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

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Abstract

Cervical cancer remains a significant public health challenge, particularly in regions where prevention programmes have not achieved optimal effectiveness. While persistent high-risk human papillomavirus infection is a necessary cause of cervical neoplasia, disease progression is influenced by a complex interplay of viral, host, microbial, and behavioural factors. This study aimed 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.

The study group included 112 consecutive new patients referred to Riga East University Hospital for colposcopy examination due to abnormal cervical cytology smear results.

The control group consisted of 120 women who underwent a gynaecological examination for other reasons (routine examination, follow-up of other gynaecological pathology). All patients included in the study were interviewed, and a custom-designed questionnaire was filled out. After that, women had gynaecological examination, pH measurement of vaginal discharge; samples were taken for wet-mount microscopy, testing of HPV DNA, mRNA, vaginal infections, and HLA class II genes. As a next step, a colposcopy was performed, and biopsies were taken if indicated.

The study revealed that smoking is a significant independent risk factor for CIN2+, whereas higher educational attainment has a protective effect. Disturbances of vaginal ecosystem, including elevated pH and abnormal microflora, were more common among women with cervical neoplasia. Specifically, bacterial vaginosis was linked to low-grade lesions.

At the same time, aerobic vaginitis – exceptionally moderate to severe cases – demonstrated a strong, independent association with high-grade cervical lesions (CIN2+), highlighting the critical role of vaginal inflammation in cervical carcinogenesis. Detection of HR-HPV DNA and E6/E7 mRNA emerged as the most important predictors of high-grade cervical lesions. Although no significant associations were found between sexually transmitted infections and CIN, *Mycoplasma hominis* ranked as the third most important independent risk factor for CIN2+. Genetic analysis identified significant associations between specific HLA class II alleles (*HLA-DRB1*11:01*, *DRB1*15:01*, *DQA1*01:03, DQA1*04:01*, *DQB1*03:01,* and *DQB1*04:01*) and an increased risk of CIN2+, while other alleles (*DRB1*14:01*, *DRB1*16:01*, and *DQA1*03:01*) appeared to confer a protective effect.

The findings underscore the limitations of universal, one-size-fits-all screening strategies and highlight the urgent need for personalised, risk-based cervical cancer prevention models that integrate viral, microbial, genetic, and epidemiological risk factors. Such individualised approaches could improve screening precision, resource allocation, and ultimately contribute to the elimination of cervical cancer as a public health concern.

Keywords: cervical intraepithelial neoplasia, high-risk human papillomavirus, personalised screening

Anotācija

Cilvēka papilomas vīrusa genotipu, maksts ekosistēmas, seksuāli transmisīvo infekciju, cilvēka imunoģenētikas un sociālo faktoru ietekme uz dzemdes kakla priekšvēža izmaiņu attīstību

Dzemdes kakla vēzis joprojām ir būtisks sabiedrības veselības izaicinājums, īpaši reģionos, kur profilakses programmas nav sasniegušas optimālu efektivitāti. Lai gan persistējoša augsta riska cilvēka papilomas vīrusa infekcija ir nepieciešams priekšnoteikums cervikālās intraepiteliālās neoplāzijas attīstībai, slimības progresu ietekmē sarežģīta vīrusa, saimniekorganisma, mikrobioma un uzvedības faktoru mijiedarbība. Šī pētījuma mērķis bija identificēt saistību starp dažādiem sociālajiem faktoriem, maksts ekosistēmu, maksts infekcijām, tostarp seksuāli transmisīvajām un augsta riska cilvēka papilomas vīrusa infekcijām, HLA II klases alēlēm un dažādām dzemdes kakla priekšvēža izmaiņu pakāpēm.

Pētījuma grupā tika iekļautas 112 pacientes, kuras tika nosūtītas uz Rīgas Austrumu klīnisko universitātes slimnīcu kolposkopijas izmeklējuma veikšanai sakarā ar izmainītām dzemdes kakla citoloģijas analīzēm. Kontroles grupu veidoja 120 sievietes, kurām tika veikta ginekoloģiskā apskate cita iemesla dēļ (profilaktiska pārbaude, citu ginekoloģisku patoloģiju novērošana). Visas pētījumā iekļautās dalībnieces tika intervētas, aizpildot speciāli izstrādātu anketu. Tālāk tika veikta ginekoloģiskā apskate, maksts izdalījumu pH noteikšana; tika paņemti paraugi natīvai mikroskopijai, cilvēka papilomas vīrusa DNS, mRNS, maksts infekciju un HLA II klases gēnu analīzei. Pēc tam tika veikta kolposkopija un, ja nepieciešams, biopsija.

Pētījuma rezultāti parādīja, ka smēķēšana ir būtisks neatkarīgs riska faktors CIN2+ attīstībai, kamēr augstāks izglītības līmenis darbojas kā aizsargfaktors. Maksts ekosistēmas traucējumi, tostarp paaugstināts pH un izmainīta mikroflora, bija biežāk sastopami sievietēm ar dzemdes kakla neoplāziju. Bakteriālā vaginoze bija saistīta ar vieglas pakāpes bojājumiem. Aerobais vaginīts – īpaši vidēji smagos un smagos gadījumos – bija neatkarīgs augstas pakāpes dzemdes kakla bojājumu (CIN2+) riska faktors. Šī saistība norāda uz maksts iekaisuma nozīmīgo lomu dzemdes kakla kanceroģenezē. Augsta riska cilvēka papilomas vīrusa DNS un E6/E7 mRNS klātbūtne izrādījās vissvarīgākie augstas pakāpes dzemdes kakla bojājumu prognostiskie faktori. Lai gan netika atrastas būtiskas saistības starp seksuāli transmisīvajām infekcijām un CIN, *Mycoplasma hominis* tika atzīts par trešo nozīmīgāko neatkarīgo riska faktoru CIN2+ attīstībā. Ģenētiskā analīze parādīja būtisku saistību starp

noteiktām HLA II klases alēlēm (*HLA-DRB1*11:01, DRB1*15:01, DQA1*01:03, DQA1*04:01, DQB1*03:01* un *DQB1*04:01*) un paaugstinātu CIN2+ attīstības risku, kamēr citas alēles (*DRB1*14:01, DRB1*16:01* un *DQA1*03:01*) demonstrēja aizsargājošu efektu.

Šī pētījuma rezultāti uzsver, ka universālajam skrīninga modelim ir ierobežojumi. Sakarā ar ko ir nepieciešams izstrādāt personalizētu skrīninga stratēģiju, kas ietvertu dažādus riska faktorus – vīrusu, mikrobiālos, ģenētiskos un epidemioloģiskos. Šādas individualizētas pieejas varētu uzlabot skrīninga precizitāti, optimizēt resursu izmantošanu un ilgtermiņā veicināt dzemdes kakla vēža izskaušanu kā sabiedrības veselības problēmu.

Atslēgvārdi: cervikālā intraepiteliālā neoplāzija, augsta riska cilvēka papilomas vīruss, personalizēts skrīnings

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Abbreviations

AI	artificial intelligence
AIDS	Acquired Immune Deficiency Syndrome
AGUS	atypical glandular cells of unknown significance
ART	antiretroviral therapy
ASC-H	atypical squamous cells – high grade not excluded
ASCUS	atypical squamous cells of unknown significance
AV	aerobic vaginitis
BV	bacterial vaginosis
CD	cluster of differentiation
CI	confidence interval
CIN	cervical intraepithelial neoplasia
CST	community state type
DNA	deoxyribonucleic acid
E	early
GWAS	genome-wide association study
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
Hpf	high-power field
HPV	human papillomavirus
HR-HPV	high-risk human papillomavirus
HSIL	high-grade squamous intraepithelial lesion
IBD	inflammatory bowel disease
IVAC	International Vaccine Access Center
L	late
LBC	liquid-based cytology
LBG	lactobacillary grades
LCR	long control region
LSIL	low-grade squamous intraepithelial lesion
MHC	major histocompatibility complex
mRNA	messenger ribonucleic acid
msAV	moderate to severe aerobic vaginitis
NVD	Nacionālais veselības dienests (National Health Service)
OR	odds ratio
PCR	polymerase chain reaction
RA	rheumatoid arthritis

SPKC	Slimību profilakses un kontroles centrs (Centre for Disease Prevention and Control (Latvia))
STI	sexually transmitted infections
Th	T helper
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 (HR-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 behaviourial 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 behaviourial 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 Literature review

1.1 Cervical cancer – situation worldwide and in Latvia

Cervical cancer is a major global healthcare problem – it is the 4th most common cancer among women worldwide. In 2022, approximately 660 000 women were diagnosed with cervical cancer, and about 350 000 died from it. (WHO, 2024) The highest age-standardised estimated incidence of cervical cancer globally, according to the International Agency for Research on Cancer, is in sub-Saharan Africa, and the lowest is in North America, Australia, and certain European (e.g., Malta, Switzerland, Finland, Luxembourg) and Western Asian countries (Figure 1.1).

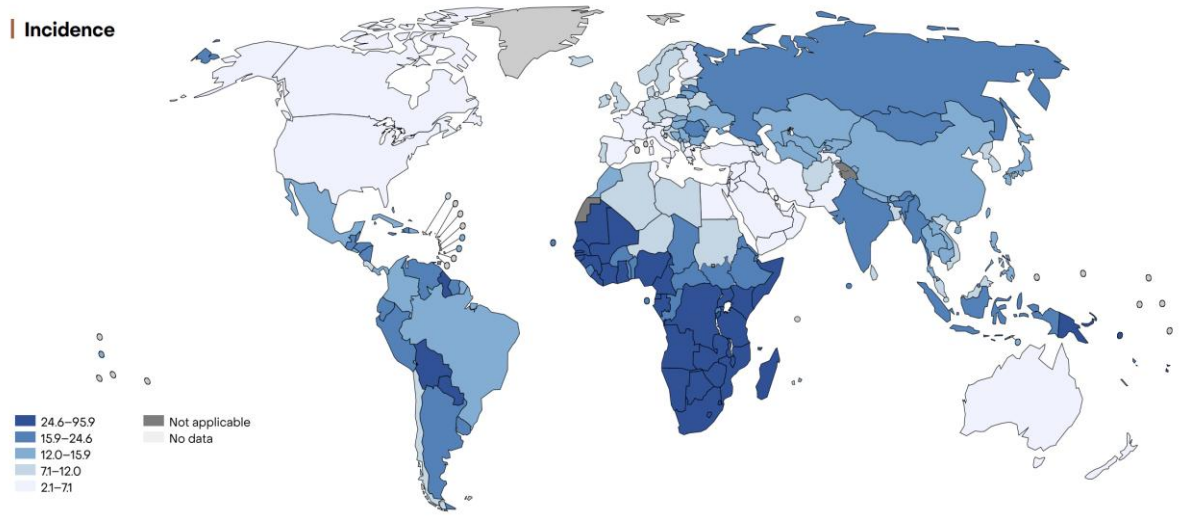


Figure 1.1 **Age-standardised incidence rates of cervical cancer in the world** (estimates for 2022) (Ferlay et al., 2024)

Cervical cancer is the 2nd most frequent tumour among women under the age of 45 and the third most common cause of cancer-related deaths among women in Latvia, following breast and lung cancer. (SPKC, 2024) According to the WHO (Figure 1.2), the incidence of cervical cancer in Latvia in 2021 was higher than the average in European Union countries and countries in the European region.

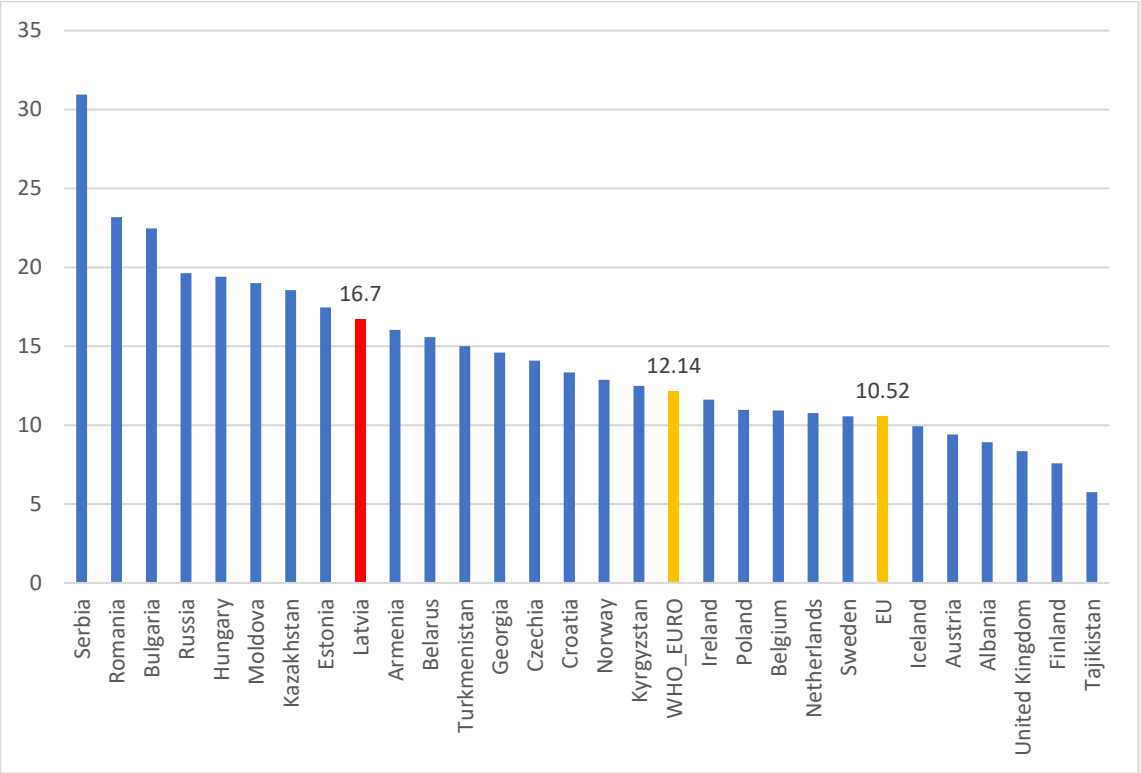


Figure 1.2 **Crude rates of European incidence of cervical cancer per 100 000 women in 2021**
(WHO European Health Information Gateway, 2024;
Slimību profilakses un kontroles centrs (SPKC), 2024

In the past 13 years, the incidence of cervical cancer in Latvia has shown only a slight decline. In 2023, cervical cancer was diagnosed in 178 women, with a crude rate of 17.7 per 100 000 women. (Figures 1.3 and 1.4) When calculation is applied to the population distribution of a standard population (age-standardised ratio, using the European new standard population), the incidence is 16.6 per 100 000 women (Figure 1.5). (SPKC, 2024)

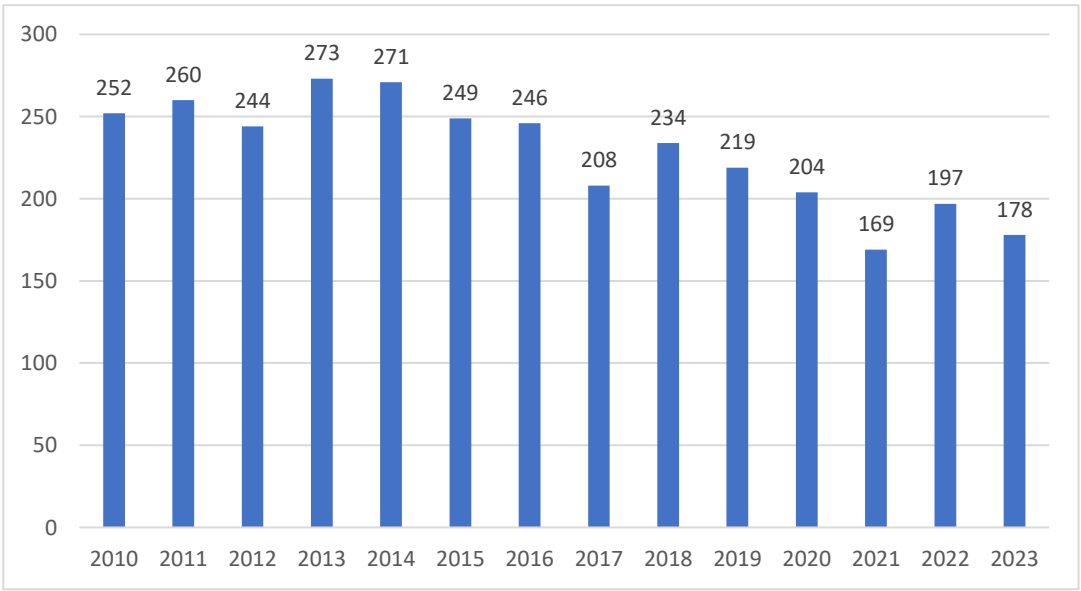


Figure 1.3 **Cervical cancer incidence in Latvia 2010–2023, absolute numbers**
(SPKC, 2024)

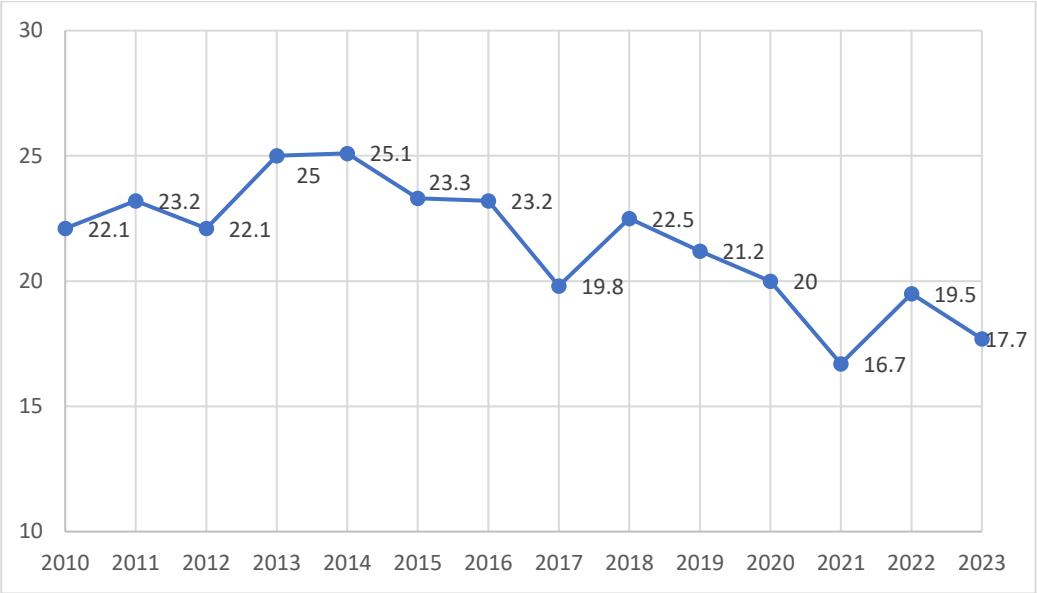


Figure 1.4 **Cervical cancer incidence in Latvia 2010–2023, crude rates per 100 000 women**

(SPKC, 2024)

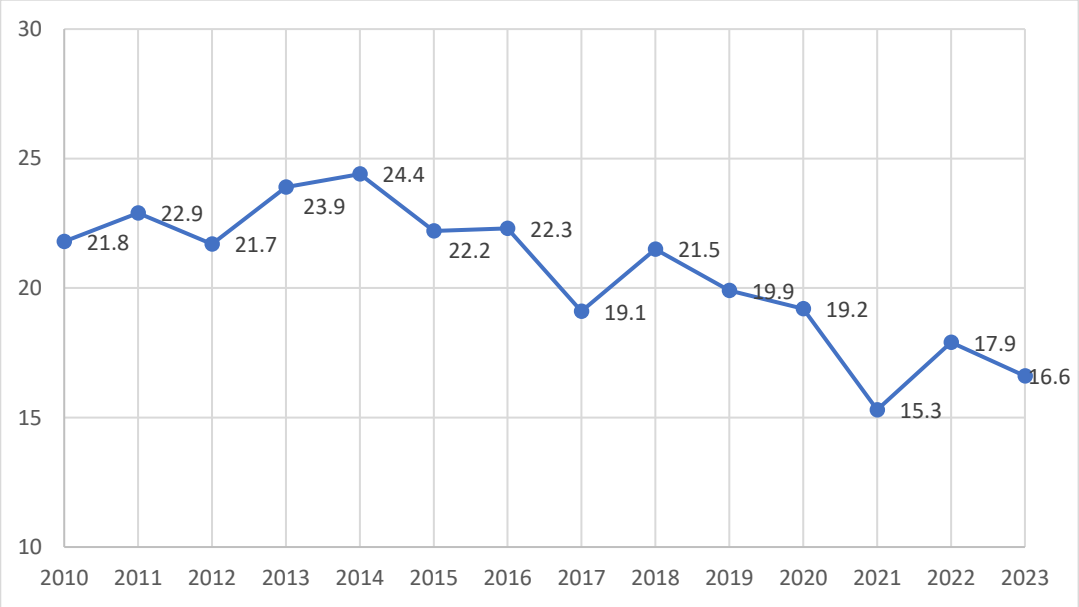


Figure 1.5 **Cervical cancer incidence in Latvia 2010–2023, age-standardised rates per 100 000 women**

(SPKC, 2024)

Mortality incidence rates also remain relatively stable over the years; crude rates per 100 000 are shown in Figure 1.6.

