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High-Risk Human Papillomavirus Prevalence and Genotypes Distribution: Baseline Findings from Cervical Cancer Screening with Alinity m among Women in Bulgaria

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Abstract

Background: Persistent high-risk human papillomavirus HPV (HR-HPV) infection is the most significant risk factor for epithelial cell changes, the development of high-grade squamous cell lesions, and the progression to cervical cancer. This study aimed to provide epidemiological data and assess the prevalence and genotype distribution of HR-HPV. **Methods:** Liquid-based cytology (LBC) samples from 190 Bulgarian women were simultaneously tested to identify HR-HPV genotypes and perform cytological analysis. HPV detection and genotyping were performed using the clinically validated Alinity m HR-HPV assay. This test provides detailed information on HPV16, HPV18, and HPV45 and categorizes the remaining 11 HR-HPV genotypes. **Results:** HR-HPV infection was detected in 16.8% of the women screened, with mean age of 38.2 (±9.8) years. Infection with HPV16 was seen in 5.3%, HPV18 positivity was 2.2% and genotype HPV45 was found in 1.0%. Abnormalities in LBC cytology were observed in 18.9% of women. HR-HPV positivity in normal cytology samples was 5.2% and 63.9% in abnormal smears. **Conclusion:** At present, there is a notable absence of official data concerning the substantial impact of HPV in Bulgaria. Presented baseline findings establish a foundational understanding of the prevalence and genotype distribution of HR-HPV within selected segments of the Bulgarian population.

Keywords

Human Papillomavirus, High-Risk HPV Types, Alinity m HPV, Primary Cervical Cancer Screening

1. Introduction

Human papillomavirus (HPV) is a double-stranded DNA virus within the Papillomaviridae family that is responsible for the most prevalent sexually transmitted infection globally, adversely affecting personal social life [1]. Concurrently, HPV accounts for nearly 5% of all oncological diseases in both women and men worldwide, thereby reducing life expectancy [2]. The Papillomaviridae family includes two subfamilies (First-papillomavirinae and Second-papillomavirinae), comprising 53 genera and over 130 species [3]. Over 200 HPV genotypes have been identified, categorized into different genera (Alpha, Nu/Mu-, Beta-, and Gammapapillomavirus) based on viral genome structure and tropism to human epithelial tissues, affecting epithelial cells at various localizations in the human body [4]. Based on their scientifically established link to carcinogenic potential, mucosal alphapillomaviruses are divided into two primary groups: non-oncogenic, low-risk HPV (LR-HPV), such as types 6 and 11, which are responsible for cutaneous and anogenital warts and respiratory tract papillomas, and oncogenic HPV (HR-HPV), which causes cervical cancer, anogenital cancers, and oropharyngeal cancers, as well as anal, vulvar, vaginal, and penile cancers [5]. Approximately 40 HPV types infect the mucosal epithelium, including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68, which are classified as oncogenic HR-HPVs [6]. International Agency for Research on Cancer (IARC) recognizes 12 HPV types as oncogenic (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) and 1 HPV type as "probably oncogenic" (HPV68) [7].

In the 1970s, Prof. Zur Hausen established the relationship between HPV and cervical cancer. A German virologist confirmed the hypothesis that the viral infection found in condyloma acuminata (genital warts) might lead to cervical neoplasia and invasive cancer development. The author was awarded the Nobel Prize in Physiology or Medicine in 2008 [8]. The replacement of cervical carcinogenesis can be summarized in four stages: 1) HPV acquisition, 2) HPV persistence (vs. clearance), 3) progression of a persistent infection to cervical precancer, and 4) invasion. The carcinogenic potential of HPVs is due to the function of viral E6 and E7 oncoproteins that enhance uncontrolled cell proliferation and block apoptosis by inactivating the cell cycle control factors p53 and pRb, respectively [9].

The prevalence of HPV infection varies geographically and demographically, and is influenced by factors such as sexual behavior, socioeconomic status, healthcare access, and vaccination coverage. HPV infects both men and women, although the disease burden is significantly higher in women due to the high susceptibility of cervical cells to HPV infection [10]. Despite the high incidence of HPV infections, 70% - 90% of infected individuals experience spontaneous resolution within 1 - 2 years, without progressing to clinically manifest disease. In a minority of cases, infection persists, evading immune system detection, suppressing innate immunity, and hindering the adaptive immune response. Persistent infection is the most significant risk factor for epithelial cell changes, the development of high-grade squamous cell lesions, and the progression to cervical cancer

and other malignancies [11].

HR-HPV genotype 16 is responsible for approximately 50% of cervical cancer cases globally, with genotypes 16 and 18 together accounting for approximately 66% of cases. Additionally, five other high-risk types, 31, 33, 45, 52, and 58, contributed to a further 15% of reported cervical cancer cases and 11% of all HPV-related cancer cases [12]. Over 50% of women were infected with HPV by the age of 21, which correlates with the peak incidence of cervical cancer occurring between the ages of 40 and 55. Between 5% and 30% of individuals infected with HPV harbor multiple virus types [13]. Due to multiple HPV genotypes, infection with one type of virus does not confer immunity against another type, potentially leading to subsequent infections. Furthermore, the post-infection immune response is often short-lived, allowing for reinfection with the same HPV type [14].

The World Health Organization has crafted a worldwide plan to eradicate cervical cancer as a public health issue. Often dubbed the "cancer of the young", cervical cancer sees about 660,000 new cases and 350,000 deaths each year. This type of cancer is largely preventable through HPV vaccination and regular screenings, as advised by national guidelines, and it can be successfully treated if caught early and managed swiftly [15]. Nations across the globe are ramping up efforts to eliminate cervical cancer, following the global 90-70-90 targets: ensuring 90% of girls are fully vaccinated with the HPV vaccine by age 15, 70% of women undergo screening by ages 35 and 45, and 90% of women with precancerous conditions or invasive cancer receive suitable treatment. In many European countries, vaccination, screening programs, and follow-up treatments have led to a decrease in cervical cancer incidence and mortality [16].

Cervical cancer is the 4th most frequent cancer among women in Bulgaria and the 2nd most frequent cancer among women between 15 and 44 years of age. Approximately 1009 new cervical cancer cases are diagnosed annually in Bulgaria. In Eastern Europe, the region of Bulgaria accounts for 9.7% of women in the general population who are estimated to harbor cervical HPV16 and HPV18 infection, and 84.7% of invasive cervical cancers are attributed to HPV16 or 18 [17] [18].

The Alinity m HR-HPV assay, developed by Abbott Molecular in the USA, received the CE mark in 2019 as part of a series of advanced molecular assays compatible with the automated continuous random-access Abbott Alinity m system. This HPV assay is designed to detect and partially detect 14 high-risk HPV genotypes. Extended genotyping for high-risk human papillomavirus types enhances diagnostic precision by identifying additional oncogenic HPV types beyond HPV 16 and 18. The assay provides detailed information on three primary high-risk HPV genotypes-HPV16, HPV18, and HPV45, and categorizes the remaining 11 targeted high-risk HPV genotypes into two groups: HPV31, 33, 52, 58 (other HR-HPV A) and HPV35, 39, 51, 56, 59, 66, 68 (other HR-HPV B) [19].

Since 1941, the Papanicolau-stained smear test (PAP test) has served as the standard method for screening premalignant lesions and cervical cancer. However, it has a false-negative rate of 5% to 20%, influenced by factors such as the

population being tested, the quality of the laboratory, and the disease threshold. Given the limitations of conventional PAP primary testing for high-risk HPV is globally recommended as a strategy for cervical cancer screening in women with normal risk levels [20]. Currently, molecular HPV tests are used in conjunction with liquid-based cytology for cervical cancer screening. These tests serve as an initial primary screening method to identify abnormal cellular changes in the cervix that could potentially develop into cervical cancer and to detect atypical squamous cells of undetermined significance or squamous intraepithelial lesions. This screening and monitoring should be tailored according to professional medical guidelines, taking into account previous screening outcomes, medical history, and other risk factors [21].

