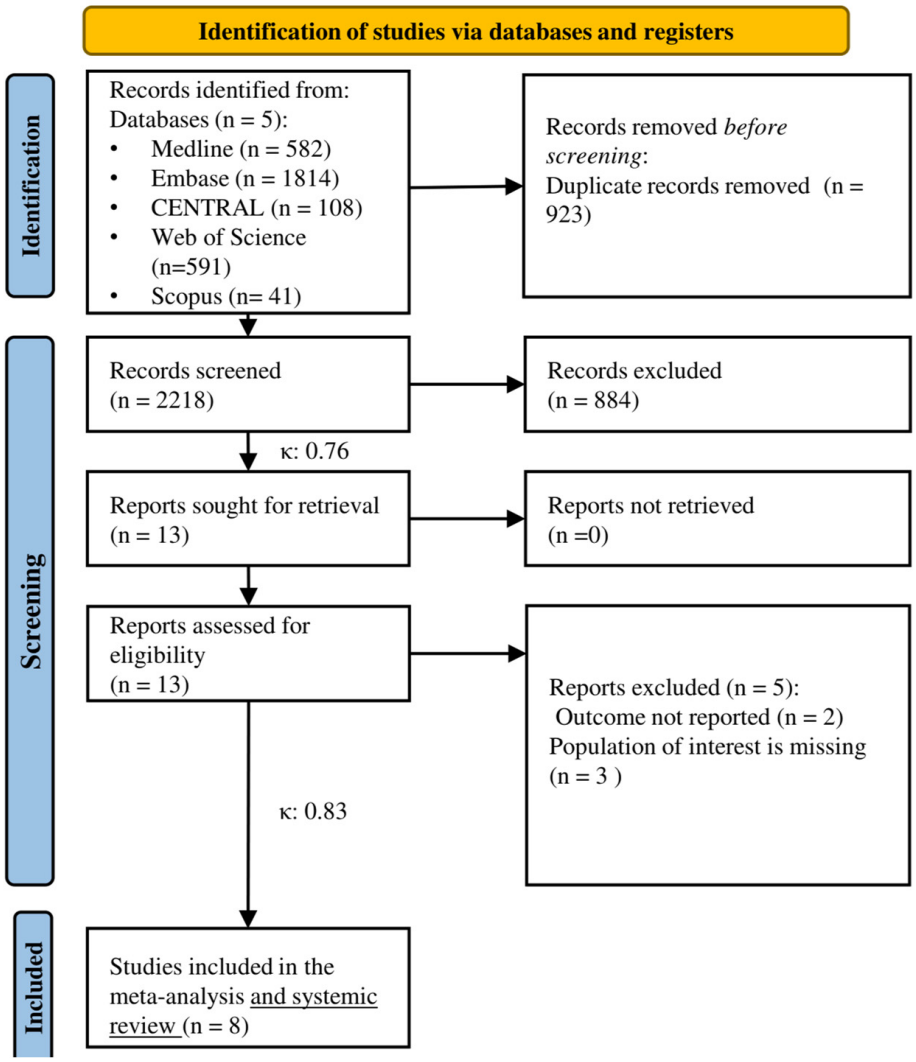

Figure 1. PRISMA flowchart of selection n: number of studies, κ: Cohen's kappa coefficient.

3.2. Basic Characteristics of Included Studies

The eligible articles were published between 2012 and 2022. Regarding demographics, the mean age of women included in the studies was 30.41 years (±2.15). The mean follow-up time was 18.62 (±12.00) months. In six studies, women had histologically proven CIN 2–3; in the seventh study, both cytology and histology were used [27]. Quantitative synthesis was possible only for a subpopulation with HPV status. HPV tests were performed in seven articles. Details about the doses and application of Imiquimod can be found in Table S2.

Altogether, 672 patients were included from the eight studies [10–13,27–30], with 398 women receiving Imiquimod treatment. Detailed baseline characteristics can be found in Table 1.

Table 1. Basic characteristics of included studies.

Author, Years	Study Type	Region	Follow Up Time (Months)	Number of Patients in Intervention	Age (Mean) Intervention, SD	Number of Patients in Control	Age (Mean) Control, SD	CIN [B]	CIN2/CIN3 Ratio	HPV [D] Type	Dose of Imiquimod/Patient	Intervention of Control Group	Adverse Event Reporting	Dropout of Patients
Grimm et al., 2012 [11]	RCT [A]	Austria	5	30	29.2 ± 6.1	29	31.8 ± 7.8	CIN 2–3	1.73	HPV 16/18, other HR [E] HPV	243.75 mg	observation	CTCAE [F] 3.0	6.70%
Hendriks et al., 2022 [12]	Non-randomized interventional	The Netherlands	6	61	33.3 ± 9.1	62	35.2 ± 7	CIN 2–3	0.69	HPV 16/18, other HR HPV	300 mg	conization	VAS [G]	22.90%
Cokan et al., 2021 [28]	RCT	Slovenia	6	52	28.3 ± 4.2	52	26 ± 4.6	CIN 2–3	0.79	NA	600 mg	conization	CTCAE 5.0	17.30%
Lin et al., 2012 [29]	Retrospective cohort analysis	Taiwan	33.4	72	51.75 [B]	20	50 [B]	NA [C]	NA	persistent HR-HPV	150 mg	observation	NA	NA
Fonseca et al., 2021 [10]	RCT	Brazil	24	45	32 [B]	45	36 [B]	CIN 2–3	0.4	NA	150 mg	observation	CTCAE 4.0	15.60%
Pachman et al., 2012 [13]	RCT	USA	37.2	28	30 ± 8.9	28	29 ± 9.7	CIN 1–2–3	1.42	HR-HPV	12.5 mg	conization, laser, cryotherapy	CTCH [H] 2.0	7.14%
Polterauer et al., 2022 [30]	RCT	Austria	24	51	31.4 [B]	42	30.1 [B]	CIN 2–3	0.28	HPV 16/18, other HR HPV	243.75 mg	conization	CTCAE 3.0	9.80%
Kim et al., 2019 [27]	retrospective cohort analysis	Republic of Korea	13.4	55	30 [B]	NA	NA	CIN 2–3	0.74	HPV 16/18, other HR HPV	100 mg	NA	NA	1.80%

[A] randomised control trial; [B] median cervical intraepithelial neoplasia; [C] Not applicable; [D] Human papillomavirus; [E] High-risk; [F] Common Terminology Criteria for Adverse Events; [G] Visual Analog Scale; [H] Common Toxicity Criteria.

