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The Influence of Human Papillomavirus Genotypes, the Vaginal Ecosystem, Co-Occurring Sexually Transmitted Infections, Host Immunogenetic, and Social Factors on the Development of Cervical Neoplasia

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Table of Contents

Abbreviations

AI	artificial intelligence
AV	aerobic vaginitis
BV	bacterial vaginosis
CI	confidence interval
CIN	cervical intraepithelial neoplasia
DNA	deoxyribonucleic acid
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HPV	human papillomavirus
HR-HPV	high-risk human papillomavirus
HSIL	high-grade squamous intraepithelial lesion
LBG	lactobacillary grades
mRNA	messenger ribonucleic acid
msAV	moderate to severe aerobic vaginitis
OR	odds ratio
PCR	polymerase chain reaction
STI	sexually transmitted infections
WHO	World Health Organization

Introduction

In 2008, German virologist Harald zur Hausen received the Nobel Prize in Physiology or Medicine for the discovery that persistent infection with high-risk human papillomavirus (HPV) types is the leading cause of cervical cancer. His research fundamentally changed the understanding of cervical carcinogenesis and provided the scientific basis for the development of prophylactic HPV vaccines. (Baumann and Doeberitz, 2024) These vaccines have since become a key part of global efforts to prevent cervical cancer, representing a significant step forward in protecting women's health.

Cervical cancer remains one of the most preventable types of cancer, due to its well-understood aetiology and the slow progression of the disease. HPV infection is a mandatory cause of cervical cancer, but not a sufficient one; the majority of infections are transient and resolve spontaneously. However, in some women, particularly those with high-risk HPV genotypes, the infection may persist and lead to precancerous disease and eventually invasive cervical cancer. This progression typically unfolds over many years, opening a wide window for detecting and treating these lesions to prevent malignisation.

Recognizing the preventability of cervical cancer, the World Health Organization (WHO) introduced a global strategy in 2020 to eliminate it as a public health problem. The plan aims to reduce the incidence to fewer than 4 cases per 100 000 women-years. To reach this goal by 2030, the WHO recommends that 90 % of girls receive full HPV vaccination by age 15, 70 % of women are screened by ages 35 and 45, and 90 % of women diagnosed with cervical disease receive appropriate treatment. (WHO, 2020)

Despite these global efforts, cervical cancer remains a significant public health challenge in many countries, including Latvia. In Latvia, cervical cancer is the second most common malignancy in women of reproductive age, and its incidence has remained stable over the last 15 years despite the implementation of national screening and HPV vaccination programmes. These trends highlight critical gaps in prevention strategies, underscoring the need for a more nuanced understanding of the factors that influence HPV persistence and disease progression.

While HPV infection is a prerequisite condition for the development of cervical cancer, it alone does not determine the clinical outcome. A growing body of evidence indicates that the progression from HPV infection to premalignant lesions and invasive cancer is influenced by a complex interplay of viral, host, environmental, and behavioural factors. These include the specific HPV genotype, host genetic susceptibility, immune response, co-infections with other sexually transmitted pathogens, the composition of the vaginal microbiota, smoking, and sexual behaviour, among others. (Kyrgiou et al., 2017; Trottier and Franco, 2006) Therefore,

there is a growing consensus that current "one-size-fits-all" screening and management strategies may not be adequate to address the diverse risks within different populations.

Particularly in high-risk HPV-positive women, accurate risk stratification is essential to avoid both overtreatment and missed diagnoses. Although universal HPV-based screening has shown higher sensitivity than cytology-based methods, (Arbyn et al., 2013) it also increases the number of women referred for unnecessary follow-up and invasive procedures, most of whom will never develop high-grade lesions. This is especially important in the context of limited healthcare resources and in vaccinated populations, where the prevalence of cervical disease is expected to decline. As a result, predictive models are needed to integrate multiple risk factors, including genetic, microbial, behavioural, and demographic characteristics, to identify women at the highest risk better and personalise prevention strategies accordingly. Identifying population-specific risk markers could improve the effectiveness of screening programmes, facilitate earlier detection of high-grade lesions, and ultimately contribute to achieving the goals of the WHO's cervical cancer elimination strategy.

Aim

To identify associations between various social factors, the vaginal ecosystem, vaginal infections, including sexually transmitted infections and high-risk human papillomavirus, human leucocyte antigen (HLA) class II alleles, and different degrees of cervical precancerous changes.

Objectives

1 To assess and compare social and behavioural factors in women with and without proven cervical neoplasia.
2 To analyse vaginal pH and vaginal microflora in women with and without cervical intraepithelial neoplasia.
3 To evaluate the frequency of high-risk HPV, sexually transmitted, and other vaginal infections in patients with and without cervical precancerous lesions.
4 To detect HLA class II alleles' genetic variants in women with and without cervical precancerous lesions.

Hypotheses

1 Women with cervical precancerous lesions are more likely to have different social and behavioural risk factors compared to individuals without cervical precancerous lesions.

2 Women with cervical intraepithelial neoplasia have an increased vaginal pH and prevalence of abnormal vaginal microflora compared to women without cervical intraepithelial neoplasia.
3 The prevalence of high-risk HPV, sexually transmitted infections, and other vaginal infections is significantly higher in women with cervical precancerous lesions than in those without.
4 Specific HLA class II alleles are associated with an increased risk of developing cervical precancerous lesions.

Novelty

This study examined a broad range of factors associated with cervical intraepithelial neoplasia (CIN), with particular attention to vaginal ecosystem disturbances and host immunogenetic variability. Although persistent high-risk human papillomavirus (HR-HPV) infection is a necessary condition for the development of cervical neoplasia, only a small proportion of infected women progress to clinically significant disease. In the context of primary HPV-based screening, improved identification of HPV-positive women at increased risk of progression remains a significant clinical challenge. The findings of this study support the value of incorporating microbial and host factors into future risk-stratification approaches.

A central finding of this research is the demonstrated association between aerobic vaginitis and histologically confirmed cervical neoplasia, particularly high-grade lesions. While previous research has focused mainly on bacterial vaginosis, this study highlights the importance of inflammatory vaginal conditions. The use of a modified *Donders-Schröder* scale for wet-mount microscopy enabled more consistent and detailed classification of vaginal microflora, allowing differentiation between bacterial vaginosis, aerobic vaginitis, and mixed patterns and reducing potential diagnostic misclassification.

In addition, this study provides the first analysis of HLA class II gene variants in Latvian women with cervical neoplasia. Several HLA alleles were identified as being associated with increased or reduced risk of high-grade cervical lesions, including variants not previously reported in this context. Given population-specific genetic variability, these findings contribute to a better understanding of how host immunogenetic factors may influence susceptibility to cervical disease and highlight the importance of studying less-represented populations in cervical cancer research.

1 Materials and methods

This study was performed in Riga East University Hospital's Outpatient Department. It was designed as a cross-sectional study. Participant recruitment and clinical examinations were conducted between January 2016 and June 2017, while laboratory analyses were performed subsequently, during the time period July 2016 – December 2022. The study group consisted of consecutive women referred for colposcopic examination because of abnormal cervical cytology, whereas the control group included women attending routine gynaecological care or follow-up for non-cervical conditions and presenting with normal cervical findings.

The sample size calculation was based on the prevalence of high-risk HPV deoxyribonucleic acid (DNA), a key etiological factor. Assuming 80 % statistical power, a 5 % significance level, a minimum HR-HPV prevalence of 30 % in the study group, and a maximum of 10 % in the control group, at least 63 participants per group were required. To account for potential dropouts, the sample size was increased by at least 20 %. Ultimately, 112 women were included in the study group and 120 women in the control group.

Inclusion criteria were age ≥ 18 years, signed informed consent, and either abnormal cervical screening results (study group) or normal cervical examination findings (control group). Exclusion criteria included age under 18 years, prior history of cervical intraepithelial neoplasia or cervical cancer, pregnancy, and refusal to participate.

Fieldwork and clinical procedures

After providing informed consent, all participants completed a structured questionnaire addressing sociodemographic characteristics and behavioural factors. A gynaecological examination with a vaginal speculum was then performed. Vaginal pH was measured, and samples from the anterior vaginal fornix were obtained for wet-mount microscopy. Cervical samples were collected and stored at −80°C for later detection of HR-HPV DNA and messenger ribonucleic acid (mRNA), sexually transmitted and other vaginal infections, and HLA class II gene analysis.

Colposcopy was performed for all participants. Biopsies were obtained from at least two cervical sites in the study group and from control group participants when colposcopic findings were suspicious for pathology.

Sociodemographic and behavioural factors

Collected variables included age, educational level, smoking status, marital status, and contraceptive use. Age was categorised into three groups (≤ 30 years, 31–49 years, ≥ 50 years). Education was classified as low (primary or secondary education) or high (a bachelor's degree

or higher). Contraceptive use was grouped into effective methods (hormonal contraception, intrauterine devices, or sterilisation), condom use, withdrawal, or no contraception.

Vaginal ecosystem assessment

Vaginal pH was measured with Machery-Nagel pH strips; values above 4.4 were considered elevated. Wet-mount phase-contrast microscopy was performed on vaginal samples. The assessment included lactobacillary grades (LBG), leukocyte counts, proportion of toxic leukocytes, presence of clue cells, red blood cells, sperm cells, parabasal cells, and background flora characteristics.

LBG classification followed a Donder's modified *Schröder* system (Donders, 1999; Donders et al., 2002):

- LBG I exhibits a dominant presence of lactobacillus morphotypes with no other bacteria.
- LBG IIa shows lactobacilli dominance alongside other bacterial species.
- LBG IIb – other microorganisms outnumber lactobacilli.
- LBG III indicates an absence of lactobacilli, with other bacteria present.