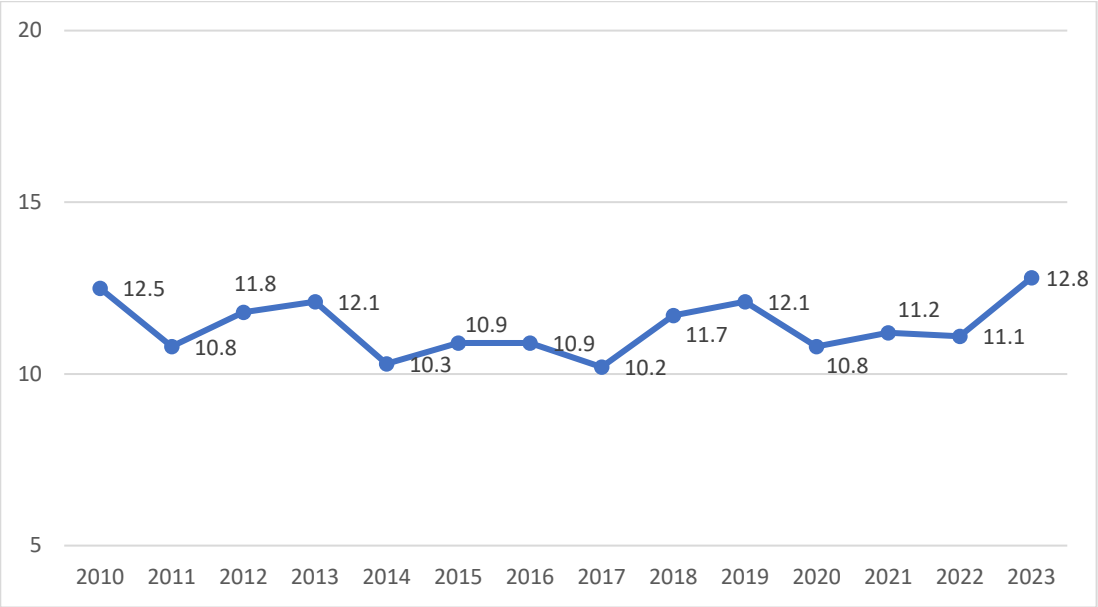


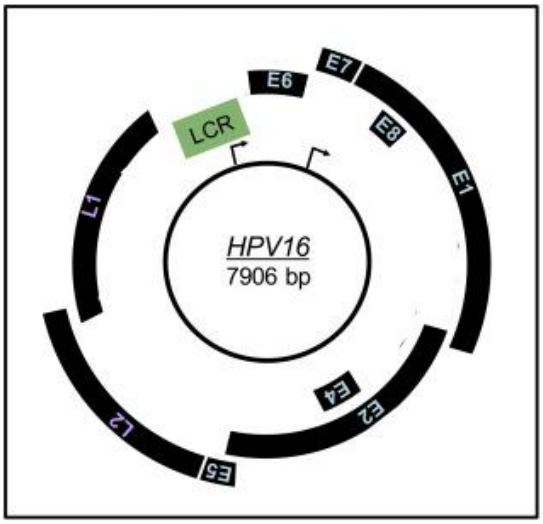
Figure 1.6 **Cervical cancer mortality in Latvia 2010–2023, crude rates per 100 000 women**

(SPKC, 2024)

1.2 Human papillomavirus structure and classification

Human papillomavirus (HPV) is recognised as a cervical cancer aetiological factor. Persistent infection with high-risk HPV types accounts for approximately 99 % of cervical cancer cases. (Walboomers et al., 1999)

It is a small, non-enveloped deoxyribonucleic acid (DNA) virus belonging to the family *Papillomaviridae.* (Bzhalava et al., 2015) Its genome consists of three major regions: the early (E) region, the late (L) region, and the long control region (LCR) (Figure 1.7). Six early proteins (E1, E2, E4, E5, E6, E7) encode regulatory and oncogenic proteins and can cause the malignant transformation of the cell. Two late proteins (L1 and L2) are responsible for capsid protein synthesis. The LCR contains regulatory elements for viral replication and transmission. (Doorbar, 2006; Harden & Munger, 2017)



EARLY REGION: proteins necessary for viral replication

LATE REGION: viral capsid proteins

LONG CONTROL REGION: sequences controlling viral replication & transcription

ORF	HPV16 PROTEIN FUNCTION
E1	origin binding protein, ATPase-dependent helicase involved in genome replication
E2	regulator of viral gene transcription, association with E1 (origin binding), viral genome partitioning
E4	expressed abundantly as E1^E4 fusion protein, cytokeratin network destabilization, virus release and transmission
E5	small transmembrane protein, interacts with EGF receptor activating mitogenic pathways
E6	drives cell cycle allowing genome amplification in upper epithelial layers, association with E6AP and degradation of p53, PDZ-protein binding, hTert activation
E7	drives cell cycle allowing genome amplification in upper epithelial layers, association with and degradation of pRB, mitotic mutator
E8	expressed as E8^E2 fusion protein, acts as a repressor of transcription and replication during the viral life cycle
L1	major capsid protein, assembles into pentameric capsids forming the icosahedral virion (prophylactic vaccines)
L2	minor capsid protein, involved in viral DNA encapsidation, facilitates viral entry and trafficking

Figure 1.7 **HPV genome structure**

(Harden and Munger, 2017)

These viruses specifically infect epithelial cells in the skin and mucous membranes. There are more than 200 different HPV types, and 40 of them specifically infect the anogenital region. (Moeinzadeh et al., 2020) HPV types are categorised into cutaneous and mucosal types based on tissue tropism. According to their ability to cause various cancers, including cervical, anogenital, and oropharyngeal cancers, HPV can be classified into low-risk and high-risk genotypes. There are 14 high-risk HPV types – 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. (WHO, 2021) Among these, HPV 16 and HPV 18 account for the majority of HPV-related cancers. (Muñoz et al., 1994; Smith et al., 2007) According to oncogenicity potential, HR-HPV types can be further subclassified as highest oncogenicity viruses (HPV 16, 18, 45), medium oncogenicity (HPV 31, 33, 52, 58), and low oncogenicity (HPV 35, 39, 51, 56, 59, 66, 68). (Arroyo Mühr et al., 2025)

1.3 Human papillomavirus prevalence

HPV is a widespread infection, but the prevalence in the population can vary based on sex, age, and geographical region.

In Europe, the prevalence of HPV among women is estimated to be approximately 12 %. (Florescu et al., 2018) Higher prevalence rates are reported in sub-Saharan Africa (24 %) and Latin America (16 %), and lower rates were observed in Western Asia (2 %) and

North America (5 %) (Bruni et al., 2010). Typically, the highest HPV prevalence, up to 40 %, is in younger ages (< 25 years). As women age, the risk of HPV infection generally decreases. (Bruni et al., 2010)(Florescu et al., 2018) At the age of 50-54 years, HR-HPV prevalence is 6 %, and after the age of 75, it is 5 % (Osmani et al., 2025) However, data show that in some regions and countries, there is a variable rebound in the prevalence rates at older ages. The second peak in Central and South America has been identified at the age > 45, and in Western Africa, at > 55. (Bruni et al., 2010) From European countries, Estonia has reported an increase in HR-HPV prevalence, especially in type 16, in the age group 67–70. (Pärna et al., 2023) The reason for this second peak is unclear. It can be explained by the reactivation of a latent infection, a new infection from a sexual partner, or a cohort effect – diverse HPV exposure in different birth cohorts.(Trottier and Franco, 2006)

In Latvia, recent data show that 11 % of women in the general population are HR-HPV positive. (Berza et al., 2024)

In males, high-risk HPV prevalence is generally higher. It was calculated that the pooled prevalence of HR-HPV in men in Europe and Latin America is 22 %, 27 % in North America, 25 % in sub-Saharan Africa, and 10 % in Asia. Unlike in females, in men, high HPV prevalence persists throughout adulthood. (Bruni et al., 2023) The prevalence of HR-HPV in males in Latvia is unknown.

In both men and women, the most common type detected is HPV 16 (Bruni et al., 2010; Florescu et al., 2018).

1.4 Human papillomavirus transmission

The primary route for HPV transmission is through sexual contact, including vaginal, anal, and oral sex. It mainly spreads via skin-to-skin contact in the genital area; therefore, condoms do not provide complete protection, as in the case of other sexually transmitted infections. (Carr and Gyorfi, 2000; Manhart and Koutsky, 2002) Results of the meta-analysis show greater female-to-male transmission risk compared to male-to-female (pooled estimate 3.01 vs. 1.60, $p < 0.01$). (Balaji et al., 2020)

HPV can also be transmitted through non-sexual routes, although it is much less common. Vertical transmission from mother to child is possible during pregnancy or delivery. HPV virus has been detected not only in the vagina and cervix of the mother but also in the placenta, amniotic fluid, umbilical cord, and even in the ovaries. (Oyouni, 2023; Sabeena et al., 2017) HPV transmission through breastfeeding has not been proven. (Sabeena et al., 2017)

Close contact, like kissing and digital contact, as well as autoinoculation, although rare, can explain some cases of HPV infection in children and sexually inactive individuals. (Sabeena et al., 2017) However, getting infected via toilet seats or swimming pools is impossible. (Puranen et al., 1996)

Nosocomial transmission through transvaginal ultrasound probes and colposcopes has also been described. (Ryndock and Meyers, 2014; Sabeena et al., 2017) Centers for Disease Prevention and Control classifies them as semicritical devices that require proper disinfection. (Rutala and Weber, 2019)

1.5 Pathogenesis of cervical intraepithelial neoplasia and cervical cancer

Over 99 % of cervical cancer cases are attributed to high-risk HPV infection. (Bosch et al., 2008; Smith et al., 2007) The natural history of HPV infection encompasses a series of events that begin with viral acquisition and, in some cases, culminate in malignancy. The majority of HPV infections are transient and asymptomatic; however, persistent infection with high-risk HPV types can lead to cervical and anogenital cancers through mechanisms such as immune evasion, viral persistence, and genetic alterations in host cells. The transformation from initial infection to invasive carcinoma typically spans 10 to 15 years. (Castellsagué, 2008)

Squamous cervical cancer originates in the cervical transformation zone, an epithelial region where the proximal single-layered columnar epithelium transitions to the distal stratified squamous epithelium. During normal development, reserve cells from the columnar epithelium undergo squamous metaplasia, differentiating into squamous cells. As HPV depends on cell replication and differentiation for its life cycle, actively dividing cells in the cervical transformation zone are particularly susceptible to HPV infection. (Doorbar, 2016; Moscicki, Schiffman, et al., 2020)

The process begins when HPV infects the basal epithelial cells of the stratified cervical epithelium (Figure 1.8), typically entering through microabrasions in the epithelial layer during sexual intercourse, where friction or mechanical injury to the cervix may occur. (Harden and Munger, 2017; Schiffman et al., 2007)

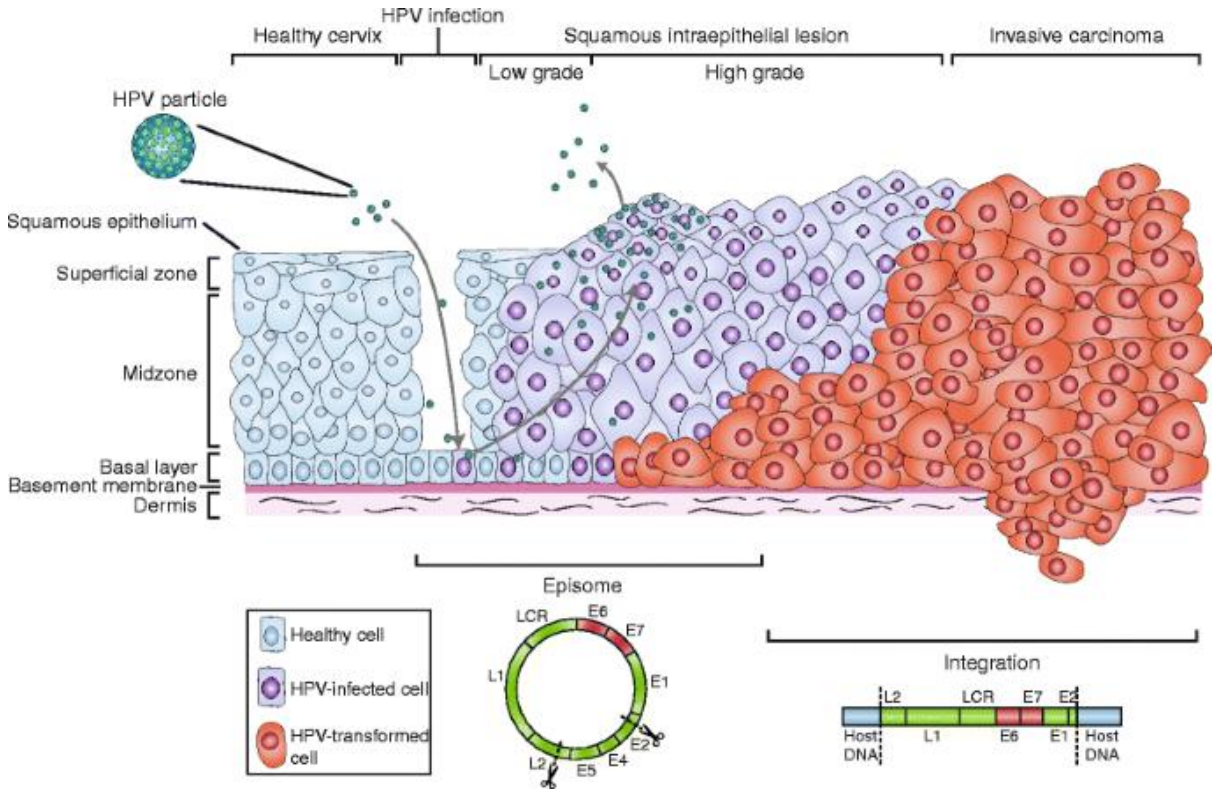


Figure 1.8 **Natural history of HR-HPV infection**

(Piersma, 2011)

Upon entry into basal epithelial cells, HPV viral particles bind to specific receptors on the host cell surface, primarily heparan sulphate proteoglycans. Following the attachment, the virus is internalised through clathrin-mediated endocytosis. Once inside the cell, the viral DNA can either remain episomal or integrate into the host genome, leading to the potential for persistent infection. (Day et al., 2003; Doorbar, 2005; Schiller et al., 2010) In the early stages, the HPV genome remains episomal, replicating alongside host cells and producing viral particles that accumulate in the upper epithelial layers as the cells differentiate. Clinically, this stage manifests as low-grade squamous intraepithelial lesions (LSIL) or cervical intraepithelial neoplasia grade 1 (CIN1), which often resolves spontaneously within a few years (Figure 1.9). (Doorbar, 2005; Gravitt and Winer, 2017; Prati et al., 2018)

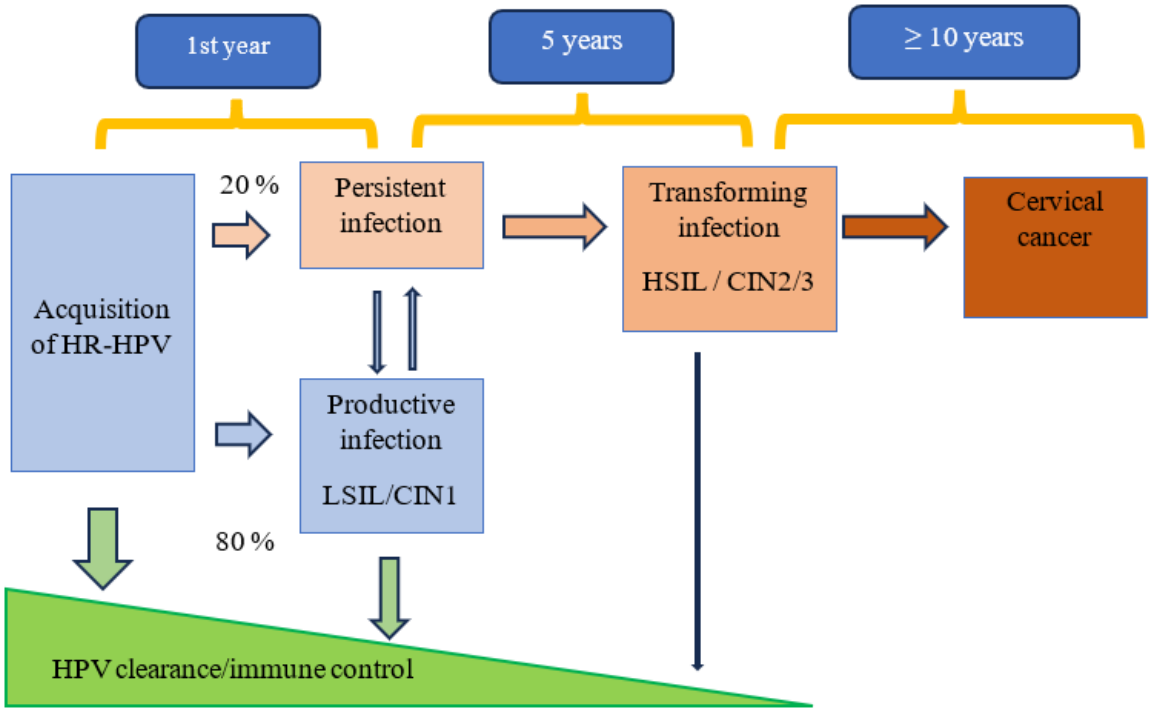


Figure 1.9 **Timeframe and outcomes of HR-HPV infection**

(Gravitt and Winer, 2017; Moscicki, Schiffman, et al., 2020)