3.3. CIN 2–3 Regression

A total of 294 women received topical Imiquimod treatment for CIN 2–3 [10,12,27,28,30]. These patients showed a regression rate of 61% (CI: 0.46–0.75; I^2: 77%) to CIN 1 or no disease after topical Imiquimod therapy (see Figure 2). The subgroup analysis based on study type revealed a histologic regression rate of 59% (CI: 0.47–0.70) in the ITT-RCTs, while the response rate in the cohort studies was 64% (CI: 0–1.00 I^2: 94) (see Figure 2). In the PP population, which consisted of 155 patients, the regression rate was 67% (CI: 0.54–0.78; I^2: 0%) (see Figure S1).

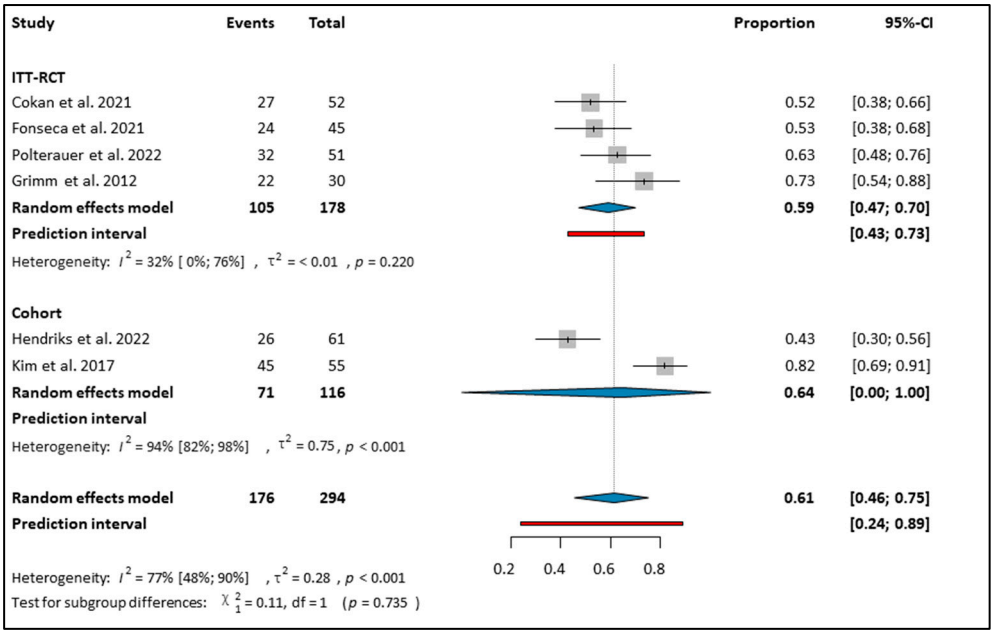

Figure 2. Forest plot of studies representing Imiquimod and CIN 2–3 regression based on study type [10–12,27,28,30]. ITT: intention to treat, RCT: randomized control trial. Effect Estimate: The effect estimate for each study is represented by a grey square, located along the x-axis. Confidence Interval (CI): A line extending from the effect estimate represents the confidence interval. This indicates the range within which the true effect size is likely to lie, with the point estimate positioned at the center of the bar. Overall Estimate: A summary effect estimate, represented by a blue diamond at the bottom of the plot, combines the results of all studies included in the meta-analysis. The center of the diamond represents the point estimate, and the width of the diamond represents the confidence interval around the summary estimate. I^2 (I-squared): A measure of heterogeneity in meta-analysis, indicating the proportion of total variation across studies that is due to heterogeneity rather than chance. p-value (Probability value): The probability of obtaining test results at least as extreme as the observed results, indicating the significance of the heterogeneity test. χ^2 (Chi-square): A test for subgroup differences, evaluating whether the observed differences between subgroups are statistically significant. df (degrees of freedom): The degrees of freedom, which represent the number of independent pieces of information used to estimate a parameter. τ^2 (Tau-squared): The variance of true effects in a random-effects model, reflecting the variability of effect sizes across studies beyond sampling error.

Two articles investigated the efficacy of topical Imiquimod [10,11]. In both studies, the RR for CIN regression was higher when Imiquimod was compared to no treatment (RR: 1.87; CI: 1.12–3.10 and RR: 2.37; CI: 1.25–4.48, respectively) (see Figure S2).

In the experimental group, 196 women received Imiquimod treatment, while 196 women were in the control group who underwent conization [10,12,28,30]. Women who underwent conization had a 38% decrease in the risk of persistence or progression in CIN compared to the women who applied Imiquimod (RR: 0.62; CI: 0.42–0.92; I^2: 64%) (see Figure 3). The subgroup analysis showed a randomized clinical trial where conization was superior to Imiquimod and had a 44% decrease in the risk of unsuccessful treatment (RR: 0.56; CI: 0.43–0.74) (see

Figure 3) [28]. Likewise, in the PP analysis, Imiquimod was not a superior intervention to conization (RR: 0.78; CI: 0.56–1.07; I^2: 0%) (see Figure S3).