2. Materials and Methods

2.1. Aim of the Study

The objective of this study was to evaluate the prevalence and genotype distribution of high-risk human papillomavirus using the Alinity m analyzer in a cohort of 190 women across Bulgaria. These individuals were tested at one of the largest medical diagnostic laboratories at the inception of the newly initiated and expanded "National Program for Primary Prevention of Cancer Diseases Caused by Human Papillomavirus 2025-2030" in Bulgaria.

2.2. Study Population

This descriptive, cross-sectional study on the prevalence of high-risk HPV was conducted over a six-month period from May 2025 to October 2025 at the Medical Diagnostics Laboratory Synevo in Bulgaria. Participants in our study were recruited exclusively from routine cervical cancer screening appointments. Cervical swab samples were collected by a gynecologist during consultations using a specialized cervical brush and placed in a container designed for liquid-based cytology (LBC) (ThinPrep PreserveCyt test collection vials, Hologic, UK). The vials containing the specimens in PreservCyt Solution were stored at room temperature and transported to the laboratory for processing. Upon arrival at Synevo, the cervical samples were tested concurrently in routine liquid-based cytology for HR-HPV genotypes and cytology from the same test collection vial, as the solution was formulated to preserve cellular integrity for both cytological evaluation and ancillary molecular testing. The respondents did not provide information on epidemiological factors such as their vaccination status, history of screenings, or behavioral risk factors.

2.3. HR-HPV Screening with Alinity m

Women participating in the HPV screening round were evaluated using the fully automated molecular diagnostics analyzer Alinity m (Abbott, USA), employing the high-risk (HR) HPV assay, in accordance with the manufacturer's instructions. The HR-HPV assay is a qualitative test that facilitates the identification of

HR-HPV genotypes and genotype groups within a highly conserved region of L1 at clinically relevant levels. Alinity m HR-HPV is recommended for cervical cancer screening and patient management in alignment with professional medical guidelines, including HPV primary screening, co-testing (adjunctive screening), and triage of atypical squamous cells of undetermined significance (ASC-US) in women to assess the risk of cervical cancer.

2.4. Principles of Alinity m HR HPV PCR Procedure

The Alinity m HR HPV assay comprises several sequential steps, including sample preparation, PCR assembly, amplification/detection, and calculation and reporting of the results. The Alinity m system automates the entire assay procedure, eliminating the need for user intervention in the intermediate processing or transfer steps [22].

1) *Sample Preparation* (*Nucleic acid extraction and purification*). Nucleic acids from specimens were extracted using the Alinity m Sample Prep Kit 1 and Alinity m System Solutions. Nucleic acid extraction (lysis) disrupted the biological matrix to release the nucleic acid materials and allowed it to adhere to the surface of magnetic microparticles. The magnetic microparticle technology facilitated nucleic acid capture, wash and elution.

2) *Nucleic acid amplification*. Purified specimen nucleic acids were amplified by real-time polymerase chain reaction (PCR) using a mix composed of thermostable DNA polymerase, dNTPs, $MgCl_2$, and short oligonucleotide primers for the 14 HR-HPV targets and an endogenous human beta-globin sequence. The endogenous human beta-globin sequence was measured in a separate channel and served as an internal control (IC) to evaluate cell adequacy, sample extraction, and amplification efficiency.

3) *Nucleic acid detection*. Within a single PCR well, the Alinity m HR HPV probes were labeled with different fluorophores that allowed for genotype specific detection of HPV16, 18, and 45 while the remaining 11 HR HPV genotypes were detected as other HR-HPV A (HPV31, 33, 52, 58 genotypes) or other HR-HPV B (HPV35, 39, 51, 56, 59, 66, 68). Amplification of beta-globin sequence was detected also reported separately using a uniquely labeled fluorescent probe.

The Alinity m System's data analysis software provided two basic types of analysis for a single assay reaction: Ct (threshold method and MaxRatio (Maximum ratio, MR) method. The MaxRatio method is an Abbott's proprietary algorithm to produce MaxRatio value that is related to PCR efficiency and is used to differentiate positive from negative reactions. Ct threshold method was defined as the cycle number at which the PCR fluorescence signal reached established threshold above the baseline fluorescence level. Every signal was defined either as "HPV detected", if the cycle number (CN) is less than or equal to a fixed cutoff cycle for that signal, or as "HPV not detected", if CN is not generated or it was possibly greater than the assay cutoff cycle.

Inclusion criteria were: 1) female sex, 2) cervical samples for liquid-based cy-

tology—ThinPrep, 3) HPV test performed on Alinity m (Abbott) in Synevo laboratory, Bulgaria and 4) valid result of the performed HPV test. Exclusion criteria were: 1) women tested for HPV with samples different from LBC (ThinPrep), although Alinity m HR HPV assay is compatible with various collection devices such as: vaginal specimens (simpli-collect™ HPV Collection Kit and Evalyn® Brush) and cervical specimens: SurePath and Alinity m Cervi-Collect Specimen Collection Kit), 2) women tested for HPV with dry swabs, 3) women without parallel LBC result, 4) women with missing data.

2.5. Liquid-Based Cytology (LBC)

Cervical specimens were taken by a specialist and immediately rinsed or agitated in a vial containing ThinPrep PreservCyt Solution (Hologic Inc., Marlborough, MA, USA) to optimally preserve cellular morphology and prevent air-drying artifacts. In the Synevo laboratory, the vials were loaded onto an automated ThinPrep processor. The processor disperses the cells, removes debris and obscuring material (such as blood or mucus), and transfers a representative sample of cells onto a glass slide in a uniform, thin layer of cells onto a glass slide for microscopic examination, according to the manufacturer's instructions. LBC samples stained with Papanicolaou dye were reported by pathology specialists using the 2014 Bethesda System [23]. Specimens were classified based on cytology as negative for intraepithelial lesions (NILM) or any atypical cytology: atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (LSIL), and high-grade intraepithelial lesions (HSIL).

2.6. Ethical Statement

The study was conducted in accordance with the principles set forth in the Declaration of Helsinki and was registered for ethical evaluation within the institutional ethical review framework of Synevo. It received approval from the Ethical Committee of Synevo Romania (Reference number: 1/December 2025), the Ethical Committee of Synevo Bulgaria (Reference number: 51/09.02.26), and the General Manager of Synevo Bulgaria. All participants provided written informed consent for testing at the Synevo clinical laboratory, where the principles of medical ethics and Bulgarian legal standards were explicitly outlined. One participant, aged 15 years, was included in the study. Parental consent was obtained in accordance with institutional guidelines, and assent was secured from the adolescent participant by providing age-appropriate information and confirming voluntary agreement. The informed consent process ensured that participants were thoroughly informed about the nature, purpose, risks, benefits, and alternatives of HR-HPV PCR testing.

2.7. Statistical Analysis

Statistical analysis was performed using the BrightStat.com software. Descriptive statistics, such as proportions, together with corresponding 95% confidence inter-

vals (CI), had been calculated. The prevalence of HPV16, HPV18, and HPV45 genotypes, as well as other HR-HPV A (HPV31, 33, 52, 58 genotypes) and HR-HPV B (HPV35, 39, 51, 56, 59, 66, 68) groups were assessed. Age data are presented as mean ± standard deviation and median. Age groups (15 - 20, 21 - 25, 26 - 30, 31 - 35, 36 - 40, 41 - 45, 46 - 50, 51 - 55, 56 - 60, 61 - 65) were categorized. The liquid-based cytology results were scored. The relationship between age groups, HPV infections, and liquid-based cytology results was investigated using the chi-square test or Fisher's exact test. The settlement distribution of the collected HPV samples was visualized using paintmaps.com. Statistical significance was set at $p < 0.05$.