Normal vaginal microbiota corresponds to LBG I and IIa, while abnormal microbiota is classified as LBG IIb and III. Abnormal microbiota was further classified into bacterial vaginosis (BV), aerobic vaginitis (AV), or mixed BV–AV patterns. AV severity was scored using a composite index incorporating LBG, leukocyte count, toxic leukocytes, background flora, and parabasal cells. Moderate to severe AV (msAV) was defined as an AV score of five or higher. (Donders et al., 1996; Donders, 1999; Donders et al., 2002)

Colposcopy and histological evaluation

Colposcopic examinations were performed by certified specialists in accordance with European quality assurance guidelines. Cervical biopsy specimens were formalin-fixed, paraffin-embedded, stained with haematoxylin and eosin, and examined by a certified pathologist. Histological diagnoses were categorised as no CIN, CIN1, CIN2, CIN3, or carcinoma. For analytical purposes, all CIN grades were combined into an "all CIN" category, and CIN2, CIN3, and carcinoma were grouped as CIN2+.

HPV DNA and mRNA detection

Cervical samples were analysed for DNA of the most prevalent high-risk HPV types (16/18, 31, 33, 45, and 58), which account for 90 % of cervical cancer cases, using polymerase chain reaction after nucleic acid extraction. Detection of E6/E7 mRNA from 14 high-risk HPV types was performed using multiplex real-time polymerase chain reaction (PCR) and automated

amplification systems. Laboratory analyses were carried out in certified microbiology and diagnostic laboratories.

Detection of sexually transmitted and other vaginal infections

Seven genital microorganisms (*C. trachomatis, N. gonorrhoeae, T. vaginalis, M. hominis, M. genitalium, U. urealyticum, U. parvum*) were detected using molecular diagnostic methods based on DNA extraction and multiplex real-time PCR. For statistical analyses, selected pathogens were grouped as "all sexually transmitted infections (STI)" or "non-STI *Mycoplasmas*."

HLA class II genotyping

Cervical samples were collected for human DNA extraction and low-resolution HLA class II genotyping. Alleles at the DRB1, DQA1, and DQB1 loci were analysed using real-time PCR. Due to budget constraints, HLA genotyping was performed for only 84 women in the study group and 57 in the control group.

Statistical analysis

Statistical analysis was conducted using Microsoft Excel 2020 and IBM SPSS 20.0. The relationship between variables was evaluated with either Pearson chi-square or Fisher's exact test. A p-value of less than 0.05 was deemed statistically significant. Both univariate and multivariate logistic regression analyses were performed to explore the association between CIN2+ and various risk factors. The multivariate analysis included only those variables that demonstrated a significant association in the univariate analysis ($p < 0.05$). The odds ratios were calculated to assess the risk of developing CIN2+ based on different risk factors.

Ethical considerations

The study was carried out in accordance with the Declaration of Helsinki. Before being included, all women read the "Information about the study" and signed an informed consent form to participate.

The study has been approved by the Riga Stradiņš University Ethical Committee, the Central Ethical Committee, and the Riga Clinical University Hospital Scientific Department

2 Results

The study group included 31 (28.2 %) women with CIN1, 57 (51.8 %) with CIN2, 21 (19.1 %) with CIN3, and 1 (0.9 %) with cervical cancer.

2.1 Sociodemographic and behavioural factors

The participants' ages ranged from 19 to 59 years. The mean age of women in the study group was 35.0 years (±9.3), while it was 37.1 years (±8.0) in the control group ($p = 0.059$).

In the age group ≤ 30 years, CIN1 and "all CIN" occurred more frequently than "no CIN" ($p = 0.032$ and $p = 0.039$, respectively). No other significant differences between age groups and CIN severity were found.

Women in the control group more frequently had higher education and did not smoke. Conversely, women with any degree of CIN were more likely to smoke.

No statistically significant differences were observed between the groups regarding marital status and contraception methods. However, 43.6 % of participants in the study group did not use any contraception, compared to 34.2 % in the control group. Additionally, 24.5 % of the study group used a barrier method, specifically a male condom, versus 34.2 % in the control group.

2.2 Vaginal ecosystem

Increased vaginal pH > 4.4 was observed more frequently in all CIN severity grades.

Abnormal vaginal microflora was significantly more often diagnosed in the study group compared to the control group (50 % vs. 35 %, $p = 0.023$) and in CIN2+ compared to healthy controls – 51.9 % vs. 35 %, respectively ($p = 0.019$)

When analysing different lactobacillary grades, LBG I and IIa were the most common in women without CIN. LBG IIb was most often identified in CIN2+ patients. The number of LBG III BV and LBG III AV cases was almost equal in women with and without CIN. LBG III mixed BV-AV was characteristic primarily of patients with CIN. However, the differences between LBGs were not statistically significant.

Any-BV was found more frequently in CIN1 patients than in the control group (25.8 % vs. 7.7 %, $p = 0.01$) or in CIN2+ cases (25.8 % vs. 10.1 %, $p = 0.036$).

A difference in the presence of any AV was observed between the CIN2+ group and women with "no CIN" (36.7 % vs. 20.5 %, $p = 0.012$). The same trend was observed between "all CIN" and the control group, though it did not reach statistical significance (31.8 % vs. 20.5 %, $p = 0.052$). However, the proportion of msAV was notably higher in CIN2+ cases compared to women without CIN (16.5 % vs. 5.1 %, $p = 0.009$), and also higher in "all CIN" compared to the control group (13.6 % vs. 5.1 %, $p = 0.038$) (Table 2.1).

Table 2.1

Vaginal pH and microflora

Factor	no CIN n = 117 n (%)	CIN1 n = 31 n (%)	CIN2+ n = 79 n (%)	all CIN n = 110 n (%)	P value no CIN vs. all CIN	P value no CIN vs CIN1	P value no CIN vs CIN2+	P value CIN 1 vs CIN2+
pH								
≤ 4.4	95 (81.2)	18 (58.1)	39 (49.4)	57 (51.8)	< 0.0001	0.007	< 0.0001	0.411
> 4.4	22 (18.8)	13 (41.9)	40 (50.6)	53 (48.2)				
Lactobacillary grade (LBG)								
LBG I	40 (34.2)	8 (25.8)	18 (22.8)	26 (23.6)	0.121	0.090	0.184	0.488
LBG IIa	36 (30.8)	9 (29.0)	20 (25.3)	29 (26.4)				
LBG IIb	21 (17.9)	4 (12.9)	22 (27.8)	26 (23.6)				
LBG III BV	5 (4.3)	2 (6.5)	3 (3.8)	5 (4.6)				
LBG III AV	12 (10.2)	3 (9.7)	10 (12.7)	13 (11.8)				
LBG III BV-AV	3 (2.6)	5 (16.1)	6 (7.6)	11 (10.0)				
Microflora								
Normal	76 (65.0)	17 (54.8)	38 (48.1)	55 (50.0)	0.023	0.300	0.019	0.525
Abnormal	41 (35.0)	14 (45.2)	41 (51.9)	55 (50.0)				
Presence of bacterial vaginosis (BV)								
No BV	108 (92.3)	23 (74.2)	71 (89.9)	94 (85.5)	0.099	0.010	0.553	0.036
Any BV	9 (7.7)	8 (25.8)	8 (10.1)	16 (14.5)				
Presence of aerobic vaginitis (AV)								
No AV	93 (79.5)	25 (80.6)	50 (63.3)	75 (68.2)	0.052	0.887	0.012	0.079
Any AV	24 (20.5)	6 (19.4)	29 (36.7)	35 (31.8)				
msAV	6 (5.1)	2 (6.5)	13 (16.5)	15 (13.6)	0.038	0.674	0.009	0.225

2.3 HR-HPV DNA and E6/E7 mRNA

60.9 % were HR-HPV DNA positive in the “all CIN” group, 51.6 % in CIN1, and 64.6 % in CIN2+ compared to 7.7 % in healthy controls.

Women with any grade of CIN were more likely to test positive for HR-HPV E6/E7 mRNA expression compared to women without CIN (Table 2.2).

Table 2.2

HR-HPV DNA and E6/E7 mRNA analysis

Factor	no CIN n = 117 n (%)	CIN1 n = 31 n (%)	CIN2+ n = 79 n (%)	all CIN n = 110 n (%)	P value no CIN vs. all CIN	P value no CIN vs CIN1	P value no CIN vs CIN2+	P value CIN 1 vs CIN2+
HR-HPV DNA of the six most common types								
Negative	108 (92.3)	15 (48.4)	28 (35.4)	43 (39.1)	< 0.0001	< 0.0001	< 0.0001	0.211
Positive	9 (7.7)	16 (51.6)	51 (64.6)	67 (60.9)				
HR-HPV E6/E7 mRNA								
Negative	103 (88)	13 (41.9)	8 (10.1)	21 (19.1)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Positive	14 (12)	18 (58.1)	71 (89.9)	89 (80.9)				

2.4 Vaginal infections

The study identified six positive cases of *C. trachomatis* and four in the control group (p = 0.160). No *N. gonorrhoeae* cases were found in either group. *T. vaginalis* was diagnosed in two cases, and *M. genitalium* in one among CIN2+ patients. *M. hominis* was more prevalent in the "all CIN" and CIN 2+ groups compared to women without CIN (8.2 % vs. 1.7 %, p = 0.016; and 8.9 % vs. 1.7 %, p = 0.032). *U. parvum* and *U. urealyticum* showed no significant difference between the groups.

Analysis of the combination of all STIs and non-STI *Mycoplasmas* didn't show statistically significant differences between the groups.

2.5 Univariate and multivariate logistic regression

When analysing different factors separately, the most significant associations with the CIN2+ were with the positive E6/E7 HPV mRNA (OR 65.3, 95 % CI 26.0–163.8, p < 0.0001), HR-HPV DNA (OR 21.9, 95 % CI 9.6–49.7, p < 0.0001). Conversely, women with higher education levels had a reduced risk of CIN2+ (OR 0.3, 95 % CI 0.17–0.58, p < 0.0001) (Table 2.3).