The first line of defence against HPV entry is healthy mucosal epithelium. It also mediates the initial immune response. Next, HPV is recognised by a complex network of innate and adaptive immune responses within the female genital tract. Innate immune cells identify pathogens through pattern recognition receptors, triggering protective responses. Antigen-presenting cells, including Langerhans cells, are crucial in linking innate and adaptive immunity, while macrophages and natural killer cells contribute to clearing HPV infections. However, altered dendritic cell function and high neutrophil levels may contribute to immune evasion and cervical cancer progression. (Ntuli et al., 2022)

Another defence mechanism is the adaptive immune response. During HPV infection, dendritic cells capture the antigen and activate adaptive immunity by releasing inflammatory cytokines. CD4+ (cluster of differentiation 4+) T cells recognise the antigen, proliferate, and stimulate B cells to mature. In contrast, T-helper (Th) 1 cells secrete cytokines that activate CD8+ T cells, resulting in cytotoxic T lymphocyte responses that eliminate infected cells. Additionally, Th2 cells promote the differentiation of B lymphocytes into plasma cells that produce HPV-specific antibodies. Some B and T cells become long-lived memory cells for future immunity. (Ntuli et al., 2022)

T-cell-mediated immune responses play an important role in controlling HPV infection. However, infected basal cells can retain low viral copy numbers, establishing

a latent infection that may reactivate if immune surveillance declines. (Doorbar, 2023; Gravitt and Winer, 2017; Lycke et al., 2025)

Approximately 80 % of newly acquired HPV infections are transient and are cleared or suppressed by the host immune system within 1–2 years. However, a minority of high-risk HPV (HR-HPV) infections persist and remain detectable for more than 12 months. (Gravitt and Winer, 2017; Schiffman et al., 2007)

HPV employs several mechanisms to evade immune detection. There is no viremia, and HPV is not cytolytic. (Stanley, 2010) The virus can alter the function of antigen-presenting cells, inhibit immune cell recruitment, and suppress cytokine production and interferon signalling. (Ntuli et al., 2022; Song et al., 2015) It can also reduce the presentation of HLA class I molecules on the cell surface and impair cytotoxic T lymphocyte responses. Increased levels of immunosuppressive cytokines (IL-10, TGF-β) and regulatory T cells further impair the function of antigen-presenting cells and T cells. (Doorbar, 2023; Ntuli et al., 2022; Song et al., 2015) The likelihood of clearing the infection decreases with its duration. (Androphy, 1994; Doorbar et al., 2015a)

Persistent HR-HPV infection can lead to viral genome integration into the host DNA, a critical event in carcinogenesis. (Schiffman et al., 2007) Viral integration disrupts the region containing the E1 and E2 genes, resulting in the loss of their expression. The loss of E2 leads to the unchecked upregulation of the viral oncoproteins E6 and E7. (Doorbar et al., 2015; Prati et al., 2018)

E6 and E7 play a crucial role in the viral life cycle by altering the cellular environment to facilitate viral genome amplification in growth-arrested, fully differentiated cells, typically incapable of DNA replication. E6 and E7 promote cell proliferation in the basal and parabasal layers, expanding the initially infected area. (Harden and Munger, 2017) E6 induces the degradation of the tumour suppressor protein p53, impairing apoptosis and allowing the survival of cells with DNA damage. Meanwhile, E7 binds to and inactivates the retinoblastoma protein (pRb), resulting in uncontrolled cell cycle progression. (Crosbie et al., 2013)

Clinically, these processes manifest as high-grade squamous intraepithelial lesions (HSIL) or CIN 2 and 3, where undifferentiated cells with genetic abnormalities replace up to two-thirds or the entire thickness of the stratified epithelium. If left untreated, these lesions can develop into invasive cervical cancer driven by continuing cellular proliferation, genomic instability, accumulation of genetic mutations, and insufficient immune response. (Doorbar et al., 2015; Gravitt and Winer, 2017)

In 2014, WHO presented a new classification of precancerous lesions of the cervix. It is a two-tiered system based on histopathology and HPV-related carcinogenesis, replacing the CIN classification. According to this classification, lesions are classified as LSIL and HSIL. Where LSIL corresponds to CIN1 in a previous system, and HSIL encompasses CIN2 and CIN3. This classification aligns with the Bethesda System used in cytology and reflects the biological behaviour of HPV-related cervical lesions. (Carcangiu et al., 2014)

The natural history of CIN varies depending on the severity of the disease. CIN1 shows the highest regression rates within 1–2 years, and the risk of progression to cervical cancer is minimal, 0.03 %, according to the results of a meta-analysis. (Loopik et al., 2021) Therefore, the management of CIN1 is conservative, with follow-up cytology and/or HR-HPV test in 12 months. (Redman et al., 2021)

The clinical course of CIN2 is heterogeneous. It can regress to normal in up to 50 % of cases, especially in women younger than 30. Progression risk to cancer is also low, 0.3 %; however, there is a 19 % risk of progression to CIN3. (Loopik et al., 2021) Expectant management of histologically confirmed CIN2 with colposcopy every 6 months is recommended in women < 30 if the lesion is small and the transformation zone is completely visible. In other situations, excisional treatment is recommended. (Redman et al., 2021)

The risk of progression to invasive cervical cancer in the case of CIN3 is 2 %, and the regression to normal is 18 %. (Loopik et al., 2021) The usual recommendation in histologically proven CIN3 is excisional treatment. (Redman et al., 2021)

High-risk HPV types, particularly HPV-16 and HPV-18, are most often linked to the progression from CIN to invasive cervical cancer. (Schiffman et al., 2007)

1.6 Risk factors of cervical intraepithelial neoplasia and cervical cancer

While HPV is a necessary cause, other factors are needed to develop cervical cancer. Both viral and host factors are significant in the development of cervical disease. Viral factors include infection with high-risk HPV types, especially 16 and 18, which cause ~70 % of cervical cancer cases, persistent HR-HPV, and co-infection by multiple types. (De Brot et al., 2017; Kyrgiou et al., 2017) Factors known to increase the persistence of HPV infection and the risk of progression to high-grade cervical lesions and cancer include different behavioural, socioeconomic, and reproductive factors, compromised immunity and genetic susceptibility, and the presence of concurrent vaginal infections. (Trottier and Franco, 2006)

1.6.1 Smoking

Smoking is a recognised risk factor for cervical cancer. Studies have demonstrated that women who smoke have a higher chance of developing cervical intraepithelial neoplasia and cervical cancer compared to non-smokers. (Collins et al., 2010; Haverkos et al., 2003; Nagelhout, Ebisch, Hel, et al., 2021; Plummer et al., 2003; Roura et al., 2014) Tobacco smoke contains carcinogens that may directly affect the cervical epithelium, promoting cellular transformation and facilitating the progression to cancer. (Louie et al., 2011) Smoking has also been shown to impair various immune system functions significantly. (Eldridge et al., 2017; Ma et al., 2023; Trottier and Franco, 2006) Studies have revealed that smoking directly suppresses T-cell activity by increasing the proportion of CD8+ T cells while reducing CD4+ T cell levels. (Aguayo et al., 2020) Regarding humoral immune responses, smokers are considered to have a higher risk of persistent HPV infection due to reduced antibody levels or impaired antibody development. (Eldridge et al., 2017) Moreover, smoking increases oxidative stress in cervical tissues, leading to DNA damage, genomic instability, and an increased likelihood of malignant transformation. (Burd, 2003)

Smoking can also show an indirect effect on HPV infection. Certain confounding factors related to differences in lifestyle between smokers and non-smokers may contribute to a higher risk of persistent HPV infection. For instance, smokers are more likely have risky sexual behaviours. (Ma et al., 2023; Vaccarella et al., 2008)

1.6.2 Socioeconomic status and education level

Socioeconomic status and education level are significant determinants of cervical cancer development risk. Individuals with low socioeconomic status more often experience higher rates of incidence and mortality from cervical cancer. (Jensen et al., 2008) These women could more often face barriers to healthcare access, leading to decreased participation in the cervical cancer screening program, resulting in delayed diagnosis of cervical cancer. (Broberg et al., 2018; Powell et al., 2018)

Women with higher socioeconomic status, particularly those with higher education, have the highest cancer screening attendance and, therefore, lower cervical cancer rates, indicating that health information plays a crucial role. (Akinyemiju et al., 2016)

The combination of low socioeconomic status and limited education increases the risk of cervical malignancy. Women in these groups often face multiple challenges, such as restricted access to healthcare, lower health literacy, and a higher probability of being exposed to risk factors like smoking and sexually transmitted infections. (Akinyemiju et al., 2016; Powell et al., 2018; Swan et al., 1999)

1.6.3 Sexual behaviour

HPV is transmitted through sexual contact, and therefore, sexual behaviour plays an essential role in the risk of developing cervical cancer, mainly by increasing the acquisition of HPV infection. First sexual intercourse at a young age, mainly before 18, is associated with a higher risk of cervical cancer. (Louie et al., 2009; Mekonnen and Mittiku, 2023) Also, having multiple sexual partners increases the risk of both non-malignant and malignant HPV-induced cervical diseases. (Liu et al., 2015).

1.6.4 Parity

Higher parity is linked to an increased risk of cervical cancer. The more children a woman has had, the greater her risk of developing cervical cancer. (Skegg, 2002; Tekalegn et al., 2022) The exact mechanism of how the number of deliveries increases the risk is not fully understood. Possible explanations are – a high rate of HPV detection and cervical abnormalities during pregnancy, modulation of immune response by increased levels of oestrogen and progesterone, and the potential role of cervical trauma during delivery. (Castellsagué and Munoz, 2003; Tekalegn et al., 2022)

1.6.5 Use of combined oral contraceptives

Prolonged use of combined oral contraceptives can increase the risk of cervical malignancy in women infected with HR-HPV. (Asthana et al., 2020; Castellsagué and Munoz, 2003) Short-term use (less than 5 years) almost doesn't influence the risk of cervical cancer. However, in women who are taking oral contraception for five or more years, the risk was 2-fold. (Asthana et al., 2020) In oral contraceptive users, the risk of adenocarcinoma is higher compared to squamous cell carcinoma (OR 1.7 vs. 1.29, respectively). It is suggested that there is no difference between past and current users, although there are studies that report declining risk with cessation of oral contraceptive use. (Asthana et al., 2020)

The precise mechanism by which oral contraceptives elevate the risk of cervical cancer remains unclear. Mouse model studies demonstrated that oestrogens may enhance the development of cervical cancer. (Brake and Lambert, 2005) Steroid hormones may also facilitate the integration of HPV DNA into the host genome, modulate gene transcription and cell apoptosis, and enhance carcinogenesis. 17β-oestradiol can alter dendritic cell function, thus promoting HPV persistence by inhibiting the elimination of infected cells by cytotoxic T cells. (Gadducci et al., 2020)

1.6.6 Vaginal ecosystem

The vaginal ecosystem is a dynamic balance between the microbiota and its environment. The microbiome influences vaginal conditions and provides defence against pathogens, and the other way round the environment determines microbial composition. (Ünlü and Donders, 2011) The dominance of different *Lactobacillus* species characterises a healthy vaginal ecosystem. *Lactobacilli* maintain an acidic pH and produce hydrogen peroxide and protective proteins, including biosurfactants and bactericin-like substances, as their primary defence mechanism against pathogens. Lactobacilli can form a biofilm that prevents the adhesion of pathogenic microorganisms to the vaginal mucosa. (Kyrgiou and Moscicki, 2022) *Lactobacillus* depleted state and overgrowth of other bacteria are considered abnormal and are associated with different gynaecological and obstetrical pathologies, including HPV infection, cervical dysplasia, and cervical cancer.

Bacterial vaginosis (BV) is a common condition and is characterised by an overgrowth of predominantly anaerobic bacteria, such as *Gardnerella vaginalis*, *Prevotella* species, *Peptostreptococcus*, *Fusobacterium* and *Mobiluncus* species. This overgrowth causes a decrease in *Lactobacillus* populations and an increase in vaginal pH. (Donders, 2010) The link between BV and HPV infection, cervical neoplasia, and cervical cancer has been extensively studied. It has been reported that the prevalence of HPV infection and CIN is higher in women with BV. (Gillet et al., 2012) A meta-analysis has calculated that the risk of HPV infection is 2.5 times and of CIN 1.5 times higher in the case of BV. (Liang et al., 2019) It is hypothesised that BV contributes to the development of cervical cancer by increasing vaginal nitrosamine levels and altering cytokine profiles. (Biswal et al., 2014) Certain bacteria associated with BV, such as *Atopobium*, can activate proinflammatory pathways, including nuclear factor-kB, tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), IL-8, and macrophage inflammatory protein 3α. (Libertucci and Young, 2019; Sharifian et al., 2023) Additionally, other BV-associated bacteria exhibit a similar proinflammatory profile, characterised by increased levels of IL-1α, IL-1β, IL-1γ, IL-8, TNF-α, and granulocyte–macrophage colony-stimulating factor. (Kyrgiou et al., 2017; Sharifian et al., 2023) Fusobacteria (*Fusobacterium* and *Sneathia*) and *G. vaginalis* produce the enzyme sialidase, contributing to mucus degradation. This process compromises the cervical epithelium, increasing susceptibility to HPV infection. (Borgdorff et al., 2016) Moreover, *Fusobacterium* species can activate the Wnt signalling pathway, which is a critical mechanism for cell survival and proliferation and is known to play a role in cervical cancer. (Sharifian et al., 2023; Üren et al., 2005)

Aerobic vaginitis (AV) is another type of abnormal vaginal microflora. It is characterised by the absence of lactobacilli, along with overgrowth of aerobic bacteria such as *E. coli, Enterobacteria, Streptococci, Staphylococci*, and different degrees of inflammation. (Donders, 2010) The association between AV, HPV infection, CIN, and cervical cancer is not well established, and data are limited. *E. faecalis* – one of the most common AV-associated pathogens – was found more often in HPV-positive women. (Carrillo-Ng et al., 2021) Some studies found that AV is more common in women with CIN compared to healthy ones. (Jahic et al., 2013; Vieira-Baptista et al., 2016) One possible explanation for this association is inflammation, which is present in AV in various degrees. A chronic pro-inflammatory environment leads to tissue damage and an accumulation of immune infiltrates, which release potential carcinogens. This process likely enhances HPV's oncogenic potential by speeding up DNA and cellular damage. (Kyrgiou and Moscicki, 2022) The detection of proinflammatory cytokines (IL-1, IL-6, IL-8) in women with AV supports the idea that these cytokines have a role in cervical cancer development. (Donders et al., 2011; Moscicki, Shi, et al., 2020) A recent study found that carbonic anhydrase IX – the enzyme that maintains alkaline intracellular pH in cancer cells that is critical for their survival – is detected together with moderate/severe AV cases and CIN3. (Grincevičienė et al., 2024)

Vaginal ecosystem diagnostic methods

Vaginal pH

The normal vaginal environment is characterised by the dominance of *Lactobacilli*. The production of lactic acid and hydrogen peroxide decreases vaginal pH, and the normal pH value in reproductive-age women is 3.8-4.4. On the other hand, when the vaginal ecosystem is disturbed, and the number of *Lactobacilli* is decreased, pH increases. Therefore, elevated vaginal pH can be used as a marker or a screening method to diagnose vaginal dysbiosis (Donders, 2007; Donders et al., 2007) However, there are many non-infectious conditions that can influence pH value – blood, semen, and the use of different vaginal products. Therefore, if vaginal pH is measured, further diagnostic methods like microscopy, or molecular-biology techniques should follow, to prove the diagnosis. (Donders, 2007)

Wet-mount microscopy

Wet-mount microscopy is a fast, bedside, and inexpensive method for describing the vaginal ecosystem. (Donders, 2007; Rumyantseva et al., 2015) The evaluation is based on the relative abundance of lactobacilli morphotypes relative to other bacteria. The conclusion is expressed as lactobacillary grades (LBG) I-III. In case of LBG I lactobacilli of variable size are dominant, and this is considered normal microflora. LBG II reflects intermediate flora,

where a combination of lactobacilli and other bacteria is found. LBG II is subdivided into LBG IIa, with lactobacilli still outnumbering other bacteria, and LBG IIb, where the number of lactobacilli is decreased, and other bacteria are abundant. LBG III reflects an abnormal environment, with the complete loss of lactobacilli, replaced by other bacterial types. LBG III is divided into subtypes – bacterial vaginosis, aerobic vaginitis, and mixed flora. In BV, large numbers of granular, uncountable bacteria are seen; AV flora is described as coarse, coccoid bacteria, accompanied by increased leucocyte counts and parabasal cells. Mixed infection is diagnosed when signs of BV and AV are seen on the same slide (Figure 1.10). (Donders, 1999, 2007)

