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.

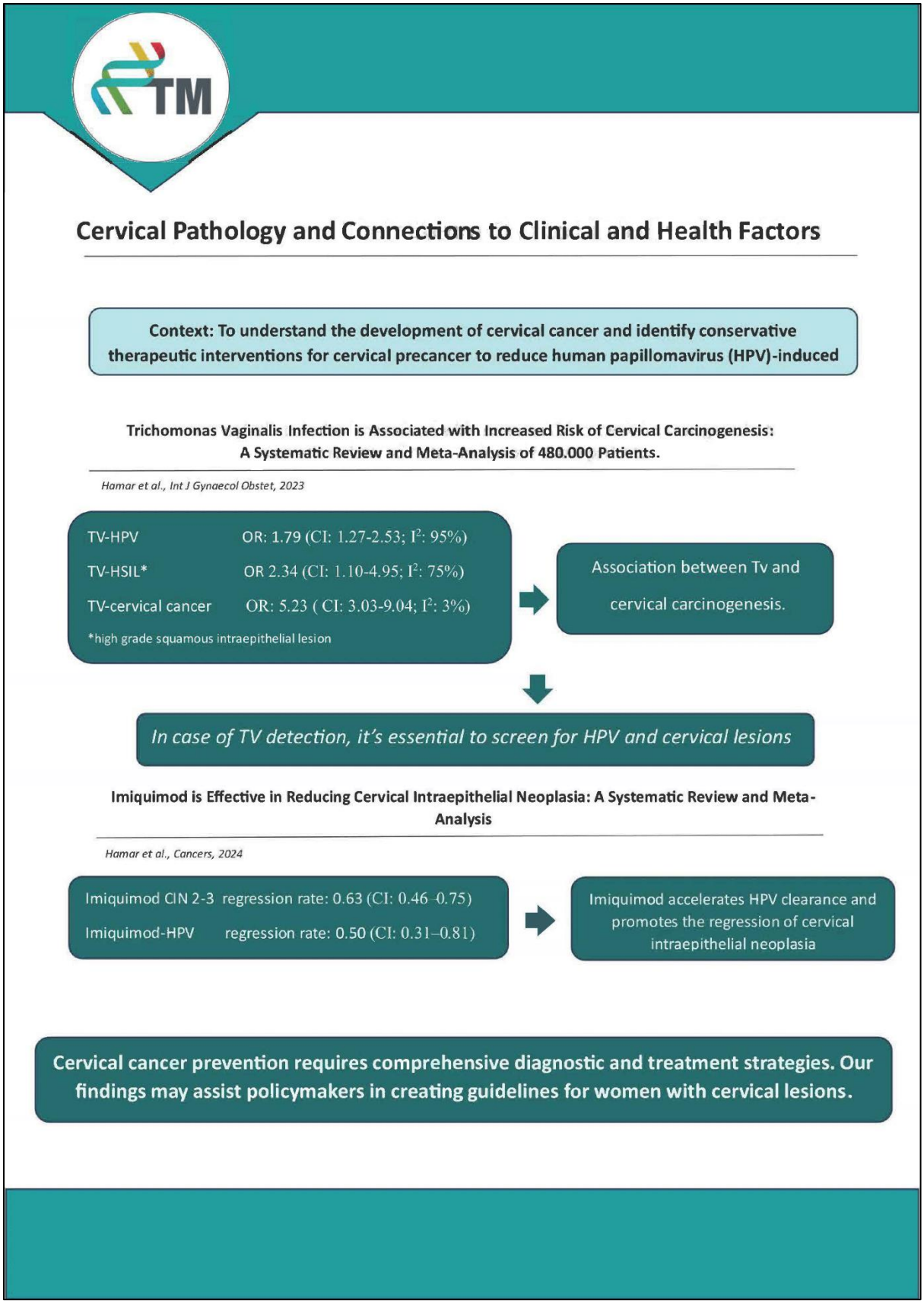
Our clinical question was whether TV can be a risk factor for the development of cervical cancer. Our second question concerned the extent to which topical Imiquimod had a positive effect on cervical intraepithelial neoplasia.