Table 2.3

Univariate logistic regression CIN2+ compared to "no CIN"

	Odds ratio	95 % Confidence Interval	P value
High education level	0.3	0.17–0.58	< 0.0001
Smoking	4.7	2.2–10.3	< 0.0001
Positive HR-HPV DNA of the six most common types	21.9	9.6–49.7	< 0.0001

Table 2.3 continued

	Odds ratio	95 % Confidence Interval	P value
Positive HR-HPV E6/E7 mRNA expression	65.3	26.0 – 163.8	< 0.0001
pH	4.4	2.3–8.4	< 0.0001
M. hominis	5.6	1.1–27.7	0.035
Abnormal microflora	2.0	1.1–3.6	0.020
anyAV	2.2	1.2–4.3	0.013
msAV	3.6	1.3–10.0	0.012

Multivariate logistic regression identified key factors linked to CIN2+, including positive HR-HPV E6/E7 mRNA expression (OR 59.4, 95 % CI 14.84–237.51, p < 0.0001) and positive HR-HPV DNA (OR 3.9, 95 % CI 1.16–13.23, p = 0.028). Additionally, a higher level of education was associated with a reduced risk of CIN2+ (OR 0.2, 95 % CI 0.07–0.71, p = 0.012) (Table 2.4).

Table 2.4

Multivariate logistic regression CIN2+ compared to "no CIN"

	Odds ratio	95 % Confidence Interval	P value
High education level	0.2	0.07–0.71	0.012
Smoking	1.7	0.46–6.49	0.414
Positive HR-HPV DNA status of the six most common types	3.9	1.16–13.23	0.028
Positive HR-HPV E6/E7 mRNA expression	59.4	14.84–237.51	< 0.0001
pH	2.2	0.66–7.35	0.199
M.hominis	1.66	0.08–32.09	0.776
Abnormal microflora	1.3	0.31–5.41	0.723
anyAV	0.73	0.15–3.57	0.696
msAV	3.4	0.37–31.31	0.283

2.6 HLA class II genotyping

The study population included 57 patients with "no CIN," 23 with histologically confirmed CIN 1, and 61 cases of CIN2+. A total of 114 alleles were analysed in women with "no CIN," 46 in those with CIN1, and 122 in women with CIN2+.

The most common alleles in the "no CIN" and CIN1 groups were DQB1*06:02-8 (30/114, 26,3 % and 18/46, 39.1 %, respectively) and DQB1*03:01 (30/114, 26,3 % and 13/46, 28,3 %, respectively). In the CIN2+ group, the prevalent alleles were DQB1*03:01 (50/122, 41,0 %) and DQA1*05:01 (33/122, 27,0 %).

CIN2+ was more frequently linked with DQA1*04:01 (OR 6.68, 95 % CI 1.47–30.29, p = 0.014), DRB*15:01 (OR 2.99, 95 % CI 1.22–7.39, p = 0.017), DQB1*04:01 (OR 2.91, 95 % CI 1.11–7.68, p = 0.03), DQA1*01:03 (OR 2.72, 95 % CI 1.02–7.21, p = 0.045),

DRB1*11:01 (OR 2.42, 95 % CI 1.10–5.33, p = 0.029), and DQB1*03:01 (OR 1.94, 95 % CI 1.12–3.38, p = 0.018).

Women with "no CIN" more frequently had DQB1*05:01 (OR 0.17, 95 % CI 0.04–0.81, p = 0.026), DRB1*16:01 (OR 0.21, 95 % CI 0.06–0.78, p = 0.019), DQA1*03:01 (OR 0.35, 95 % CI 0.14–0.87, p = 0.024), and DRB1*14:01 (OR 0.59, 95 % CI 0.01–0.46, p = 0.007) (Table 2.5).

Table 2.5

Risk estimates of CIN2+ compared to "no CIN"

Alleles	Odds ratio (OR)	95 % Confidence Interval	P value
DRB1*11:01	2.42	1.10–5.33	0.029
DRB1*14:01	0.59	0.01–0.46	0.007
DRB1*15:01	2.99	1.22–7.39	0.017
DRB1*16:01	0.21	0.06–0.78	0.019
DQA1*01:03	2.72	1.02–7.21	0.045
DQA1*03:01	0.35	0.14–0.87	0.024
DQA1*04:01	6.68	1.47–30.29	0.014
DQB1*03:01	1.94	1.12–3.38	0.018
DQB1*04:01	2.91	1.11–7.68	0.03
DQB1*05:01	0.17	0.04–0.81	0.026

Discussion

The results of this study confirm the central role of HR-HPV infection, particularly HR-HPV DNA and E6/E7 mRNA expression, in the development of CIN2+. Importantly, aerobic vaginitis emerged as a strong independent factor associated with high-grade cervical lesions. In addition, specific HLA class II alleles were associated with either increased susceptibility or a protective effect against CIN2+. The strength of this study lies in the histological verification of all CIN diagnoses and the integrated analysis of viral, microbial, genetic, and social determinants of cervical neoplasia.

Sociodemographic and behavioural factors

Low education level emerged as one of the most critical factors associated with CIN2+, with women in the control group more likely to have higher education. This finding aligns with previous research, which has identified lower education as a key risk factor for cervical neoplasia, with a higher prevalence of CIN across all grades among women with limited formal education. (Caiyan et al., 2012; Parikh et al., 2003; Tao et al., 2014) A lower level of education is often associated with reduced health literacy, which can lead to decreased awareness of cervical cancer risk factors, symptoms, and the significance of regular screening. (Low et al., 2012) Additionally, women with less education may be more likely to have high-risk sexual behaviours, like an earlier age at sexual debut, multiple sexual partners, and inconsistent contraception use (Rada, 2014), which significantly increases the risk of HR-HPV infection. (Hariri et al., 2011; Trottier and Franco, 2006)

National Latvian data further support these associations. Among women who received cervical cancer screening invitations, 74 % responded; however, the proportion of screened women was lower among those with only primary education. (Ķīvīte-Urtāne, 2023) Another indicator of health literacy in Latvia is knowledge about sexually transmitted infections, particularly HIV. National data reveal that only one-third of respondents possess a minimally satisfactory understanding of HIV, with the lowest knowledge levels found among individuals with lower education and income, as well as among youth. Alarmingly, since 2003, the proportion of young people with moderate knowledge about HIV has declined (Ķīvīte-Urtāne, 2023), possibly due to the removal of health education as a separate school subject.

Although health literacy and sexual behaviour were not directly assessed in this study, observed patterns of contraceptive use provide indirect evidence. Women with CIN were less likely to use condoms and more often reported no contraception, aligning with national trends.

Smoking is a major cervical precancer and cancer risk factor. This has been proven in many studies, including meta-analysis. (Nagelhout et al., 2021; Plummer et al., 2003; Roura

et al., 2014) In our research, smoking was also more common among women with cervical precancerous disease. As a recognised risk factor for cervical cancer, smoking impacts both humoral and cellular immunity in the vagina and cervix. A disrupted cellular response can impair HPV clearance, leading to persistent infection and progression to more severe conditions. (Eldridge et al., 2017; Haverkos et al., 2003; Plummer et al., 2003; Sopori, 2002) Additionally, smoking could serve as an indirect marker of education level.

Vaginal ecosystem

Growing evidence indicates that alterations in the vaginal microbiome contribute to HPV persistence and cervical neoplasia. Healthy vaginal flora is typically dominated by *Lactobacillus species*, whereas cervical lesions are associated with reduced lactobacilli and increased microbial diversity (Mitra et al., 2015; Norenhag et al., 2020; Chen et al., 2020). High-grade lesions, in particular, are associated with increased abundance of anaerobic and aerobic bacteria, such as *Sneathia, Anaerococcus*, and *Peptostreptococcus* (Mitra et al., 2015; Wen et al., 2025). However, the question remains whether the alterations in the microbiome are causing the progression of HPV infection toward precancer and cancer, or if the causality is reversed around. *Mitra et al.* found that women who underwent excisional treatment for high-grade squamous intraepithelial lesion (HSIL) maintained the same vaginal microbiota profile before and after treatment, supporting the notion that an altered vaginal microbiome is a crucial CIN risk factor. (Mitra et al., 2021)

This study demonstrated that elevated vaginal pH and abnormal vaginal microflora were associated with CIN2+. These findings are consistent with previous reports linking increased vaginal pH to HPV detection and high-grade cervical lesions (Clarke et al., 2012; Teng & Hao, 2020). Although elevated pH reflects changes in the vaginal environment rather than acting as a direct carcinogenic factor, recent experimental data suggest that higher pH may facilitate HPV adherence to epithelial cells (Liu & Li, 2024).

According to our results, abnormal vaginal microflora itself is a CIN2+ risk factor. However, different types of it could be associated with CIN differently. The role of bacterial vaginosis as a risk factor for cervical squamous lesions has been a subject of debate for many years. Despite numerous studies, the results are contradictory. Some have reported a significantly increased incidence of CIN in women with bacterial vaginosis, although not all studies address the associations with the severity of CIN. (Gillet et al., 2012; Liang et al., 2019; Nam et al., 2009) In contrast, one study reported that BV was not only not associated with cervical precancer but could even prevent it. (Long et al., 2023) A meta-analysis identified BV as a significant risk factor for HPV infection (OR 2.62, 95 % CI 1.84–3.73, $p < 0.05$), but only

a modest risk factor for CIN (OR 1.56, 95 % CI 1.21–2.00, $p < 0.05$). (Liang et al., 2019) This study observed a correlation between BV and CIN1 but not with CIN2+. Low-grade CIN can result from both high-risk and low-risk HPV types. (Moscicki et al., 2001; Steben & Duarte-Franco, 2007) Additionally, engaging in sexual behaviour, especially with a new partner, is the primary risk factor for HPV infection. (Moscicki et al., 2001) These findings imply that BV might act as an indirect indicator of HPV sexual transmission instead of directly contributing to HPV infection progression. However, since the study did not evaluate participants' sexual activity and behaviours, we cannot confirm this assumption.