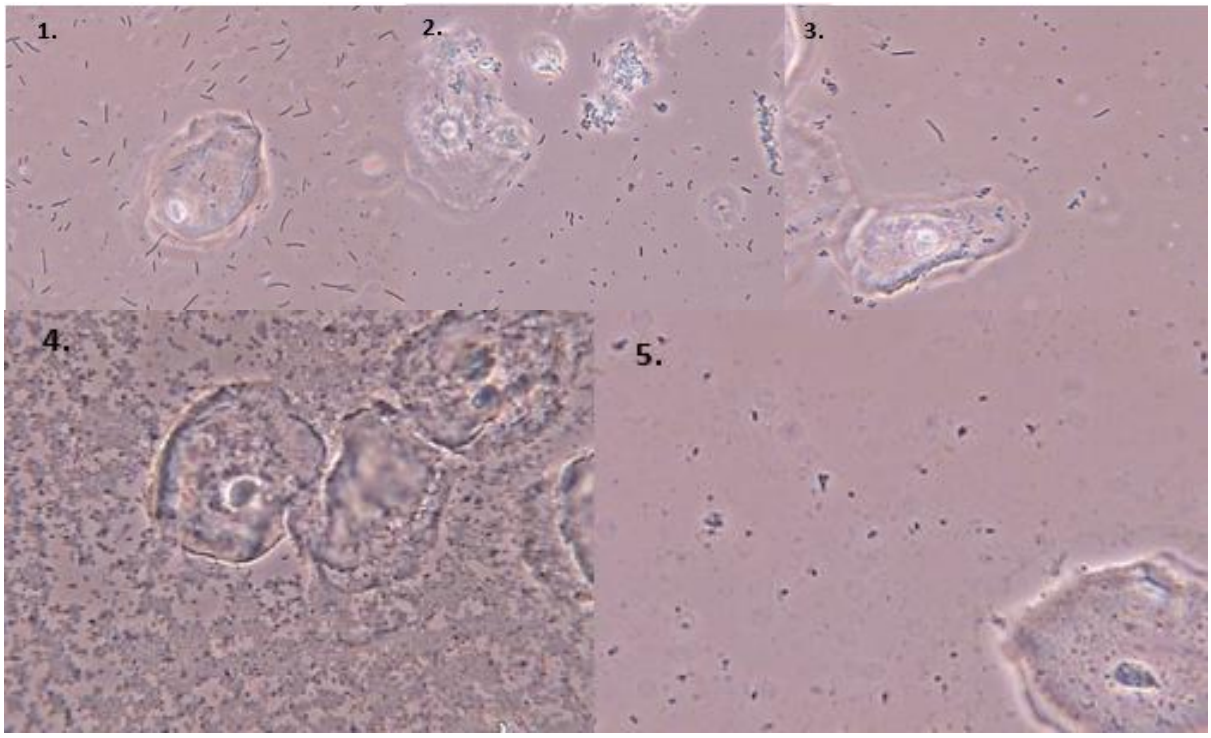


Figure 1.10 **Lactobacillary grades**

1. LBG I; 2. LBG IIa; 3. LBG IIb; 4. LBG III BV; 5. LBG III AV
(Courtesy to prof. G. Donders) (Donders, 2007)

Wet-mount microscopy, like any microscopic method, is subjective and operator-dependent. However, with appropriate training and the use of standardised criteria, it has high diagnostic accuracy and can help minimise diagnostic errors and reduce both overtreatment and undertreatment of vaginal conditions. (Donders et al., 2015; Vieira-Baptista et al., 2021) Using wet-mount microscopy to identify lactobacillary grades and clue cells showed excellent intra- and interobserver reproducibility (Donders et al., 2009). Because of high diagnostic accuracy and relatively simple evaluation, the greater use of wet-mount microscopy to identify bacterial morphotypes has been proposed not only among gynaecologists but also in family practices. (Schmidt & Hansen, 2000)

Microscopy of Gram-stained specimen

Microscopy of Gram-stained vaginal specimens is commonly used to diagnose various vaginal infections. The standard procedure for evaluating these smears is Nugent's score, which quantifies certain bacterial types, including *Lactobacillus*, *Gardnerella*, *Bacteroides*, and *Mobiluncus.* Score 0–3 represents Normal microflora with the dominance of lactobacilli, 4–6 is intermediate flora, and 7–10 represents bacterial vaginosis (Table 1.1). (Verhelst et al. 2005; Nugent et al. 1991)

Table 1.1

Nugent's score
(Nugent et al. 1991)

Score	*Lactobacilli*	*Gardnerella* and *Bacteroides*	*Mobiluncus*
0	4+	0	0
1	3+	1+	1–2+
2	2+	2+	3–4+
3	1+	3+	–
4	0	4+	–

The method requires time and skill and also does not evaluate other bacterial types, leukocytes, or other cell types. Therefore, it is highly effective at diagnosing bacterial vaginosis but may miss other forms of dysbiosis, such as aerobic vaginitis. (Verhelst et al. 2005; Donders et al., 1996; Donders 2007).

Molecular biology techniques

Advanced laboratory techniques, such as molecular biology methods, enable precise identification of vaginal microbial composition at the species level. Moreover, certain methods, such as quantitative real-time polymerase chain reaction (PCR), enable quantification and relative abundance of different microorganisms. Using these methods, previously unknown microorganisms were identified, and the importance of certain species (*Atopobium vaginae, Sneathia, Megasphaera*) was highlighted. (Donders et al. 2017; Ravel et al. 2011) It was proven that a healthy vaginal microbiome is characterised by a limited number of species, usually 3–4, and a classification system dividing all microbiome types into 5 community state types (CST) has been introduced. CST I, II, and V represent certain lactobacillus species dominance (*L. crispatus, L. gasseri* and *L. jensenii* respectively) and are characterised by strong protection against infections. CST III is dominated by *L. iners* which is less stable and prone to dysbiosis. CST IV is a non-lactobacilli dominant microflora, mainly with different anaerobes (*Gardnerella, Atopobium*), and is associated with bacterial vaginosis, and increased risk of STI. (Ravel et al. 2011) Furthermore, molecular biology methods are the only ones allowing precise diagnosis of STI. However, it should be noted that these

methods are much more expensive than microscopy techniques; the detection of certain species does not necessarily indicate abnormality (e. g., *Ureaplasma spp*.), and the organism's response cannot be identified (e. g., inflammation, epithelial desquamation). (Donders et al. 2017)

1.6.7 Sexually transmitted infections

Sexually transmitted infections (STI) are a global healthcare burden. The common STI are human immunodeficiency virus (HIV) infection, *Chlamydia trachomatis* (*C. trachomatis*), *Neisseria gonorrhoeae* (*N. gonorrhoeae*), *Trichomonas vaginalis* (*T. vaginalis*), *Mycoplasma genitalium* (*M. genitalium*), and herpes simplex virus (HSV). Several viral and bacterial STI have been associated with the higher risk of cervical cancer progression in women with HPV. (Kombe Kombe et al., 2021)

C.trachomatis is a Gram-negative, obligatory intracellular bacteria that can cause genital tract and ocular infections. (Franchini et al., 2022; Silva et al., 2014; Yang et al., 2021) It is the most prevalent bacterial STI worldwide. In 2020, there were approximately 129 million cases worldwide. (WHO Sexually Transmitted Infections (STIs), 2024) *C. trachomatis* is related to different negative healthcare outcomes: pelvic inflammatory disease, ectopic pregnancy, infertility, HPV infection, and cervical cancer. (Silva et al., 2014) *C. trachomatis* can cause chronic inflammation and squamous metaplasia, with metaplastic cells being a target for HPV, thus increasing the risk of getting infected with HPV. (Silva et al., 2014) Like other intracellular pathogens, *Chlamydia* can alter gene expression and protein production within host cells. These alterations can lead to host genome instability and apoptosis inhibition and contribute to the observed epidemiological link between *C. trachomatis* and cervical cancer. (Franchini et al., 2022; Yang et al., 2021) *Chlamydia* also has some immunomodulating abilities – it can lower the number of antigen-presenting cells in the cervical mucosa, thus decreasing the ability of the host immunity to recognise pathogens, including HPV. (Franchini et al., 2022; Silva et al., 2014)

N.gonorrhoeae is a Gram-negative diplococcus responsible for genital tract infection. In 2020, there were 82 million estimated cases worldwide. (WHO STIs, 2024) The association between *N. gonorrhoeae* and cervical cancer has not been sufficiently studied. Nonetheless, there is data that *N. gonorrhoeae* can effect *p53* (controls cell division and death) and *cIAP2* (inhibits apoptosis) gene expression and theoretically increases the risk of cervical cancer. (Kazaal, 2024)

T.vaginalis is a flagellate protozoa and the most common cause of non-viral STI worldwide. In 2020, there were 156 million estimated cases globally. (WHO STIs, 2024) *T. vaginalis* is linked to an increased risk of infertility, preterm birth, HPV infection, and

cervical cancer. Yang et al., 2018) The latest meta-analysis found that *T. vaginalis*-positive women had higher odds of being HPV-infected and developing cervical cancer. (Hamar et al., 2023) *T. vaginalis* can create micro-lesions in the cervical epithelium, disrupt the protective vaginal mucus layer, and trigger the release of proinflammatory cytokines (IL-8, macrophage inflammatory protein 3α). These effects can facilitate the entry of HPV into the basal epithelial layer, increasing the likelihood of persistent HPV infection and possibly carcinogenesis. (Castle et al., 2001; Costa et al., 2005; Hamar et al., 2023)

1.6.8 Role of Mycoplasmataceae

Mycoplasmataceae are the smallest known bacteria. The family *Mycoplasmataceae* consists of the genera *Mycoplasma* and *Ureaplasma*. (Klein et al., 2020) Among these *M. genitalium* is emerging as a sexually transmitted pathogen associated with various inflammatory reproductive tract conditions in women, such as cervicitis, and pelvic inflammatory disease. (McGowin and Anderson-Smits, 2011) However, *M. hominis, U. urealyticum, and U. parvum* are commonly found in asymptomatic persons without disease and should not be considered, routinely diagnosed, and treated as STI. (Horner et al., 2018) Nonetheless, there is data that these bacteria could be associated with an increased risk of HPV infection and even cervical cancer. *M.hominis* was significantly associated with HR-HPV infection and high-grade cervical lesions. (Adebamowo et al., 2017; Klein et al., 2020) A meta-analysis published in 2018 found that the presence of *M. genitalium* and *U. urealyticum* significantly increased the risk of HR-HPV. Moreover, *M. genitalium*, *U. urealyticum*, and *U. parvum* were related to a higher risk of abnormal cytological smear. (Ye et al., 2018)

The mechanism through which *Mycoplasma* species may promote HR-HPV infection or contribute to cervical carcinogenesis is still unclear. Being intracellular, *Mycoplasma* infection may directly impact HPV replication within epithelial cells and shape the immune microenvironment by modulating cytokine expression. (Adebamowo et al., 2017; Klein et al., 2020)

1.6.9 Immunosuppression

Immunosuppression, for various reasons, is a recognised risk factor for cervical cancer. (Moscicki et al., 2019) HIV-infected women, solid organ transplant recipients, patients with autoimmune disorders, and individuals undergoing immunosuppressive treatment face a considerably higher risk of HPV infection and cervical cancer. (Dugué et al., 2013; Moscicki et al., 2024)

Immunosuppression caused by HIV infection results from various factors, such as disrupted innate signalling pathways, higher viral load, the progressive loss of CD4+ T cells,

and the depletion of T lymphocytes at mucosal sites. These factors collectively lead to progressive immune deficiency and acquired immunodeficiency syndrome. (Elfaki, 2014) Cervical cancer was listed as an acquired immune deficiency syndrome (AIDS)-defining disease in 1993. (Castro et al., 1993) HIV-positive women have a sixfold increased risk of cervical malignancy compared to healthy individuals and, therefore, are recommended for more intensive screening. (Bowden et al., 2023; Dugué et al., 2013; Stelzle et al., 2021) However, studies report that the risk of cervical neoplasia may be related more to the CD4+ T cell count than to HIV infection itself. The risk of CIN2+ significantly increases when there are < 500 CD4+ T cells/µl (RR 3.0, 95 % CI 1.6–5.5) and even more if < 200 CD4+ T cells/µl (RR 5.6, 95 % CI 2.1–14.7). On the contrary, if the number of CD4+ cells is ≥ 500 cells/µl, the CIN2+ risk is unchanged (RR 0.8, 95 % CI 0.4–1.7). (Silverberg et al., 2018) Nowadays, many women are using antiretroviral therapy (ART) to suppress viral replication and preserve or improve immune functions. (Dugué et al., 2013) Early initiation of ART combined with sustained adherence is considered to enhance HPV clearance and decrease the incidence and progression of CIN, eventually reducing the risk of invasive cervical cancer by maintaining elevated levels of CD4+ T cells. (Kelly et al., 2018)

Solid organ transplant recipients are more often infected with HR-HPV and have a 3-fold increased risk for developing squamous intraepithelial lesions and a 2 to 6-fold increased risk of cervical cancer. (Moscicki et al., 2019)

Inflammatory bowel disease (IBD) patients have the same HPV infection rates overall. However, the risk of infection with types 16 and 18 is higher than in healthy individuals, especially in IBD patients receiving immunosuppressive treatment. However, in most of the studies, the risk of HSIL and cervical cancer was not increased. (Moscicki et al., 2024)

Patients with systemic lupus erythematosus have higher HR-HPV infection and HSIL rates, and the risk of cervical cancer is increased 2–3-fold. (Moscicki et al., 2024)

Data about the cervical cancer risk in rheumatoid arthritis (RA) patients are contradictory. An umbrella review published in 2023 described a weak association between cervical cancer and RA. (Bowden et al., 2023) On the contrary, recently published cervical cancer screening recommendations for immunosuppressed women reported that patients with rheumatoid arthritis had a standardised incidence ratio of 9.5 for cervical cancer. (Moscicki et al., 2024) Possibly, it is not rheumatoid arthritis *per se*, but immunosuppressive medications and disease-modifying antirheumatic drugs used to treat the disease, that can play a role and modify cancer risk. (Bowden et al., 2023)

1.6.10 Genomic risk factors

Most cervical cancer cases result from persistent infection with high-risk HPV types, but genetic predisposition also plays a role in determining who is more likely to develop cancer after HPV exposure. Cancer registry studies have reported that the 1st line relatives have a 1.5–2.3 relative risk for developing cervical cancer. (Ramachandran and Dörk, 2021) The most extensively studied are human leucocyte antigen genes.

The host immune response is essential in the natural history of HPV infection. (Song et al., 2015) B lymphocytes (via antibodies) are key in preventing infection, facilitated by the extended lag between virus attachment and subsequent cell entry. In contrast, T cells mainly control established infections, partly through the presentation of viral particles by the Major Histocompatibility Complex (MHC). (Nelson and Mirabello, 2023) MHC encodes cell-surface glycoproteins that bind intracellular and extracellular peptides. Located on chromosome 6, the human MHC contains over 200 genes and produces human leukocyte antigens ((HLA) encoded by HLA class I and II genes), which present peptides to T cells. Therefore, the HLA system is crucial in determining the immune system's ability to recognise and respond to infections. MHC exhibits extensive polymorphism, which poses a challenge in finding matched pairs but improves the immune system's capacity to identify diverse invading pathogens. (Mosaad, 2015) Because of that, hosts may vary in their ability to control infection depending on the specific viral type or variant. Genome-wide association studies (GWAS) have identified particular HLA class I (presenting to CD8+ T cells) and class II (presenting to CD4+ T cells) alleles linked to an increased or decreased risk of cervical cancer. (Nelson and Mirabello, 2023)

Several studies have been performed to identify specific HLA alleles and haplotypes possibly affecting a woman's risk of developing cervical neoplasia and cancer. HLA-DRB1*15:01 has been consistently associated with cervical cancer in multiple populations, including Asian and European cohorts. (Bao et al., 2018; Chen et al., 2013; Hu et al., 2014b; Krul et al., 1999; Leo et al., 2017; Rathika et al., 2016) In the British population, DRB1*11:01 has been linked to an increased risk of high-grade cervical lesions (Odunsi et al., 1996), while DQB1*03:01 has been associated with high-grade disease and cervical cancer in British, Spanish, and Norwegian populations. (Cuzick et al., 2000; Lie et al., 1999; Montoya et al., 1998; Odunsi et al., 1996) HLA alleles *HLA-B**07:02, *HLA-B**15:01, *HLA-DRB1**13:01, *HLA-DRB1**15:01, *HLA-DQA1**01:03, *HLA-DQB1**06:03, *HLA-DQB1**06:02, and *HLA-C**07:02 showed significant association with cervical neoplasia and cancer in the Swedish population. (Chen et al., 2018) The *HLA-DQB1*05:01 and HLA-DRB3*99:01* were identified as risk alleles in several other

studies in European and African-American populations. (Ramachandran and Dörk, 2021) Conversely, protective effects have been reported for DQB1*05:01 in British populations (Cuzick et al., 2000; Odunsi et al., 1996), as well as for DRB1*13:01 and DQB1*06:03 across multiple ethnic groups. (Chen et al., 2013; Chen and Gyllensten, 2015; de Araujo Souza et al., 2009; de Freitas et al., 2012; Leo et al., 2017; Zoodsma et al., 2005)

Extensive population studies, including GWAS, found that haplotypes HLA-B*0702-DRB1*15:01/HLADQB1*06:02, HLA-DRB1*15/HLA-DQB1*06:02/HLA-DQA1*01:02, HLA-B*07:02/HLA-C*07:02, and HLA-DRB1*04:01/HLA-DQA1*03:01 are associated with increased risk, but HLA-B*15:01/HLA-DRB1*13:01/HLA-DQA1*01:03/HLA-DQB1*06:03 and HLA-DRB1*13-DQB1*06:03-DQA1*01:03 with the decreased risk. (Brown and Leo, 2019; D. Chen et al., 2013; Leo et al., 2017; Madeleine et al., 2008; Wang et al., 2002)

Non-HLA-related associations with cervical cancer have also been identified through polymorphisms in numerous genes in candidate gene studies, including T53, Toll-like receptors, and genes related to DNA repair. However, none of these have yet been strongly linked to cervical dysplasia or cancer. (Leo et al., 2017)

1.7 Cervical cancer prevention

Among all malignancies, cervical cancer can be effectively prevented. An optimal cervical cancer prevention strategy includes primary prevention through vaccination to protect against high-risk oncogenic HPV types, along with risk reduction measures such as safe sexual practices, smoking cessation, and overall lifestyle modifications to support immune health. Secondary prevention emphasises early detection and treatment of precancerous lesions to prevent the development of the malignancy.