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



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)

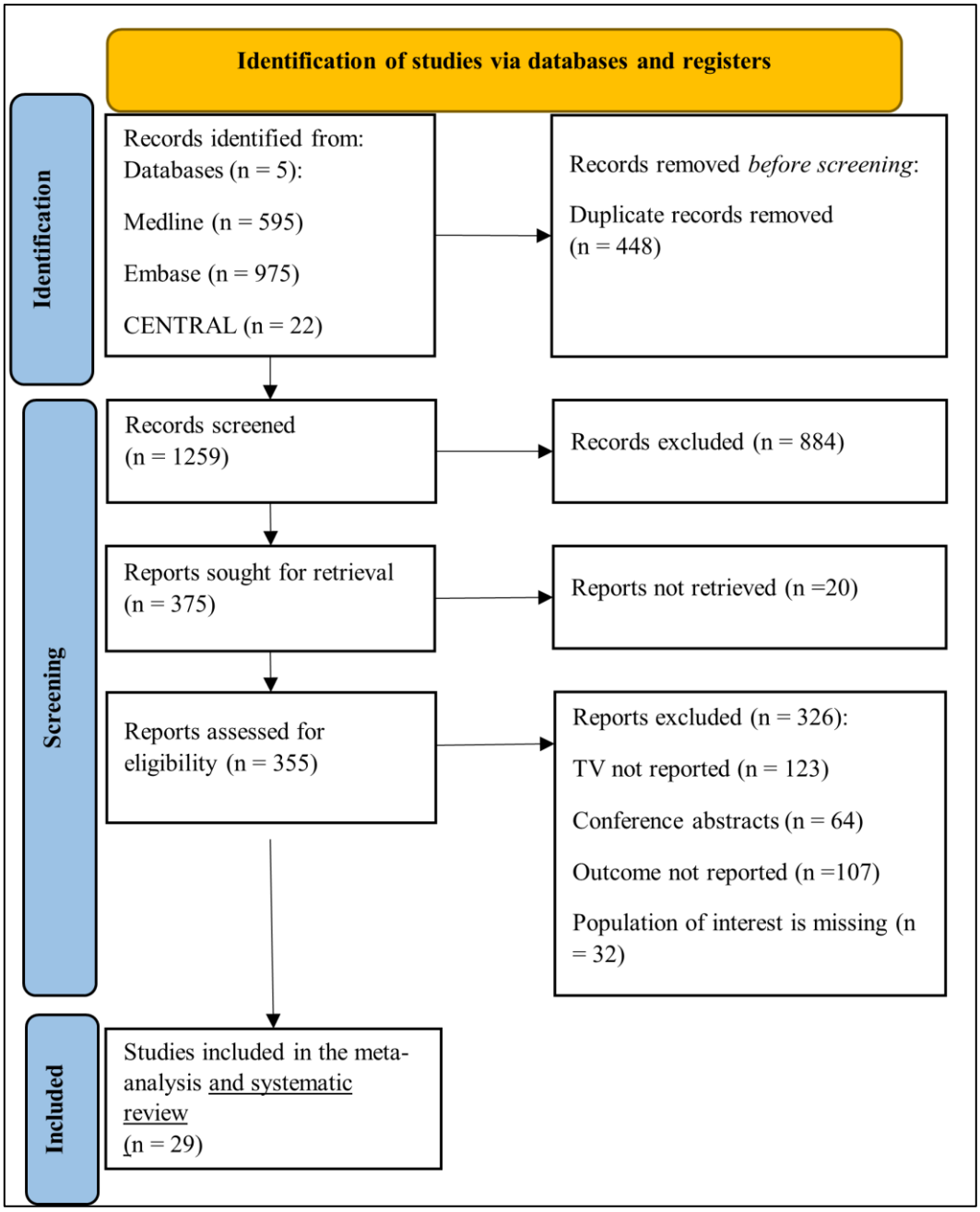


Figure 1. PRISMA 2020 flowchart representing the study selection process.

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)