Aerobic vaginitis is a relatively lesser-known form of abnormal vaginal microflora, and its relationship with cervical disease remains underexplored. This study identified an association between AV-related bacterial changes and histologically confirmed CIN. Notably, the severity of AV was correlated with the grade of cervical lesions, with moderate to severe AV strongly associated with CIN2+, whereas BV showed no similar link. Several other studies have also observed a connection between AV and abnormal cervical conditions cytology. (Jahic et al., 2013; Vieira-Baptista et al., 2016) *Vieira-Baptista et al.* have also described an essential link between inflammation caused by AV and cervical neoplasia. (Vieira-Baptista et al., 2016) The inflammatory features common to msAV and HPV-induced cervical dysplastic lesions are crucial in the progression toward invasive cancer. Inflammation is a key mechanism of HPV-driven carcinogenesis (Boccardo et al., 2010), as evidenced by elevated levels of inflammatory interleukins in individuals with CIN. (Iwata et al., 2015; Kemp et al., 2010). Moreover, cervical inflammation contributes to genotoxic damage through oxidative stress and changes in the cervical tissue microenvironment. (Castle & Giuliano, 2003; Matos et al., 2018) Similarly, AV is marked by varying degrees of inflammation, including an increased presence of vaginal leukocytes, immune-active "toxic leukocytes," and significantly elevated levels of IL-1β and IL-6. (Donders et al., 2002) In this study, aside from the connection with msAV, a positive but non-significant relationship was also noted between the extent of leucocytosis and CIN2+ patients. The most effective diagnostic method for aerobic vaginitis is wet-mount microscopy, which was evaluated using the modified *Donders-Schroeder* scale, incorporating a composite AV score. (Donders et al., 2002; Wilson et al., 2018) While classical Gram-stained microscopy is highly effective for diagnosing BV and vulvovaginal candidiasis, it lacks specific diagnostic criteria for AV. (Wilson et al., 2018) As a result, relying solely on Gram-staining to assess the vaginal ecosystem may lead to missed AV diagnoses. Furthermore, other studies have emphasised the need for greater attention to AV and its potential association with cervical neoplasia. (Bi et al., 2023)

High-risk HPV DNA and E6/E7 mRNA

HR-HPV infection is a necessary prerequisite for cervical cancer development (Bosch et al., 1995). This study identified a nearly fourfold increased risk of CIN2+ among women positive for HR-HPV DNA. However, approximately one-third of CIN2+ cases were HR-HPV DNA-negative, likely because the analysis was limited to only the six most common HR-HPV types. These genotypes (16, 18, 31, 33, 45, 58) are responsible for over 90 % of invasive cervical cancer cases. (Wei et al., 2024) Another possible explanation is that low-risk HPV types may also play a role in the development of CIN1-2 lesions. (Peto et al., 2004) In the "no CIN" group, nearly 8 % of individuals tested HR-HPV DNA positive, which could represent women with either newly acquired infections or those in whom cellular transformation has yet to occur. Other studies suggest that 5.1–29.3 % of cytologically normal smears may test positive for HR-HPV, varying based on the population and geographic region. (Peto et al., 2004)

Detection of E6/E7 mRNA emerged as the strongest marker associated with CIN2+, reflecting transcriptionally active HPV infection. Expression of E6 and E7 oncogenes disrupts p53 and pRB tumor suppressor pathways, driving cellular transformation (Hoppe-Seyler et al., 2018; Gupta et al., 2022). Numerous studies support the clinical utility of E6/E7 mRNA testing for identifying transforming infections and triaging HPV-positive women (Campbell et al., 2013; Ren et al., 2018). Although the present study demonstrated a strong association, wide confidence intervals suggest limited statistical precision due to sample size.

Vaginal infections

Chlamydia trachomatis was the most common sexually transmitted infection in our study population, but no significant differences were observed between groups. However, previous studies have reported a link between *C. trachomatis* infection and cervical precancerous lesions. (Escarcega-Tame et al., 2020; Lehtinen et al., 2011; Madaan et al., 2019) The findings of this study did not replicate these results. However, the higher prevalence observed in the CIN1 group might be due to the transient nature of HPV infection at this stage, as well as the strong association between sexual behaviour and high-risk HPV acquisition. (Moscicki et al., 2001) The study of *Jensen et al*. reports similar results and finds no link between the detection of *C.trachomatis* DNA and the risk of HSIL among HPV-positive women. However, repeated chlamydiosis was related to a higher risk of CIN3+. (Jensen et al., 2014) Nonetheless, a systematic review concluded that *C.trachomatis* co-infection can increase cervical cancer risk, and therefore, it would be recommended to test HPV-positive women for *Chlamydia* infection. (Karim et al., 2018)

T. vaginalis and *M. genitalium* cases in this study were found exclusively in the CIN2+ group, but their low numbers preclude meaningful correlations. In the literature, *T. vaginalis* is identified as a risk factor for cervical cancer. (Hamar et al., 2023; Yang et al., 2018) Data on *M. genitalium* remains inconclusive. A 2018 meta-analysis found a link between *M. genitalium* and high-risk HPV infection, although it did not find an association with cervical cytopathology. (Ye et al., 2018)

Among *Mycoplasma* species, *M. hominis* showed a significant univariate association with CIN2+, though this did not persist in multivariate analysis. Previous research indicates that *Mycoplasma* species may facilitate HPV persistence, but their independent role in cervical carcinogenesis remains unclear (Lopez-Arias et al., 2019; Ye et al., 2018). Shared sexual behavioural risk factors likely contribute to the co-occurrence of HPV and vaginal infections, though biological interactions cannot be excluded. Therefore, screening for these infections in the HPV-positive population could be advisable to identify high-risk women. Whether treatment of STI can lower disease progression and facilitate HPV clearance requires future research.

HLA class II genes

This study is the first to investigate the link between HLA class II genotypes and various grades of cervical intraepithelial neoplasia among Latvian women, making it one of the initial analyses of this kind in the Baltic region. The findings suggest a strong association between *HLA-DRB1*11:01*, *DRB1*15:01*, *DQA1*01:03, DQA1*04:01*, *DQB1*03:01, and DQB1*04:01* and CIN2+. In contrast, *HLA-DRB1*14:01*, *DRB1*16:01*, and *DQA1*03:01* were more frequently observed in the "no CIN" group, suggesting a potential protective role.

Consistent with previous studies, *HLA-DRB1*15:01* has been associated with cervical cancer across various populations. (Bao et al., 2018; Chen et al., 2013; Hu et al., 2014; Krul et al., 1999; Leo et al., 2017; Rathika et al., 2016) This allele was notably more prevalent among patients with CIN1 and CIN2+ in this research. Notably, the *DQA1*04:01* allele was six times more prevalent in the CIN2+ group than the "no CIN" group, though the wide 95 % confidence interval suggests the small sample size could impact this. This allele has not been linked to cervical disease before. Similarly, *DQB1*04:01* was identified as significantly associated with CIN2+, with only one prior study documenting its presence in 2 out of 47 healthy individuals. (Rathika et al., 2016) Additionally, *DRB1*11:01* was associated with more than a twofold increased risk of CIN2+, a finding also found in the British population. (Odunsi et al., 1996) The *DQB1*03:01* allele, previously linked to high-grade disease and cervical cancer in British, Spanish, and Norwegian populations, was also more often observed in our CIN2+ cohort

compared to healthy participants. (Cuzick et al., 2000; Lie et al., 1999; Montoya et al., 1998; Odunsi et al., 1996) In contrast to previous genome-wide association studies that identified *DQB1*06:02* as a risk allele, we found this allele to be nearly equally distributed between the "no CIN" and CIN2+ groups, with a higher prevalence in CIN1 patients. (Chen et al., 2013; Leo et al., 2017)

While other European studies have reported associations between *DRB1*04:01, DRB1*04:03, DQB1*03:02, DQB1*04:02*, and *DQB1*06:03* with increased risk of CIN and cervical cancer, we did not observe similar trends in our study population. (Cuzick et al., 2000; Montoya et al., 1998; Odunsi et al., 1996) However, we identified *DRB1*14:01, DRB1*16:01*, and *DQA1*03:01* notably more frequently in the "no CIN" group, suggesting a potential protective role that was not previously documented. Additionally, *DQB1*05:01* demonstrated a protective effect against CIN2 or worse, a finding consistent with studies about the British population. (Cuzick et al., 2000; Odunsi et al., 1996) Conversely, *DRB1*13:01* and *DQB1*06:03*, which have been widely reported as protective alleles in previous studies, did not show a similar effect in the present study. (Chen et al., 2013; Chen & Gyllensten, 2015; de Araujo Souza et al., 2009; de Freitas et al., 2012; Leo et al., 2017; Zoodsma et al., 2005) Unexpectedly, *DQA1*01:03*, previously described as a protective allele, was 2.5 times more common in the CIN2+ group compared to the "no CIN" group in this study. (Chen et al., 2013; Leo et al., 2017)

HLA polymorphisms are highly population-specific and reflect inherited immunogenetic susceptibility rather than modifiable risk (Trowsdale, 2011). These differences underscore the importance of studying underrepresented populations and suggest that population-specific genetic risk profiles may inform long-term risk stratification.