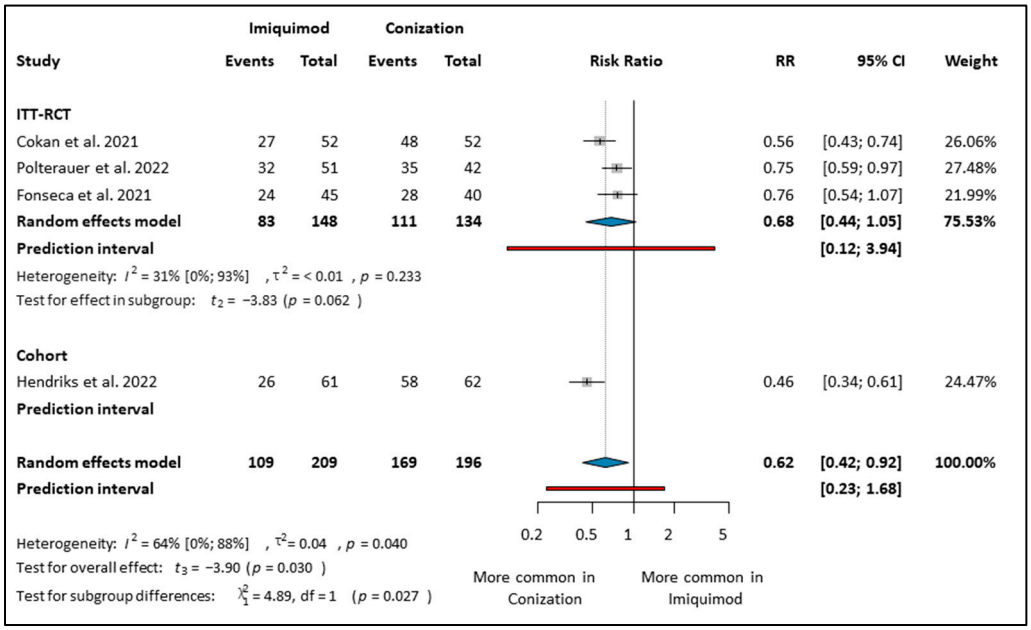

Figure 3. Forest plot of studies representing the Imiquimod group compared to conization on CIN 2–3 regression based on study type [10,12,28,30]. ITT: intention to treat, RCT: randomized control trial. Effect Estimate: The effect estimate for each study is represented by a grey square, located along the x-axis. Confidence Interval (CI): A line extending from the effect estimate represents the confidence interval. This indicates the range within which the true effect size is likely to lie, with the point estimate positioned at the center of the bar. Overall Estimate: A summary effect estimate, represented by a blue diamond at the bottom of the plot, combines the results of all studies included in the meta-analysis. The center of the diamond represents the point estimate, and the width of the diamond represents the confidence interval around the summary estimate. I^2 (I-squared): A measure of heterogeneity in meta-analysis, indicating the proportion of total variation across studies that is due to heterogeneity rather than chance. *p*-value (Probability value): The probability of obtaining test results at least as extreme as the observed results, indicating the significance of the heterogeneity test. χ^2 (Chi-square): A test for subgroup differences, evaluating whether the observed differences between subgroups are statistically significant. df (degrees of freedom): The degrees of freedom, which represent the number of independent pieces of information used to estimate a parameter. τ^2 (Tau-squared): The variance of true effects in a random-effects model, reflecting the variability of effect sizes across studies beyond sampling error. Test for overall effect (t_3): A statistical test used to assess whether the observed effect size is significantly different from zero, indicating the presence of an overall effect in the meta-analysis.

3.4. Imiquimod on HPV Clearance

Among the 254 patients who received Imiquimod treatment, 50% (CI: 0.35–0.64; I^2: 64) of women had HPV clearance (see Figure 4) [10,13,27,29,30]. We performed a subgroup analysis according to the grade of cervical dysplasia (see Figure 4). When CIN 2–3 was the diagnosis, the HPV clearance rate was 42% (CI: 0.29–0.56; I^2: 49%); when CIN 1–3 was the diagnosis, the HPV clearance rate was 68% (CI: 0.48–0.84). Finally, when there was no CIN, only HPV positivity, the HPV clearance rate was 65% (CI: 0.44–0.83). However, we had only one study for each outcome. The subgroup analysis regarding the study types showed a 56% (CI: 0.28–0.80; I^2: 59%) HPV clearance in the ITT-RCTs and 44% (CI: 0.17–0.75; I^2: 73%) in the cohort studies (see Figure S4). Moreover, in the PP population of 100 patients, the HPV clearance rate was higher at 60% (CI: 0.35–0.84; I^2: 57%) (see Figure S5). The subgroup analysis in the PP for CIN 2–3 showed a 54% HPV clearance (CI: 0.06–0.96; I^2: 47%), and for CIN 1–3, the HPV clearance was 73%

(CI: 0.52–0.88) (see Figure S6). The HPV tests were conducted on average 2.33 (SD: ±1.91) months after finishing Imiquimod treatment.

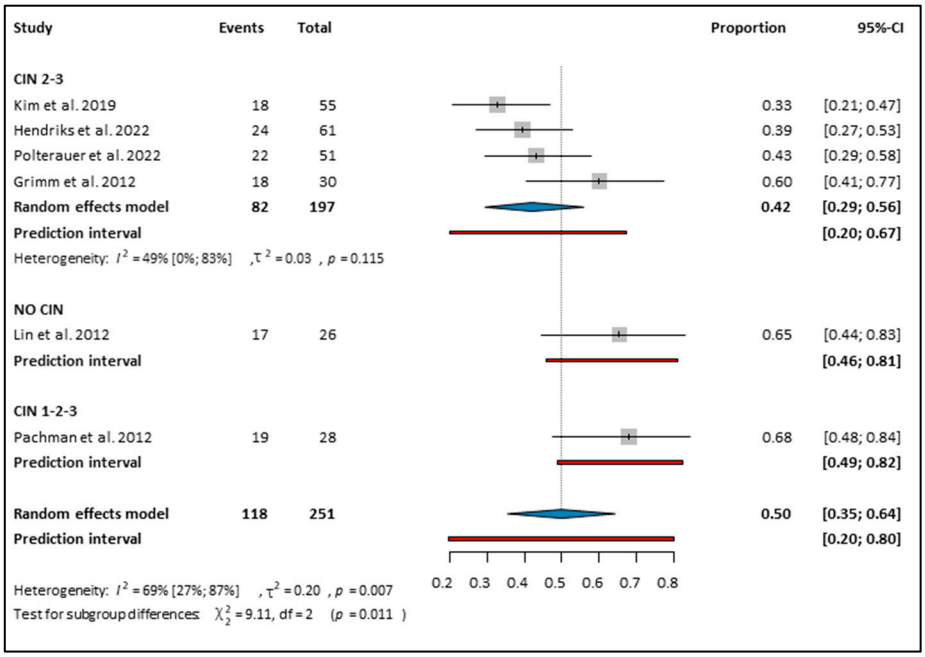

Figure 4. Forest plot of studies representing Imiquimod on HPV clearance based on the CIN status [11–13,27,29,30]. CIN: cervical intraepithelial neoplasia. Effect Estimate: The effect estimate for each study is represented by a grey square, located along the x-axis. Confidence Interval (CI): A line extending from the effect estimate represents the confidence interval. This indicates the range within which the true effect size is likely to lie, with the point estimate positioned at the center of the bar. Overall Estimate: A summary effect estimate, represented by a blue diamond at the bottom of the plot, combines the results of all studies included in the meta-analysis. The center of the diamond represents the point estimate, and the width of the diamond represents the confidence interval around the summary estimate. I^2 (I-squared): A measure of heterogeneity in meta-analysis, indicating the proportion of total variation across studies that is due to heterogeneity rather than chance. p-value (Probability value): The probability of obtaining test results at least as extreme as the observed results, indicating the significance of the heterogeneity test. χ^2 (Chi-square): A test for subgroup differences, evaluating whether the observed differences between subgroups are statistically significant. df (degrees of freedom): The degrees of freedom, which represent the number of independent pieces of information used to estimate a parameter. τ^2 (Tau-squared): The variance of true effects in a random-effects model, reflecting the variability of effect sizes across studies beyond sampling error.