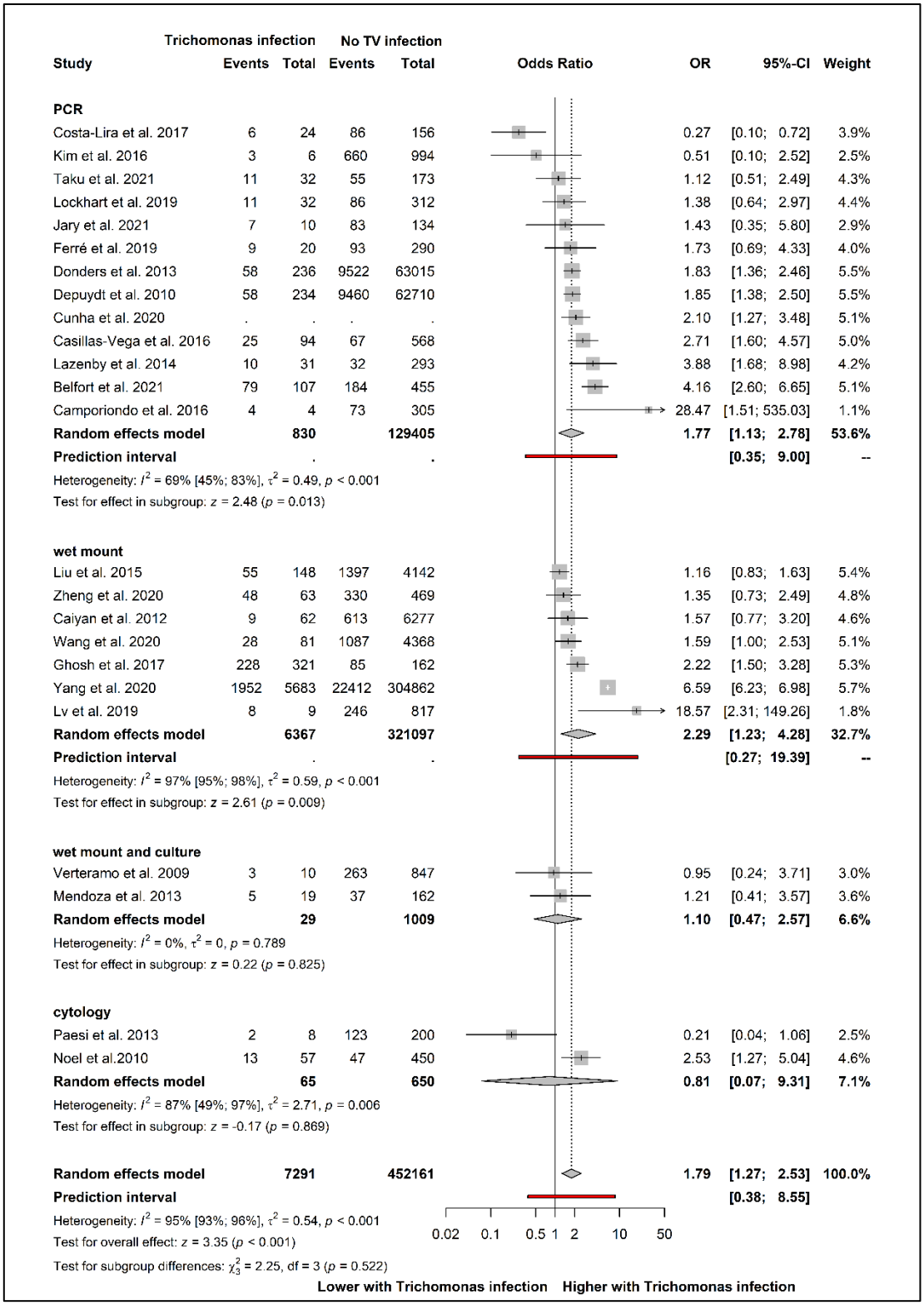


Figure 2. Forest plot of studies representing an association between **TV** and **HPV** infection.

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).