Personalised screening and artificial intelligence

The chance of developing cervical cancer after an HPV infection is relatively low. (Woodman et al., 2007) Identifying women at the highest risk is therefore essential. Evidence increasingly supports a multifactorial model of cervical carcinogenesis involving viral, microbial, host genetic, and behavioural factors. (Kyrgiou et al., 2017) Therefore, relying on HPV status alone is insufficient. Integrating various determinants – such as virus genotype, smoking, immune response, and co-infections – is crucial for developing comprehensive risk prediction models. Artificial intelligence (AI) has recently become a promising tool in this context. Artificial intelligence-based models integrating these variables show promise for individualised risk assessment and precision screening. (Aballéa et al., 2020; Mehmood et al., 2021)

Risk-based screening strategies are already being implemented in some countries, such as the United States, where management is guided by estimated CIN3+ risk rather than test results alone. (Perkins et al., 2020) HPV vaccination further necessitates adaptation of screening algorithms, as genotype prevalence shifts in vaccinated populations. (Gray et al., 2025) For countries with persistently high cervical cancer incidence, such as Latvia, personalised, risk-adapted screening could improve prevention effectiveness and resource allocation. (Stankunas et al., 2022)

Limitations

The main limitation of this study is its relatively small sample size, primarily due to financial constraints, which may affect the reliability and statistical power of the results, however, sample size calculations indicate that the number of participants remains sufficient to achieve statistically significant results. All patients underwent colposcopy by certified specialists, and all cases with suspected cervical neoplasia were subsequently subjected to histological examination, thereby increasing diagnostic precision. The cross-sectional design also restricts the ability to assess lesion progression over time. The analysis was limited to the six HR-HPV genotypes, but these types are most important in the pathogenesis of CIN. Moreover, the absence of data on sexual behaviour and infection risk factors hinders a complete understanding of the relationship between vaginal infections and HPV. Though this does not significantly influence the relationship between aerobic vaginitis and CIN, it is one of the most important findings in this study. Wet-mount microscopy, being a relatively subjective method, exhibits intra- and inter-observer variability that can limit its reliability. The method can be influenced by the technique, device, scoring system used, and the performer's skills. (Vieira-Baptista et al. 2021) However, in this study, Donder's modification of Schröder's scale was used to describe vaginal microflora. It provides a structural approach to vaginal composition and allows distinction between lactobacillary-dominated flora and abnormal microflora, including bacterial vaginosis, aerobic vaginitis, and mixed infection. (Donders, 1999; Donders et al., 2002) Use of standardised criteria improves diagnostic accuracy. (Donders et al., 2015). Moreover, randomly selected slides were double-checked by a more experienced microscopy specialist assoc. prof. Jana Zodzika, which improves diagnostic quality.

Conclusions

1 Women diagnosed with cervical precancer in this study exhibit a higher prevalence of increased vaginal pH and disturbances in the vaginal microbiome compared to healthy individuals. Notably, bacterial vaginosis demonstrates a significant correlation with low-grade precancerous lesions, while aerobic vaginitis – especially in moderate to severe stages – is strongly associated with CIN2+.

2 The presence of high-risk HPV DNA and E6/E7 mRNA is the most important factor for high-grade cervical neoplasia in the current investigation. No statistically significant associations were identified between sexually transmitted infections and CIN. However, among other vaginal infections, *M. hominis* emerged as the third most important independent risk factor for CIN2+.

3 A notable link was found in this research between certain HLA alleles – specifically, *HLA-DRB1*11:01, DRB1*15:01, DQA1*01:03, DQA1*04:01, DQB1*03:01*, and *DQB1*04:01* – and a higher risk of CIN2+. Conversely, alleles like *HLA-DRB1*14:01, DRB1*16:01*, and *DQA1*03:01* were more common in women without CIN, indicating they might offer some protection against cervical neoplasia.

4 Smoking is significantly associated with cervical intraepithelial neoplasia across all severity grades and is one of the most important independent risk factors for CIN2+ in the present study. Conversely, a higher education level appears to be a protective effect against the development of CIN.

Proposals

1 Develop and implement personalised, risk-based cervical cancer screening models that incorporate viral, microbial, genetic, and behavioural factors to enhance diagnostic accuracy and optimise resource use.

2 Enhance public health efforts to promote smoking cessation, sexual health awareness, and education on the significance of regularly participating in cervical screening programmes.

3 Further investigate pH measurement and assessment of abnormal flora, such as aerobic vaginitis, alongside HPV testing in women at increased risk of cervical neoplasia, and explore the potential for reducing the risk by restoring a normal vaginal microenvironment.

4 Investigate the application of HLA class II genotyping in assessing cervical cancer risk to identify women with a genetic predisposition to high-grade cervical lesions and aid in developing population-specific genetic risk profiles.

5 Conduct longitudinal and mechanistic studies on the roles of aerobic vaginitis, *Mycoplasma hominis*, and HLA polymorphisms in cervical carcinogenesis to develop future personalised prevention strategies.

List of publications, reports and patents on the topic of the Thesis

Publications:

1. Plisko, O., Zodzika, J., Jermakova, I., Pcolkina, K., Prusakevica, A., Liepniece-Karele, I., Zariņa M., Storozenko, J., Rezeberga, D. (2024). Predicition of high-grade cervical precancerous abnormalities: the role of personal factors, vaginal microflora, sexually transmitted infections, and high-risk human papillomavirus. *PLoS ONE* 19(11): e0313004. doi:10.1371/journal.pone.0313004.
2. Plisko, O., Zodzika, J., Jermakova, I., Liepniece-Karele, I., Eglite, J., Rezeberga, D. (2024). Human Leucocyte Antigen Class II Risk and Protective Alleles in Women with Cervical Intraepithelial Neoplasia. *Acta Medica Lituanica* 31(1):16–22. doi:10.15388/Amed.2024.31.1.1.
3. Plisko, O., Zodzika, J., Jermakova, I., Pcolkina, K., Prusakevica, A., Liepniece-Karele, I., Donders, G.G.G., Rezeberga, D. (2021). Aerobic vaginitis – underestimated risk factor for cervical intraepithelial neoplasia. *Diagnostics* 11(1), 97. doi:10.3390/diagnostics11010097.

Reports and theses at international congresses and conferences:

1. Plisko, O., Zodzika, J., Jermakova, I., Pcolkina, K., Araja, D., Kunicina, D., Rezeberga, D. 2023. Association between high-risk HPV E6/E7 mRNA expression and the severity of cervical precancerous lesions. Rīga Stradinš University International Research Conference on Medical and Health Sciences: Knowledge for Use in Practice. 29.–31.03.2023. Medicina (Kaunas) 2023;59(Supplement 2):52
2. Plisko, O., Zodzika, J., Jermakova, I., Osite, J., Priedite, M., Storozenko, J., Rezeberga, D. 2022. Association between high-risk HPV E6/E7 expression, sexually transmitted infections, and urogenital mycoplasmas in women with cervical intraepithelial neoplasia. IX European Federation for Colposcopy Congress: 08.–11.06.2022.
3. Plisko, O., Zodzika, J., Rezeberga, D., Jermakova, I., Liepniece-Karele, I., Kunicina, D., Araja, D., Eglite, J. 2021. Human Leucocyte Antigen class II risk and protective alleles in women with cervical intraepithelial neoplasia. 7th European Federation for Colposcopy Satellite Meeting: 08.05.2021.
4. Plisko, O., Zodzika, J., Rezeberga, D., Jermakova, I., Liepniece-Karele, I., Araja, D., Kunicina, D., Prusakevica, A., 2021. Association between vaginal microflora, high-risk HPV infection and HPV E6/E7 expression in high grade cervical intraepithelial neoplasia. 27th EBCOG European Congress of Obstetrics and Gynaecology: 02.–04.09.2021. European Journal of Obstetrics and Gynecology and Reproductive Biology, Volume 270, e31
5. Plisko, O., Zodzika, J., Rezeberga, D., Jermakova, I., Pcolkina, K., Prusakevica, A., Liepniece-Karele, I. 2021. Relation between aerobic vaginitis and histologically proven cervical intraepithelial neoplasia. Rīga Stradinš University International Research Conference on Medical and Health Sciences: Knowledge for Use in Practice: Abstracts 24.–26.03.2021, 27.
6. Plisko, O., Zodzika, J., Rezeberga, D., Jermakova, I., Liepniece-Karele, I., Kunicina, D., Sivina, D., Kasjko, D., Eglite, J. 2019. Human leukocyte antigen class II alleles and risk of cervical precancerous lesions. 03rd ISIDOG Congress: 31.10.–02.11.2019.
7. Plisko, O., Zodzika, J., Jermakova, I., Rezeberga, D., I., Sivina, D., Kunicina, D., Uspele, L. 2019. Association between sexually transmitted infections and cervical precancerous lesions in selected Latvian population. 03rd ISIDOG Congress: 31.10.–02.11.2019.
8. Plisko, O., Zodzika, J., Rezeberga, D., Jermakova, Eglite, J., Kasjko, D., I., Liepniece-Karele, I., Kunicina, D., Sivina, D. 2019. Association between Human Leukocyte Antigen Genes and Cervical Precancerous Lesions. Rīga Stradinš University International Research Conference on Medical and Health Sciences: Knowledge for Use in Practice: Abstracts, 01.–03.04.2019., 69.
9. Plisko, O., Zodzika, J., Rezeberga, D., Jermakova, I., Kroiza, J., Eglzte, L., Liepniece-Karele, I., Sivina, D., Kunicina, D., Senfelde., I. 2019. Association of different vaginal flora types with high risk human papillomavirus infection. Rīga Stradinš University International Research Conference on Medical and Health Sciences: Knowledge for Use in Practice: Abstracts, 01.–03.04.2019., 246.

