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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

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ABSTRACT

Objectives: Free-of-charge vaccination against human papillomavirus (HPV) of 12–13-year-old teenagers was introduced on the 1st of June 2023 in Poland. Data on baseline HPV genotype cervical distribution are crucial to evaluate potential changes after full implementation of the vaccination program. We aimed at evaluating the status of HPV infection and distribution of HPV genotypes in cervical cytology of gynecological patients tested in one of the largest HPV laboratories in Poland.

Material and methods: Data on all HPV tests performed in ALAB Laboratoria z o.o. in Poland in 2018–2021 were analyzed, focusing on tests that identified genotypes: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 44, 45, 51–54, 56, 58, 59, 61, 62, 66–68, 70, 73, 81–83, 89. Distribution of HPV genotypes was assessed among HPV-positive women.

Results: Among 11,151 medical records retrieved in women with valid HPV test results, 5,681 were positive (50.9%), of whom 2,929 were infected with a single genotype (51.6%). At least one high-risk (HR) genotype was detected in 4,351 women (76.6%). Among all HPV-positive women, the most common HR genotypes were HPV-16, HPV-31 and HPV-66 (24.0%, 11.3%, 11.3%, respectively). HPV-53 was the most prevalent among non-HR types (10.5%).

Conclusions: HPV-16 followed by HPV-31 and HPV-66 were the most frequent genotypes in the studied cohort. These results may be compared with the same methodology after full roll-out of HPV vaccination program in the future to track potential changes in HPV genotype distribution.

Keywords: human papillomavirus genotypes; human papillomavirus vaccine

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INTRODUCTION

High-risk (HR) human papillomavirus (HPV) is the etiological agent of more than 99% of cases of cervical cancer (CC) worldwide [1]. The majority of HPV positive women will never develop cancer or precancerous lesions since most HPV infections are transient and regress spontaneously [2]. However persistent HPV infection may trigger cervical carcinogenesis. Persistence and progression rates vary depending on the virus genotype [3, 4]. Currently, more than 200 genotypes of HPV have been isolated with different oncogenic potential [5]. International Agency for Research on Cancer (IARC) classified 12 High Risk (HR) types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), 17 as likely to be oncogenic (25, 53, 66–68 , 70, 73, 82) and other with low oncogenic potential (6, 11, 34, 40, 42, 43, 53, 54, 73) [6]. The distribution of HPV genotypes in populations and contribution to the development of precancerous lesions and CC differs and depends on the age, sex, population, and the geographical location of the patients [2, 7].

Although there is no specific treatment for HPV, vaccination, screening for and treatment of cervical precancerous lesions is highly successful in preventing cervical cancer [8, 9]. In Poland, HPV testing is still not a part of reimbursed population-based cervical cancer screening. Most HPV tests have been performed in the private healthcare sector, mainly for triage of abnormal cytology and screening as a part of co-testing and for other indications.

In Poland, two HPV vaccines are currently available: Cervarix (against genotypes 16 and 18), and Gardasil 9 (against genotypes 6, 11, 16, 18, 31, 33, 45, 52, 58). Free-of-charge HPV vaccination of 12–13 year old teenagers was introduced into Population-based Immunization Program in Poland on the 1st of June 2023 with the bivalent and nonavalent vaccine which could be chosen by the parents. The goal of the immunization program within the National Oncology Strategy (NOS) is to reach 60% vaccination coverage among adolescents until 2028 [10, 11].

Cervico-vaginal prevalence of HPV and HPV-genotype distribution differ greatly depending on age, geographic region, population and current cytological and histological status of the cervix. HPV genotype distribution was studied *e.g.* in Brazilian women with and without cervical lesions in 2009–2011. HPV-56 was the most prevalent type in women with NILM (no intraepithelial lesion or malignancy) cytology and LSIL (low-grade squamous intraepithelial lesion) and HPV-16 was the most common genotype in women with ASC-US (atypical squamous cells of undetermined significance), HSIL (high grade squamous intraepithelial lesion) and invasive CC [12]. Population-based study before introduction of an HPV-vaccination program was conducted in Denmark. HPV-16 was the dominant type independent of cytological results and HPV-18 was increased in more advanced lesions. HPV-31, 51 and 52 were frequent in both LSIL and HSIL [13].