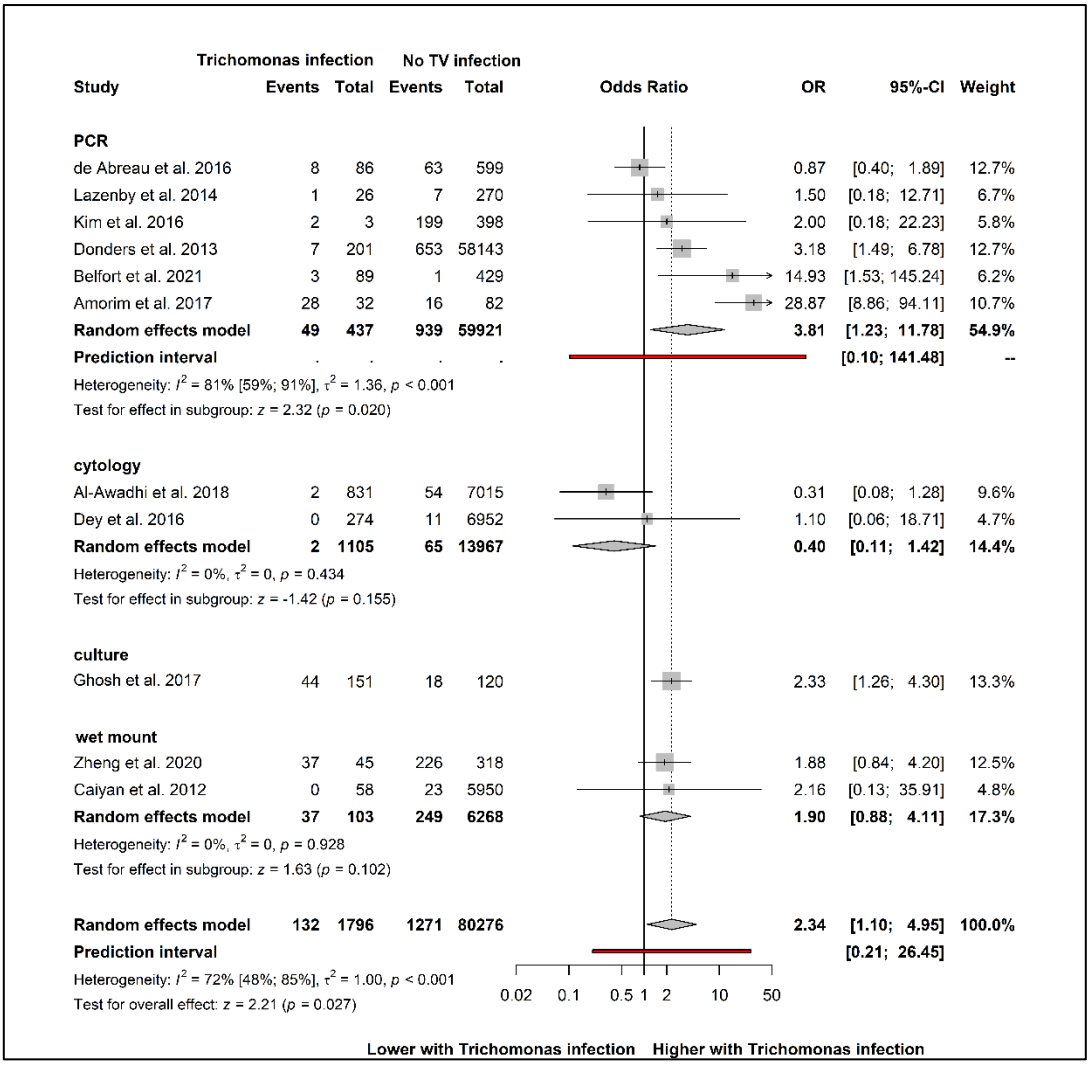


Figure 3. Forest plot of studies showing the association between **TV** infection and **HSIL**.

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.

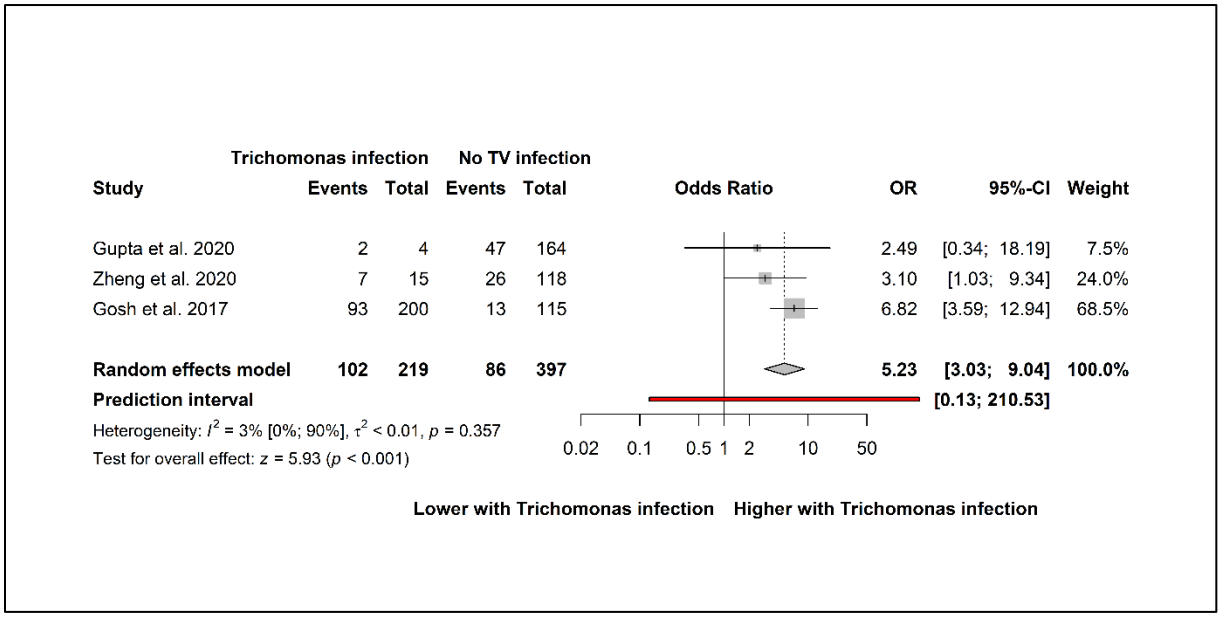


Figure 4. Forest plot of studies showing the association between **TV** infection and **cervical cancer**.