3. Results

3.1. The Prevalence of HR-HPV Infection

A total of 190 Bulgarian women, aged 15 to 64 years, participated in the study (n = 190). The mean age of the participants was 37.6 years (SD = 5.2), with a median age of 37.0 years. HR-HPV infection was detected in 32 of the 190 women screened, corresponding to an overall infection rate of 16.8% (95% CI: 11.8% to 22.9%, n = 32) (Figure 1).

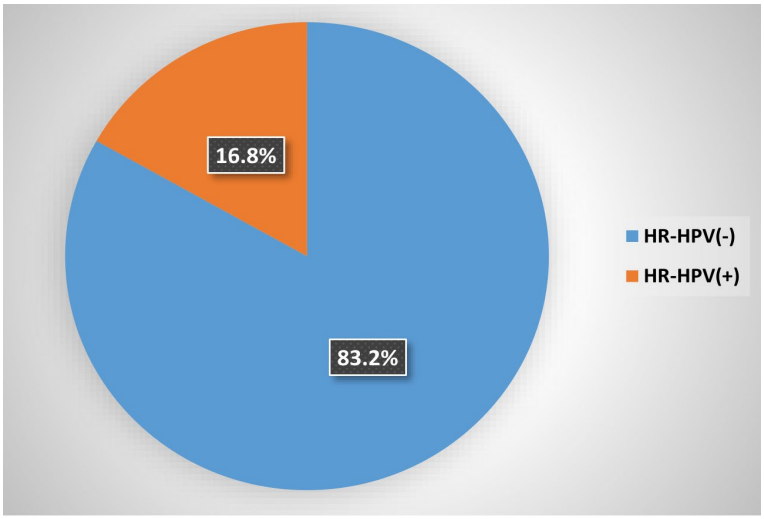

Figure 1. The prevalence of HR-HPV in the tested group.

The HPV genotype distribution results obtained from the conducted study showed that 83.2% (95% CI: 77.1% to 88.2%, n = 158) of the tested women were HR-HPV negative (Figure 2).

Infection with HPV16 could be seen in 5.3% (95% CI: 2.5% to 9.5%, n = 10), HPV18 positive were 2.2% (95% CI: 0.6% to 5.3%, n = 4) and genotype HPV45 was found in 1.0% (95% CI: 0.1% to 3.7%, n = 2). Women positive for one or more HPV genotypes from group A and B were registered—2.6% (95% CI: 0.8% to 6.3%, n = 5) and 4.7 % (95% CI: 2.2% to 8.8%, n = 9), respectively. Two outpatients were found to be group A and group B HPV carriers—1.0% (95% CI: 0.1% to

3.7%, n = 2). The most prevalent HR-HPV genotype in the HPV-positive, HR-HPV-positive subgroup (n = 32) was HPV16—31.2% (95% CI: 16.1% to 50.0%, n = 10) (Figure 3).

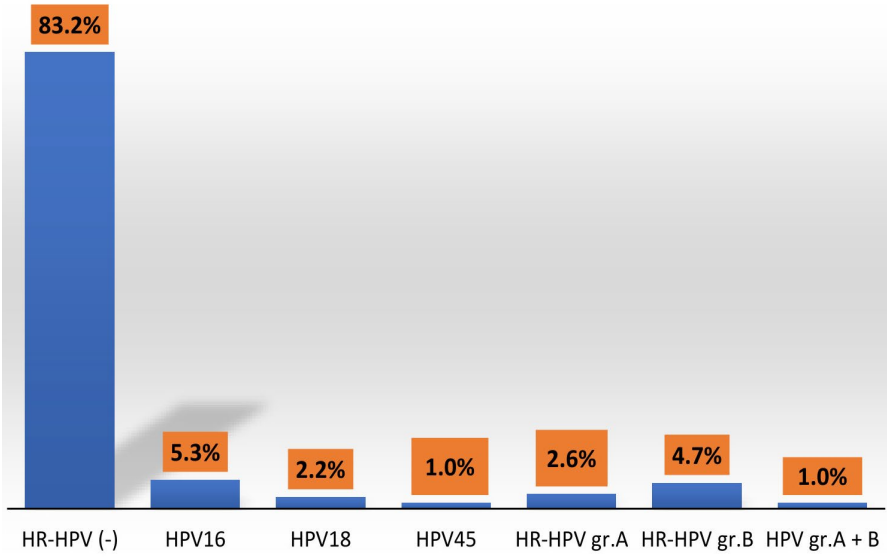

Figure 2. High-Risk HPV genotype distribution in the studied cohort (n = 190).

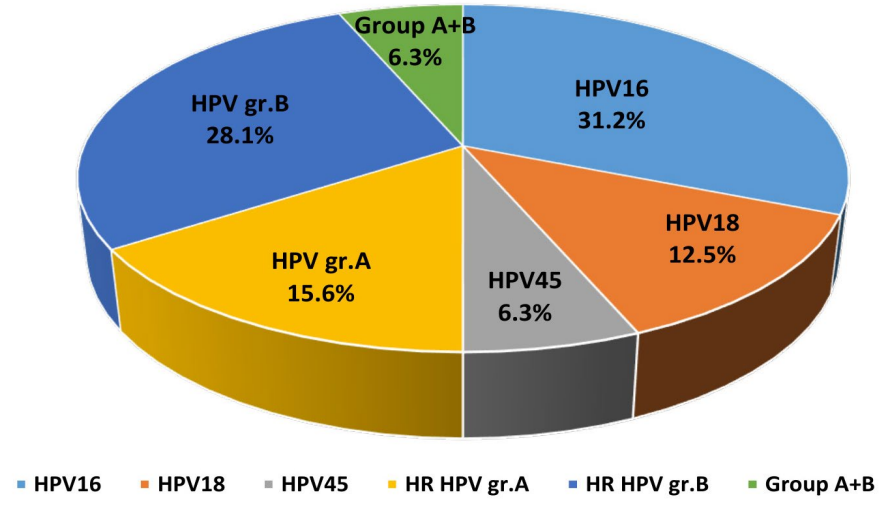

Figure 3. HR-HPV genotype prevalence in the studied HPV-positive group (n = 32).

The prevalence of HPV18 among women was 12.5% (95% CI: 3.5% to 28.9%, n = 4), while HPV45 was found in 6.3% (95% CI: 0.7% to 20.8%, n = 2). The HR-HPV group A had a rate of 15.6% (95% CI: 5.2% to 32.8%, n = 5), and HR-HPV group B showed a rate of 28.1% (95% CI: 13.7% to 46.7%, n = 9). Infection with HPV genotypes from both groups A and B was observed in 6.3% (95% CI: 0.7% to 20.8%, n = 2). High-risk HPV types 16, 18, and 45 constituted 50.0% (95% CI: 31.9% to 68.1%, n = 16) of all HPV genotypes tested. Among the HR-HPV-positive subgroup, HR-HPV infection from other groups A and B was 43.7% (95% CI:

26.4% to 62.3%, n = 14), which could involve single or multiple HPV genotypes.