HPV-vaccination programs and the use of different vaccines may have an impact of HPV types of prevalence and genotype distribution. HPV-6 and HPV-11 have decreased significantly and HPV-16 has had a slight decrease after 10 years after implementation of the vaccination program in Spain. However, distribution of HPV-31, HPV-52, and HPV-45 have increased in this population [14]. The similar changes have also been observed in France [15].

Therefore, data on type-specific HPV prevalence patterns before implementing HPV-vaccination program should be collected before roll-out of a vaccination program. This information can aid in the selection of HPV vaccine types and formulations that would provide the greatest impact on reducing HPV-related diseases. It may also allow to evaluate HPV prevalence after vaccination and help monitor changes in genotype distribution and evaluate the impact of the vaccination program in future [16].

Objectives

The aim of the study was to assess cervical prevalence and distribution of HPV genotypes in a cohort of gynecological patients from all over the country tested in one of the largest HPV laboratories before the implementation of the population-based HPV immunization program in Poland.

MATERIAL AND METHODS

This was a retrospective cohort study based on cervical cytology material referred to ALAB Laboratoria Sp. z o.o. between 2018–2021. ALAB is a large diagnostic laboratory performing diagnostic tests for both reimbursed and private healthcare sectors from all over the country. The cervical cytology samples were collected by gynecologists and midwives in 556 sites across Poland located all over the country in all sixteen voivodeships in a process of cervical cancer screening, triage, surveillance and follow-up after treatment and other cervical-related procedures. Additionally, the following data was retrieved from ALAB database: date of sampling; age at sampling; voivodeship of women's place of residence; result, including detected genotypes; type of HPV test used. There were several HPV assays used by the provider: Amplisens HPV HCR genotype-titre-FRT PCR Kit and Alinity m HR HPV Assay/Abbot, BD Onclarity, Linear Array HPV Genotyping Test, Roche; Ampliquality HPV-type express v3.0, AB Analitica; INNO-LIPA Genotyping Extra II, Fuji Rebio. Only results of tests with complete genotyping were included in analysis, *i.e.* Linear Array HPV Genotyping Test, Roche; Ampliquality HPV-type express v3.0, AB Analitica; INNO-LIPA Genotyping Extra II, Fuji Rebio (all tests detect genotypes: 6, 11, 16, 26, 18, 31, 33, 35, 39,