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).

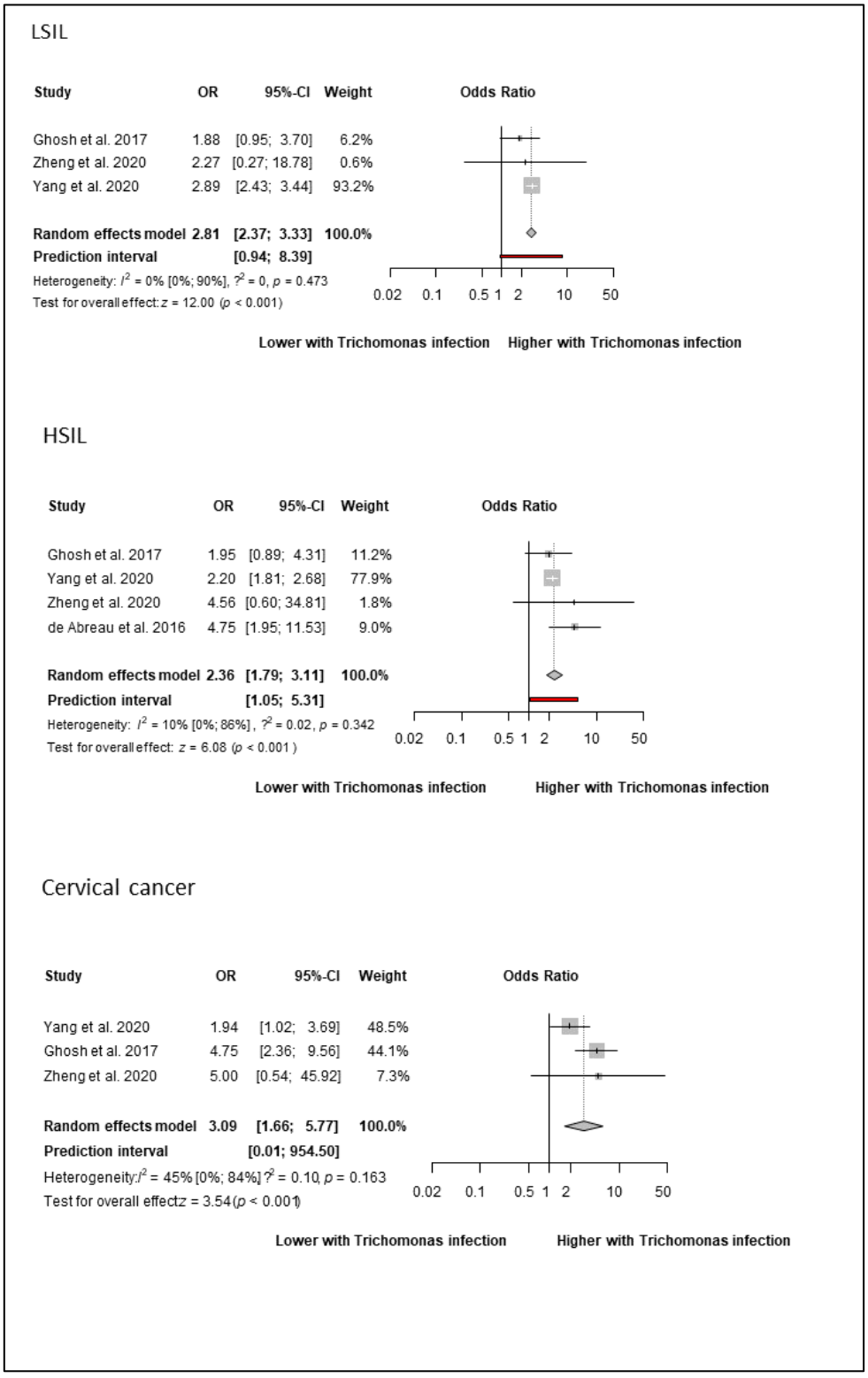


Figure 5: Forest plot of studies showing the association between **TV** and **LSIL, HSIL,** and **cervical cancer** in the **HPV-positive population**.