3.2. Settlement Distribution of the HPV Samples

Cervical swab samples were taken by a gynecologist from different areas in Bulgaria, and majority of them-77.4% (95% CI: 70.7% to 83.1%, n = 147) were from the three largest cities in the country—Sofia, Varna, and Plovdiv (Figure 4). The settlement distribution as per HPV specimens investigated in the study was evaluated as follows: Varna—33.2% (95% CI: 26.5% to 40.3%, n = 63); Plovdiv—28.0% (95% CI: 21.6% to 34.8%, n = 53); Sofia—16.3% (95% CI: 11.4% to 22.5%, n = 31); Stara Zagora—8.4% (95% CI: 4.9% to 13.3%, n = 16); Pazardjik—6.8% (95% CI: 3.7% to 11.4%, n = 13); Burgas—3.2% (95% CI: 1.2% to 6.7%, n = 6); Haskovo—2.6% (95% CI: 0.8% to 6.0%, n = 5); Dobrich—1.0% (95% CI: 0.1% to 3.7%, n = 2) and Blagoevgrad—0.5% (95% CI: 0.01% to 2.9%, n = 1). This multi-site recruitment approach aimed to enhance the representativeness and generalizability of our findings.

Figure 4. HPV samples obtained across Bulgaria.

3.3. The Prevalence of HR-HPV Infection in the Defined Age Groups

The distribution of HR-HPV among different age groups is shown in Table 1. According to the HPV PCR test results, the mean age of the patients was 38.2 (±9.8) years in the HR-HPV positive group and 34.4 (±9.9) years in the HR-HPV negative group. The women were divided into 10 age groups: 15 - 20; 21 - 25; 26 - 30; 31 - 35; 36 - 40; 41 - 45; 46 - 50; 51 - 55; 56 - 60 and 61 - 65 years old.

The majority of HR-HPV-positive results were recorded in women between 31 and 45 years—56.0% (95%: 37.6% to 73.6%, n = 18). Among them, the largest proportion of HPV (+) samples belonged to the 36 - 40 years old group, with a

Table 1. The overall prevalence of HR-HPV among the age groups.

Age group	15 - 20 n (%)	21 - 25 n (%)	26 - 30 n (%)	31 - 35 n (%)	36 - 40 n (%)	41 - 45 n (%)	46 - 50 n (%)	51 - 55 n (%)	56 - 60 n (%)	61 - 65 n (%)	Total n (%)
Samples	8 (4.2)	14 (7.4)	24 (12.6)	36 (18.9)	39 (20.5)	26 (13.7)	22 (11.6)	12 (6.3)	7 (3.7)	2 (1.1)	190 (100.0)
HR-HPV infection	4 (50.0)	3 (21.4)	3 (12.5)	7 (19.4)	8 (20.5)	3 (11.5)	2 (9.1)	1 (8.3)	0 (0)	1 (50.0)	32 (16.8)
					HR-HPV genotypes						
HPV16	0 (0)	1 (7.1)	1 (4.2)	3 (8.3)	4 (10.3)	1 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)	10 (31.2)
HPV18	1 (12.5)	0 (0)	0 (0)	0 (0)	1 (2.6)	1 (3.8)	0 (0)	1 (8.3)	0 (0)	0 (0)	4 (12.5)
HPV45	0 (0)	0 (0)	0 (0)	1 (2.8)	1 (2.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (6.3)
Group A	0 (0)	0 (0)	1 (4.2)	0 (0)	1 (2.6)	0 (0)	2 (9.1)	0 (0)	0 (0)	1 (50.0)	5 (15.6)
Group B	1 (12.5)	2 (14.3)	1 (4.2)	3 (8.3)	1 (2.6)	1 (3.8)	0 (0)	0 (0)	0 (00	0 (0)	9 (28.1)
Group A + B	2 (25.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (00	0 (0)	0 (0)	2 (6.3)

prevalence of HR-HPV infection of 20.5% (95%: 9.3% to 36.5%, n = 8). The age group in second place was 31 - 35 years old, at 19.4% (95% CI: 8.2% to 36.0%, n = 7). The majority of HPV16-positive samples were registered in the 36 - 40 years old group—10.3% (95% CI: 2.8% to 24.2%, n = 4). All of the HR-HPV group A together with group B positive results were found in the youngest age group: 15 - 20 years of age-25.0% (95%: 3.2% to 65.1%, n = 2). In addition, the cases were allocated into two age groups: ≤40 years and >40 years. No significant difference was found between the age groups in terms of HR-HPV positivity according to the chi-square test (p = 0.6). It was observed that HPV infection rates decreased with increasing age, and HPV-positive results were not registered in the age group 50 - 56 years.

3.4. The Prevalence of HR-HPV Infection and LBC Results

Cytological examination was performed for all 190 samples (Table 2). Negative for intraepithelial lesion (NILM) results were defined in 81.0% (95%: 74.7% to 86.4%, n = 154). Abnormalities in LBC cytology were observed in 18.9% (95%: 13.6% to 25.2%, n = 36) of women.

Table 2. Bethesda system report of HR-HPV genotypes according to liquid-based cytology results.

	NILM n (%)	ASC-US n (%)	LSIL n (%)	HSIL n (%)	Total n (%)
HR-HPV (-)	145 (94.3%)	13 (43.4%)	0 (0%)	0 (0%)	158 (83.2%)
HPV16	2 (1.3%)	6 (20.0%)	1 (33.3%)	1 (33.3%)	10 (5.3%)
HPV18	3 (1.9%)	1 (3.3%)	0 (0%)	0 (0%)	4 (2.1%)
HPV45	1 (0.6%)	1 (3.3%)	0 (0%)	0 (0%)	2 (1.0%)

Continued

HR-HPV gr. (A)	2 (1.3%)	3 (10.0%)	0 (0%)	0 (0%)	5 (2.6%)
HR-HPV gr. (B)	1 (0.6%)	5 (16.7%)	2 (66.7%)	1 (33.3%)	9 (4.7%)
HR-HPV (gr.A + B)	0 (0%)	1 (3.3%)	0 (0%)	1 (33.3%)	2 (1.0%)
Total n (%)	154 (81.0%)	30 (15.8%)	3 (1.6%)	3 (1.6%)	190 (100%)

Atypical squamous cells of undetermined significance 15.8% (95%: 10.9% to 21.7%, n = 30) showed the highest frequency among all tested women, followed by low-grade intraepithelial lesion (LSIL) 1.6% (95%: 0.3% to 4.5%, n = 3), and high-grade intraepithelial lesion (HSIL) 1.6% (95%: 0.3% to 4.5%, n = 3). With regard HPV genotype distribution according to cervical cytology, HPV16 was the most common genotype in cases with atypical squamous cells of undetermined significance—20.0% (95%: 7.7% to 38.6%, n = 6). Although HR-HPV positivity in normal cytology samples was 5.2% (95%: 2.3% to 9.9%, n = 9), it was 63.9% (95%: 46.2% to 79.2%, n = 23) in abnormal smear cytology results ($p < 0.00001$).

4. Discussion

To construct an accurate regional map, we evaluated the prevalence of high-risk human papillomavirus (HR-HPV) using the clinically validated Alinity m HR-HPV Assay among women in a sample population of 190 Bulgarian women. This initiative coincides with the commencement of the newly expanded "National Program for Primary Prevention of Cancer Diseases Caused by Human Papillomavirus 2025-2030" in Bulgaria. It is noteworthy that the prevalence of high-risk human papillomavirus infection in Bulgaria is significant, with rates among women exhibiting normal cytology ranging from approximately 29.8% to 38.8%, as reported in extensive population-based studies. Persistent infection with high-risk human papillomavirus genotypes is recognized as the primary etiological factor in 99% of cervical cancer cases, suggesting that such cases may be preventable through HPV screening [24] [25].