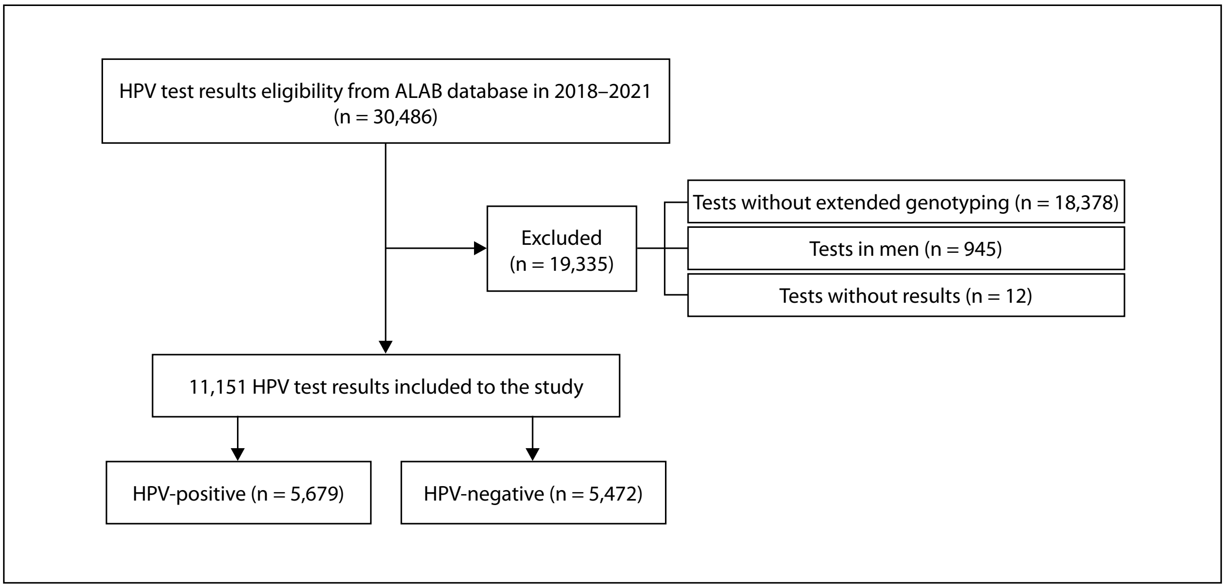

Figure 1. The flowchart of HPV test results enrolled in the study

40, 42, 45, 51–54, 56, 58, 59, 61, 62, 66–68, 70, 73, 81–83, and 89).

Inclusion criteria were: (1) female sex, (2) HPV test performed in ALAB Laboratoria in 2018–2021 and (3) valid result of performed HPV test. Exclusion criteria were: (1) no HPV genotype reported in the database despite positive result of the test and (2) test without complete genotyping performed.

The distribution of specific HPV genotypes was calculated among all infected women as well as among those single-infected only and by the following age groups: less than 30, 30–39, 40–49, 50–59, at least 60 years of age. The Cochran–Armitage test was performed to verify the trends in infections with specific genotypes across age groups. The prevalence of 14 HR genotypes, *i.e.* 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 was estimated in each age group.

Stata 14 software [17] was used to perform analyses and figures were prepared in the R environment [18]. Women with missing data were excluded from analysis.

RESULTS

We retrieved 30.486 HPV test results from ALAB in the studied period. We excluded 18,378 of them as results of tests without extended genotyping (60.3%); subsequent 945 reported in men and 12 without final result (3.1%). The remaining 11,151 results in women with complete genotyping were included in analysis (36.6%) (Fig. 1).

The mean age of the analyzed cohort of women was 34 years. Following HPV test types were performed: Ampliquality/AB Analitica (5.819; 52.2%), INNO-LiPA®/Fujirebio (146; 1.3%) and Linear Array/Roche (5.186; 46.5%). Of 30 HPV genotypes detected by all listed tests, 14 genotypes belonged to HR HPV group (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), and 16 were other than HR HPV (6, 11, 26, 40, 42, 53, 54, 61, 62, 67, 70, 73, 81–83, and 89). Cervical material was sampled in 2018–2021, with most of tests performed in 2019 (47.0%) and 2021 (46.3%), in varying fractions in all 16 voivodeships of Poland (Fig. 2).

HPV-positive women

We identified 5,679 women infected with an HPV positive result (50.9%), including 4,351 cases with HR and 3,066 with non-HR HPV genotypes (39.0% and 27.5%, respectively). HPV-16 was the most common amongst HPV-positive women (1,362; 24.0%). Five following genotypes had similar frequency: HPV-31 (641; 11.3%), HPV-66 (639; 11.3%), HPV-53 (596; 10.5%), HPV-51 (566, 10.0%) and HPV-61 (564; 9.9%) (Fig. 3).

HR HPV-positive women

When restricting analysis to the sample of 4.351 HR HPV-positive women only, the most common genotype was also HPV-16 (31.3%), followed by HPV-31 (14.7%), HPV-66 (14.7%), HPV-51 (13.0%) and HPV-56 (11.0%) (Fig. 4). In each age group the most common genotype was HPV-16, and in second and third places HPV-31 and HPV-66 on alternating combinations (Fig. 5).

Single infections & distribution of HPV types by age

In single-infections, constituting 69.0% of all HR HPV-positive cases (3.001/4,351), listed HR genotypes were the most common as well (20.8%, 7.9%, 7.0% for HPV-16, HPV-31 and HPV-66, respectively).