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

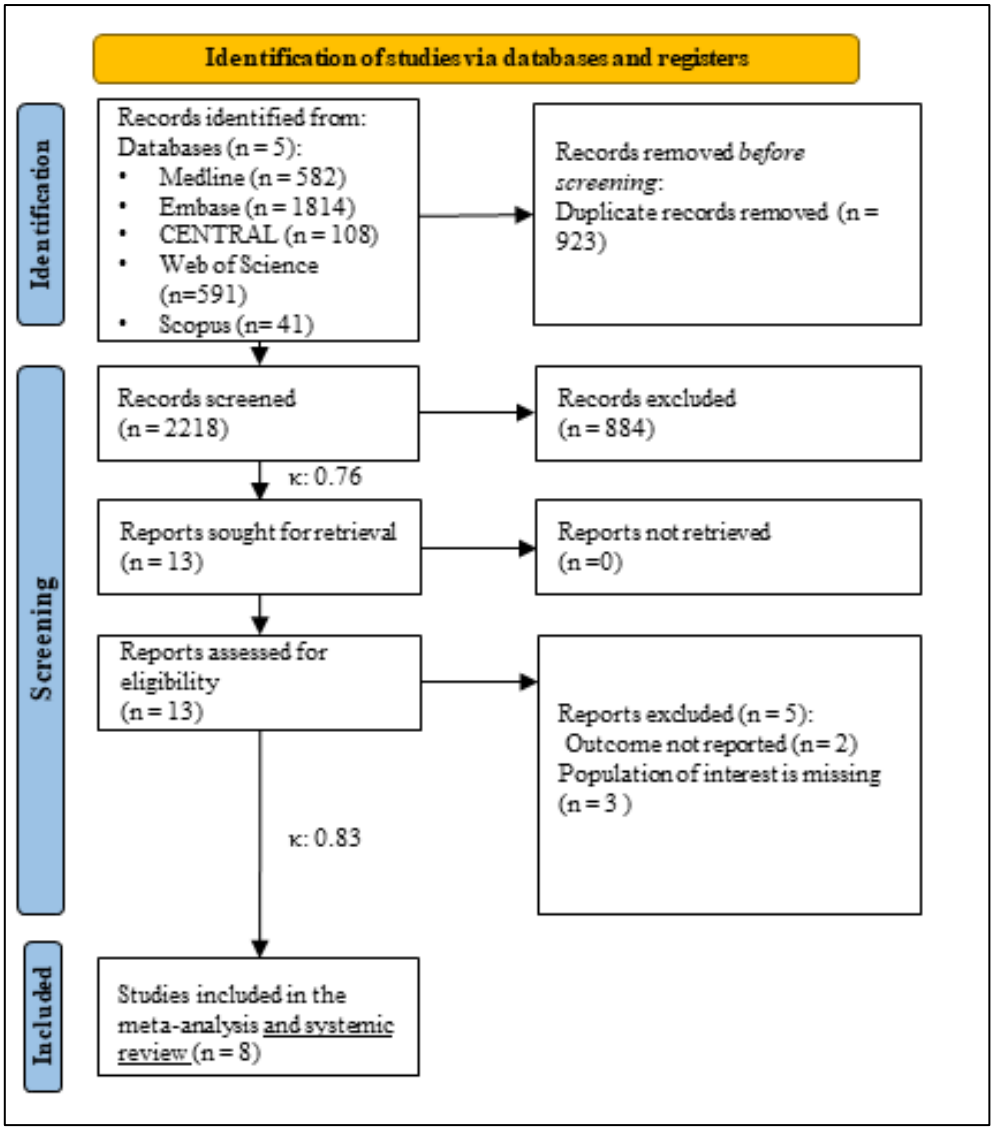


Figure 6. PRISMA flowchart of selection n: number of studies, κ: cohen's kappa coeficient

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).