Geographical variations in the prevalence of high-risk human papillomavirus infection have been documented. Numerous studies have reported varying findings regarding the prevalence and distribution of high-risk HPV types. HPV prevalence ranges from 11% to 12%, with Africa exhibiting the highest rate at 21.1%, followed by Europe at 14.2%, America at 11.5%, and Asia at 9.4%. We propose that these differences may be attributed to factors such as geographic location, the methodologies employed for HPV PCR detection, the educational background of patients in healthcare settings, and their awareness of HPV infections. Eastern Europe continues to report the highest rates of cervical cancer and HPV infection in Europe [26]. The epidemiological distribution of HPV in Eastern Europe is re-

ported to be 21.4%, whereas in Western Europe, it is 9.0%, ranging from 2% in Spain to approximately 12% in France and Belgium. Poland has a prevalence of 14.4%, the Czech Republic 25.6%, and Lithuania 24.2% [27]. Data from a Latvian study indicated an overall HR-HPV prevalence of 11% in the general population [28]. In Greece, HPV prevalence ranges from 2.5% to 50.7%, whereas in Iran, it varies from 0.6% to 37.9% [1]. Studies from Türkiye reported HR-HPV prevalence between 2.4% and 47.7% [29]-[31]. Overall, the HPV-DNA positivity rate in Italy was 35.9% among women [32]. A meta-analysis found that HPV DNA prevalence among women with normal cytology (ages 17 - 77) ranged from 5.3% to 35.6%, with a mean prevalence of 2.6% [33]. The variation in HPV prevalence reported may stem from differences in the age distribution, sampling methods, screening history, and vaccination status of the population studied. The HR-HPV positivity rate found in this research is consistent with global figures, with 16.8% of women testing positive for HR-HPV.

In every instance, HPV genotypes 16, 18, and 45 were the most prevalent, with their proportions significantly rising in women with cervical cancer compared to those with normal cytology or low-grade cervical lesions. In our study, HPV16, HPV18, and HPV45 accounted for 50% of all genotypes. Research involving over 6000 women diagnosed with CIN2 or invasive cervical cancer from 17 European countries identified HPV16, HPV18, and HPV45 as the most prevalent types in women with invasive cervical cancer, accounting for 63.3%, 15.2%, and 5.3%, respectively [34] [35]. HPV 16, 18, and 45 are associated with 75% of all squamous cell carcinomas and 94% of all adenocarcinomas, with HPV 45 alone responsible for 12% of all adenocarcinomas [36]. A study conducted on over 4000 Slovenian women with normal cytology found HPV16 and HPV18 to be the most frequently detected HPV types, followed by HPV31, HPV39, HPV51, HPV52, and HPV59 [37].

Bulgaria has a population of 3.06 million women aged 15 years and older, who are at risk of developing cervical cancer [38]. Current data suggest that each year, 1009 women are diagnosed with cervical cancer and 503 of them die from the disease. Cervical cancer screening coverage among women aged 20 - 69 was 57% in 2019 (lower than the EU average of 71% in the same year), but it has increased by 21% since 2008, when coverage was 47% [39]. A recent study conducted in Bulgaria from 2018 to 2020 that analyzed the economic impact of cervical cancer, the most common complication of sexually transmitted HPV infections, calculated the economic burden of HPV-related malignancies amounted to be 51.3 million euros. These findings indicate that nationwide actions are needed to reduce the ongoing consequences of neoplasms related to HPV [40]. The country has one of the highest rates of cervical cancer incidence and mortality within the European Union, with HPV16 and HPV18 identified in approximately 77% of invasive cervical carcinomas, followed by HPV56, HPV33, and HPV31. HPV16 is detected in approximately 13% - 17% of Bulgarian women in general population studies, a stark contrast to the distribution in the general population in Europe (2.8%), such

as in Germany (2.5%) and France (3.9%). Among women with high-risk HPV infections, HPV16 was reported to be 46% [25]. The distribution of high-risk HPV has been consistently observed in extensive population-based studies of Bulgarian women with normal cytology, as well as in women with abnormal cytology and in high-risk groups, such as female sex workers [41] [42]. In our study, the prevalence of HPV16 was identified as 5.3% in the sample population and 31.2% within the HR-HPV positive group (HR-HPV-positive subgroup). HPV18 was found in 2.2% of the studied cohort and 12.5% of the HR-HPV positive group. HPV45 was detected in 1.0% of all tested women and 6.3% of the HR-HPV-positive subgroup.

Clinically validated HR-HPV tests, like the Alinity m HR-HPV assay, can significantly enhance patient risk stratification by offering extended genotyping beyond HPV16 and HPV18. In the study, HR-HPV positivity rates were 2.6% in group A and 4.7% in group B. Within the (HR-HPV-positive subgroup, infections involving either single or multiple HPV genotypes were observed at rates of 15.6% in HR-HPV group A, 28.1% in the HR-HPV group B, and 6.3% with HPV genotypes from both groups A and B. Among the HR-HPV positive cohort, infections from other groups A and B were noted at 43.7%. These two groups included HPV56, HPV33, and HPV31, aligning with existing literature on Bulgaria. Furthermore, women in groups A and B were infected with single or multiple HPV genotypes. Numerous molecular epidemiological and diagnostic studies have frequently reported co-infections with multiple high-risk HPV types, with some research suggesting that these co-infections may contribute to the progression of cervical neoplasia [19].

Population-based studies have elucidated the distribution of high-risk human papillomavirus genotypes within Bulgarian urban centers, with a primary focus on cities, such as Sofia, Plovdiv, Varna, Burgas, Pleven, and Vidin, as well as smaller communities [24] [25]. Our research indicated that 77.4% of the female samples were derived from the three largest cities in Bulgaria—Sofia, Varna, and Plovdiv—with HPV16 consistently emerging as the most prevalent genotype. While the majority of samples were collected in urban Bulgarian areas, this does not completely rule out the inclusion of rural regions and economically disadvantaged populations, as some individuals may have sought medical attention at city-based gynecological clinics.

Reports indicate that HR-HPV prevalence is highest among adolescents and young adults and then declines with age, with regional differences in the extent and shape of the age curve. In women, HR-HPV prevalence typically peaks in those under 25 years old, with estimates around 16.9% in this group, then steadily declines after age 35 in most populations. In some regions, a second, smaller peak may occur in women over 55 years of age, but this varies according to location and sexual behavior patterns [43] [44]. According to our test results, the average age of patients in the HR-HPV positive group was 38.2 (±9.8) years, compared to 34.4 (±9.9) years in the HR-HPV negative group. Most HR-HPV-positive results were found in women aged 31 to 45 (56.0%). Of these, the largest proportion of

HPV (+) samples (20.5%) belonged to the 36 - 40-year-old group. The second most common age group was 31 - 35 years old (19.4%). The HPV16 genotype was most prevalent in the 36 - 40-year-old group, accounting for 10.3%. All results from HR-HPV groups A and B were found in the youngest age group of 15 to 20 years. Studies have reported no significant link between the risk of developing abnormal cervical cytology and increasing age. Similarly, this study found that increasing the age of HPV-positive women did not significantly increase the risk of high cervical cytology scores (0.6). We observed that HPV infection rates decreased with age and no HPV-positive results were recorded in women aged 50 - 56 years. The decrease in HPV prevalence with increasing age can be attributed to fewer new sexual partners and acquired immunity. In our study, the small sample sizes within age groups may limit the statistical power needed for definitive conclusions. Although the data indicate certain trends, they should be interpreted cautiously, and we have refrained from over-interpreting the results.