1.7.1 Primary prophylaxis

Primary prevention aims to prevent disease before it occurs. Vaccination against HR-HPV types – the main cause of cervical cancer – is an effective primary prophylaxis method. The primary goal of vaccination is to prevent cervical cancer and precancerous conditions and to provide effective and long-term protection against HPV infection. In 2006 and 2007, the European Medicines Agency (EMA) registered and approved two vaccines for use in Europe, which were indicated for preventing precancerous cervical lesions and cervical cancer directly linked to HPV infection. These are the recombinant Human Papillomavirus Quadrivalent (Types 6, 11, 16, 18) Vaccine, recombinant Silgard/Gardasil, and the recombinant Human Papillomavirus Bivalent (Types 16 and 18) Vaccine, recombinant Cervarix. (EMA Gardasil 2025, 2025; EMA Cervarix, 2025) In 2015, a new vaccine was

registered with the EMA – the Human Papillomavirus Nonavalent Vaccine (types 6, 11, 16, 18, 31, 33, 45, 52, 58 (Human Papillomavirus 9-Valent Vaccine, recombinant, adsorbed)). (EMA Gardasil 9, 2025)

Vaccines contain virus-like particles (VLPs) of the L1 protein of the HPV capsid. The bivalent vaccine contains VLPs of HPV 16 and 18 and protects against these two HR-HPV types. The nonavalent protects against seven high-risk HPV types – 16, 18, 31, 33, 45, 52, 58, and two low-risk types – 6 and 11. Both vaccines use aluminium-based adjuvants to enhance the immune response. Bivalent – AS04 (aluminium hydroxide and 3-deacylated monophosphoryl lipid A), nonavalent – aluminium hydroxyphosphate sulfate. Vaccines do not contain the viral genome and are unable to cause the disease. (WHO, 2024; Schiller et al., 2012)

After administration, the VLPs are recognised by dendritic cells and macrophages and presented to other immune system components, including T and B cells. B cell activation activates humoral immunity and results in the production of protective antibodies. T cells are responsible for the cellular immunity responsible for early defence mechanism. (McFall-Boegeman and Huang, 2022; Pasmans et al., 2022)

HPV vaccines demonstrate high immunogenicity, with seropositivity rates approaching 100 % following vaccination. The vaccine elicits a significantly stronger serological response than natural infection. (Hoes et al., 2022; WHO, 2024) Although antibody levels slightly decline over the years, they remain significantly high and provide long-term protection. Studies report follow-up to 14 years. (Hoes et al., 2022) Booster doses are not recommended after initial vaccination. (WHO, 2024)

Numerous studies have confirmed the efficacy of HPV vaccination against high-grade lesions and cervical cancer. (Huh et al., 2017; Kjaer et al., 2021; Lei et al., 2020; Toh et al., 2019) The main target group for HPV vaccination is girls who have not yet become sexually active, as they exhibit the highest clinical efficacy. (von Karsa et al., 2015) In studies focusing on HPV disease endpoints – like cervical intraepithelial neoplasia grade 2 or higher (CIN2+) and adenocarcinoma in situ (AIS) – HPV vaccines have shown high efficacy in populations that are HPV-naïve, with effectiveness nearly reaching or achieving full protection 100 %. (WHO, 2024) Real-world effectiveness studies show that women vaccinated at < 17 years had no invasive cervical cancer cases during > 10 years of follow-up. (Kjaer et al., 2021; Lei et al., 2020)

Catch-up vaccination of girls and young women until age 26 can accelerate the impact of vaccination programmes. (von Karsa et al., 2015) Vaccination of this age group also provides highly effective and long-term protection against cervical disease. A recent study

reports a 90 % effectiveness against CIN2+ for at least 10 years in HR-HPV-negative women vaccinated at 16–26 years of age. (Kjaer et al., 2024)

Vaccinating boys and young males up to age 26 helps reduce the prevalence of HPV infection in the population, adds cross-protection for unvaccinated women, therefore lowers cervical cancer rates, and protects men against HPV-related cancers. (Naidoo et al., 2024)

All HPV vaccines are considered safe. The Global Advisory Committee on Vaccine Safety routinely reviews their safety. There is no evidence linking HPV vaccines to serious adverse effects such as Guillain-Barré syndrome, complex regional pain syndrome, postural orthostatic tachycardia syndrome, premature ovarian insufficiency, primary ovarian failure, or venous thromboembolism. (Global Advisory Committee on Vaccine Safety, 2017)

The United States of America was the first country to implement a national HPV vaccination programmein 2006, followed by Australia and several European countries (Belgium, France, Germany, and Spain) in 2007. In 2025, 147 countries had included HPV vaccination in their national immunisation programmes (Figure 1.11). (International Vaccine Access Center (IVAC), 2025)

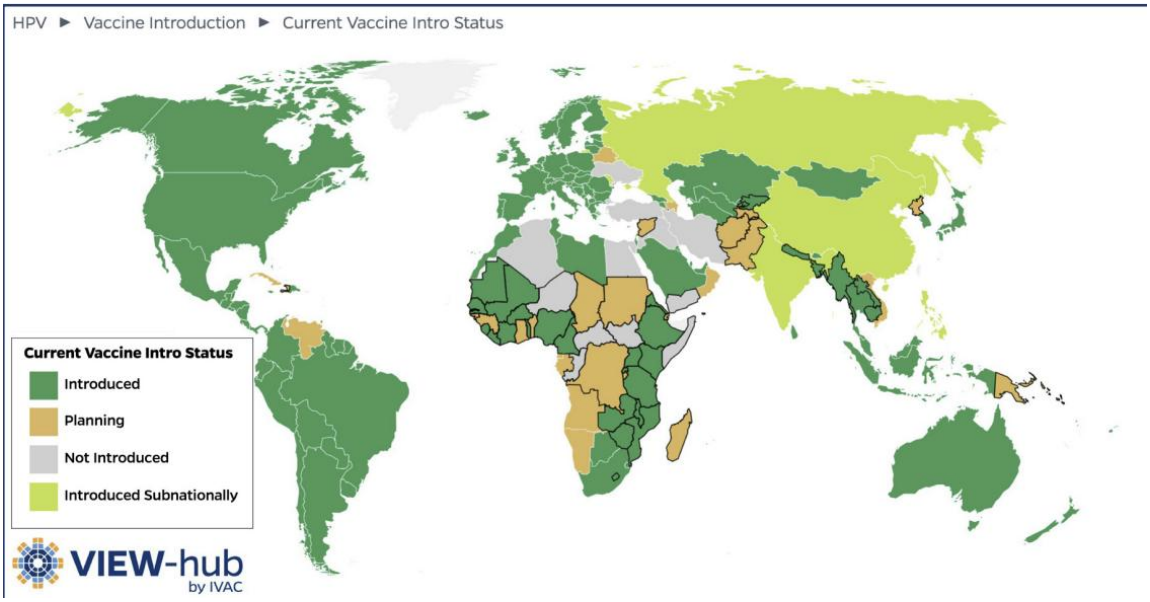


Figure 1.11 **Countries with national HPV immunisation programmes in the world**
(IVAC, 2025)

Vaccine uptake differs significantly between regions and countries despite being introduced in many countries. Globally, it is estimated that only 27 % of girls have received at least one dose of a vaccine (WHO Immunisation Data portal, 2025). The highest coverage rates are in Scandinavian countries, North America, Australia, Oceania, and some Southern American and Southern African countries (Figure 1.12).

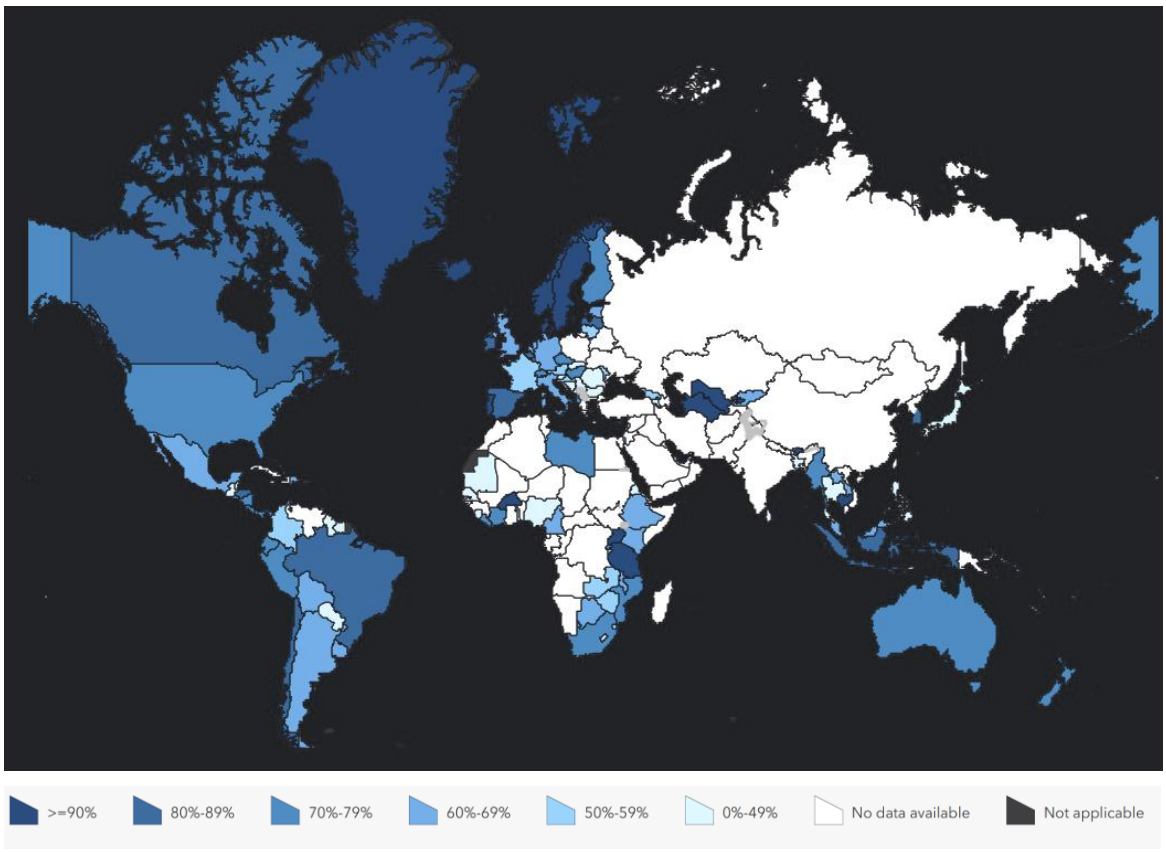


Figure 1.12 **Vaccination programme coverage with the first dose in females**

(WHO Immunisation Data portal, 2025)

Vaccination against HPV in Latvia started in 2010. Since 2023, it has been recommended for girls and boys aged 12 to 17. The nine-valent vaccine is used. The vaccination schedule for immunocompetent persons is two vaccines six months apart. For immunocompromised individuals, a three-dose schedule is recommended: the second dose is administered one month after the first, and the third dose follows six months after the second dose. (SPKC, Pārskati par imunizāciju, 2025) Vaccination coverage is seen in Figure 1.13.

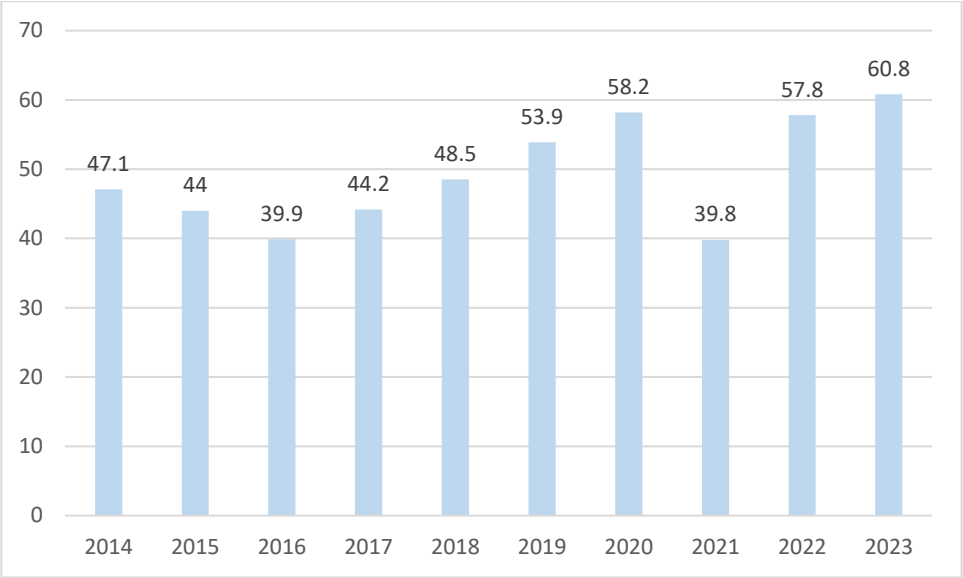


Figure 1.13 **Timely received HPV vaccination with 1st dose in 12-year-old girls in Latvia**

(SPKC, Pārskati par imunizāciju, 2025)

1.7.2 Secondary prophylaxis

Precancerous changes in the cervix are a prerequisite for the further development of malignant disease. Malignant transformation typically takes several years (averaging 10–15 years) to occur in normal epithelial cells. Precancerous changes in the cervix do not cause complaints. Therefore, it is essential to detect these changes during preventive examinations.

It has been proven that cervical precancerous changes can be detected most effectively and then prevented on time if prophylactic tests are performed in an organised population screening program. Countries that have implemented an organised cervical cancer screening programme show a significant decline in disease compared with those that have opportunistic or no screening. Organised screening is also more cost-effective than opportunistic. (Arbyn et al., 2008)

The screening test should be easy to perform, available at a reasonable cost, with minimal discomfort during test collection, and have high sensitivity and specificity for detecting CIN. (Arbyn et al., 2008) Organised cervical cancer screening is a multi-step process that involves:

- Identifying the target population
- Recruiting eligible women for screening
- Collecting samples
- Examining and reporting test results

- Providing reassurance to women with normal results and informing them about the timing of their next screening
- Recalling women with unsatisfactory or inadequate samples for repeat testing
- Following up on abnormal results through diagnostic procedures and treatment if necessary, ensuring a fail-safe system is in place
- Registering, monitoring, and evaluating the entire screening program. (Arbyn et al., 2008)

Currently, two screening methods are available in Europe – cervical cytology and detection of high-risk human papillomavirus. (Arbyn et al., 2008)

For many decades, cytology has been used as the primary screening test. Two currently available cytology methods are conventional cytology (Papanicolaou test, Pap test) and liquid-based cytology (LBC). LBC has higher sensitivity (70 %) than the Pap test (50–70 %) and allows an HR-HPV test to be performed on the same sample.

Primary HR-HPV testing increases the diagnostic sensitivity of CIN to 95 %, but the specificity is only slightly lower than the Papanicolaou method (91 % vs. 98 %, respectively). Studies show that HR-HPV clinically validated tests are much more effective than cytology for primary screening of cervical precancer and cancer in individuals over 30, allowing for safe extension of screening intervals to five years when using HPV tests. (Arbyn et al., 2012; Hoste et al., 2013)

Not all women with positive HR-HPV test will develop precancerous or malignant changes. In many cases, non-progressive CIN2+ or CIN3+ changes will be found in the case of a positive HR-HPV test. To ensure that colposcopy is performed only in the case of high-risk changes, several triage tests are available after a primary positive HPV test: (Arbyn et al., 2008):

1) Cytology – women with primary HR HPV-positive results can undergo cytology testing. Cytology, used as a triage method after a primary positive HR-HPV test, improves CIN3+ detection compared to cytology alone. (Kitchener et al., 2009; Leinonen et al., 2012) Women with results of atypical squamous cells – high-grade not excluded (ASC-H), HSIL, or more severe changes require immediate colposcopy. Individuals with atypical squamous cells of unknown significance (ASCUS), atypical glandular cells of unknown significance (AGUS), or low-grade squamous intraepithelial lesion (LSIL) may be recommended to undergo a repeat HPV test in 6–12 months or to undergo colposcopy. (von Karsa et al., 2015) HR HPV-positive, cytology-negative women should repeat HR-HPV testing after 6–12 months. If persistent HR-HPV infection is detected, cytology is reassessed. If

abnormalities (≥ ASCUS) appear, a colposcopy is necessary. If cytology remains negative, options include repeating the HR-HPV test after 12 months, undergoing colposcopy, or returning to routine screening. (von Karsa et al., 2015)

2) HPV 16/18 genotyping – over 70 % of cervical cancers in Europe are caused by HPV 16 and 18. (Smith et al., 2007) Identifying these genotypes helps stratify patient risk and improves the detection of CIN3+ lesions, especially in women over 25. (Castle et al., 2011) HPV 16/18 genotyping is more sensitive for CIN3+ than cytology triage. (Agorastos et al., 2015) It has also proven a cost-effective triage tool, allowing for earlier diagnosis of CIN3+ and optimizing resource utilisation. (Huh et al., 2015)
3) Dual p16/Ki-67 staining – this test identifies abnormal cell cycle regulation associated with HPV oncogenes. p16 overexpression correlates with disease severity, whereas Ki-67 is a cell proliferation marker. (Li et al., 2015; Tsoumpou et al., 2009) Dual staining provides higher sensitivity for detecting CIN2+ than cytology alone. If dual staining is negative, the risk of CIN2+ is significantly lower. This also allows for a longer follow-up interval (3 years). (Clarke et al., 2019; Wentzensen et al., 2019)
4) Methylation testing – DNA methylation regulates gene expression and plays a role in cervical cancer progression. The specialised cell maintains a stable and unique DNA methylation pattern that controls tissue-specific gene expression transcription. (Moore et al., 2013) Methylation can be detected both for the HPV virus and the host. Studies show that methylation levels significantly increase in CIN2+/CIN3+ compared to ≤ CIN1. The specificity of the methylation test is higher than that of cytology (≥ ASCUS) and sensitivity is greater than HPV 16/18 genotyping. (Kelly et al., 2019) It may help predict high-risk lesions likely to progress to invasive cancer.
5) Test combinations – combining triage tests may improve detection accuracy. HPV 16/18 genotyping plus cytology or dual staining enhances CIN3+ risk identification compared to a single test. (Wentzensen et al., 2019) Adding DNA methylation to HPV 16/18 genotyping improves specificity by ~7 %. (Verhoef et al., 2014) While combinations appear superior, no single best approach is currently recommended.

Regardless of which test is used to triage the primary positive HR-HPV test, colposcopy is indicated in case of abnormal results. Colposcopy is used to evaluate women with abnormal cervical screening results or those with clinically suspicious cervical tissues findings. (Redman et al., 2021) The objectives of colposcopy are: (Redman et al., 2021)

- Assess cervical, vaginal, vulvar, and perianal epithelium, distinguishing normal from abnormal.
- Determine the cervical transformation zone's exact location.
- Identify atypical squamous epithelium for histological evaluation.
- Detect or rule out cervical glandular disease.
- Identify or exclude invasive cancer.
- Aid in treating intra-epithelial lesions, ensuring colposcopy precedes treatment.
- Monitor lesion progression or regression.

Colposcopy is a medically and economically justified procedure. It is performed on an outpatient basis, requires no anaesthesia, lasts about 15 minutes, and its findings align with histological diagnoses. When combined with targeted cytology, biopsy, or HR-HPV testing, colposcopy enables early and accurate detection of cervical precancerous changes, facilitating timely treatment while preserving reproductive function and quality of life and reducing cervical cancer incidence. (Arbyn et al., 2008)

High-quality colposcopy assessment requires a thorough understanding of female genital anatomy and the dynamic tissue changes occurring at different life stages. This knowledge enables specialists to differentiate between normal cervical changes and pathological conditions, thereby improving the accuracy and effectiveness of the examination. (Arbyn et al., 2008)

Only trained, preferably certified colposcopists or supervised trainees should perform colposcopy. (Redman et al., 2021)

During colposcopy, the specialist determines whether a biopsy is necessary. Cervical biopsies can be obtained via targeted biopsy, electroexcision, or endocervical curettage. Colposcopically guided biopsies should: (Arbyn et al., 2008; Redman et al., 2021)

- Be sufficient for histological analysis.
- Improve sensitivity when at least two samples are taken.
- Be uninformative in Type 3 transformation zones.
- Be optional for LSIL with low-risk findings but still recommended.
- Be repeated if histologically uninformative.
- Be replaced by diagnostic excision if microinvasion is suspected.