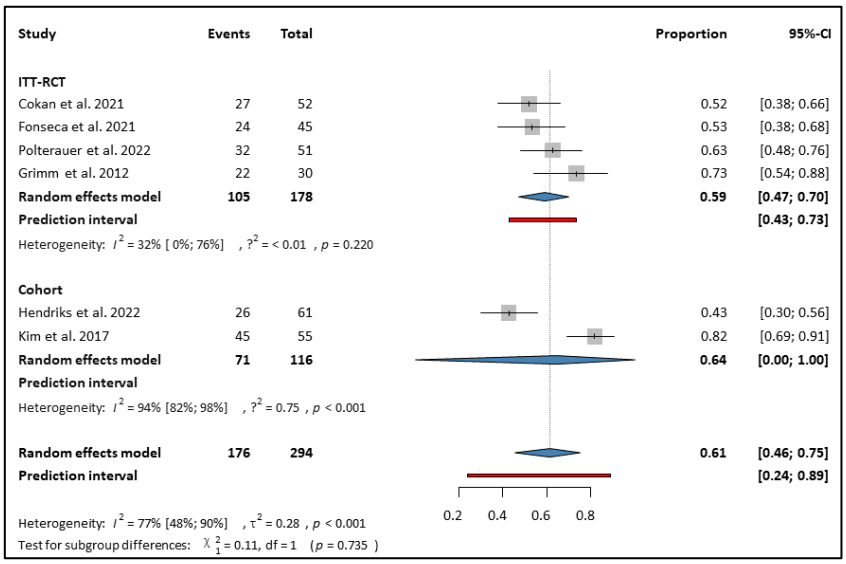


Figure 7. Forest plot of studies showing **Imiquimod** and **CIN 2-3 regression based on study type.** ITT: intention to treat, RCT: randomized control trial

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).

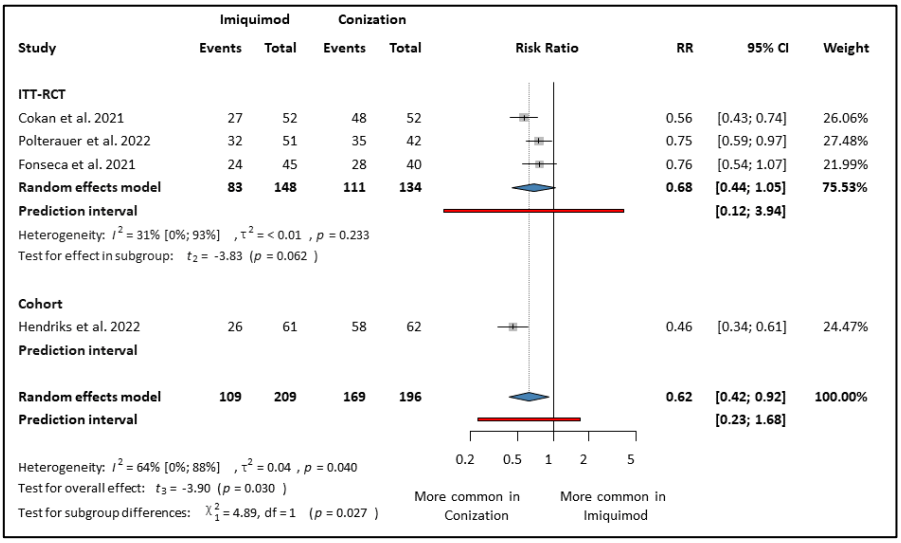


Figure 8. Forest plot of studies showing the **Imiquimod group** compared to **conization** on **CIN 2-3 regression based on study type.** ITT: intention to treat, RCT: randomized control trial

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.

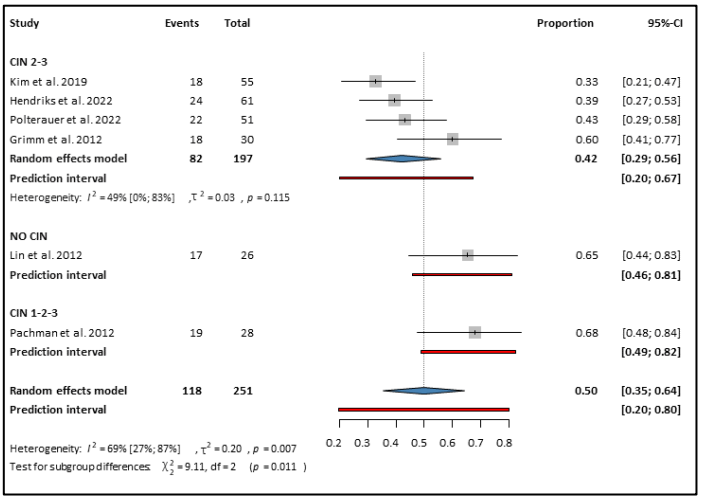


Figure 9. Forest plot of studies representing **Imiquimod on HPV clearance based on the CIN status** CIN: cervical intraepithelial neoplasia

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).