The Imiquimod group had 196 patients, while the control arm had 180 patients. Imiquimod treatment did not result in better HPV clearance compared to that of the control group (RR: 1.29; CI: 0.52–3.21; I^2: 80%) (see Figure 5). In the control group, the treatment differed between the studies. When the control group received conization, it was more effective than Imiquimod (RR: 0.67; CI: 0.46–0.99) [30]. However, when no intervention was implemented in the control group and HPV infection was persistent, Imiquimod was more effective (RR: 4.20; CI: 1.62–10.89) [11]. In another study, when there was only persistent HPV positivity and no cervical dysplasia, and the control arm received no intervention, Imiquimod was more effective (RR: 2.18; CI: 1.06–4.05) [29]. In one study, the control arm had surgical interventions (conization, cryotherapy, laser) [13]. We found that in this article, Imiquimod treatment did not result in better HPV clearance than the control group (RR: 1.19; CI: 0.79–1.79) [13]. When we examined the PP group, we concluded that Imiquimod treatment did not result in a higher HPV clearance rate compared to the control group (see Figure S7).

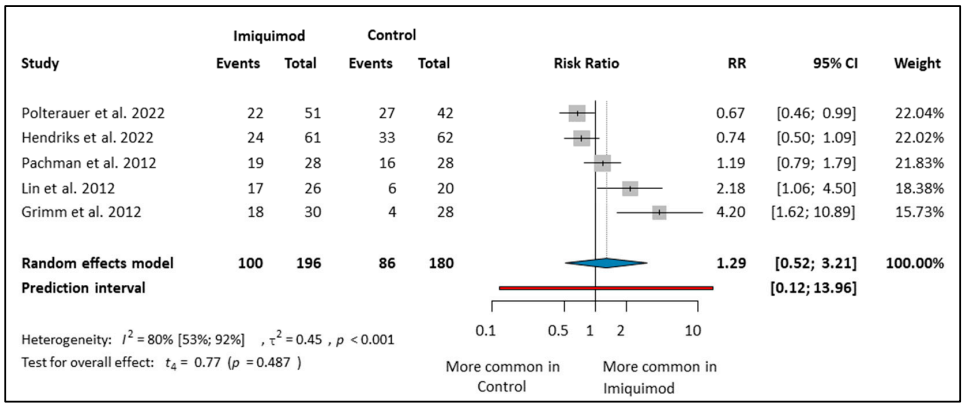

Figure 5. Forest plot of studies representing the Imiquimod group compared to control on HPV clearance [11–13,29,30]. Effect Estimate: The effect estimate for each study is represented by a grey square, located along the x-axis. Confidence Interval (CI): A line extending from the effect estimate represents the confidence interval. This indicates the range within which the true effect size is likely to lie, with the point estimate positioned at the center of the bar. Overall Estimate: A summary effect estimate, represented by a blue diamond at the bottom of the plot, combines the results of all studies included in the meta-analysis. The center of the diamond represents the point estimate, and the width of the diamond represents the confidence interval around the summary estimate. I^2 (I-squared): A measure of heterogeneity in meta-analysis, indicating the proportion of total variation across studies that is due to heterogeneity rather than chance. *p*-value (Probability value): The probability of obtaining test results at least as extreme as the observed results, indicating the significance of the heterogeneity test. τ^2 (Tau-squared): The variance of true effects in a random-effects model, reflecting the variability of effect sizes across studies beyond sampling error. Test for overall effect (t_4): A statistical test used to assess whether the observed effect size is significantly different from zero, indicating the presence of an overall effect in the meta-analysis.

Among the 186 patients who received Imiquimod, we investigated HPV 16/18 clearance compared to the clearance of other high-risk HPV (HR-HPV) types [11,12,27,30]. Our findings indicate no significant difference between HPV 16/18 clearance and clearance of other HR-HPV types (RR: 0.89; CI: 0.58–1.37; I^2: 0) (see Figure S8).

3.5. Adverse Events

In five studies, we were able to quantitatively synthesize the adverse events in patients who received Imiquimod, as they all used a very similar grading system (see Table 1) [10,11,13,28,30]. They graded side effects on a scale of one to five, where the grades ranged from mild to moderate, serious, life-threatening, and death (see Figure 6 and Figures S9–S18).

The most common systemic side effects were flu-like symptoms and myalgia, while from the local side effects, vaginal pruritus was the most frequent.

Grade 3 side effects occurred 8 times, with two articles [10,28] reporting abdominal pain and two [28,30] reporting headache. The remaining four occurrences of grade 3 side effects were one flu-like symptom [11], one fever [30], one myalgia [30], and one vaginal inflammation [28].

3.6. Risk of Bias Assessment and GRADE

For randomized controlled studies, the ROB2 showed some concern for the risk of bias in five outcomes and a low risk of bias in two outcomes. In non-randomized clinical trials, the ROBINSON tool showed a moderate and serious risk of bias in two outcomes. The latter study was a retrospective cohort analysis that used a historical control group for comparison and had methodological concerns. When we analyzed the response rates according to the JBI critical appraisal checklist, we found that the most frequent issue was regarding sample size (see Figures S19–S21).