10. Plisko, O., Zodzika, J., Rezeberga, D., Jermakova, I., Kroica, J., Eglite, L., Sivina, D., Kunicina, D. 2018. Associations between sexually transmitted infections and cervical precancerous lesions in Latvian women. 26th EBCOG European Congress of Obstetrics and Gynaecology: 08.–10.03.2018. European Journal of Obstetrics and Gynecology and Reproductive Biology, Volume 234, e153
11. Plisko, O., Jermakova, I., Rezeberga, D., Zodzika, J., Kroica, J., Eglite, L., Sivina, D., Kunicina, D. 2017. Association of abnormal cervical cytology with different types of vaginal microflora and HPV. 02nd ISIDOG Congress: 26.–29.10.2017.
12. Plisko, O., Zodzika, J., Rezeberga, D., Jermakova, I., Kroica, J., Eglite, L., Sivina, D., Kunicina, D. 2017. Associations between sexually transmitted infections and abnormal cervical cytology results in patients with cervical precancerous lesions. 25th EBCOG European Congress of Obstetrics and Gynaecology

Reports and theses at local congresses and conferences:

1. Plisko, O., Žodžika, J., Rezeberga, D., Jermakova, I., Kroiča, J., Eglīte, L., Sīviņa, D., Kunicina, D., Utorova, M., Pavlovska, I. 2018. Saistība starp *Mycoplasma genitalium* infekciju un dzemdes kakla priekšvēža saslimšanām Rīgas Kolposkopijas references centra pacientēm. Rīgas Stradiņa Universitātes 2018.gada Zinātniskā Konference: Tēzes grāmata, 22.–23.03.2018., 13.
2. Plisko, O., Žodžika, J., Rezeberga, D., Jermakova, I., Kroiča, J., Sīviņa, D., Kunicina, D., Eglīte, L., Utorova, M., Pavlovska, I., Šenfelde., I. 2018. Saistība starp multiplu cilvēka papillomas vīrusa infekciju un dzemdes kakla priekšvēža saslimšanām. 8. Latvijas dzemdību speciālistu un ginekologu kongress: 20.–21.04.2018.
3. Plisko, O., Žodžika, J., Rezeberga, D., Jermakova, I., Kroiča, J., Sīviņa, D., Kunicina, D., Eglīte, L., Utorova, M., Pavlovska, I., Šenfelde., I. 2018. Maksts vides īpatnības pacientēm ar dzemdes kakla priekšvēža saslimšanām. 8. Latvijas dzemdību speciālistu un ginekologu kongress: 20.–21.04.2018.
4. Plisko, O., Jermakova, I., Rezeberga, D., Žodžika, J., Eglīte, L., Utorova, M., Pavlovska, I. 2017. Maksts ekosistēmas īpatnības pacientēm ar izmainītu dzemdes kakla citoloģiju Rīgas Austrumu Klīniskās Universitātes Slimnīcas Kolposkopijas references centrā. RSU Zinātniskā konference: 06.–07.04.2017.

References

1. Aballéa, S., Beck, E., Cheng, X., Demarteau, N., Li, X., Ma, F., Neine, M., Zhao, F.-H. (2020). Risk factors for cervical cancer in women in China: A meta-model. *Women's Health*, 16, 1745506520940875. doi: 10.1177/1745506520940875

2. Arbyn, M., Roelens, J., Simoens, C., Buntinx, F., Paraskevaidis, E., Martin-Hirsch, P. P., Prendiville, W. J. (2013). Human papillomavirus testing versus repeat cytology for triage of minor cytological cervical lesions. *The Cochrane Database of Systematic Reviews*, 2013(3), CD008054. doi:10.1002/14651858.CD008054.pub2

3. Bao, X., Hanson, A. L., Madeleine, M. M., Wang, S. S., Schwartz, S. M., Newell, F., Pettersson-Kymmer, U., Hemminki, K., Tiews, S., Steinberg, W., Rader, J. S., Castro, F., Safaeian, M., Franco, E. L., Coutlée, F., Ohlsson, C., Cortes, A., Marshall, M., Mukhopadhyay, P., Leo, P. J. (2018). HLA and KIR Associations of Cervical Neoplasia. *The Journal of Infectious Diseases*, 218(12), 2006–2015. doi:10.1093/infdis/jiy483

4. Baumann, M., Doeberitz, M. von K. (2024). Obituary: Nobel Laureate Harald zur Hausen (1936–2023) – Who dedicated his life to unraveling the role of infectious agents in the pathogenesis of human cancers. *European Journal of Cancer*, 202. doi:10.1016/j.ejca.2024.113586

5. Bi, H., Zhang, D., Xiao, B. (2023). Association between human papillomavirus infection and common sexually transmitted infections, and the clinical significance of different Mycoplasma subtypes. Frontiers in Cellular and Infection Microbiology, 13, 1145215. doi: 10.3389/fcimb. 2023.1145215

6. Boccardo, E., Lepique, A. P., Villa, L. L. (2010). The role of inflammation in HPV carcinogenesis. In *Carcinogenesis*, 31(11), 1905–1912. doi:10.1093/carcin/bgq176

7. Bosch, F. X., Manos, M. M., Muñoz, N., Sherman, M. E., Jansen, A. M., Peto, J., Schiffman, M., Moreno, V., Kurman, R. J., Shah, K. V. (1995). Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *Journal of the National Cancer Institute*, 87(11), 796–802. doi: 10.1093/jnci/87.11.796

8. Caiyan, X., Weiyuan, Z., Minghui, W., Songwen, Z. (2012). Prevalence and risk factors of lower genital tract infections among women in Beijing, China. *Journal of Obstetrics and Gynaecology Research*, 38(1), 310–315. doi:10.1111/j.1447-0756.2011.01624.x

9. Campbell, L. M., Pitta, D. R., De Assis, A. M., Derchain, S. F. M., Campos, E. A., Sarian, L. O. Z. (2013). Retrieval of HPV oncogenes E6 and E7 mRNA from cervical specimens using a manual open technology protocol. *SpringerPlus*, 2(1), 473. doi:10.1186/2193-1801-2-473

10. Castle, P. E., Giuliano, A. R. (2003). Chapter 4: Genital tract infections, cervical inflammation, and antioxidant nutrients – assessing their roles as human papillomavirus cofactors. In Journal of the National Cancer Institute. Monographs. doi:10.1093/oxfordjournals.jncimonographs.a003478

11. Chen, D., Gyllensten, U. (2015). Lessons and implications from association studies and post-GWAS analyses of cervical cancer. *Trends in Genetics*, 31(1), 41–54. doi:10.1016/ j.tig.2014.10.005

12. Chen, D., Juko-Pecirep, I., Hammer, J., Ivansson, E., Enroth, S., Gustavsson, I., Feuk, L., Magnusson, P. K. E., McKay, J. D., Wilander, E. (2013). Genome-wide association study of susceptibility loci for cervical cancer. *Journal of the National Cancer Institute*, 105(9), 624–633. doi: 10.1093/jnci/djt051

13. Chen, Y., Qiu, X., Wang, W., Li, D., Wu, A., Hong, Z., Di, W., Qiu, L. (2020). Human papillomavirus infection and cervical intraepithelial neoplasia progression are associated with increased vaginal microbiome diversity in a Chinese cohort. *BMC Infectious Diseases*, 20(1), 629. doi:10.1186/s12879-020-05324-9

14. Clarke, M. A., Rodriguez, A. C., Gage, J. C., Herrero, R., Hildesheim, A., Wacholder, S., Burk, R., Schiffman, M. (2012). A large, population-based study of age-related associations between vaginal pH and human papillomavirus infection. *BMC Infectious Diseases*, 12(1), 33. doi: 10.1186/1471-2334-12-33

15. Cuzick, J., Terry, G., Ho, L., Monaghan, J., Lopes, A., Clarkson, P., Duncan, I. (2000). Association between high-risk HPV types, HLA DRB1* and DQB1* alleles and cervical cancer in British women. *British Journal of Cancer*, 82(7), 1348–1352. doi:10.1054/bjoc.1999.1103

16. de Araujo Souza, P. S., Sichero, L., Maciag, P. C. (2009). HPV variants and HLA polymorphisms: the role of variability on the risk of cervical cancer. *Future Oncology*, 5(3), 359–370. doi:10.2217/fon.09.8

17. de Freitas, A. C., Gurgel, A. P. A. D., Chagas, B. S., Coimbra, E. C., do Amaral, C. M. M. (2012). Susceptibility to cervical cancer: An overview. *Gynecologic Oncology*, 126(2), 304–311. doi:10.1016/j.ygyno.2012.03.047

18. Donders, G. G. (1999). Microscopy of the bacterial flora on fresh vaginal smears. *Infectious Diseases in Obstetrics and Gynecology*, 7(4), 177–179. doi:10.1155/S1064744999000290

19. Donders, G. G. G., Marconi, C., Bellen, G., Donders, F., Michiels, T. (2015). Effect of Short Training on Vaginal Fluid Microscopy (Wet Mount) Learning. *Journal of Lower Genital Tract Disease* 19(2):165-169. doi: 10.1097/LGT.0000000000000052

20. Donders, G. G. G., Vereecken, A., Bosmans, E. (2002). Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. *BJOG: An International Journal of Obstetrics and Gynaecology*, 109(1), 34–43. doi:10.1111/j.1471-0528.2002.00432.x

21. Donders, G. G., Vereecken, A., Salembier, G., Van Bulck, B., Spitz, B. (1996). Assessment of vaginal lactobacillary flora in wet mount and fresh or delayed gram's stain. *Infectious Diseases in Obstetrics and Gynecology*, 4(1), 2–6. doi:10.1155/S1064744996000026

22. Eldridge, R. C., Schiffman, M., Wentzensen, N., Pawlita, M., Waterboer, T., Wilson, L., Castle, P. E., Gravitt, P. E. (2017). Smoking and subsequent human papillomavirus infection: a mediation analysis. *Annals of Epidemiology*, 27(11), 724–730.e1. doi:10.1016/j.annepidem.2017.10.004

23. Escarcega-Tame, M. A., López-Hurtado, M., Escobedo-Guerra, M. R., Reyes-Maldonado, E., Castro-Escarpulli, G., Guerra-Infante, F. M. (2020). Co-infection between genotypes of the human papillomavirus and Chlamydia trachomatis in Mexican women. *International Journal of STD & AIDS*, 31(13), 1255–1262. doi:10.1177/0956462420947587

24. Gillet, E., Meys, J. F. A., Verstraelen, H., Verhelst, R., de Sutter, P., Temmerman, M., Broeck, D. Vanden. (2012). Association between Bacterial Vaginosis and Cervical Intraepithelial Neoplasia: Systematic Review and Meta-Analysis. *PLoS ONE*, 7(10), e45201. doi:10.1371/journal. pone.0045201

25. Gray, P., Wang, J., Kleppe, S. N., Elfström, K. M., Dillner, J. (2025). Population-based age-period-cohort analysis of declining Human Papillomavirus prevalence. *The Journal of Infectious Diseases*, 15;231(4):e638–e649. doi: 10.1093/infdis/jiaf032.