In 2018, the World Health Organization (WHO) advocated for a global initiative to eliminate cervical cancer through prevention and early detection, which are recognized as highly cost-effective strategies. The WHO delineates three principal pillars for cervical cancer prevention: primary prevention via HPV vaccination ideally administered prior to the onset of sexual activity. In conjunction with vaccination, one of the strategic pillars is cervical cancer screening utilizing well-established and widely adopted methods such as traditional cytology (Pap smear), Liquid-Based Cytology, and molecular techniques for detecting the HR HPV virus. The WHO emphasizes that HPV-based screening offers superior protection against cervical cancer compared to cytology. For tertiary prevention, given the absence of a virus-specific treatment for HPV infection, precancerous lesions can be effectively managed through ablative methods (destroying abnormal tissue by burning or freezing) or excision, which provides an effective treatment for CIN [45] [46].

Efforts to reduce this cervical cancer burden in Bulgaria have included national vaccination campaigns and screening programs until 2017 with the "National Program for Primary Prevention of Cervical Cancer in the Republic of Bulgaria", but coverage remains low and political instability has hindered consistent implementation. Implementation of the new "National Program for Primary Prevention of Cancer Diseases Caused by Human Papillomavirus 2025-2030" aims to expand organized HPV screening and provide free immunization upon request to girls aged 10 - 18 years and boys aged 10 - 14 years. The program is a key element in efforts to reduce the incidence of HPV-related cancers, among which cervical cancer is the leading cancer [47]. The screening program also provides medical and diagnostic tests to establish infection with HPV from a cervical sample. The target group included women between the ages of 25 and 65, regardless of their health insurance status and family burden [48]. Recognizing the need to differentiate between vaccinated and unvaccinated individuals in the study is crucial, as without this distinction, any definitive comparisons of genotype frequencies, especially for

vaccine-covered types, remain incomplete and require further investigation.

Introduced in Bulgaria in 1974, the conventional PAP test has been a widely employed screening method, with eligibility for women aged 30 to 40 being determined by general practitioners [48]. Currently, Bulgaria is transitioning from the conventional PAP test to liquid-based cytology HPV screening. In the present study, cytological examination was conducted for all respondents. Across multiple studies, HR-HPV prevalence was consistently higher in specimens with abnormal cytology than in those with normal cytology. The prevalence of high-risk human papillomavirus in cervical specimens with abnormal cytology varies widely depending on the degree of cytological abnormality, the population studied, and geographic region. Global data indicate that the prevalence of high-risk human papillomavirus is in the range 37% - 52% in cases of atypical squamous cells of undetermined significance, 76% - 79% in low-grade squamous intraepithelial lesions, and 85% in high-grade squamous intraepithelial lesions [49]. This finding correlates with the presented data in our study, where HR-HPV prevalence in the abnormal cytology smears was accounted for 63.9%. In the present study, atypical squamous cells of undetermined significance (15.8%) showed the highest frequency among abnormal cytology categories, followed by equally distributed LSIL and HSIL intraepithelial lesion—1.6%. As regards HPV genotype distribution according to cervical cytology, HPV16 was the most common genotype in cases with atypical squamous cells of undetermined significance—20.0%. Our results showed that although HR-HPV positivity in normal cytology samples was 5.2%, it was 63.9% in abnormal smear cytology samples ($p < 0.00001$). According to the Bethesda system, patients diagnosed with low-grade squamous intraepithelial lesions or atypical squamous cells of uncertain malignant potential are recommended for further investigation, including colposcopy and biopsy, to exclude the presence of cervical intraepithelial neoplasia 2 [23].

The present study outlines several limitations that merit attention, especially concerning future research challenges related to cohort sample sizes, selection bias, incomplete vaccination data, behavioral risk factors data, and the lack of longitudinal outcomes. Although small sample sizes within categories can result in insufficient statistical power, our study still provides significant insights into HPV testing and underscores the necessity for larger samples in future research endeavors. The cohort was assembled using a laboratory-based, opportunistic sampling strategy, primarily in urban clinical settings where gynecologists practice, rather than employing a random sampling method from the Bulgarian general population. This recruitment strategy suggests that while the cohort might include individuals from rural areas seeking gynecological services in urban settings, it does not fully represent the entire general population, particularly those in rural and socioeconomically disadvantaged groups who may have limited access to specialists. The lack of information on participants' HPV vaccination status poses a limitation in analyzing HPV genotype distribution and prevalence, especially considering the ongoing national vaccination initiatives in Bulgaria. Including compre-

hensive data on HPV immunization would enhance the reliability and interpretability of conclusions related to HPV epidemiology. The absence of longitudinal outcomes limits the ability to observe HPV persistence over time in the cohort. Collecting accurate behavioral data often requires extensive resources, which were not available in the recent study due to resource constraints and the potential burden on participants. Despite these limitations, the findings presented offer valuable evidence and inform future research directions aimed at enhancing methodological rigor, improving data quality, and developing comprehensive study designs to advance knowledge in the field of HPV management in Bulgaria.

5. Conclusion

At present, there is a notable absence of official data concerning the substantial impact of HPV on the general population in Bulgaria. The implementation of the "National Program for Primary Prevention of Cancer Diseases Caused by Human Papillomavirus 2025-2030" in Bulgaria is critically important, as it directly addresses the country's persistently high incidence and mortality rates of cervical cancer, which rank among the highest in the European Union. The data presented in the manuscript indicate that cervical cancer screening becomes more accessible through the use of the HPV PCR test, which demonstrates high sensitivity by detecting the presence of HPV in cervical cells, rather than identifying precancerous changes that may result from chronic infection. It is imperative that this test undergo clinical validation and receive approval. The high sensitivity of HPV testing reduces the risk of undetected disease and permits extended intervals between screenings. The current study aims to establish a foundational understanding of the prevalence and genotype distribution of HR-HPV within selected segments of the Bulgarian population. These baseline findings may serve as a basis for future research endeavors focused on developing an effective model for preventing HPV transmission, examining its epidemiological patterns, establishing screening guidelines, and evaluating and enhancing vaccination efforts.

Institutional Review Board Statement

The study received ethics approval from Ethical Committee of Synevo Romania (Reference number: 1/December 2025), the Ethical Committee of Synevo Bulgaria (Reference number: 51/09.02.26), and the General Manager of Synevo Bulgaria.

Informed Consent Statement

All study participants provided written informed consent prior to enrollment.

Data Availability Statement

The data that support the findings of this study are not freely available due to reasons of sensitivity and are available from corresponding author upon reasonable request. Data are located in controlled access data storage at Medical diagnos-

tic laboratory "Synevo", Bulgaria.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Kombe Kombe, A.J., Li, B., Zahid, A., Mengist, H.M., Bounda, G., Zhou, Y., *et al.* (2021) Epidemiology and Burden of Human Papillomavirus and Related Diseases, Molecular Pathogenesis, and Vaccine Evaluation. *Frontiers in Public Health*, **8**, Article 552028. https://doi.org/10.3389/fpubh.2020.552028

[2] Wang, W., Sawleshwarkar, S. and Piraveenan, M. (2025) Computational Approaches of Modelling Human Papillomavirus Transmission and Prevention Strategies: A Systematic Review. *Journal of Biological Dynamics*, **19**, Article ID: 2436376. https://doi.org/10.1080/17513758.2024.2436376

[3] Zerbini, F.M., Crane, A., Kuhn, J.H., Simmonds, P. and Lefkowitz, E.J. (2025) Summary of Taxonomy Changes Ratified by the International Committee on Taxonomy of Viruses (ICTV)—General Taxonomy Proposals, 2025. *Journal of General Virology*, **106**, Article ID: 002116. https://doi.org/10.1099/jgv.0.002116

[4] Hanft, W., Stankiewicz Karita, H., Khorsandi, N., Vohra, P. and Plotzker, R. (2025) Sexually Transmitted Human Papillomavirus and Related Sequelae. *Clinical Microbiology Reviews*, **38**, e00085-23. https://doi.org/10.1128/cmr.00085-23