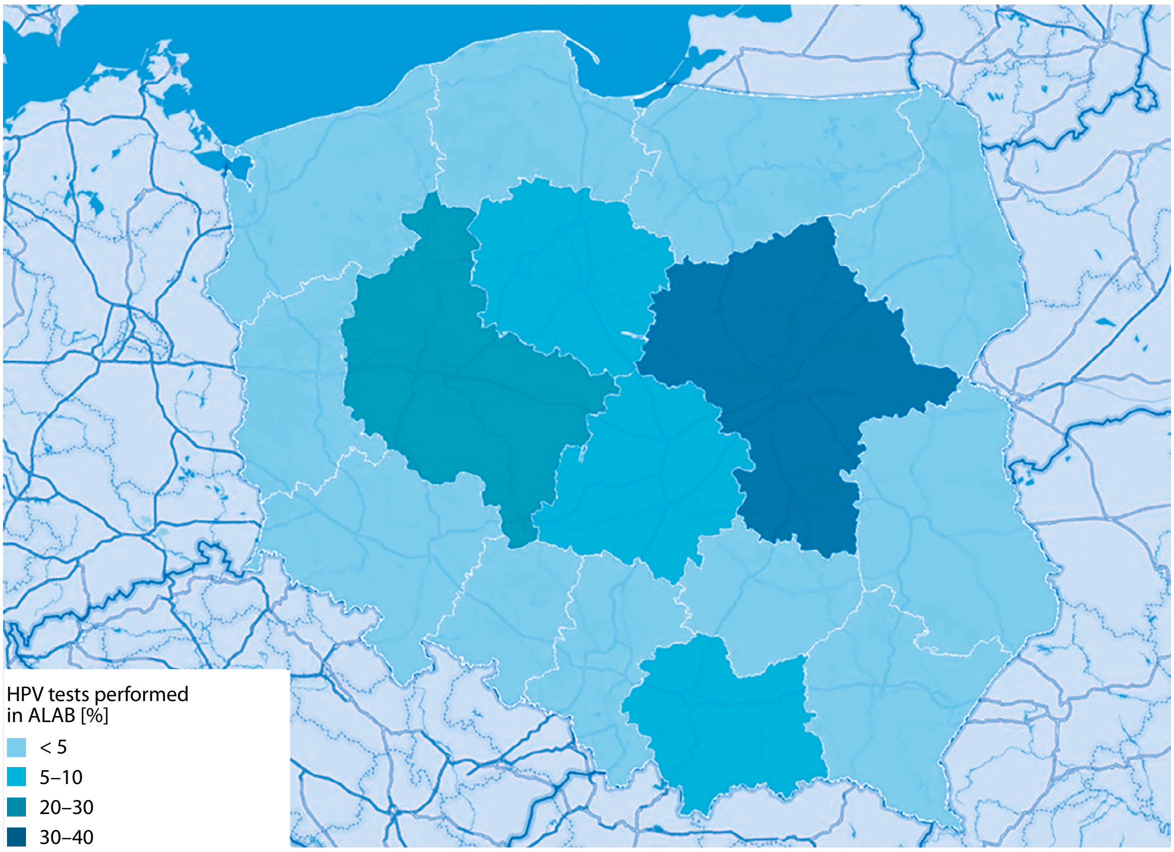

Figure 2. Distribution of HPV tests performed in ALAB Laboratoria Sp. z o.o. in 2018–2021 by voivodeships in Poland

Comparing HR HPV genotypes prevalence in age groups of all HR HPV-positive women, some fluctuations were visible, including significant trends for HPV-16, HPV-18, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56 and HPV-59 (p for trend: < 0.001, 0.015, < 0.001, 0.044, < 0.001, 0.010, 0.012 and 0.015, respectively) (Fig. 5).

DISCUSSION

The prevalence of genital HPV genotypes in women is diverse across regions of the world and depends on many factors such as age, sexual activity, number of past and current sexual partners, HPV prevalence and type-distribution in men and absence/presence of cervical disease. Recent data indicate impact of fully implemented HPV-vaccination programs both on genital HPV prevalence and genotype distribution [19–21]. Many countries in the world have already introduced or are in the process of introduction of prophylactic HPV population-based vaccination. Currently available vaccines cover from 2 to 9-HPV genotypes and their use may impact both HPV prevalence and genotype distribution in the vaccinated cohorts dramatically [14, 15, 22]. This study was undertaken to generate baseline data on the distribution of HPV genotypes in a cohort of women before implementation of population-based HPV-vaccination program in Poland. To our knowledge, this is largest study covering over 30 thousand of HPV test results performed in 2018–2021 in women from all over Poland. Among 11,151 women qualified for the study, we identified 5.679 patients with HPV infection (50.9%), including 4,351 (39.0%) with HR genotypes. As expected, the most frequent HPV genotype in our study cohort was 16. It was most prevalent in all age groups, especially in young women (< 30 years, 26.7%). The two following genotypes in terms of frequency were: HPV-31 and HPV-66 in both groups (all type HPV/HR HPV). Among the HR HPV-positive women, the prevalence of 16 and/or 18 was 37.3%, while the prevalence of any vaccine HR genotype (16, 18, 31, 33, 45, 52, 58) was 69.5%.