Depending on the colposcopy and biopsy results, the patient can be assigned for follow-up, treatment procedure, or return to routine screening. (Arbyn et al., 2008) The aim of treating cervical precancerous changes is to lower the occurrence of cervical cancer and decrease deaths caused by this disease by preventing HSIL changes in the cervix. Treatment methods include excision procedures and local tissue destruction. Histologically proven

CIN2/CIN3 and cervical adenocarcinoma in situ are indications for excisional treatment, which aims to remove the lesion completely. (Kyrgiou et al., 2020) Local tissue destruction can be used in persistent CIN1 cases to destroy CIN with tissue-destructive therapies. (Perkins et al., 2020)

Post-treatment follow-up is crucial, as treated women have a 2 to 5 times higher risk of developing cervical cancer within the next 20 years. (Redman et al., 2021) Women receiving treatment for high-grade disease should have cytology testing and colposcopy 6 months after the treatment. Then, cytology alone at 12 and 24 months, followed by annual cytology for 5 subsequent years. If treated for low-grade disease, cytology follow-up at 6, 12, and 24 months. (Arbyn et al., 2008) HR-HPV detection as a test of cure has considerably greater sensitivity in forecasting residual or recurrent CIN. (Arbyn et al., 2008) Therefore, HR-HPV (with or without LBC) can be taken at the 6-month follow-up visit. If both cytology does not find an intraepithelial lesion, and the HR-HPV test is negative, the patient returns to the routine screening program. The colposcopic examination is indicated if cytology shows ASCUS or more pronounced changes and/or HR-HPV is positive. (Redman et al., 2021)

Cervical Cancer Screening Programmes Globally and in Latvia

By 2021, 139 countries globally had implemented cervical cancer screening recommendations, with cytology as a primary test in 109 countries and the HR-HPV test in 48 countries. Despite that, it is estimated that 67 % of women aged 20–70 have never been screened. Screening coverage is closely tied to a country's income level. High-income countries exhibit the highest estimated screening rates, with 84 % of women aged 30–49 having been screened in their lifetime. In turn, in low-income countries, it is only 11 %. (Bruni et al., 2022)

Organised cervical cancer screening in Latvia started in 2009 through the National Health Service, with invitations sent to women via letter. The algorithm has been modified and updated according to the latest European cervical cancer screening recommendations over the years. Since 01.07.2022, cervical cancer screening in Latvia has differed between two age groups: 25–29 (screening interval 3 years, Table 1.2) and 30–70 (screening interval 5 years, Figure 1.14). The primary test for the 25–29 age group is the LBC, and for the 30–70 age group, it is the HR-HPV test.

Table 1.2

Diagnostic algorithm according to screening test results for women aged 25–29
(Nacionālais Veselības Dienests (NVD), 2024)

Test result	Action
Testing without result	The test must be repeated in 3 months.
No intraepithelial lesion	Wait 3 years for the following invitation.
ASCUS, HR-HPV negative	Wait 3 years for the following invitation.
ASCUS, HR -HPV positive	Colposcopy
ASC-H	Colposcopy
LSIL, HR-HPV negative	Wait for the following invitation in 3 years
LSIL, HR-HPV positive	Colposcopy
HSIL	Colposcopy
AGUS, HR-HPV negative	Wait 3 years for the following invitation (if pathological uterine bleeding – perform appropriate examinations – ultrasound, endometrial biopsy)
AGUS, HR-HPV positive	Colposcopy
Signs of malignancy	Consultation with an oncogynecologist

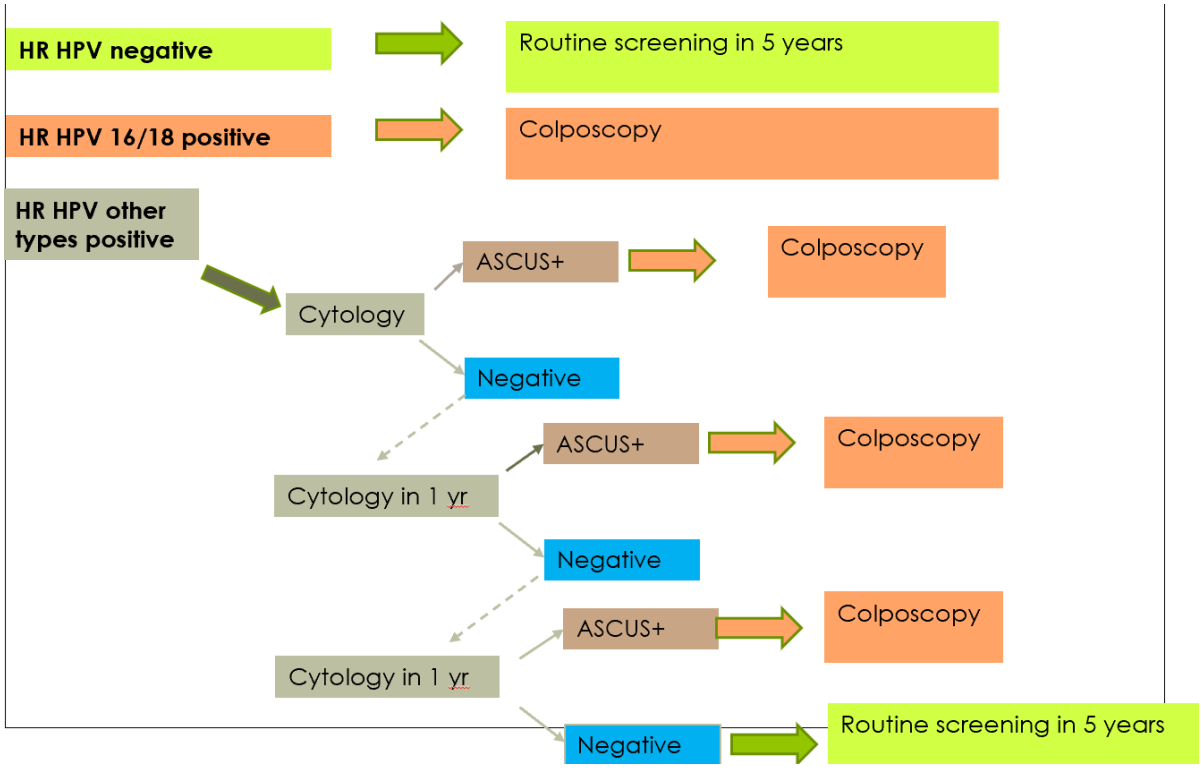


Figure 1.14 **Diagnostic algorithm according to screening test results for women aged 30–70**
(Nacionālais Veselības Dienests (NVD), 2024)

Even though an organised cervical cancer screening programme in Latvia has been available for 15 years, response rates are still far from the WHO goals (Figure 1.15).

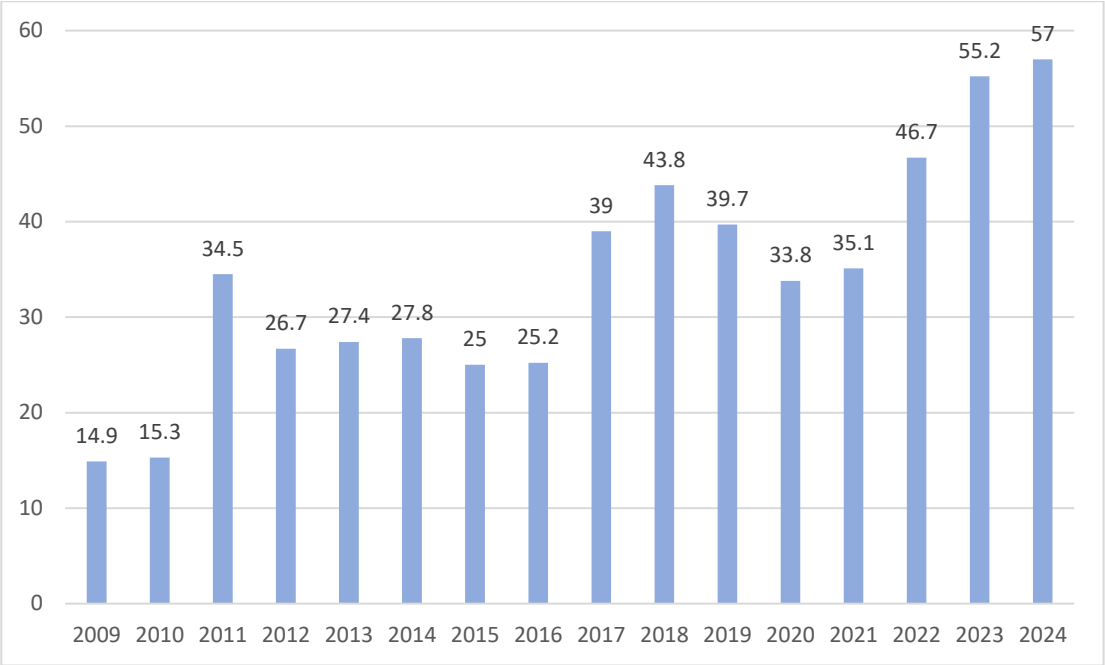


Figure 1.15 **Response rates to cervical cancer screening in Latvia**

(NVD, 2025)

Reasons for non-attendance include not receiving an invitation letter, visiting a gynaecologist recently for another reason, lack of time, organisational difficulties in visiting the clinic, and others. (Zodzika et al., 2021) Some improvements have been made to assess these reasons; for example, the invitation letter has been sent electronically through the national electronic address since May 2024. Although response rates are increasing, the prevalence of non-attenders remains high, and further actions are needed. Some possible solutions could involve general practitioners, nurses, and midwives taking screening samples and introducing HPV self-sampling. (Zodzika et al., 2021) The meta-analysis's results indicate that HPV self-sampling is a viable alternative for all individuals, regardless of age, income, or geographical region. (Nishimura et al., 2021) Moreover, using HPV self-sampling can increase response rates in long-term non-attenders. (Aasbø et al., 2022)

Recently, there has been discussion about the superiority of personalised risk-based cancer screening. The public and healthcare professionals support personalised, risk-based screening as a natural step in early cancer detection, offering customised risk assessments and tailored screenings to enhance patient-centred care (Tan et al., 2025). For example, artificial intelligence (AI) can improve an individual's risk by analysing multiple risk factors, improving diagnosis accuracy, and providing a personalised management plan. (Kyrgiou et al., 2016; Rodriguez et al., 2023)

The development of cervical premalignant disease is driven by a complex interplay of viral, microbial, immunogenetic, and social factors, yet their combined impact remains insufficiently characterised. Although persistent high-risk HPV infection is mandatory for

cervical cancer development, alone it is not enough. The influence of changes in the vaginal ecosystem and genetic factors needs further investigation. This study addresses these critical gaps by systematically analysing HR-HPV status, vaginal ecosystem, sexually transmitted infections, and HLA class II alleles in women with cervical precancer, offering insights for better risk estimation and personalised cancer prevention.

2 Materials and methods

2.1 Design of the study

It was a cross-sectional study.

Study periods:

- Recruitment of participants (interviews, collection of vaginal and cervical samples for wet-mount microscopy, collection and freezing of cervical and vaginal samples for subsequent analysis (HLA genotyping, detection of HPV DNA, mRNA and 7 vaginal infections), colposcopy, histological examination) – January 2016 – June 2017.
- Wet mount microscopy – July 2016 – June 2017.
- HPV DNA and mRNA detection, analysis of 7 vaginal infections – January 2019 – December 2020.
- HLA genotyping – April 2022 – December 2022 (Figure 2.1).

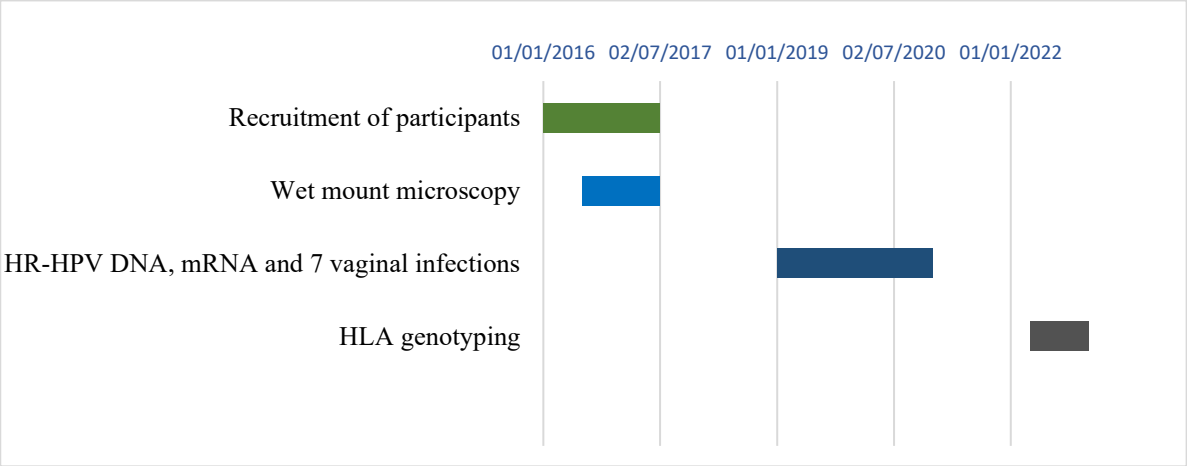


Figure 2.1 **Timeline of the research**

HR-HPV DNA prevalence, as an established significant aetiological factor, was used to calculate the study sample size.

In order to provide 80 % power of the study with the statistical significance level 5 % and assuming the HR-HPV prevalence of at least 30 % in the study group (Sjoeborg, 2010) and a maximum of 10 % in the control group (Bruni, 2010), 63 participants per group are required. Given the potential dropout rate, the study sample was increased by at least 20 %.

The study group included 112 consecutive new patients referred to Riga East University Hospital for colposcopy examination due to abnormal cervical cytology smear results. Participants were included between January 2016 and January 2017.

The control group consisted of 120 women who underwent a gynaecological examination for other reasons (routine examination, follow-up of other gynaecological pathology). Participants were included between January 2016 and June 2017.

Inclusion criteria:

1 Age ≥ 18 years old.
2 Agreed to take part in the study and signed an informed consent form.
3 Abnormal cervical cancer screening result – for inclusion in the study group.
4 Normal cervical examinations – for inclusion in the control group.

Exclusion criteria:

1 Age < 18 years old.
2 Personal history of cervical intraepithelial neoplasia and/or cervical cancer.
3 Pregnant women.
4 Refusal to participate in the study.

2.2 Fieldwork

Eligible women were invited to participate in the study and signed an informed consent.

All patients included in the study were interviewed, and a custom-designed questionnaire was filled out (Annex 1). After that, women had gynaecological *per speculum* examination, pH measurement of vaginal discharge; sample from the anterior vaginal fornix was taken for wet-mount microscopy, samples from the cervix were taken and frozen at −80 °C for subsequent detection of HR-HPV DNA, mRNA, vaginal infections, and HLA class II genes. as a next step, a colposcopy was performed, and biopsies were taken if indicated (Figure 2.2).

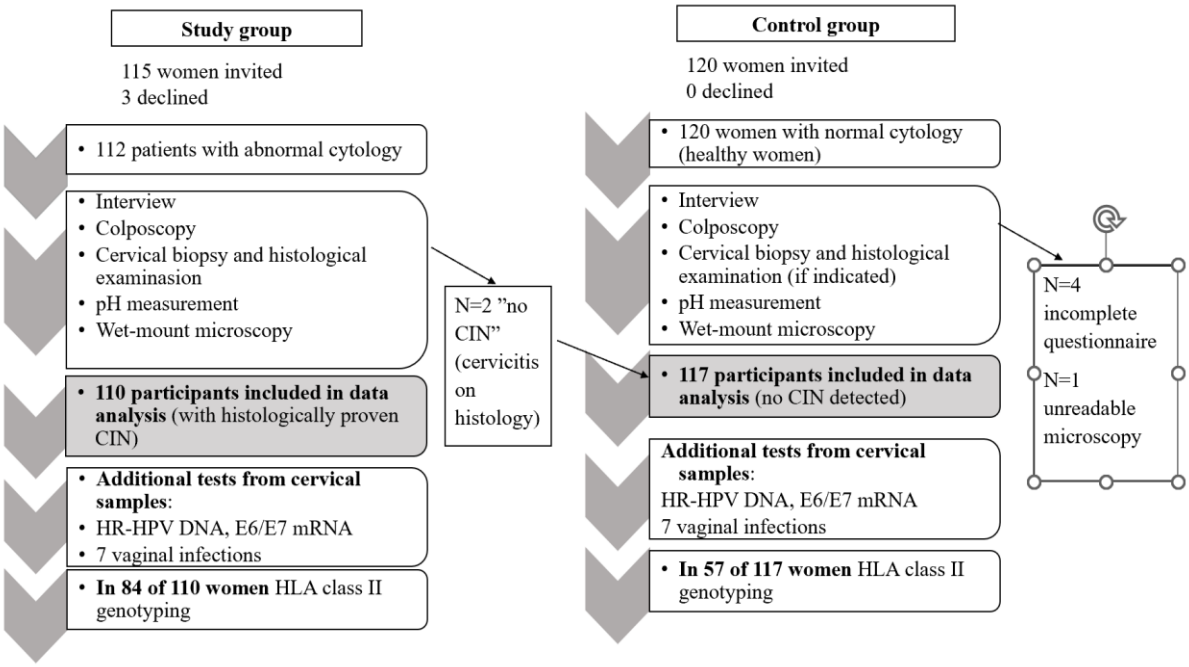


Figure 2.2 **Structure of the study**

2.3 Analysis of sociodemographic and behaviourial factors

The patients provided information on their age, education level, smoking habits, marital status, and contraception type. Three age categories were established: 1) ≤ 30 years, 2) 31–49 years, and 3) ≥ 50 years. Women were considered to have a low education level if they had primary education (nine years) or secondary education (twelve years), while a high education level was assigned if they held at least a bachelor's degree. Contraceptive methods were classified as "effective method" (including combined hormonal contraceptives, progestin-only contraception, intrauterine devices, hormonal intrauterine systems, and sterilisation for males and females), "condom", "withdrawal" (interrupted intercourse), and "no contraception."

2.4 Examination of vaginal ecosystem - vaginal pH measurement and wet-mount microscopy

During the gynecological exam, an unmoistened vaginal speculum was employed. Samples from the anterior vaginal fornix were collected for pH testing and wet-mount microscopy. Vaginal pH was determined using Machery-Nagel pH strips on a glass slide, within a range of 3.1–7. (G. G. G. Donders et al., 2007) A pH level > 4.4 was considered increased. For wet-mount microscopy, the material was spread on a glass slide, air-dried, and later rehydrated with a drop of normal saline. (Donders et al., 2009) Olga Plisko, who attended native microscopy courses in 2012 led by Prof. G. Donders, performed phase-contrast microscopy. The slides were randomly selected and verified by assoc.prof. Jana Zodzika (experienced in native microscopy). A Leica DM 1000 microscope (Warburg, Germany) was

used with phase contrast at 400 times magnification. Microscopic examinations involved assessing lactobacillary grades, counting leucocytes (less than 10 per high power field (hpf); more than 10/hpf but fewer than 10 per epithelial cell; or 10 or more per epithelial cell), evaluating the proportion of toxic leucocytes (none or sporadic, up to 50 %, or more than 50 %), detecting the presence of "clue cells," red blood cells, and sperm cells, measuring the proportion of parabasal cells (none or less than 1 %, up to 10 %, or more than 10 %), and examining background flora (unremarkable, cytolysis, small coliform bacilli, cocci, or other bacteria) chains). (Donders et al., 2002) LBG were classified based on the ratio of lactobacillus to other bacteria, following Donders' modification of Schröder's classification. (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.