[5] Ouh, Y.T., Kim, H.Y., Yi, K.W., Lee, N.W., Kim, H.J. and Min, K.J. (2024) Enhancing Cervical Cancer Screening: Review of p16/Ki-67 Dual Staining as a Promising Triage Strategy. *Diagnostics*, **14**, Article 451. https://doi.org/10.3390/diagnostics14040451

[6] Einstein, M.H., Schiller, J.T., Viscidi, R.P., Strickler, H.D., Coursaget, P., Tan, T., *et al.* (2009) Clinician's Guide to Human Papillomavirus Immunology: Knowns and Unknowns. *The Lancet Infectious Diseases*, **9**, 347-356. https://doi.org/10.1016/s1473-3099(09)70108-2

[7] Galati, L., Chiantore, M.V., Marinaro, M. and Di Bonito, P. (2024) Human Oncogenic Viruses: Characteristics and Prevention Strategies—Lessons Learned from Human Papillomaviruses. *Viruses*, **16**, Article 416. https://doi.org/10.3390/v16030416

[8] Lipsick, J. (2021) A History of Cancer Research: Tumor Viruses. *Cold Spring Harbor Perspectives in Biology*, **13**, a035774. https://doi.org/10.1101/cshperspect.a035774

[9] Kusakabe, M., Taguchi, A., Sone, K., Mori, M. and Osuga, Y. (2023) Carcinogenesis and Management of Human Papillomavirus-Associated Cervical Cancer. *International Journal of Clinical Oncology*, **28**, 965-974. https://doi.org/10.1007/s10147-023-02337-7

[10] Karaoğlan, B.B. and Ürün, Y. (2024) Unveiling the Role of Human Papillomavirus in Urogenital Carcinogenesis a Comprehensive Review. *Viruses*, **16**, Article 667. https://doi.org/10.3390/v16050667

[11] Gu, Y., Li, T., Zhang, M., Chen, J., Shen, F., Ding, J., *et al.* (2024) The Display between HPV Infection and Host Immunity in Cervical Cancer. *Frontiers in Bioscience-Landmark*, **29**, Article 426. https://doi.org/10.31083/j.fbl2912426

[12] de Sanjosé, S., Serrano, B., Tous, S., Alejo, M., Lloveras, B., Quirós, B., *et al.* (2018) Burden of Human Papillomavirus (HPV)-Related Cancers Attributable to HPVs 6/11/16/18/31/33/45/52 and 58. *JNCI Cancer Spectrum*, **2**, pky045.

https://doi.org/10.1093/jncics/pky045

[13] Franco, E.L., Schlecht, N.F. and Saslow, D. (2003) The Epidemiology of Cervical Cancer. *The Cancer Journal*, **9**, 348-359. https://doi.org/10.1097/00130404-200309000-00004

[14] Ntuli, L., Mtshali, A., Mzobe, G., Liebenberg, L.J. and Ngcapu, S. (2022) Role of Immunity and Vaginal Microbiome in Clearance and Persistence of Human Papillomavirus Infection. *Frontiers in Cellular and Infection Microbiology*, **12**, Article 927131. https://doi.org/10.3389/fcimb.2022.927131

[15] Cangelosi, G., Sacchini, F., Mancin, S., Petrelli, F., Amendola, A., Fappani, C., *et al.* (2025) Papillomavirus Vaccination Programs and Knowledge Gaps as Barriers to Implementation: A Systematic Review. *Vaccines*, **13**, Article 460. https://doi.org/10.3390/vaccines13050460

[16] Fashedemi, O., Ozoemena, O.C., Peteni, S., Haruna, A.B., Shai, L.J., Chen, A., *et al.* (2025) Advances in Human Papillomavirus Detection for Cervical Cancer Screening and Diagnosis: Challenges of Conventional Methods and Opportunities for Emergent Tools. *Analytical Methods*, **17**, 1428-1450. https://doi.org/10.1039/d4ay01921k

[17] Blanco, R. and Muñoz, J.P. (2025) Molecular Insights into HR-HPV and HCMV Co-Presence in Cervical Cancer Development. *Cancers*, **17**, Article 582. https://doi.org/10.3390/cancers17040582

[18] World Health Organization (2024) WHO Guideline for Screening and Treatment of Cervical Pre-Cancer Lesions for Cervical Cancer Prevention. World Health Organization. https://doi.org/10.2471/b09020

[19] Goldstein, D.Y., Yang, T., Lucic, D., Zhang, Y., Cullum, R., Kostera, J., *et al.* (2025) Multi-center Evaluation of the Alinity M HR HPV Assay with Liquid-Based Cytology Cervical Specimens in the United States. *Microbiology Spectrum*, **13**, e01918-24. https://doi.org/10.1128/spectrum.01918-24

[20] Banerjee, D., Mittal, S., Mandal, R. and Basu, P. (2022) Screening Technologies for Cervical Cancer: Overview. *Cytojournal*, **19**, Article 23. https://doi.org/10.25259/cmas_03_04_2021

[21] Martinelli, M., Giubbi, C., Di Meo, M.L., Perdoni, F., Musumeci, R., Leone, B.E., *et al.* (2023) Accuracy of Human Papillomavirus (HPV) Testing on Urine and Vaginal Self-Samples Compared to Clinician-Collected Cervical Sample in Women Referred to Colposcopy. *Viruses*, **15**, Article 1889. https://doi.org/10.3390/v15091889

[22] Huang, S., Gentil, L.G., Schmidt, C., Cullum, R., Zhang, Y., Nelson, K., *et al.* (2025) Comparative Clinical Performance of Alinity M HR HPV, Cobas 4800 HPV, and Cobas 6800 HPV for Cervical Cancer Screening. *Microbiology Spectrum*, **13**, e00589-25. https://doi.org/10.1128/spectrum.00589-25

[23] Nayar, R. and Wilbur, D.C. (2017) The Bethesda System for Reporting Cervical Cytology: A Historical Perspective. *Acta Cytologica*, **61**, 359-372. https://doi.org/10.1159/000477556

[24] Kovachev, S., Slavov, V. and Slavova, K. (2013) Prevalence of Human Papillomavirus Infection in Women in Some Cities and Regions of Bulgaria. *Journal of Medical Virology*, **85**, 1577-1584. https://doi.org/10.1002/jmv.23652

[25] Kovachev, S. and Slavov, V. (2018) Prevalence of Human Papillomavirus Infection in Women in Bulgaria: A 2017 Update. *Journal of Medical Virology*, **90**, 1142-1149. https://doi.org/10.1002/jmv.25050

[26] Yordanov, A., Vasileva-Slaveva, M., Galai, N., Faraggi, D., Kubelac, M.P., Tripac-Iacovleva, I., *et al.* (2023) Cancer of the Cervix in Bulgaria: Epidemiology of a Crisis.