26. Gupta, S. M., Warke, H., Chaudhari, H., Mavani, P., Katke, R. D., Kerkar, S. C., Mania-Pramanik, J. (2022). Human Papillomavirus E6/E7 oncogene transcripts as biomarkers for the early detection of cervical cancer. *Journal of Medical Virology*, 94(7), 3368–3375. doi: 10.1002/jmv.27700

27. Hamar, B., Teutsch, B., Hoffmann, E., Hegyi, P., Váradi, A., Nyirády, P., Hunka, Z., Ács, N., Lintner, B., Hermánné, R. J., Melczer, Z. (2023). Trichomonas vaginalis infection is associated with increased risk of cervical carcinogenesis: A systematic review and meta-analysis of 470 000 patients. *International Journal of Gynecology & Obstetrics*, 163(1), 31–43. doi: 10.1002/ijgo.14763

28. Hariri, S., Unger, E. R., Sternberg, M., Dunne, E. F., Swan, D., Patel, S., Markowitz, L. E. (2011). Prevalence of genital human papillomavirus among females in the United States, the National Health And Nutrition Examination Survey, 2003-2006. *The Journal of Infectious Diseases*, 204(4), 566–573. doi:10.1093/infdis/jir341

29. Haverkos, H. W., Soon, G., Steckley, S. L., Pickworth, W. (2003). Cigarette smoking and cervical cancer: Part I: a meta-analysis. *Biomedicine & Pharmacotherapy*, 57(2), 67–77. doi:10.1016/ S0753-3322(03)00196-3

30. Hoppe-Seyler, K., Bossler, F., Braun, J. A., Herrmann, A. L., Hoppe-Seyler, F. (2018). The HPV E6/E7 Oncogenes: Key Factors for Viral Carcinogenesis and Therapeutic Targets. *Trends in Microbiology*, 26(2), 158–168. doi:10.1016/j.tim.2017.07.007

31. Hu, J. M., Sun, Q., Li, L., Liu, C. X., Chen, Y. Z., Zou, H., Pang, L. J., Zhao, J., Yang, L., Cao, Y. W., Cui, X. Bin, Qi, Y., Liang, W. H., Zhang, W. J., Li, F. (2014). Human leukocyte antigen-DRB1*1501 and DQB1*0602 alleles are cervical cancer protective factors among Uighur and Han people in Xinjiang, China. *International Journal of Clinical and Experimental Pathology*, 7(9), 6165–6171.

32. Iwata, T., Fujii, T., Morii, K., Saito, M., Sugiyama, J., Nishio, H., Morisada, T., Tanaka, K., Yaguchi, T., Kawakami, Y., Aoki, D. (2015). Cytokine profile in cervical mucosa of Japanese patients with cervical intraepithelial neoplasia. *International Journal of Clinical Oncology*, 20(1), 126–133. doi:10.1007/s10147-014-0680-8

33. Jahic, M., Mulavdic, M., Hadzimehmedovic, A., Jahic, E., Clinic, G. (2013). Association Between aerobic vaginitis, bacterial vaginosis and squamous intraepithelial lesion of low grade. *Medical Arhives*, 67(2), 94–96. doi:10.5455/medarh.2013.67.94-96

34. Jensen, K. E., Thomsen, L. T., Schmiedel, S., Frederiksen, K., Norrild, B., van den Brule, A., Iftner, T., Kjær, S. K. (2014). Chlamydia trachomatis and risk of cervical intraepithelial neoplasia grade 3 or worse in women with persistent human papillomavirus infection: a cohort study. *Sexually Transmitted Infections*, 90(7), 550. doi:10.1136/sextrans-2013-051431

35. Karim, S., Souho, T., Benlemlih, M., Bennani, B. (2018). Cervical cancer induction enhancement potential of Chlamydia trachomatis: a systematic review. *Current Microbiology*, 75(12), 1667–1674. doi: 10.1007/s00284-018-1439-7

36. Kemp, T. J., Hildesheim, A., García-Piñeres, A., Williams, M. C., Shearer, G. M., Rodriguez, A. C., Schiffman, M., Burk, R., Freer, E., Bonilla, J., Herrero, R., Pinto, L. A. (2010). Elevated systemic levels of inflammatory cytokines in older women with persistent cervical human papillomavirus infection. *Cancer Epidemiology Biomarkers and Prevention*, 19(8), 1954–1959. doi:10.1158/1055-9965.EPI-10-0184

37. Ķīvīte-Urtāne, A., Lazdāne, G., Demčenko, K., Gobiņa, I., Isajeva, L., Ivanovs, A., Kezika, S., Kursīte, M., Libora, I., Miķelsone, A. I., Ozoliņa, K., Penka, E., Rakša, V., Štolcere, I. E., Zīverte, M. L. (2023). Pētījums par Latvijas iedzīvotāju seksuālās un reproduktīvās veselības ietekmējošiem faktoriem un paradumiem. Pētījuma ziņojums. *Rīga: LR Veselības ministrija*.

38. Krul, E. J. T., Schipper, R. F., Schreuder, G. M. T., Fleuren, G. J., Kenter, G. G., Melief, C. J. M. (1999). HLA and susceptibility to cervical neoplasia. *Human Immunology*, 60(4), 337–342. doi:10.1016/S0198-8859(98)00127-X

39. Kyrgiou, M., Mitra, A., Moscicki, A.-B. (2017). Does the vaginal microbiota play a role in the development of cervical cancer? *Translational Research*, 179, 168–182. doi: 10.1016/ j.trsl.2016.07.004

40. Lehtinen, M., Ault, K. A., Lyytikainen, E., Dillner, J., Garland, S. M., Ferris, D. G., Koutsky, L. A., Sings, H. L., Lu, S., Haupt, R. M., Paavonen, J., for the FUTURE I, Group, I. I. S. (2011). Chlamydia trachomatis infection and risk of cervical intraepithelial neoplasia. *Sexually Transmitted Infections*, 87(5), 372–376. doi:10.1136/sti.2010.044354

41. Leo, P. J., Madeleine, M. M., Wang, S., Schwartz, S. M., Newell, F., Pettersson-Kymmer, U., Hemminki, K., Hallmans, G., Tiews, S., Steinberg, W. (2017). Defining the genetic susceptibility to cervical neoplasia—A genome-wide association study. *PLoS Genetics*, 13(8), e1006866. doi: 10.1371/journal.pgen.1006866

42. Liang, Y., Chen, M., Qin, L., Wan, B., Wang, H. (2019). A meta-analysis of the relationship between vaginal microecology, human papillomavirus infection and cervical intraepithelial neoplasia. *Infectious Agents and Cancer*, 14, 29. doi:10.1186/s13027-019-0243-8

43. Lie, A. K., Skarsvåg, S., Haugen, O. A., Skjeldestad, F. E., Olsen, A. O., Skovlund, E., Rønningen, K. S. (1999). Association between the HLA DQB1*0301 gene and human papillomavirus infection in high-grade cervical intraepithelial neoplasia. *International Journal of Gynecological Pathology*, 18(3), 206–210. doi:10.1097/00004347-199907000-00003

44. Liu, Y., Li, Z. (2024). Vaginal pH value can affect the susceptibility to human papillomavirus infection. *BMC Infectious Diseases*, 24(1), 176. doi: 10.1186/s12879-024-09074-w

45. Long, T., Zhang, C., He, G., Hu, Y., Lin, Z., Long, L. (2023). Bacterial Vaginosis Decreases the Risk of Cervical Cytological Abnormalities. *Cancer Prevention Research*, 16(2), 109–117. doi:10.1158/1940-6207.CAPR-22-0288

46. Lopez-Arias, M., Vazquez-Jimenez, S., Martinez-Abundis, E., Gomez-Crisostomo, N. P., Chavez-Blanco, A., Contreras-Paredes, A., De la Cruz-Hernandez, E. (2019). Genital association of human papillomavirus with *Mycoplasma* and *Ureaplasma spp*. in Mexican women with precancerous lesions. *International Journal of STD & AIDS*, 30(10), 969–977. doi:10.1177/0956462419855508

47. Low, E. L., Simon, A. E., Lyons, J., Romney-Alexander, D., Waller, J. (2012). What do British women know about cervical cancer symptoms and risk factors? *European Journal of Cancer*, 48(16), 3001–3008. doi:10.1016/j.ejca.2012.05.004

48. Madaan, N., Pandhi, D., Sharma, V., Bhattacharya, S. N., Guleria, K., Mishra, K., Bharadwaj, M. (2019). Association of abnormal cervical cytology with coinfection of human papillomavirus and Chlamydia trachomatis. *Indian Journal of Sexually Transmitted Diseases and AIDS*, 40(1), 57–63. doi:10.4103/ijstd.IJSTD_9_19

49. Matos, A., Silva, A. P. da, Medeiros, R., Bicho, M., Bicho, M. C. (2018). Microenvironment in Vagina as a Key-Player on Cervical Cancer: Interaction of Polymorphic Genetic Variants and Vaginal Microbiome as Co-Factors. *InTech*. doi:10.5772/intechopen.73108

50. Mehmood, M., Rizwan, M., Gregus Ml, M., Abbas, S. (2021). Machine Learning Assisted Cervical Cancer Detection. *Frontiers in Public Health*, 9, 788376. doi:10.3389/fpubh.2021.788376

51. Mitra, A., MacIntyre, D. A., Lee, Y. S., Smith, A., Marchesi, J. R., Lehne, B., Bhatia, R., Lyons, D., Paraskevaidis, E., Li, J. V., Holmes, E., Nicholson, J. K., Bennett, P. R., Kyrgiou, M. (2015). Cervical intraepithelial neoplasia disease progression is associated with increased vaginal microbiome diversity. *Scientific Reports* 5, 16865. doi:10.1038/srep16865