In a meta-analysis by Bruni et al. [23] covering 194 studies published between 1995–2009 based on analysis of 1.016,719 women with normal cytological findings, the dominant types were oncogenic, namely, HPV-16, 18, 52, 31, 58, 39, 51, and 56. The authors indicated that HPV-31 was especially frequent in Europe and HPV-52 in Northern America, Africa, and Asia. HPV-18 usually was second after HPV-16 in the pooled analyses. Our results are partially in line with

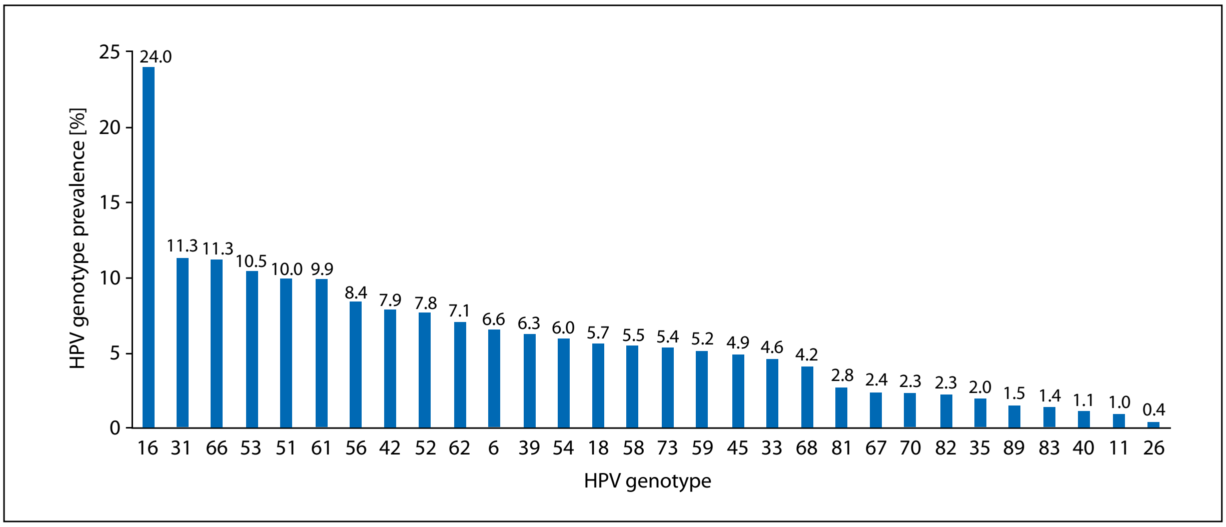

Figure 3. HPV genotypes prevalence in the study cohort

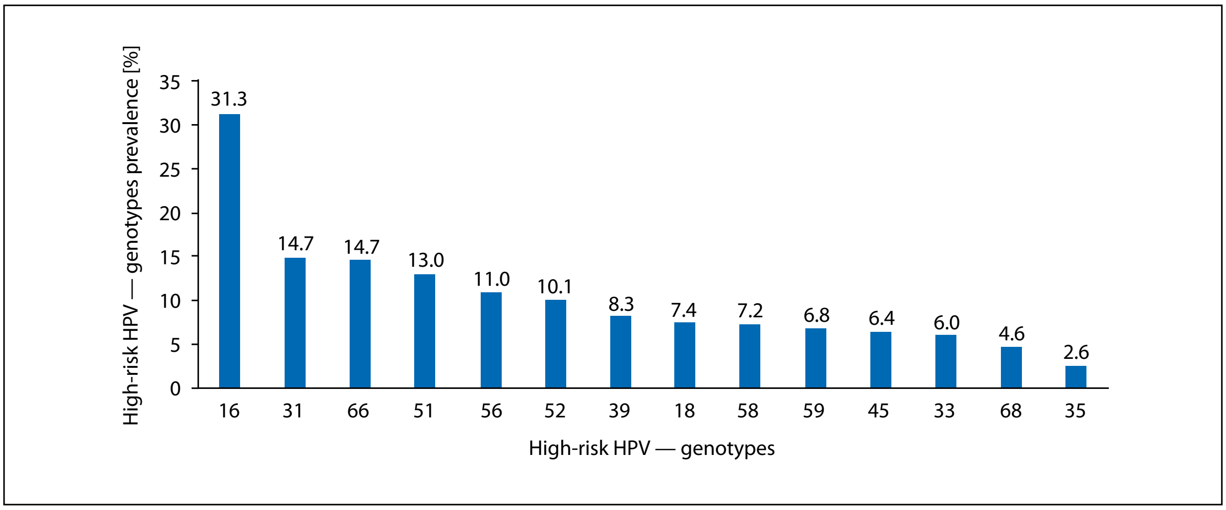

Figure 4. HR-HPV genotypes prevalence in the study cohort, restricted to HR HPV positive women

these observations. In our study, HR types (HPV-16 — 24%, 31 — 11.3%), 66 (11.3%), 51 (10%) were the most frequent genotypes but non-HR types (HPV-53 — 10.5%), 61 (9.9%) were also common. In the cited meta-analysis, HPV-6 was the most frequent low risk type in both Latin and Northern America, but neither in Asia nor in Africa, where HPV-83, 72, 70, and 51 occurred more frequently. In our analysis, HPV-6 was not among the top 3 of low-risk genotype and it was less common than types 53, 61, 42.

In another meta-analysis, de Sanjosé et al. [24] analyzed distribution of HPV genotypes in women with normal cytology. The study covered the period of 1995–2005 and included 78 studies on 157,879 women. Five most common HPV types in women worldwide were HPV-16, 18, 31, 58, and 52, representing 50% of all HPV infections. The most common type was HPV-16, followed by HPV-18. However, authors reported variations in types by regions. In Japan, Taiwan and Eastern Africa, HPV-52 was the most common. HPV-18 was one of the most frequent HPV types in all regions apart from Southern Europe, and HPV-53 was among the five most common HPV types detected in Eastern Africa and Central and Northern America. Poljak et al. [25] presented data about the HPV genotypes type distribution in sixteen Central and Eastern European countries (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Montenegro, Poland, Romania, Serbia, Slovakia, Slovenia and the Former Yugoslav Republic of Macedonia). The analysis covered 8,610 women with normal cervical cytology and showed that HPV-16 was the most common genotype. This result confirmed data presented in previous study on 4,431 Slovenian women aged 20–64 years, in which, irrespective of Pap test results, the most prevalent