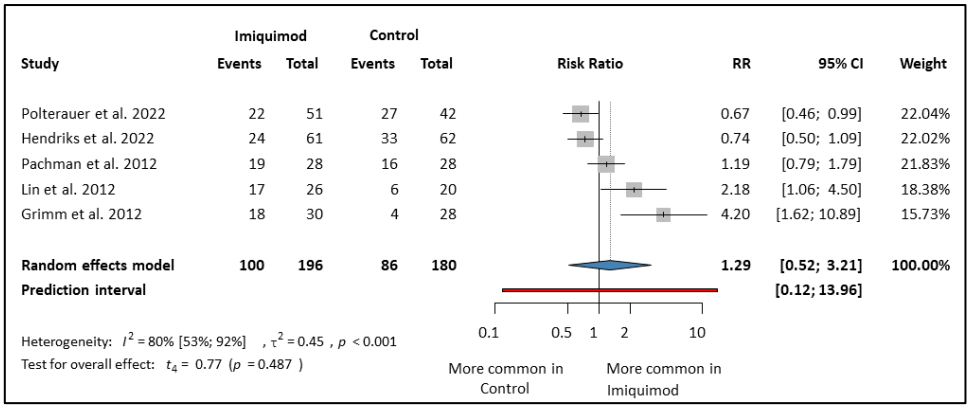


Figure 10. Forest plot of studies showing the **Imiquimod group** compared to the **control group** in terms of **HPV clearance**

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).

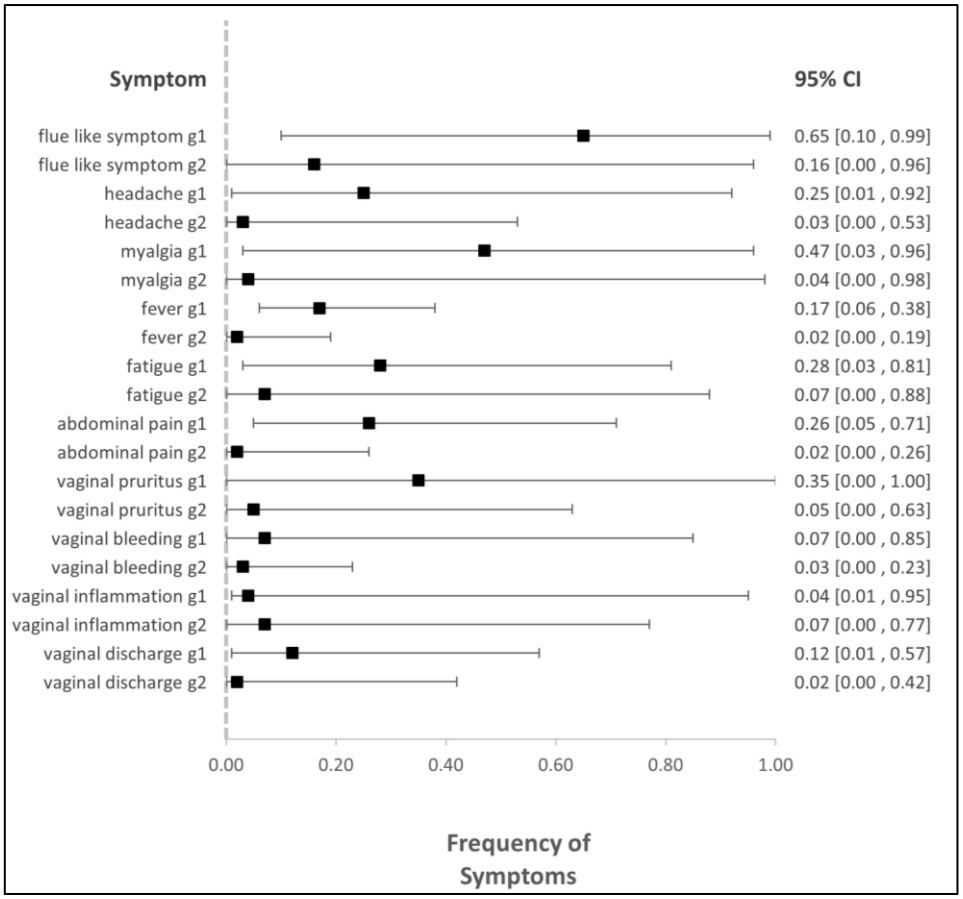


Figure 11. Forest plot of studies showing the frequency of all symptoms g1: grade1, g2: grade2, CI: confidence interval.

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.

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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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18. PUBLICATIONS