In LBG IIb, other microorganisms outnumber lactobacilli. LBG III indicates an absence of lactobacilli, with other bacteria present. This group is subdivided into BV, AV, and mixed BV-AV flora. Full BV is characterised by a widespread granular microflora with numerous bacteria and over 20 % of "clue cells." Mixed areas with streaks of BV-like microflora or occasional "clue cells" alongside other microflora types are classified as partial BV. (Donders et al., 1996) Normal vaginal microbiota corresponds to LBG I and IIa, while abnormal microbiota is classified as LBG IIb and III.

The leucocyte count was rated as: 0 points for fewer than 10 per high power field; 1 point for more than 10 per high power field but less than 10 per epithelial cell; and 2 points for 10 or more per epithelial cell. (Donders, 1999)

Leucocytes were assessed for lysozymic activity, resulting in a granular appearance (the proportion of "toxic" leucocytes: score 0 if none or few such leucocytes were present, score 1 if up to 50 % of leucocytes were granular, and score 2 if more than 50 % were granular. (Donders, 1999)

The presence of specific cell types was assessed, including "clue cells," red blood cells, and sperm cells; the proportion of parabasal cells; and background flora, which could be unremarkable, show cytolysis, contain small coliform bacilli, cocci, or chains. Parabasal cell counts were scored as: 0 for none or less than 1 %, 1 for 10 % or less, and 2 for more than 10 %. (Donders, 1999)

To assess how a particular type of abnormal microbiota influences CIN development, pathological microbiota was classified into: 'any AV flora,' which includes LBG III AV, mixed AV-BV, and LBG IIb with AV signs; and 'any BV,' encompassing LBG III BV, mixed AV-BV, and LBG IIB with BV signs (partial BV). (Donders, 1999)

The severity of AV was evaluated using the AV score outlined by Donders. The score parameters included LBG, leucocyte count, proportion of toxic leucocytes, background microflora, and parabasal epitheliocytes. An AV score below 3 indicated no AV, 3–4 signified mild AV, 5–6 reflected moderate AV, and above 6 denoted severe AV. An AV score of 5 or higher was categorised as moderate to severe AV (msAV). (Donders et al., 2002)

2.5 Colposcopy and histological examination

All study participants had colposcopy examinations conducted by certified colposcopy specialists (Jana Žodžika, Irina Jermakova, Kristīne Pčolkina) following European guidelines for quality assurance in cervical cancer screening. (Arbyn et al., 2008) Biopsies were taken from at least two sites for each patient in the study group, and, if there was visual suspicion of cervical pathology, from the control group patients. A histological examination was conducted at the Riga East University Hospital Pathology Center. Formalin-fixed paraffin-embedded biopsy tissue sections were placed on microscope slides and stained with haematoxylin-eosin using an automatic Daco CoverStainer. All slides were examined under a light microscope (4×, 10×, 40× objectives) by a certified pathologist (Inta Liepniece-Karele).

The results were classified into negative ("no CIN"), CIN1, CIN2, CIN3, and carcinoma. Cases with CIN1-2 or CIN2-3 lesions were upgraded and included in the CIN2 or CIN3 groups. In the control group, if colposcopy was adequate and no signs of precancerous lesions were observed, this was considered "no CIN." For data analysis, we combined all CIN severity groups into a single category labeled "all CIN." Additionally, CIN2, CIN3, and carcinoma cases were analysed as CIN2+. Results were compared across the groups: "no CIN," CIN1, CIN2+, and "all CIN."

2.6 HPV DNA and mRNA detection

To detect DNA of the six most common high-risk HPV types – 16/18, 31, 33, 45, and 58, which account for 90 % of cervical cancer cases – samples from the cervix were analysed. These samples were stored at −80 °C until testing. HPV DNA detection was performed using the SENSOQUEST Labcycler Thermoblock 96 device, and DNA was extracted with the Qiagen DNA Mini nucleic acid extraction kit. All tests took place at the Department of Microbiology, Rīga Stradiņš University.

To detect E6/E7 viral messenger RNA (mRNA) from 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), a multiplex real-time PCR assay was used with the Anyplex (TM) II HPV HR Detection assay (Seegene, CAT. No. HP7E00X). Subsequently, E6/E7 mRNA levels were determined using an automated hybridisation and amplification system that includes a fully automated sample-to-answer instrument, the Panther System

(Hologic), along with the Panther Run Kit (Hologic, CAT. No. 303096), and the Aptima HPV assay (Hologic, CAT. No. 303093). All tests were performed at "E. Gulbja Laboratorija".

2.7 Sexually transmitted and other vaginal infections

Seven genital infections (*C. trachomatis, N. gonorrhoeae, T. vaginalis, M. hominis, M. genitalium, U. urealyticum, U. parvum*) were identified using molecular diagnostics: DNA extraction followed by real-time PCR. Swab samples were taken from the cervix and stored in MSwab® (COPAN, CAT. No. 6E011N) transport media. These samples were frozen at −80 °C and kept until analysis. DNA was extracted with the MICROLAB Nimbus IVD automated liquid handler (HAMILTON) and the STARMag 96 X 4 Universal Cartridge Kit (Seegene, CAT. No. 7444300.4.UC384), which uses reversible nucleic acid binding to paramagnetic beads in appropriate buffers. The purified DNA was then amplified using a multiplex real-time PCR assay based on proprietary DPO™ and TOCE™ technologies, specifically the AnyPlexTM II STI-7e panel (Seegene, CAT. No. SD7701X) and the CFX 96 C1000 real-time PCR system (Bio-Rad). All testing was performed at the "Centrālā Laboratorija".

C. trachomatis, N. gonorrhoeae, T. vaginalis, and M. genitalium were analysed together as "all STI". As well as *M. hominis, U. urealyticum,* and *U. parvum* were combined and analysed as "non-STI *Mycoplasmas*".

2.8 HLA class II gene analysis

After inserting an unmoistened speculum, a cotton swab was used to collect material from the cervix, which was then transferred to an EDTA anticoagulant medium. Samples were stored at −80 °C until analysis. The analysis involved extracting human DNA, followed by amplification and typing of HLA genes. DNA extraction was performed with the QIAamp® DNA Kit (Qiagen). HLA genotyping of 13 DRB1*, 8 DQA1*, and 12 DQB1* alleles was conducted using a programmed thermocycler (DTLite, DNA-Technology). The second polymorphic exon of HLA class II genes was amplified, and low-resolution real-time PCR was used for genotyping. All tests were performed at the Rīga Stradiņš University, Joint Laboratory of Clinical Immunology and Immunogenetics. Due to budget constraints, HLA genotyping was only done for 84 women in the study group and 57 women in the control group.

2.9 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.

2.10 Ethical aspects and approvals

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 (Annex 2).

The study has been approved by the Rīga Stradiņš University Ethical Committee, the Central Ethical Committee, and the Riga Clinical University Hospital Scientific Department (Annexes 3, 4, 5, and 6).

3 Results

Out of 235 women invited to participate in the study, 232 agreed. Two study group participants' histological examination of cervical biopsy did not prove cervical neoplasia, so they were added to the control group. One control group participant had an unreadable microscopy result and an incomplete questionnaire, so they were excluded from the analysis. In the final analysis, 110 cases with CIN (study group) and 117 cases without CIN (control group) were included. (see Flowchart of study, p. 48). Given the estimated 63 participants per group, the number of patients included is representative.

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

3.1 Sociodemographic and behaviourial 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 (Table 3.1).

Table 3.1

Sociodemographic and behavioural characteristics of the groups

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+
Mean age	37.1 (± 8.0)	34.4 ± 1.7	35.5 ± 1.0	35.0 (± 9.3)	0.059	0.103	0.118	0.684
Age group								
< 30	27 (23.1)	13 (41.9)	26 (32.9)	39 (35.5)	0.032	0.039	0.093	0.661
31–49	87 (74.4)	16 (51.6)	48 (60.8)	64 (58.2)				
≥ 50	3 (2.6)	2 (6.5)	5 (6.3)	7 (6.3)				

Table 3.1 continued

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+
Education level								
lower	25 (21.4)	15 (48.4)	37 (46.9)	52 (47.3)	< 0.0001	0.003	< 0.0001	0.083
higher	92 (78.6)	16 (51.6)	42 (53.1)	58 (52.7)				
Relationship								
Registered marriage	60 (51.3)	18 (58.0)	43 (54.4)	61 (55.5)	0.785	0.877	0.847	0.950
Non-registered marriage	44 (37.6)	10 (32.3)	29 (36.7)	39 (35.5)				
Lonely	13 (11.1)	3 (9.7)	7 (8.9)	10 (9.0)				
Contraception group								
Effective	29 (24.8)	7 (22.6)	22 (27.8)	29 (26.4)	0.344	0.287	0.628	0.702
Condoms	40 (34.2)	6 (19.4)	21 (26.6)	27 (24.5)				
Withdrawal	8 (6.8)	2 (6.4)	4 (5.1)	6 (5.5)				
No contraception	40 (34.2)	16 (51.6)	32 (40.5)	48 (43.6)				
Smoking								
No	106 (90.6)	19 (61.3)	53 (67.1)	72 (65.5)	< 0.0001	< 0.0001	< 0.0001	0.565
Yes	11 (9.4)	12 (38.7)	26 (32.9)	38 (34.5)				

3.2 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). There were no differences observed between the groups in terms of vaginal leucocytosis (Table 3.2).

Table 3.2

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)				
Vaginal leucocytosis								
< 10/hpf	85 (72.7)	25 (80.7)	51 (64.5)	76 (69.1)	0.824	0.463	0.476	0.160
< 10/epithelial cell	19 (16.2)	5 (16.1)	16 (20.3)	21 (19.1)				
> 10/epithelial cell	13 (11.1)	1 (3.2)	12 (15.2)	13 (11.8)				
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)				

Table 3.2 continued

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+
Vaginal leucocytosis								
< 10/hpf	85 (72.7)	25 (80.7)	51 (64.5)	76 (69.1)	0.824	0.463	0.476	0.160
< 10/epithelial cell	19 (16.2)	5 (16.1)	16 (20.3)	21 (19.1)				
> 10/epithelial cell	13 (11.1)	1 (3.2)	12 (15.2)	13 (11.8)				
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

3.3 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 3.3).

Table 3.3

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)				

3.4 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 (Table 3.4).

Table 3.4

Analysis of seven vaginal infections

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+
C. trachomatis	4 (3.4)	3 (9.7)	3 (3.8)	6 (5.4)	0.529	0.160	1.000	0.348
N. gonorrhoeae	0	0	0	0	–	–	–	–
T. vaginalis	0	0	2 (2.5)	2 (1.8)	0.234	–	0.161	1.000
M. genitalium	0	0	1 (1.3)	1 (0.9)	0.485	–	0.403	1.000
All STI	4 (3.4)	3 (9.7)	6 (7.6)	9 (8.2)	0.157	0.160	0.206	0.710
M. hominis	2 (1.7)	3 (9.7)	7 (8.9)	10 (9.1)	0.016	0.062	0.032	1.000
U. parvum	35 (29.9)	13 (41.9)	29 (36.7)	42 (38.2)	0.189	0.204	0.320	0.612
U. urealyticum	8 (6.8)	1 (3.2)	9 (11.4)	10 (9.1)	0.530	0.685	0.266	0.277
Non-STI *Mycoplasmas*	41 (35.0)	15 (48.4)	36 (45.6)	51 (46.4)	0.083	0.173	0.139	0.790

3.5 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 (odds ratio (OR) 65.3, 95 % confidence interval (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 3.5).

Table 3.5

Univariate logistic regression CIN2+ compared to "no CIN"

Factor	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
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 3.6).

Table 3.6

Multivariate logistic regression CIN2+ compared to "no CIN"

Factor	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

3.6 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 %) (Table 3.7).

Table 3.7

HLA-DRB1*/DQA1*/DQB1* distribution

Alleles	no CIN, n = 57 (%)	CIN1, n = 23 (%)	CIN2+, n = 61 (%)	P value no CIN vs. CIN1	P value no CIN vs. CIN2+	P value CIN1 vs. CIN2+
*DRB1*01:01*	16 (14)	9 (19.6)	21 (17.2)	0.38	0.50	0.72
*DRB1*04:01*	17 (14.9)	4 (8.7)	27 (22.1)	0.29	0.16	0.045
*DRB1*07:01*	6 (5.3)	3 (6.5)	9 (7.4)	0.71	0.51	1.00
*DRB1*08:01*	5 (4.4)	0	2 (1.6)	0.32	0.27	1.00
*DRB1*09:01*	2 (1.8)	0	0	1.00	0.23	–
*DRB1*10:01*	2 (1.8)	0	0	1.00	0.23	–
*DRB1*11:01*	10 (8.8)	7 (15.2)	23 (18.9)	0.26	0.03	0.58
*DRB1*12:01*	5 (4.4)	1 (2.2)	1 (0.8)	0.67	0.11	0.47
*DRB1*13:01*	7 (6.1)	1 (2.2)	2 (1.6)	0.44	0.09	1.00
*DRB1*14:01*	14 (12.3)	0	1 (0.8)	0.01	< 0.0001	1.00
*DRB1*15:01*	7 (6.1)	12 (26.1)	20 (16.4)	< 0.0001	0.01	0.15
*DRB1*16:01*	12 (10.5)	0	3 (2.5)	0.02	0.01	0.56
*DRB1*17:01*	10 (8.8)	9 (19.6)	13 (10.7)	0.06	0.63	0.13
*DQA1*01:01*	14 (12.3)	8 (17.4)	14 (11.5)	0.39	0.85	0.31
*DQA1*01:02*	23 (20.2)	17 (37)	23 (18.9)	0.03	0.80	0.01
*DQA1*01:03*	6 (5.3)	2 (4.3)	16 (13.1)	1.00	0.04	0.16
*DQA1*02:01*	20 (17.5)	5 (10.9)	16 (13.1)	0.29	0.34	0.69
*DQA1*03:01*	17 (14.9)	1 (2.2)	7 (5.7)	0.02	0.02	0.45
*DQA1*04:01*	2 (1.8)	1 (2.2)	13 (10.7)	1.00	0.01	0.12
*DQA1*05:01*	29 (25.4)	12 (26.1)	33 (27.0)	0.93	0.78	0.90
*DQA1*06:01*	3 (2.6)	0	0	0.56	0.11	–
*DQB1*02:01-2*	19 (16.7)	11 (23.9)	17 (13.9)	0.29	0.56	0.12
*DQB1*03:01*	30 (26.3)	13 (28.3)	50 (41.0)	0.80	0.02	0.13
*DQB1*03:02*	7 (6.1)	1 (2.2)	6 (4.9)	0.44	0.68	0.68
*DQB1*03:03*	5 (4.4)	0	2 (1.6)	0.32	0.27	1.00
*DQB1*04:01-2*	6 (5.3)	2 (4.3)	17 (13.9)	1.00	0.03	0.08
*DQB1*05:01*	10 (8.8)	1 (2.2)	2 (1.6)	0.18	0.01	1.00
*DQB1*05:02-4*	4 (3.5)	0	0	0.58	0.05	–
*DQB1*06:01*	3 (2.6)	0	2 (1.6)	0.56	0.68	1.00

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 3.8).

Table 3.8

Risk estimates of CIN2+ compared to "no CIN"

Alleles	Odds ratio	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

4 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)

Recent data on the sexual and reproductive health of Latvian residents further support this association. Among women who received cervical cancer screening invitations, 74 % responded; however, the proportion of screened women was lower among those with only primary education. One of the most frequently cited reasons for non-participation in screening was the perception that it was unnecessary. (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 removing health education as a separate school subject.

National data indicate that among Latvian women aged 25–49, 30.5 % do not use any contraception, 29 % rely on condoms, 22.3 % use the withdrawal method, and only 18.3 %

opt for effective methods such as hormonal or intrauterine contraception. Additionally, 6.2 % of women report being uninformed about contraception. (Ķīvīte-Urtāne, 2023)

Although the present study did not directly investigate health literacy and sexual behaviour, the observed differences in contraception use provide some indirect insights. Our findings align with national trends, as approximately one-third of participants reported not using contraception, and another third relied on condoms. Notably, women in the "all CIN" group were less likely to use condoms and more frequently reported using no contraception at all. However, our study also observed a higher prevalence of effective contraception use and a lower reliance on withdrawal compared to national data. This difference may be attributed to the broader age range of our cohort, which included both younger and menopausal women, potentially influencing contraceptive choices.

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 disease. (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

Recent evidence suggests that the vaginal microbiome plays a significant role in the pathogenesis of HPV infection and the development of cervical neoplasia. Whereas a healthy vaginal ecosystem is characterised by the dominance of *Lactobacilli*, the vaginal microbiome of women with squamous lesions has a decreased relative abundance of lactobacteria and an increased number of other bacilli. (Chen et al., 2020; Ma et al., 2023; Mancilla et al., 2024; Mitra et al., 2015; Norenhag et al., 2020) Disruptions in the vaginal microbiome are associated with an overgrowth of anaerobic bacteria and an increased presence of aerobic bacteria in HPV-related cervical diseases. (Wu et al., 2022) Moreover, increasing disease severity is linked with a more diverse vaginal microbiome. High-grade lesions are particularly associated with increased levels of *Sneathia sanguinegens, Anaerococcus tetradius,* and *Peptostreptococcus anerobius.* (Mitra et al., 2015) The recent meta-analysis reported similar results – as cervical lesions advanced, *Lactobacillus* species became notably less prevalent, while pathogenic bacteria – such as *Anaerococcus, Peptostreptococcus, Porphyromonas, Prevotella,* and *Sneathia* – showed a substantial

increase. (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.* have found that women who underwent excisional treatment for HSIL had the same vaginal microbiota profile before and after the treatment, supporting the evidence that an altered vaginal microbiome is a crucial CIN risk factor. (Mitra et al., 2021)

The conclusions of microbiome studies are based on the results of time-consuming and expensive molecular biology methods such as polymerase chain reactions and next-generation sequencing. These methods are of limited use in everyday clinical practice. Clinicians use simpler, faster, and cheaper methods to evaluate the vaginal ecosystem. Measurement of vaginal pH and wet-mount microscopy are rapid and cost-effective point-of-care diagnostic tools for assessing the vaginal ecosystem. (Donders, 2007; Rumyantseva et al., 2015) While their diagnostic accuracy is comparable to molecular biology techniques, they are significantly more affordable and practical for routine clinical use. (Rumyantseva et al., 2016)

In this study, we identified an association between elevated vaginal pH and abnormal vaginal microflora with CIN2+. Other studies report a positive link between increased vaginal pH and detection of HPV, as well as an increased risk of high-grade lesions. (Clarke et al., 2012; Teng & Hao, 2020) Elevated vaginal pH is a marker and is primarily linked to alterations in the vaginal environment and infections, including sexually transmitted infections. However, various non-infectious factors can also influence vaginal pH, such as low oestrogen levels, the use of vaginal products, hygiene practices like vaginal douching, and the presence of blood or sperm. (Donders et al., 2016) The direct effect of pH value on HPV is unknown. However, it was discovered that an increased pH level affects the adherence of the HPV pseudovirus to the cell surface *in vivo*. (Liu and Li, 2024) The mechanism is still to be described.