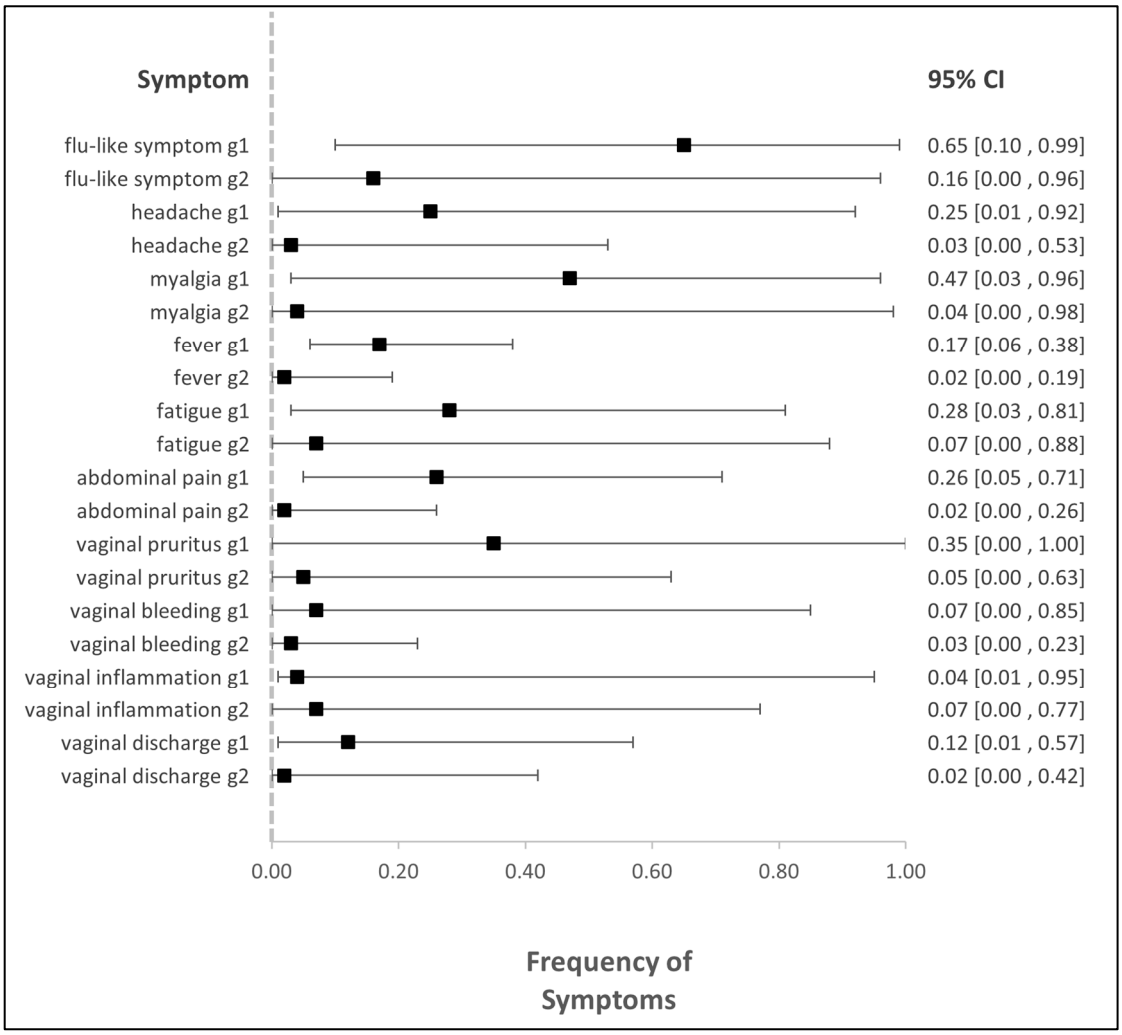

Figure 6. Forest plot of studies representing the frequency of all symptoms. g1: grade1, g2: grade2, CI: confidence interval.

Our summary of findings consisted of five outcomes where we included a control group. The quality of evidence was assessed as high for two outcomes, while it was deemed low for the remaining three outcomes (see Tables S3–S5).

4. Discussion

We investigated the safety and efficacy of topical Imiquimod on cervical intraepithelial neoplasia and HPV clearance. After Imiquimod treatment in CIN 2–3 patients, the regression rate was 61%. Regarding efficacy, we analyzed the biopsies of CIN 2–3 patients and concluded that women who received Imiquimod treatment had a higher rate of CIN regression compared to those who did not receive Imiquimod. We found that conization is more effective than Imiquimod in treating CIN 2–3 patients, as there was a 38% increase in successful treatment in the conization arm.

Imiquimod treatment in HPV-positive women showed a 50% HPV clearance rate and was 60% for women who completed the treatment. Overall, Imiquimod treatment did not result in better HPV clearance compared to the control group's treatment. When Imiquimod was compared to conization in one study, HPV clearance was higher in the conization group [30]. However, in another study comparing Imiquimod to placebo, HPV clearance was higher in the Imiquimod arm [11].

Regarding side effects, most side effects were mild, and hospitalization was not required in the majority of cases.

Increasing the dosage of Imiquimod did not result in a higher rate of CIN 2–3 regression. Regardless of the dose of Imiquimod, women who completed the treatment had a similar rate of dysplasia regression. In two studies, the remission rates were higher than in other studies [11,27]. In one study, the higher CIN 2/CIN 3 ratio could explain this. CIN 2 has a higher rate of spontaneous regression than CIN 3 and is considered a milder lesion [31,32]. The other study used cytological confirmation of CIN regression [27]. Although cytology is not a reproducible method of detecting cervical dysplasia [33], Imiquimod has been shown to be effective in reducing vulvar intraepithelial neoplasia and vaginal intraepithelial neoplasia [34,35]. The American College of Obstetricians and Gynecologists recognizes the off-label use of Imiquimod for vulvar intraepithelial neoplasia [36]. Our findings on Imiquimod in cervical dysplasia regression align with previous findings on other lower genital intraepithelial neoplasia [34,35]. Imiquimod is effective in reducing cervical CIN.

When comparing Imiquimod with the already existing surgical therapy for CIN 2–3, we found Imiquimod inferior. However, when the topical immunomodulator was used before conization, the positive margins of the resected tissue were lower than the average in the literature [10,37]. A logical explanation could be that Imiquimod reduced the lesion's depth and width and made it more suitable for surgical excision [10]. For selected patients, Imiquimod can be a choice; for example, women with future pregnancy desires have a higher demand for conservative treatment [38], given that conization increases the risk of miscarriage and preterm birth by causing cervical incompetence [5]. The ASCCP guideline recommends diagnostic evaluation of the cervix after six months in case of positive surgical excision margins [4]. Implementing Imiquimod in positive margin cases could lower the need for additional surgical excision. This could be highly desirable in women considering future pregnancy, as repeated surgical intervention of the cervix increases the risk of preterm birth compared to one surgical excision of the cervix [39]. A recent study showed that patients who respond to Imiquimod treatment could be selected priorly with an immunohistochemical method, as the immune microenvironment predicts whether the patient will respond to Imiquimod treatment [40]. This could personalize Imiquimod treatment, as not all patients respond to it.

When examining the HPV clearance for HPV 16 and 18 and other HR-HPV types, we conclude that the clearance rate is not worse for HPV 16 and 18. This is interesting since HPV 16 and 18 are known to be more aggressive and accountable for 70% of all cervical cancer [41]. We investigated the best three HPV clearance rates in the studies. We found that in one article there were only persistent HPV infections without CIN lesions [29]. In another study, CIN 2/CIN 3 rate was the highest among all publications [11]. In the third study, the CIN 2/CIN 3 rate was high, and CIN 1 also occurred [13]. Moreover, in this study, the HPV tests were taken after 6 months of Imiquimod discontinuation, which is a long time considering the natural clearance of HPV [42]. Patients with CIN 2+ lesions are known to be, molecularly, a vastly heterogeneous population; with progression, the cellular changes are more extensive, and the spontaneous regression of CIN and HPV declines [43]. Our findings support this observation, as when more CIN 3 lesions occur, HPV clearance rates are lower with Imiquimod. Higher Imiquimod doses did not result in a higher HPV clearance rate. When Imiquimod was compared to the control group, we did not experience a better HPV clearance rate. However, it should be mentioned here that the control differed between studies: surgical excision of the HPV-infected area [30] and only expectant management with no intervention [11]. In the previous case, the surgical solution was comparable or more effective than Imiquimod; however, in the latter case, Imiquimod was more effective than expectant management.