52. Mitra, A., MacIntyre, D. A., Paraskevaidi, M., Moscicki, A.-B., Mahajan, V., Smith, A., Lee, Y. S., Lyons, D., Paraskevaidis, E., Marchesi, J. R. (2021). The vaginal microbiota and innate immunity after local excisional treatment for cervical intraepithelial neoplasia. *Genome Medicine*, 13, 1–18. doi: 10.1186/s13073-021-00977-w

53. Montoya, L., Saiz, I., Rey, G., Vela, F., Clerici-Larradet, N. (1998). Cervical carcinoma: human papillomavirus infection and HLA-associated risk factors in the Spanish population. *European Journal of Immunogenetics*, 25(5), 329–337. doi:10.1046/j.1365-2370.1998.00108.x

54. Moscicki, A.-B., Hills, N., Shiboski, S., Powell, K., Jay, N., Hanson, E., Miller, S., Clayton, L., Farhat, S., Broering, J., Darragh, T., Palefsky, J. (2001). Risks for Incident Human Papillomavirus Infection and Low-Grade Squamous Intraepithelial Lesion Development in Young Females. *JAMA*, 285(23), 2995–3002. doi:10.1001/jama.285.23.2995

55. Nagelhout, G., Ebisch, R. M., Hel, O. Van Der, Meerkerk, G.-J., Magnée, T., Bruijn, T. De, Straaten, B. Van. (2021). Is smoking an independent risk factor for developing cervical intra-epithelial neoplasia and cervical cancer? A systematic review and meta-analysis. *Expert Review of Anticancer Therapy*, 21(7), 781–794. doi:10.1080/14737140.2021.1888719

56. Nam, K. H., Kim, Y. T., Kim, S. R., Kim, S. W., Kim, J. W., Lee, M. K., Nam, E. J., Jung, Y. W. (2009). Association between bacterial vaginosis and cervical intraepithelial neoplasia. *J Gynecol Oncol*, 20(1), 39–43. doi: 10.3802/jgo.2009.20.1.39

57. Norenhag, J., Du, J., Olovsson, M., Verstraelen, H., Engstrand, L., Brusselaers, N. (2020). The vaginal microbiota, human papillomavirus and cervical dysplasia: a systematic review and network meta-analysis. *BJOG: An International Journal of Obstetrics & Gynaecology*, 127(2), 171–180. doi: 10.1111/1471-0528.15854

58. Odunsi, K., Terry, G., Ho, L., Bell, J., Cuzick, J., Ganesan, T. S. (1996). Susceptibility to human papillomavirus-associated cervical intra-epithelial neoplasia is determined by specific HLA DR-DQ alleles. *International Journal of Cancer*, 67(5), 595–602. doi:10.1002/(SICI)1097-0215(19960904)67:5<595::AID-IJC1>3.0.CO;2-P

59. Parikh, S., Brennan, P., Boffetta, P. (2003). Meta-analysis of social inequality and the risk of cervical cancer. *International Journal of Cancer*. 105(5):687–91. doi:10.1002/ijc.11141

60. 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. (2020). 2019 ASCCP risk-based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors. *Journal of Lower Genital Tract Disease*, 24(2), 102–131. doi: 10.1097/LGT.0000000000000628

61. Peto, J., Gilham, C., Deacon, J., Taylor, C., Evans, C., Binns, W., Haywood, M., Elanko, N., Coleman, D., Yule, R., Desai, M. (2004). Cervical HPV infection and neoplasia in a large population-based prospective study: the Manchester cohort. *British Journal of Cancer*, 91(5), 942–953. doi:10.1038/sj.bjc.6602049

62. Plummer, M., Herrero, R., Franceschi, S., Meijer, C. J. L. M., Snijders, P., Bosch, F. X., de Sanjosé, S., Muñoz, N. (2003). Smoking and cervical cancer: pooled analysis of the IARC multi-centric case–control study. *Cancer Causes & Control*, 14(9), 805–814. doi:10.1023/B:CACO.000 0003811.98261.3e

63. Rada, C. (2014). Sexual behaviour and sexual and reproductive health education: a cross-sectional study in Romania. *Reproductive Health*, 11(1), 48. doi:10.1186/1742-4755-11-48

64. Rathika, C., Murali, V., Dhivakar, M., Kamaraj, R., Malini, R. P., Ramgopal, S., Balakrishnan, K. (2016). Susceptible and Protective Associations of HLA Alleles and Haplotypes with Cervical Cancer in South India. *Asian Pacific Journal of Cancer Prevention*, 17(5), 2491–2497.

65. Ren, C., Zhu, Y., Yang, L., Zhang, X., Liu, L., Ren, C. (2018). Diagnostic performance of HPV E6/E7 mRNA assay for detection of cervical high-grade intraepithelial neoplasia and cancer among women with ASCUS Papanicolaou smears. *Archives of Gynecology and Obstetrics*, 297(2), 425–432. doi:10.1007/s00404-017-4588-1

66. Roura, E., Castellsague, X., Pawlita, M., Travier, N., Waterboer, T., Margall, N., Bosch, F. X., De Sanjosé, S., Dillner, J., Gram, I. T. (2014). Smoking as a major risk factor for cervical cancer and pre-cancer: results from the EPIC cohort. *International Journal of Cancer*, 135(2), 453–466. doi: 10.1002/ijc.28666

67. Sopori, M. (2002). Effects of cigarette smoke on the immune system. *Nature Reviews Immunology*, 2(5), 372–377. doi:10.1038/nri803

68. Stankūnas, M., Pärna, K., Tisler, A., Ķīvīte-Urtāne, A., Kojalo, U., Zodzika, J., Baltzer, N., Nygard, J., Nygard, M., Uuskula, A. (2022). Cervical cancer in the Baltic states: can intelligent and personalized cancer screening change the situation? *Acta Medica Lituanica*, 29(1), 19. doi: 10.15388/Amed.2022.29.1.18

69. Steben, M., Duarte-Franco, E. (2007). Human papillomavirus infection: epidemiology and pathophysiology. *Gynecologic Oncology*, 107(2), S2–S5. doi: 10.1016/j.ygyno.2007.07.067

70. Tao, L., Han, L., Li, X., Gao, Q., Pan, L., Wu, L., Luo, Y., Wang, W., Zheng, Z., Guo, X. (2014). Prevalence and risk factors for cervical neoplasia: a cervical cancer screening program in Beijing. *BMC Public Health*, 14(1), 1185. doi:10.1186/1471-2458-14-1185

71. Teng, P., Hao, M. (2020). A population-based study of age-related associations between vaginal pH and the development of cervical intraepithelial neoplasia. *Cancer Medicine*, 9(5), 1890–1902. doi: 10.1002/cam4.2845

72. Trottier, H., Franco, E. L. (2006). The epidemiology of genital human papillomavirus infection. *Vaccine*, 24 Suppl 1, S1-S15. doi:10.1016/j.vaccine.2005.09.054

73. Trowsdale, J. (2011). The MHC, disease and selection. *Immunology Letters*, 137(1), 1–8. doi:10.1016/j.imlet.2011.01.002

74. Vieira-Baptista, P., Lima-Silva, J., Pinto, C., Saldanha, C., Beires, J., Martinez-de-Oliveira, J., Donders, G. (2016). Bacterial vaginosis, aerobic vaginitis, vaginal inflammation and major Pap smear abnormalities. *European Journal of Clinical Microbiology and Infectious Diseases*. 35(4), 657–64. doi:10.1007/s10096-016-2584-1

75. Wei, F., Georges, D., Man, I., Baussano, I., Clifford, G. M. (2024). Causal attribution of human papillomavirus genotypes to invasive cervical cancer worldwide: a systematic analysis of the global literature. *The Lancet*, 404(10451), 435–444. doi:10.1016/S0140-6736(24)01097-3

76. Wen, Q., Wang, S., Min, Y., Liu, X., Fang, J., Lang, J., Chen, M. (2025). Associations of the gut, cervical, and vaginal microbiota with cervical cancer: a systematic review and meta-analysis. *BMC Women's Health*, 25, 65. doi: 10.1186/s12905-025-03599-1

77. Wilson, J., Donders, G., Mendling, W., Jensen, J. S. (2018). European (IUSTI/WHO) International Union against sexually transmitted infections (IUSTI) World Health Organization (WHO), Guideline on the management of vaginal discharge. *Int J STD AIDS*, 29(13), 1258–1272. doi: 10.1177/0956462418785451

78. Woodman, C. B. J., Collins, S. I., Young, L. S. (2007). The natural history of cervical HPV infection: unresolved issues. *Nature Reviews Cancer*, 7(1), 11–22. doi:10.1038/nrc2050

79. World Health Organization. (2020). Global strategy to accelerate the elimination of cervical cancer as a public health problem. Geneva. Available from: https://www.who.int/publications/i/item/978924001410

80. Yang, S., Zhao, W., Wang, H., Wang, Y., Li, J., Wu, X. (2018). Trichomonas vaginalis infection-associated risk of cervical cancer: A meta-analysis. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 228, 166–173. doi:10.1016/j.ejogrb.2018.06.031

81. Ye, H., Song, T., Zeng, X., Li, L., Hou, M., Xi, M. (2018). Association between genital mycoplasmas infection and human papillomavirus infection, abnormal cervical cytopathology, and cervical cancer: a systematic review and meta-analysis. *Archives of Gynecology and Obstetrics*, 297, 1377–1387. doi: 10.1007/s00404-018-4733-5

82. Zoodsma, M., Nolte, M. I., Te Meerman, J. G., de Vries, G. E. E., van der Zee, G. J. A. (2005). HLA genes and other candidate genes involved in susceptibility for (pre)neoplastic cervical disease. *International journal of oncology*, 26(3), 769–784. doi:10.3892/ijo.26.3.769