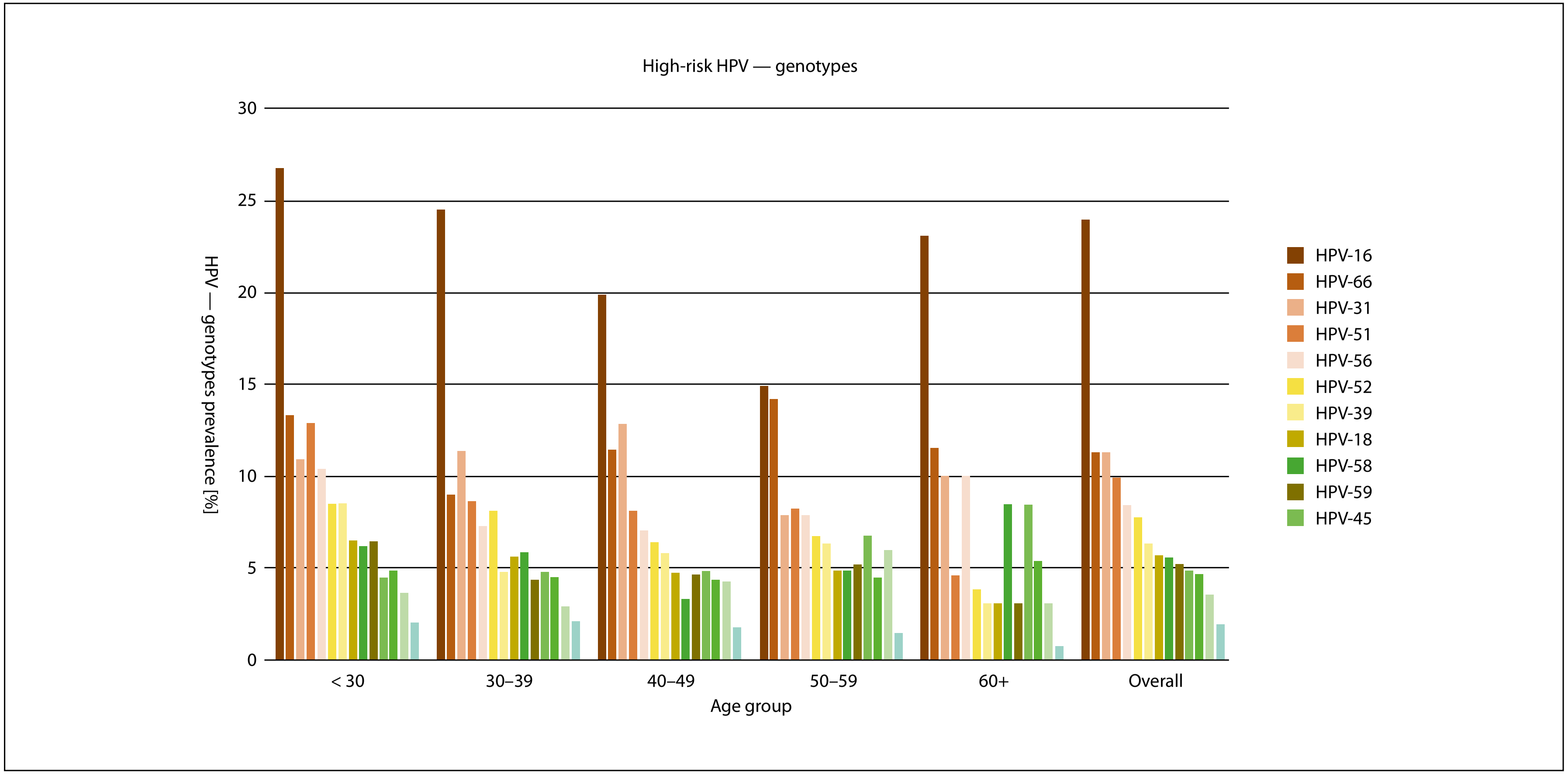

Figure 5. HR-HPV genotypes prevalence in study cohort restricted to HR HPV-positive women by age group

HPV HR genotype was HPV-16, with subsequent HPV-31, 51, 39, and 59 [26]. In a large population-based study in Denmark analyzing HPV type distribution in 11,617 women; 94.0% had normal cytology. HPV prevalence increased from 19.2% in women with normal cytology to 100% in women with CIN3+. The HPV prevalence was 26.4% with a peak in women 20–24 years (50.2%) [27]. The most frequent HPV type detected in all women was HPV-16, less common were HPV-31, 52 and 51, followed by 68, 18, 39, 53, 66, 33, 45, 56 and 6.

Population-based studies on the prevalence of HPV in Poland remain limited. A study on 834 women aged 18–59 years from the general population was carried out in 2008 by IARC collaboration with The Maria Sklodowska--Curie Memorial Cancer Centre and Institute of Oncology, Warsaw, Poland. The HPV prevalence was 16.6% and the most common type was HPV-16 (3.7% of study population, *i.e.* 22.3% of HPV-positive women) followed by HPV-56, 45, 31, and 52. HPV-16 and 18 together accounted for 23% of HPV infections in women with normal cytology. The highest HPV prevalence was detected in women of 25–34 years old (24.2%), especially among singles (37.3%). In total, 97 women had single and 41 had mixed infections (10.8% and 4.6%, respectively) [28].