Systemic and local side effects were frequent but mostly mild, and the symptoms could have been reduced with non-steroid anti-inflammatory drugs [11]. This variability can be attributed to the systemic side effects associated with Imiquimod, which other common infections or health-related conditions can influence. Additionally, local side effects caused

by Imiquimod are commonly observed in general gynecological practice. This variability in side effects can help explain the differences observed among the studies.

Dropouts can happen for several reasons (long travel, financial reasons, dissatisfaction); however, it should be mentioned that in the two studies where the highest dose of Imiquimod was implemented, the two highest rates of dropout were also observed [12,28]. Severe side effects occurred just eight times, with high doses of Imiquimod used in seven of these cases. In two studies, besides the lower doses of Imiquimod used, the application was implemented by doctors. Accordingly, direct application could contribute to the low and mild side effects [10,27].

4.1. Strengths and Limitations

This meta-analysis is the first to synthesize the findings on Imiquimod use in cervical dysplasia and HPV-positive patients.

However, several limitations should be noted. Firstly, many studies had poor patient recruitment, and efforts were made to enroll more women. Secondly, the patients were mostly selected based on specific criteria, limiting the generalizability of the study's implications to all cervical dysplasia patients. Thirdly, in some cases, the comparison was made with control groups that used different methods. Fourthly, many studies lacked longer follow-up intervals, raising questions about the durability of dysplasia remission. Fifthly, the endpoint and timing of different outcome measures were often inconsistent, which is problematic given the spontaneous tendency of these lesions to regress. Sixthly, the clinical and statistical heterogeneity was substantial in several cases.

4.2. Implications for Practice and Research

Our findings show Imiquimod is safe and effective in reducing CIN and facilitating HPV clearance. While Imiquimod is inferior to conization, it could still be considered for use in selected patients, particularly after positive margins of conization, to avoid subsequent surgical excision of the cervix.

Personalized treatment strategies hold promise for enhancing the therapeutic efficacy of Imiquimod, as the immune environment serves as a predictive factor for treatment success [40]. Further refinement of immunohistochemical methods to identify specific biomarkers could lead to even greater therapeutic responses, potentially revolutionizing the approach to CIN treatment. Investigating the microenvironment and molecular profiles in greater detail through focused studies has the potential to transform the utilization of Imiquimod in clinical practice. Additionally, exploring combined therapies involving Imiquimod and other agents, such as 5-Fluorouracil, may synergistically augment the reduction of CIN lesions [44]. This approach not only offers the possibility of reducing Imiquimod dosage but also of lowering potential side effects, thereby improving medication adherence. Determining the optimal dosage of Imiquimod is paramount, with further studies warranted to elucidate this aspect. Such investigations are motivated by the desire to minimize costs associated with Imiquimod, an expensive medication, while simultaneously mitigating side effects. Prior to the incorporation of Imiquimod into treatment guidelines, conducting cost–effectiveness analyses is imperative. Assessing the cost–benefit ratio compared to traditional treatments is essential for informing healthcare resource allocation decisions. A comprehensive understanding of these factors will be instrumental in optimizing the utilization of Imiquimod in the management of cervical dysplasia. Further interventional studies are needed in this field to better understand how Imiquimod can reduce the burden of cervical dysplasia. Particularly, investigations with extended follow-up periods are warranted, as current studies often lack prolonged monitoring. Consequently, elucidating the duration of Imiquimod's effects remains an unanswered question that requires attention.

5. Conclusions

In conclusion, Imiquimod is not a substitute for cone biopsy; however, it can be a valuable treatment option for high-grade cervical dysplasia.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/cancers16081610/s1, Figure S1: Forest plot of studies representing Imiquimod and CIN 2–3 regression in the PP analysis; Figure S2: Forest plot of studies representing Imiquimod compared to no intervention on CIN 2–3 regression; Figure S3: Forest plot of studies representing Imiquimod group compared to conization on CIN 2–3 regression in the PP analysis; Figure S4: Forest plot of studies representing Imiquimod on HPV clearance according to study type; Figure S5: Forest plot of studies representing Imiquimod on HPV clearance in the PP analysis; Figure S6: Forest plot of studies representing Imiquimod on HPV clearance according to subgroup analysis of CIN status in the PP analysis; Figure S7: Forest plot of studies representing the Imiquimod group compared to control on HPV clearance in the PP analysis; Figure S8: Forest plot of studies representing the HPV 16/18 clearance compared to other HR-HPV clearance in the Imiquimod group; Figure S9: Forest plot of studies representing the occurrence of headaches in patients treated with Imiquimod; Figure S10: Forest plot of studies representing the occurrence of myalgia in patients treated with Imiquimod; Figure S11: Forest plot of studies representing the occurrence of fatigue in patients treated with Imiquimod; Figure S12: Forest plot of studies representing the occurrence of flu-like symptoms in patients treated with Imiquimod; Figure S13: Forest plot of studies representing the occurrence of fever in patients treated with Imiquimod; Figure S14: Forest plot of studies representing the occurrence of abdominal pain in patients treated with Imiquimod; Figure S15: Forest plot of studies representing the occurrence of vaginal pruritus in patients treated with Imiquimod; Figure S16: Forest plot of studies representing the occurrence of vaginal bleeding in patients treated with Imiquimod; Figure S17: Forest plot of studies representing the occurrence of vaginal discharge in patients treated with Imiquimod; Figure S18: Forest plot of studies representing the occurrence of inflammation of the vagina in patients treated with Imiquimod; Figure S19: Risk of bias assessment of randomized control studies; Figure S20: Risk of bias assessment of non-randomized controlled studies; Figure S21: JBI Critical Appraisal Checklist for response rate outcomes; Table S1: PRISMA 2020 checklist; Table S2: Imiquimod administration; Table S3: Grade for outcomes that assessed Imiquimod compared to conization; Table S4: Grade for outcomes that assessed Imiquimod compared to control on HPV clearance; Table S5: Grade on HPV 16/18 clearance compared to other HR-HPV clearance. References [10–13,27–30] are cited in the Supplementary Materials.