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 and 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 and 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

High-risk HPV infection is a prerequisite for developing cervical cancer. (Bosch et al., 1995) Therefore, different HR-HPV types are frequently detected in high-grade cervical lesions. (Muñoz et al., 1994) This study revealed an almost fourfold higher risk of CIN2+ in individuals who tested positive for HR-HPV DNA. According to European guidelines for quality assurance in cervical cancer screening, HR-HPV DNA detection is recommended as the primary screening method for women over 30. (von Karsa et al., 2015) Many European countries have already implemented this strategy. Our study found that about a 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)

HPV is a common infection that resolves spontaneously in most cases. (Castellsagué, 2008) Therefore, it is crucial to identify women positive for HR-HPV who could develop high-grade disease. It is known that the expression of E6 and E7 oncogenes disables the tumour-suppressor proteins p53 and pRB in host cells, facilitating malignant transformation. (Erin et al., 2012; Gupta et al., 2022; Hoppe-Seyler et al., 2018; Yeo-Teh et al., 2018) Detecting E6/E7 mRNA expression holds significant promise as a biomarker for distinguishing between transient and transforming HR-HPV infections. Studies have highlighted this test's diagnostic value. (Campbell et al., 2013; Cao et al., 2022; Hui et al., 2021; Ren et al., 2018) E6/E7 mRNA testing shows strong potential for use as either a triage tool or a primary HPV screening method. It has demonstrated high sensitivity (Paolo et al., 2017; Ren et al., 2018) and is particularly effective in triaging ASCUS patients. (Ren et al., 2018; Zhu et al., 2019) In this study, HR-HPV E6/E7 mRNA positivity was closely linked to CIN2+ lesions and had the highest odds ratio among the markers examined. However, the broad 95 % Confidence Interval reduces the statistical strength of this finding, possibly due to the relatively small participant sample.

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) Our findings 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. A meta-analysis has identified *T. vaginalis* as a risk factor for cervical cancer (Hamar et al., 2023; Yang et al., 2018), whereas data on *M. genitalium* remains inconclusive. While most research on *Mycoplasmataceae* has focused on *M. hominis*, *U. parvum*, and *U. urealyticum* in cervical dysplasia, some studies suggest *M. genitalium* may also contribute to CIN risk. (Lima et al., 2018) 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 various *Mycoplasma* species, *M. hominis* was significantly linked to CIN2+, making it the third most important risk factor for CIN2+ in univariate analysis. However, this association did not hold in the multivariate model. While several studies link *Mycoplasma* species to high-risk HPV infection (A et al., 2023; Lopez-Arias et al., 2019; Ye et al., 2018), their precise role in cervical carcinogenesis remains unclear. A study by *A et al.* suggests that different *Mycoplasma* types may vary in their association with HPV, highlighting the potential need for detailed *Mycoplasma* typing in clinical practice. (A et al., 2023)

One of the significant explanations why different vaginal infections and HPV coexist is due to similar sexual behavioural risk factors. However, many studies prove the association between vaginal infections, including STI, cervical neoplasia, and cancer. (Hamar et al., 2023; Karim et al., 2018; Lima et al., 2018) 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., 2014b; 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 and 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 our study. (Chen et al., 2013; Leo et al., 2017)

While specific HLA alleles were consistently identified across different studies, considerable variability remains. HLA gene polymorphisms are highly population-specific and influenced by ethnic background. (Ramachandran and Dörk, 2021; Trowsdale, 2011) These discrepancies may explain variations in risk and protective allele distributions across populations. Despite extensive prior research, conducting similar studies in underrepresented populations remains essential. While some HLA alleles may be universally associated with cervical neoplasia risk, others may be unique to specific ethnic groups, necessitating further investigation into population-specific genetic susceptibility to cervical cancer.

HLA class II gene polymorphism is genetically inherited and a non-modifiable host factor that remains stable throughout life and influences individual immune responses to infectious agents, including HPV. (Trowsdale, 2011) HLA-associated susceptibility reflects baseline immunogenetic risk rather than a target for preventive intervention, but it may be helpful in long-term risk stratification.

Personalised screening approach and use of artificial intelligence

The chance of developing cervical cancer after an HPV infection is relatively low. (Woodman et al., 2007) However, identifying the small proportion of cases that progress to high-grade lesions is critical for timely treatment and cancer prevention, mainly as these cases primarily affect reproductive-age women. (Gargano et al., 2019; Ting et al., 2010) With HR HPV-based screening showing greater sensitivity than cytology (Arbyn et al., 2013), distinguishing the subset of HPV-positive individuals at the highest risk of cervical cancer is essential. As a result, stratifying patients by risk has become increasingly important in guiding appropriate examination, treatment, and follow-up.

A growing body of evidence indicates that HPV-related disease outcomes are shaped by a multifactorial interplay of viral, microbial, host, and behavioural factors. (Hariri et al., 2011; Kyrgiou et al., 2017; Trottier and Franco, 2006) Therefore, relying on HPV status alone is insufficient. Integrating various determinants – such as 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. AI-based models have already been developed and tested to combine clinical, demographic, virological, and genetic data for individualised risk assessment. (Aballéa et al., 2020; B. Hu et al., 2014a; Lu et al., 2020; Mehmood et al., 2021; Rothberg et al., 2018) Integrating such models into

clinical practice would represent a shift from a generalised screening strategy to a personalised, risk-adapted approach, aligning with the principles of precision medicine. AI is revolutionizing the medical field, particularly in diagnostic and therapeutic practices. One of its key strengths is integrating diverse variables to generate risk scores, offering prognostic insights, and the potential to identify novel biomarkers. (Dellino et al., 2024) However, the broad use of AI poses certain risks. Firstly, it must be carefully and thoroughly trained, using extensive and accurate data. For example, AI models in clinical validation for cytology still struggle to achieve 90 % sensitivity, though they significantly improve slide reading speed and perform comparably to junior cytologists in SIL detection. (Wu et al., 2024) Secondly, AI-based healthcare systems must clearly define responsibility for diagnosis and subsequent patient management. Therefore, expert oversight is needed to ensure AI complements rather than replaces clinical expertise. (Dellino et al., 2024) Finally, this technology requires careful implementation and strict regulations to safeguard patient privacy, provide informed consent, and maintain ethical integrity, thereby preventing potential misuse. (Wu et al., 2024)

In parallel with technological developments, global screening strategies are also evolving. Some countries are already reconsidering the traditional one-size-fits-all model in favour of risk-based approaches that better reflect individual disease pathways. For example, in 2019, the USA changed its cervical cancer screening guidelines from results-based to risk-based. The risk of immediate CIN3+ is estimated based on history and screening results. If it is equal to or greater than 4 %, immediate action is needed – either colposcopy or treatment. If the initial risk is below 4 %, the five-year CIN3+ risk is calculated to establish the follow-up intervals. (Perkins et al., 2020)

The broad implementation of vaccination against HR-HPV will also significantly impact the screening programmes. The burden of different HR-HPV genotypes will likely change in countries with high vaccination coverage. For example, in Sweden, which introduced organised school-based vaccination with the quadrivalent vaccine in 2012, the prevalence of HPV types 16 and 18 has decreased tremendously. Therefore, the number of women who need to be screened to detect one HPV-16-associated invasive cervical cancer case increased 9.4-fold in vaccinated cohorts compared to older birth cohorts of the prevaccination era (229 377 vs. 24 518 accordingly). A similar trend was observed for HPV type 18. (Gray et al., 2025) It means that screening programmes that aim to detect these HPV types become useless and need to be changed. However, detecting HPV types in the vaccines cannot be abandoned unless 100 % of the population is vaccinated. Hence, the one-size-fits-all algorithm is no longer appropriate.

These issues are particularly relevant for countries like Latvia, which continues to report one of the highest rates of cervical cancer incidence in Europe. (WHO European Health Information Gateway, 2024) Despite introducing screening and HPV vaccination programmes more than 15 years ago, incidence rates have not significantly declined. (SPKC, 2024) There is a pressing need to update and enhance cervical cancer prevention efforts. A generalised screening approach may overlook specific risk factors prevalent in local populations, such as regional HPV genotype distribution, sociodemographic disparities, and variations in access to care. Adapting these programmes to incorporate individualised risk assessments could help optimise resource use, reduce unnecessary procedures, and improve health outcomes. (Stankūnas et al., 2022)

Future research should focus on refining predictive models and optimizing screening algorithms to improve individualised prevention strategies. Given geographic and ethnic variations in HR-HPV prevalence, genomic factors, comorbidities, and behavioural patterns, global datasets are essential for training machine learning models and creating an accurate, universally applicable risk assessment tool. Harnessing genomics and microbiome research advancements could further enhance personalised cervical cancer screening, improving early detection, reducing unnecessary interventions, and enhancing overall healthcare efficiency.

Limitations of the study

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 relation between aerobic vaginitis and CIN, 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 the distinction of lactobacillary-dominated flora from abnormal microflora types, 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.

Publications and reports on topics 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.
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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
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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.
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I want to express my deepest gratitude to my family and friends for their endless support, patience, and understanding. Their belief in me and encouragement throughout this journey made all the difference.

Annexes

Annex 1

Questionnaire

Identification Number _____

Last cytology smear (date): _____

1. **Age: _____**
2. **Education:**
   1. primary
   2. secondary
   3. higher
3. **Partnership status:**
   1. registered marriage
   2. unregistered marriage
   3. no permanent partner
4. **Last cytology smear result:**
   1. ASCUS
   2. LSIL
   3. HSIL
   4. glandular atypia
5. **Current contraception method:**
   1. COC (combined oral contraception)
   2. POC (progestin-only oral contraception)
   3. LNG-IUS (hormonal intrauterine system)
   4. Other hormonal contraception
   5. Non-hormonal intrauterine device
   6. Condom
   7. Spermicides
   8. Sterilisation
   9. None
6. **Sexual activity in the past 3 months:**
   1. Yes
   2. No
7. **Number of childbirths:**
   1. 1
   2. 2
   3. 3 and more
8. **Other pregnancies:**
   1. Yes
   2. No
9. **Pregnancy in the last 6 months:**
   1. Yes
   2. No

10. Chronic diseases:

1. cardiovascular
2. endocrine
3. renal
4. other _____

11. History of malignant tumors:

1. reproductive system
2. others _____

12. Immunosuppression status:

1. HIV
2. HCV
3. iatrogenic cause

13. Smoking:

1. Yes, how much cigarettes/day _____
2. No

14. Reproductive system infections in the past 6 months:

No.	Infection	Yes	No
14.1	Chlamydia infection		
14.2	Genital herpes		
14.3	Trichomoniasis		
14.4	Fungal infection		
14.5	Mycoplasma infection		

15. Therapy used in the last month:

No.	Therapy	Yes	No
15.1	Oral antibiotics		
15.2	Vaginal antibiotics		
15.3	Oral probiotics		
15.4	Vaginal probiotics		

Annex 2

Written invitation for women to participate in a study

Study Title: **Association of vaginal environment and genital infections with cervical precancerous lesions.**

Location: Department of Obstetrics and Gynaecology, Riga Stradiņš University
Riga East Clinical University Hospital

Patient Identification Number:

.....

The human papillomavirus (HPV) has been proven to be a causative factor of cervical cancer. Every woman who has ever had sexual intercourse is at risk of HPV infection. Although HPV is the most common sexually transmitted infection among women of reproductive age, only a minority of infections persist and progress to cervical cancer. Research shows that altered vaginal microflora increases the risk of developing cervical changes, but the exact relationship remains unclear. Evidence that altered vaginal microflora and sexually transmitted infections are significant factors promoting cervical precancerous changes would allow for the introduction of early diagnosis and treatment of vaginal infections as a preventive measure against cervical precancer and cancer in clinical practice.

In this study, we aim to investigate the vaginal environment and the prevalence of genital infections among patients with cervical precancerous conditions.

As part of the study, you are offered additional examinations, which will not negatively affect your future medical care and are free of charge. If you join the study, your further care will not differ from the standard practice outlined in the guidelines of the Latvian Association of Gynaecologists and Obstetricians. During the visit, under colposcopy control, a laboratory sample will be taken from your cervical cells. Sample collection is not painful or traumatic; a special brush will be used, similar to a regular gynaecological visit.

Participation in this study is voluntary. You can withdraw from the study at any time and for any reason.

All information about you in this study will be identified only by your assigned identification number. Your name or other identifiable information will not be used. The study team will securely store participant information, results, and other data, making it inaccessible. Your name will be known only to your treating physician. All information obtained and used in the study will be used solely for scientific and medical purposes. You have the right to inquire from your physician about which analyses are being performed, their purpose, and their results.

You have the right not to sign the study's consent form and withdraw from the study at any time.

If you decide to participate, you must sign the consent form for study participation. You may receive verbal information and explanations from your physician regarding any questions.

Study Participant Consent.

I, ...
(full participant name and surname)

confirm that I have read and understood all the written information about the study "Association of vaginal environment and genital infections with cervical precancerous lesions."
The information about the study and planned activities has been explained to me verbally. I confirm that I had the opportunity to ask questions and am satisfied with the answers provided. I had sufficient time to read the information before making a decision. I voluntarily consent to participate in this study. I understand that I have the right to refuse participation and to withdraw at any time, and that this will not affect my healthcare.

Participant's signature: .....

Date: .....

RSU Ētikas komitejas lēmums

Veidlapa Nr. E-9 (2)

RSU ĒTIKAS KOMITEJAS LĒMUMS NR. 24 / 29.03.2018.

Rīga, Dzirciema iela 16, LV-1007
Tel. 67061596

	Komitejas sastāvs	Kvalifikācija	Nodarbošanās
1.	Profesors Olafs Brūvers	Dr.theo.	teologs
2.	Asoc.prof. Santa Purviņa	Dr.med.	farmakologs
3.	Asoc.prof. Voldemārs Arnis	Dr.biol.	rehabilitologs
4.	Profesore Regīna Kleina	Dr.med.	patalogs
5.	Profesors Guntars Pupelis	Dr.med.	ķirurgs
6.	Asoc.prof. Viesturs Liguts	Dr.med.	toksikologs
7.	Docente Iveta Jankovska	Dr.med.	
8.	Docents Kristaps Circenis	Dr.med.	
9.	Lektore Ilvija Razgale	Mg.soc.d.	

Pieteikuma iesniedzējs: **Olga Plisko**
Medicīnas fakultāte, doktorantūra

Pētījuma nosaukums: "Maksts vides un dzimumorgānu infekciju slimība ar dzemdes kakla priekšvēža saslimšanām"

Iesniegšanas datums: 28.03.2017.

Pētījuma protokols: Izskatot augstāk minētā pētījuma pieteikuma materiālus (protokolu) ir redzams, ka pētījuma mērķis tiek sasniegts veicot ar pacientiem, bez kāda apdraudējuma veselībai, drošībai un dzīvībai, klīnisku pētījumu (audu paraugu-materiālu ņemšanu un izdarot atbilstošas analīzes, pārbaudes, mērījumus) pacientu medicīniskās dokumentācijas (slimības vēstures) izpēti, pacientu/dalībnieku aptauju-intervēšanu, iegūto datu apstrādi un analīzi, kā arī izsakot priekšlikumus. Personu (pacientu, dalībnieku) datu aizsardzība, informēta brīvprātīga piedalīšanās un konfidencialitāte ir ievērota un nodrošināta. Līdz ar to pieteikums atbilst pētījuma ētikas prasībām.

Izskaidrošanas formulārs: ir

Piekrišana piedalīties pētījumā: ir

Komitejas lēmums: **piekrist** pētījumam

Komitejas priekšsēdētājs Olafs Brūvers ĒTIKAS KOMITEJA Tituls: Dr. miss., prof.

Paraksts

Ētikas komitejas sēdes datums: 29.03.2018.

Centrālās medicīnas ētikas komitejas lēmums

Centrālā medicīnas ētikas komiteja

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Rīgā

Datums skatāms laika zīmogā Nr. 01-29.1.2/1671
Uz 09.03.2022. Nr. [illegible]

Rīgas Stradiņa universitātes
Medicīnas fakultātei
dr.olga.plisko@gmail.com

Par atļaujas saņemšanu pētījumam
"Imunoģenētisko faktoru saistība
ar dzemdes kakla priekšvēža slimībām"

Centrālā medicīnas ētikas komiteja izskatīja Rīgas Stradiņa universitātes Medicīnas fakultātes 2021.gada 4.augusta precizētu pieteikumu par pētījumu *"Imunoģenētisko faktoru saistība ar dzemdes kakla priekšvēža slimībām"* (reģistrēts Veselības ministrijā 2022.gada 9.martā Nr.3159).

Atbilstoši Centrālā medicīnas ētikas komitejas 2022.gada 24.marta sēdes protokola Nr.2022-3 I.daļas *"PRECIZĒTIE IESNIEGUMI"* 2.punktam tiek sniegts atzinums, ka pētījums nav pretrunā bioētikas normām.

Centrālās medicīnas ētikas
komitejas priekšsēdētājs (paraksts*)
V. Sīlis

Annex 5

Rīgas Austrumu klīniskās universitātes slimnīcas atļauja akadēmiskā pētījuma veikšanai

aslimnīca
RĪGAS AUSTRUMU KLĪNISKĀ UNIVERSITĀTES SLIMNĪCA

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Reģistrācijas Nr.: 40003951628
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Pielikums Nr. 5

APSTIPRINĀTS
ar SIA „Rīgas Austrumu klīniskā universitātes slimnīca" valdes 2014. gada 17. aprīļa lēmumu Nr. V1/01-01/14/192

Rīgā

2018.gada 23.aprīlī
Nr. ZD/08-06/01-18/119

Rīgas Stradiņa Universitātes
studentei
Olgai Plisko

ATĻAUJA AKADĒMISKĀ PĒTĪJUMA VEIKŠANAI

Zinātnes daļa ir izskatījusi Jūsu iesniegto akadēmiskā pētījuma "Maksts vides un dzimumorgānu infekciju saistība ar dzemdes kakla priekšvēža saslimšanām" dokumentāciju, kas reģistrēta Zinātnes daļā ar numuru AP-54/18 un apstiprina akadēmiskā pētījuma veikšanu SIA „Rīgas Austrumu klīniskā universitātes slimnīca" (turpmāk – Iestāde) stacionārā "Gaiļezers".

Atbildīgais par pētniecības norisi Iestādē ir Jana Žodžika.

Zinātnes daļā iesniegti un izskatīti:

1. Iesniegums par akadēmiskā pētījuma AP-54/18 veikšanu
2. Pētījuma protokols ar pielikumu
3. Olgas Plisko konfidencialitātes apliecinājums
4. Ētikas komitejas atzinums, izsniegts 2018.gada 29.martā.

Dr.med. Daiga Šantare

(paraksts)

Speciāliste akadēmisko pētījumu jautājumos
Zinātnes daļa