The studies by Przybylski et. al. [29, 30] reflected the genotype distribution in patients with abnormal pap smear results. In the first study, material was obtained from 674 women who registered to Specialist Medical Practice in the years 2008–2020. 53% of patients tested positive for HPV. The positivity rate decreased with age. The following HPV types were the most common: HPV-16 (24.5%), HPV--53 (13.1%), HPV-31 (10.3%), HPV-51 (9.7%), HPV-56 (9.5%). The second cited study assessed the HR HPV prevalence and distribution in women undergoing screening for cervical cancer in the Wielkopolska region of Poland. HPV genotype distribution was assessed among women with available histological results of cervical tissue. HPV genotypes 16, 31, 52, 66, 53, and 51 were the most frequent types in the studied population. Genotypes 16 and 31 accounted for nearly one-fifth of HPV positive results. HPV- 16, 31, and 52 were found in nearly 80% of HSIL CIN 2 and CIN 3 lesions.

In our study single-type infections constituted 51,6% among all HPV-infected women and 69.0% among women infected with HR HPV. In material analyzed by Bruni et al. [23] approximately 3.2% of women tested were found to have infections with multiple HPV types. Multiple infections were more prevalent than single HPV infections in the Danish analysis [27]. In the mentioned Polish population study, in total, 10.8% women had single-type and 4.6% had multiple--type HPV infections [28]. It is difficult to compare directly our results to previous Polish and international data due to different study design and populations. Our cohort might have included women with very diverse indications for HPV testing depending on clinical practice in sites all over the country. Also, we assume that study cohort's health awareness and socioeconomic status might have been higher compared to the average Polish female population since private-based HPV testing is quite costly in Poland and reimbursed HPV-testing is not readily available. Additionally, the sampling sites were mainly placed in cities and the population of rural regions may be underrepresented in our cohort. Therefore, the distribution of HPV genotypes in the study cohort might not be fully representative for the whole Polish female population. Our study does not allow for a direct analysis of the potential impact of different vaccine preparations on the development of precancerous lesions associated with specific genotypes. However, it can be hypothetically estimated that the percentage of women requiring diagnostic work-up due to HPV-positive test results would be lower by 71.4%, 37.3%, and 29.7%, respectively, if the 9-valent, 4-valent, and 2-valent vaccines was used in this cohort. Strengths of the present study include a very large sample size and the use of a HPV complete genotyping assays allowing comparisons with similar studies around the world. The population included in study covered — albeit unevenly — all regions of Poland.

CONCLUSIONS

In our study on over 30 thousand of HPV test results performed in 2018–2021 by one of the largest medical laboratories in Poland, HPV-16 was the most prevalent genotype, followed by HPV-31 and HPV-66 in cervical cytology material of a large cohort of women tested for many clinical indications by gynecologists and midwives in over 500 sites all over Poland. Although the study cohort might not be fully representative for a total population of Polish women, it enables investigation of the impact of routine teenage HPV vaccination on HPV genotype distribution using the same cohort selection and methodology in the future. This data also allows for analyses of the potential impact of different HPV vaccine formulations and potential reductions in type-specific HPV infections in a similar cohort of gynecological patients if they had been vaccinated as teenagers.

Article information and declarations

Data availability statement

The data presented in this study are available on reasonable request from the corresponding author.

Author contributions

The authors contribution has been mentioned in the attached and signed statement for the Editors concerning the manuscript.

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Bioethics Committee of National Research Institute of Oncology (protocol code 82/2022, date of approval: 17.11.2022). The data was anonymized according to the protection of personal data law. For that reason, obtaining informed consent from study participants was not possible.

Funding

The sponsor of the study was MSD Polska. This study has been executed in collaboration with Polish Society of Gynecological Oncology based on the data provided by ALAB Laboratoria.

Conflict of interest

A. Nowakowski reports honoraria for lectures from GSK and for lecturers, educational activities and support for participation in scientific meetings by MSD. R Jaworski is an employee of MSD Polska and took part in designing the research and creating the study protocol. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or material discussed in the manuscript apart from those disclosed.

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