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References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. [CrossRef] [PubMed]
2. Okunade, K.S. Human papillomavirus and cervical cancer. *J. Obstet. Gynaecol.* **2020**, *40*, 602–608. [CrossRef] [PubMed]
3. Loopik, D.L.; Bentley, H.A.; Eijgenraam, M.N.; IntHout, J.; Bekkers, R.L.M.; Bentley, J.R. The Natural History of Cervical Intraepithelial Neoplasia Grades 1, 2, and 3: A Systematic Review and Meta-analysis. *J. Low. Genit. Tract. Dis.* **2021**, *25*, 221–231. [CrossRef] [PubMed]

4. Perkins, R.B.; Guido, R.S.; Castle, P.E.; Chelmow, D.; Einstein, M.H.; Garcia, F.; Huh, W.K.; Kim, J.J.; Moscicki, A.B.; Nayar, R.; 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*, 102–131. [CrossRef] [PubMed]
5. Weinmann, S.; Naleway, A.; Swamy, G.; Krishnarajah, G.; Arondekar, B.; Fernandez, J.; Myers, E. Pregnancy Outcomes after Treatment for Cervical Cancer Precursor Lesions: An Observational Study. *PLoS ONE* **2017**, *12*, e0165276. [CrossRef] [PubMed]
6. Lieb, J.A.; Mondal, A.; Lieb, L.; Fehm, T.N.; Hampl, M. Pregnancy outcome and risk of recurrence after tissue-preserving loop electrosurgical excision procedure (LEEP). *Arch. Gynecol. Obstet.* **2023**, *307*, 1137–1143. [CrossRef] [PubMed]
7. Bogani, G.; Di Donato, V.; Sopracordevole, F.; Ciavattini, A.; Ghelardi, A.; Lopez, S.; Simoncini, T.; Plotti, F.; Casarin, J.; Serati, M.; et al. Recurrence rate after loop electrosurgical excision procedure (LEEP) and laser Conization: A 5-year follow-up study. *Gynecol. Oncol.* **2020**, *159*, 636–641. [CrossRef]
8. Food and Drug Administration (FDA). Available online: https://www.fda.gov/drugs/drug-and-biologic-approval-and-ind-activity-reports/2021-first-generic-drug-approvals (accessed on 6 July 2023).
9. Stary, G.; Bangert, C.; Tauber, M.; Strohal, R.; Kopp, T.; Stingl, G. Tumoricidal activity of TLR7/8-activated inflammatory dendritic cells. *J. Exp. Med.* **2007**, *204*, 1441–1451. [CrossRef] [PubMed]
10. Fonseca, B.O.; Possati-Resende, J.C.; Salcedo, M.P.; Schmeler, K.M.; Accorsi, G.S.; Fregnani, J.H.T.G.; Antoniazzi, M.; Pantano, N.P.; Santana, I.V.V.; Matsushita, G.M.; et al. Topical Imiquimod for the Treatment of High-Grade Squamous Intraepithelial Lesions of the Cervix: A Randomized Controlled Trial. *Obstet. Gynecol.* **2021**, *137*, 1043–1053. [CrossRef]
11. Grimm, C.; Polterauer, S.; Natter, C.; Rahhal, J.; Hefler, L.; Tempfer, C.B.; Heinze, G.; Stary, G.; Reinthaller, A.; Speiser, P. Treatment of cervical intraepithelial neoplasia with topical imiquimod: A randomized controlled trial. *Obstet. Gynecol.* **2012**, *120*, 152–159. [CrossRef]
12. Hendriks, N.; Koeneman, M.M.; Van De Sande, A.J.M.; Penders, C.G.J.; Piek, J.M.J.; Kooreman, L.F.S.; Van Kuijk, S.M.J.; Hoosemans, L.; Sep, S.J.S.; De Vos Van Steenwijk, P.J.; et al. Topical Imiquimod Treatment of High-grade Cervical Intraepithelial Neoplasia (TOPIC-3): A Nonrandomized Multicenter Study. *J. Immunother.* **2022**, *45*, 180–186. [CrossRef]
13. Pachman, D.R.; Barton, D.L.; Clayton, A.C.; McGovern, R.M.; Jefferies, J.A.; Novotny, P.J.; Sloan, J.A.; Loprinzi, C.L.; Gostout, B.S. Randomized clinical trial of imiquimod: An adjunct to treating cervical dysplasia. *Am. J. Obstet. Gynecol.* **2012**, *206*, 42.e1–42.e7. [CrossRef] [PubMed]
14. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ* **2021**, *372*, n71. [CrossRef] [PubMed]
15. Cumpston, M.; Li, T.; Page, M.J.; Chandler, J.; Welch, V.A.; Higgins, J.P.; Thomas, J. Updated guidance for trusted systematic reviews: A new edition of the Cochrane Handbook for Systematic Reviews of Interventions. *Cochrane Database Syst. Rev.* **2019**, *10*, Ed000142. [CrossRef]
16. Munn, Z.; Stern, C.; Aromataris, E.; Lockwood, C.; Jordan, Z. What kind of systematic review should I conduct? A proposed typology and guidance for systematic reviewers in the medical and health sciences. *BMC Med. Res. Methodol.* **2018**, *18*, 5. [CrossRef] [PubMed]
17. McHugh, M.L. Interrater reliability: The kappa statistic. *Biochem. Med.* **2012**, *22*, 276–282. [CrossRef]
18. National Cancer Institute (NIH). Available online: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm (accessed on 8 July 2023).
19. Sterne, J.A.C.; Savović, J.; Page, M.J.; Elbers, R.G.; Blencowe, N.S.; Boutron, I.; Cates, C.J.; Cheng, H.Y.; Corbett, M.S.; Eldridge, S.M.; et al. RoB 2: A revised tool for assessing risk of bias in randomised trials. *BMJ* **2019**, *366*, l4898. [CrossRef]
20. Sterne, J.A.; Hernán, M.A.; Reeves, B.C.; Savović, J.; Berkman, N.D.; Viswanathan, M.; Henry, D.; Altman, D.G.; Ansari, M.T.; Boutron, I.; et al. ROBINS-I: A tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* **2016**, *355*, i4919. [CrossRef]
21. Joanna Briggs Institute (JBI). Critical Appraisal Checklist. 2017. Available online: https://jbi.global/sites/default/files/2019-05/JBI_Critical_Appraisal-Checklist_for_Prevalence_Studies2017_0.pdf (accessed on 7 August 2023).
22. GradePro. Available online: https://www.gradepro.org (accessed on 11 May 2023).
23. Clopper, C.J.; Pearson, E.S. The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. *Biometrika* **1934**, *26*, 404–413. [CrossRef]
24. Higgins, J.P.; Thompson, S.G. Quantifying heterogeneity in a meta-analysis. *Stat. Med.* **2002**, *21*, 1539–1558. [CrossRef]
25. Borenstein, M.; Hedges, L.; Higgins, J.; Rothstein, H. An Introduction to Meta-Analysis. In *Introduction to Meta-Analysis*; Wiley: Hoboken, NJ, USA, 2009; Volume 19.
26. Harrer, M.; Cuijpers, P.; Furukawa, T.; Ebert, D. *Doing Meta-Analysis with R: A Hands-On Guide*; CRC Press: Boca Raton, FL, USA, 2021.
27. Kim, J.H.; Kim, D.Y. Imiquimod as an alternative option for young women with high-grade cervical intraepithelial neoplasia. *Eur. J. Gynaecol. Oncol.* **2019**, *40*, 943–947.
28. Cokan, A.; Pakiž, M.; Serdinšek, T.; Dovnik, A.; Kodrič, T.; Fokter, A.R.; Kavalar, R.; But, I. Comparison of conservative treatment of cervical intraepithelial lesions with imiquimod with standard excisional technique using lletz: A randomized controlled trial. *J. Clin. Med.* **2021**, *10*, 5777. [CrossRef] [PubMed]