Healthcare, **11**, Article 318. https://doi.org/10.3390/healthcare11030318

[27] Bruni, L., Diaz, M., Castellsagué, X., Ferrer, E., Bosch, F.X. and de Sanjosé, S. (2010) Cervical Human Papillomavirus Prevalence in 5 Continents: Meta-Analysis of 1 Million Women with Normal Cytological Findings. *The Journal of Infectious Diseases*, **202**, 1789-1799. https://doi.org/10.1086/657321

[28] Berza, N., Zodzika, J., Kivite-Urtane, A., Baltzer, N., Curkste, A., Pole, I., *et al.* (2024) Understanding the High-Risk Human Papillomavirus Prevalence and Associated Factors in the European Country with a High Incidence of Cervical Cancer. *European Journal of Public Health*, **34**, 826-832. https://doi.org/10.1093/eurpub/ckae075

[29] Eroglu, S. and Asgin, N. (2020) Frequency and Genotype Distribution of High-Risk Human Papilloma Virus Types in Karabuk Province, Turkey: A Hospital Based Cross-Sectional Study. *Annals of Medical Research*, **27**, 765-769. https://doi.org/10.5455/annalsmedres.2019.12.787

[30] Hancer, V., Buyukdogan, M., Bylykbashi, I., Oksuz, B. and Acar, M. (2018) Prevalence of Human Papilloma Virus Types in Turkish and Albanian Women. *Journal of Cytology*, **35**, 252-254. https://doi.org/10.4103/joc.joc_162_17

[31] Taskin, M., Nursal, A., Oruc, M. and Kariptas, E. (2022) Genotype Distribution and Prevalence of High-Risk Human Papillomavirus Infection among Women in Samsun Province of Turkey. *Asian Pacific Journal of Cancer Prevention*, **23**, 2477-2482. https://doi.org/10.31557/apjcp.2022.23.7.2477

[32] Piana, A., Sotgiu, G., Castiglia, P., Pischedda, S., Cocuzza, C., Capobianco, G., *et al.* (2011) Prevalence and Type Distribution of Human Papillomavirus Infection in Women from North Sardinia, Italy. *BMC Public Health*, **11**, Article No. 785. https://doi.org/10.1186/1471-2458-11-785

[33] Bergman, H., Henschke, N., Villanueva, G., Loke, Y.K., Golder, S.P., Dwan, K., *et al.* (2022) Human Papillomavirus (HPV) Vaccination for the Prevention of Cervical Cancer and Other HPV-Related Diseases: A Network Meta-Analysis. *Cochrane Database of Systematic Reviews*, **11**, CD015364. https://doi.org/10.1002/14651858.cd015364

[34] Glinska, P., Macios, A., Jaworski, R., Bobinski, M., Pruski, D., Przybylski, M., *et al.* (2024) Baseline Data on Distribution of Human Papillomavirus (HPV) Genotypes in Cervical Samples of Gynecological Patients before Implementation of Population-Based HPV Vaccination Program in Poland. *Ginekologia Polska*, **95**, 870-878. https://doi.org/10.5603/gpl.101436

[35] Elfstrom, K.M., Smelov, V., Johansson, A.L.V., Eklund, C., Naucler, P., Arnheim-Dahlstrom, L., *et al.* (2014) Long Term Duration of Protective Effect for HPV Negative Women: Follow-Up of Primary HPV Screening Randomised Controlled Trial. *BMJ*, **348**, g130-g130. https://doi.org/10.1136/bmj.g130

[36] Holl, K., Nowakowski, A.M., Powell, N., McCluggage, W.G., Pirog, E.C., Collas De Souza, S., *et al.* (2015) Human Papillomavirus Prevalence and Type-distribution in Cervical Glandular Neoplasias: Results from a European Multinational Epidemiological Study. *International Journal of Cancer*, **137**, 2858-2868. https://doi.org/10.1002/ijc.29651

[37] Učakar, V., Poljak, M., Oštrbenk, A. and Klavs, I. (2014) Pre-Vaccination Prevalence of Infections with 25 Non-High-Risk Human Papillomavirus Types among 1,000 Slovenian Women in Cervical Cancer Screening. *Journal of Medical Virology*, **86**, 1772-1779. https://doi.org/10.1002/jmv.23997

[38] Merdzhanova-Gargova, Y., Ivanova, M., Mollova-Kysebekirova, A., Mihaylova, A., Parahuleva-Rogacheva, N., Uchikova, E., *et al.* (2025) Advancements in Cervical Can-

cer Screening: Enhancing HPV Testing and Triage Strategies for Improved Risk Assessment. *Biomedicines*, **13**, Article 1768.
https://doi.org/10.3390/biomedicines13071768

[39] OECD (2024) Beating Cancer Inequalities: Current Trends and Key Policy Directions. Organisation for Economic Cooperation Development.

[40] Lebanova, H., Stoev, S., Naseva, E., Getova, V., Wang, W., Sabale, U., *et al.* (2023) Economic Burden of Cervical Cancer in Bulgaria. *International Journal of Environmental Research and Public Health*, **20**, Article 2746.
https://doi.org/10.3390/ijerph20032746

[41] Shikova, E., Todorova, I., Ganchev, G., Kouseva-Dragneva, V. and Kalascheva-Zaimova, P. (2011) Prevalence of Human Papillomavirus Infection among Female Sex Workers in Bulgaria. *International Journal of STD & AIDS*, **22**, 278-280.
https://doi.org/10.1258/ijsa.2009.009362

[42] Grozdanov, P., Zlatkov, V., Ganchev, G., Karagiosov, I., Toncheva, D. and Galabov, A.S. (2014) HPV Prevalence and Type Distribution in Women with Normal or Abnormal Pap Smear in Bulgaria. *Journal of Medical Virology*, **86**, 1905-1910.
https://doi.org/10.1002/jmv.24020

[43] Rahangdale, L., Mungo, C., O'Connor, S., Chibwesha, C.J. and Brewer, N.T. (2022) Human Papillomavirus Vaccination and Cervical Cancer Risk. *BMJ*, **379**, e070115.
https://doi.org/10.1136/bmj-2022-070115

[44] Spinillo, A., Dal Bello, B., Gardella, B., Roccio, M., Dacco', M.D. and Silini, E.M. (2009) Multiple Human Papillomavirus Infection and High Grade Cervical Intraepithelial Neoplasia among Women with Cytological Diagnosis of Atypical Squamous Cells of Undetermined Significance or Low Grade Squamous Intraepithelial Lesions. *Gynecologic Oncology*, **113**, 115-119. https://doi.org/10.1016/j.ygyno.2008.12.037

[45] Zhang, Y., Zhao, F.H., Hu, S.Y., Zhao, X.L. and Qiao, Y.L. (2021) Interpretation of "WHO Guideline for Screening and Treatment of Cervical Pre-Cancer Lesions for Cervical Cancer Prevention, Second Edition. *Chinese Journal of Medical History*, **101**, 2653-2657. (In Chinese)

[46] Luu, X.Q., Jun, J.K., Suh, M., Oh, J., Yu, S. and Choi, K.S. (2025) Cervical Cancer Screening, HPV Vaccination, and Cervical Cancer Elimination. *JAMA Network Open*, **8**, e2526683. https://doi.org/10.1001/jamanetworkopen.2025.26683

[47] Zhou, L., Li, Y., Wang, H., Qin, R., Han, Z. and Li, R. (2025) Global Cervical Cancer Elimination: Quantifying the Status, Progress, and Gaps. *BMC Medicine*, **23**, Article No. 67. https://doi.org/10.1186/s12916-025-03897-3

[48] Todorova, I., Panayotova, Y., Kotzeva, T., Greenley, R. and McKee, M. (2026) Cervical Cancer in Bulgaria since EU Accession in 2007: A Struggle in the Face of Political Instability. *Health Policy*, **164**, Article ID: 105497.
https://doi.org/10.1016/j.healthpol.2025.105497

[49] Clarke, M.A., Risley, C., Stewart, M.W., Geisinger, K.R., Hiser, L.M., Morgan, J.C., *et al.* (2021) Age-Specific Prevalence of Human Papillomavirus and Abnormal Cytology at Baseline in a Diverse Statewide Prospective Cohort of Individuals Undergoing Cervical Cancer Screening in Mississippi. *Cancer Medicine*, **10**, 8641-8650.
https://doi.org/10.1002/cam4.4340