29. Lin, C.T.; Qiu, J.T.; Wang, C.J.; Chang, S.D.; Tang, Y.H.; Wu, P.J.; Jung, S.M.; Huang, C.C.; Chou, H.H.; Jao, M.S.; et al. Topical imiquimod treatment for human papillomavirus infection in patients with and without cervical/vaginal intraepithelial neoplasia. *Taiwan. J. Obstet. Gynecol.* **2012**, *51*, 533–538. [CrossRef] [PubMed]
30. Polterauer, S.; Reich, O.; Widschwendter, A.; Hadjari, L.; Bogner, G.; Reinthaller, A.; Joura, E.; Trutnovsky, G.; Ciresa-Koenig, A.; Ganhoer-Schimboeck, J.; et al. Topical imiquimod compared with conization to treat cervical high-grade squamous intraepithelial lesions: Multicenter, randomized controlled trial. *Gynecol. Oncol.* **2022**, *165*, 23–29. [CrossRef] [PubMed]
31. Chan, J.K.; Monk, B.J.; Brewer, C.; Keefe, K.A.; Osann, K.; McMeekin, S.; Rose, G.S.; Youssef, M.; Wilczynski, S.P.; Meyskens, F.L.; et al. HPV infection and number of lifetime sexual partners are strong predictors for 'natural' regression of CIN 2 and 3. *Br. J. Cancer* **2003**, *89*, 1062–1066. [CrossRef] [PubMed]
32. Tainio, K.; Athanasiou, A.; Tikkinen, K.A.O.; Aaltonen, R.; Cárdenas, J.; Glazer-Livson, S.; Jakobsson, M.; Joronen, K.; Kiviharju, M.; Louvanto, K.; et al. Clinical course of untreated cervical intraepithelial neoplasia grade 2 under active surveillance: Systematic review and meta-analysis. *BMJ* **2018**, *360*, k499. [CrossRef] [PubMed]
33. Kitchener, H.C.; Castle, P.E.; Cox, J.T. Chapter 7: Achievements and limitations of cervical cytology screening. *Vaccine* **2006**, *24* (Suppl. S3), 63–70. [CrossRef] [PubMed]
34. Terlou, A.; van Seters, M.; Ewing, P.C.; Aaronson, N.K.; Gundy, C.M.; Heijmans-Antonissen, C.; Quint, W.G.; Blok, L.J.; van Beurden, M.; Helmerhorst, T.J. Treatment of vulvar intraepithelial neoplasia with topical imiquimod: Seven years median follow-up of a randomized clinical trial. *Gynecol. Oncol.* **2011**, *121*, 157–162. [CrossRef] [PubMed]
35. Tranoulis, A.; Laios, A.; Mitsopoulos, V.; Lutchman-Singh, K.; Thomakos, N. Efficacy of 5% imiquimod for the treatment of Vaginal intraepithelial neoplasia-A systematic review of the literature and a meta-analysis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **2017**, *218*, 129–136. [CrossRef]
36. Committee Opinion No. 675 Summary: Management of Vulvar Intraepithelial Neoplasia. *Obstet. Gynecol.* **2016**, *128*, 937–938.
37. O'Shea, A.S.; Stockdale, C.K. The impact of LEEP margin status on subsequent abnormal cervical cytology. *Proc. Obstet. Gynecol.* **2014**, *4*, 1–8. [CrossRef]
38. Koeneman, M.M.; Essers, B.A.; Gerestein, C.G.; van de Sande, A.J.M.; Litjens, R.; Boskamp, D.; Goossens, M.F.J.; Beekhuizen, H.J.; Kruitwagen, R.; Kruse, A.J.; et al. Treatment of Cervical Intraepithelial Neoplasia: Patients Preferences for Surgery or Immunotherapy with Imiquimod. *J. Immunother.* **2017**, *40*, 148–153. [CrossRef] [PubMed]
39. Kyrgiou, M.; Athanasiou, A.; Kalliala, I.E.J.; Paraskevaidi, M.; Mitra, A.; Martin-Hirsch, P.P.; Arbyn, M.; Bennett, P.; Paraskevaidis, E. Obstetric outcomes after conservative treatment for cervical intraepithelial lesions and early invasive disease. *Cochrane Database Syst. Rev.* **2017**, *11*, Cd012847. [CrossRef] [PubMed]
40. Abdulrahman, Z.; Hendriks, N.; Kruse, A.J.; Somarakis, A.; van de Sande, A.J.; van Beekhuizen, H.J.; Piek, J.M.; de Miranda, N.F.; Kooreman, L.F.; Slangen, B.F.; et al. Immune-based biomarker accurately predicts response to imiquimod immunotherapy in cervical high-grade squamous intraepithelial lesions. *J. Immunother. Cancer* **2022**, *10*, e005288. [CrossRef] [PubMed]
41. Da Silva, R.L.; da Silva Batista, Z.; Bastos, G.R.; Cunha, A.P.A.; Figueiredo, F.V.; de Castro, L.O.; Dos Anjos Pereira, L.; da Silva, M.; Vidal, F.C.B.; Barros, M.C.; et al. Role of HPV 16 variants among cervical carcinoma samples from Northeastern Brazil. *BMC Women's Health* **2020**, *20*, 162. [CrossRef] [PubMed]
42. Gravitt, P.E.; Winer, R.L. Natural History of HPV Infection across the Lifespan: Role of Viral Latency. *Viruses* **2017**, *9*, 267. [CrossRef] [PubMed]
43. Steenbergen, R.D.; Snijders, P.J.; Heideman, D.A.; Meijer, C.J. Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. *Nat. Rev. Cancer* **2014**, *14*, 395–405. [CrossRef]
44. Rahangdale, L.; Lippmann, Q.K.; Garcia, K.; Budwit, D.; Smith, J.S.; van Le, L. Topical 5-fluorouracil for treatment of cervical intraepithelial neoplasia 2: A randomized controlled trial. *Am. J. Obstet. Gynecol.* **2014**, *210*, 314.e1–314.e8. [CrossRef]