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RESEARCH ARTICLE

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Impact of high-risk Human Papillomavirus genotyping in cervical disease in the Northern region of Portugal: Real-world data from regional cervical cancer screening program

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Abstract

Cervical cancer prevention is based on primary prevention with vaccines against Human Papillomavirus (HPV) and secondary prevention by screening with High-Risk-HPV (Hr-HPV) detection. Since 2017, cervical cancer screening in women aged 25–60 years has been performed in Portugal using Hr-HPV detection, followed by cytology in Hr-HPV-positive cases. Herein we report the prevalence of Hr-HPV genotypes and cytological abnormalities among 462 401 women (mean age: 43.73 ± 10.79; median age: 45; range: 24–66 years) that participated in the Regional Cervical Cancer Screening Program of the Northern Region of Portugal, performed between August 2016 and December 2021. Overall, we describe a prevalence rate of 12.50% for Hr-HPV varying from 20.76% at age 25% to 8.32% at age 64. The five most common Hr-HPV genotypes identified were HPV-68 (16.09%), HPV-31 (15.30%), HPV-51 (12.96%), HPV-16 (11.06%), and HPV-39 (11.01%). The prevalence of Hr-HPV included in the nonavalent vaccine (HPV-9valent) was 55.00% ranging from 47.78% to 59.18% across different age groups. Considering positive Hr-HPV cases, 65.68% had a Negative for Intraepithelial Lesion or Malignancy (NILM) cytology, 20.83% atypical squamous cells of undetermined significance (ASC-US), 8.85% Low-Grade Squamous Intraepithelial Lesion (LSIL), 1.65% High-Grade Squamous Intraepithelial Lesion (HSIL), 2.85% ASC-H, 0.09% Atypical Glandular Cells, 0.02% Adenocarcinomas, and 0.02% Squamous Cell Carcinoma (SCC). Our analysis revealed that HPV-9val genotypes were responsible for 52.13% NILM, 59.21% ASC-US, 55.06% LSIL, 90.14% HSIL, 83.50% ASC-H, and 100.00% SCC. Furthermore, multiple Hr-HPV infections (risk ratio [RR] = 1.46; 95% confidence interval [CI] 1.34–1.58), HPV-16/18 (RR = 5.16; 95% CI 4.75–5.93), or HPV-9val genotypes (RR = 5.23; 95% CI 4.68–5.85) were associated with a

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significant risk of developing > HSIL ($p < 0.001$). To date, this is the largest study on Hr-HPV genotyping in cervical cancer screening that includes data from a complete cycle of the screening program. Our findings suggest a high prevalence of HPV-9valent genotypes and a significant association with an increased risk of developing > HSIL. This constitutes important data for health authorities, which may help define the future of vaccination and cervical cancer screening strategies.

KEYWORDS
cervical cancer, genotyping, Human Papillomavirus, prevalence, screening, vaccine

1 | INTRODUCTION

Worldwide, cervical Squamous Cell Carcinoma (SCC) is one of the most incident cancers in women, being responsible for 604 127 new cases and 341 381 related deaths in 2020, according to Globocan.[1] In Portugal, in 2020 it was estimated a total of 865 new cases and 379 deaths, with age-standardized incidence and mortality rates of 10.7 and 3.7 per 100 000 women, respectively.[1]

Since the 1970s, Human Papillomavirus (HPV) was identified as the etiological factor of most SCC, and since 2005, The International Agency for Research on Cancer (IARC) acknowledged 14 different HPVs as carcinogens, which are now designated as High-Risk HPVs (Hr-HPVs).[2–5] It is well known that persistent Hr-HPVs infection is associated with the development of high-grade cervical lesions that may progress to invasive cancer.[5,6] Despite there are over 150 different HPV genotypes,[3] only 14 Hr-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are considered of carcinogenic potential, with HPV-16 and HPV-18 being responsible for the majority of cervical cancer cases worldwide, and collectively with HPV-31, −33, −45, −52, and −58 representing over 90.0% of all cases.[6–10] The identification of the different Hr-HPV genotypes may be valuable for the development of future HPV vaccines or cervical cancer prevention strategies.[11–13]

Cervical cancer is a preventable cancer since there are both primary prevention measures, through the implementation of vaccines against HPV, and secondary prevention by cervical cancer screening strategies.[6,14–16] In the last 10, years many countries have changed their cervical cancer screening programs based on evidence that Hr-HPV testing was more sensitive.[17–22] In Portugal, cervical cancer screening was introduced back in 1978 as an opportunistic strategy and in 1990 it started to be organized in the Center region of the country.[23] Later, the cervical cancer screening program was progressively implemented in Alentejo, Algarve, Azores, and the Northern region of Portugal. In the later, organized screening started in 2009 and progressively extended to the whole region, using liquid-based cytology (LBC) and reflex HPV testing in cases of atypical squamous cells of undetermined significance (ASC-US). In 2016, a pilot study was implemented in the North Region of Portugal, using genotyping of all 14 Hr-HPV as the primary method for cervical cancer screening.[24]

In this study, we report the extended results of the Hr-HPV genotyping and cytological analysis of the Regional Cervical Cancer Screening Program in the Northern Region of Portugal and discuss potential impacts on cervical cancer prevention strategies.

2 | MATERIAL AND METHODS

2.1 | Study population

In the Regional Cervical Cancer Screening Program of the North Region of Portugal, LBC samples are collected in ThinPrep™ Pap Test vials containing PreservCyt Solution™ (HOLOGIC™ Inc.) from all healthy women aged 25–60 years old (with extension up to 64 years old, for women without negative cytology in the last 3 years) at 5-year intervals. Women are excluded from the program if had a previous history of cervical cancer or hysterectomy. According to the screening protocol, Hr-HPV detection is followed by cytological triage for the detection of cell abnormalities in positive cases.

Between August 1st 2016 and December 31st 2021, a total of 462 401 women (mean age 43.73 ± 10.79 years old; median age: 45; range 24–66 years) were enrolled. All samples were obtained during the routine enrollment in the Regional Cervical Cancer Screening Program. The age and geographical distribution of women are depicted in Table 1. The study was approved by the Institutional Ethical Committee (Comissão de Ética para a Saúde) of IPO Porto (ref. CES-IPO:146/022).

2.2 | HPV genotyping

Sample processing was performed in an automated workflow using STARlet IVD, an automated system for nucleic acid (NA) isolation (Seegene®); CFX96™ Dx Real-time PCR System, a Real-Time Polymerase Chain Reaction (PCR) system (Bio-Rad Laboratories, Inc.); and Seegene Viewer™, a data analysis software (Seegene®). Hr-HPV genotyping was performed according to the manufacturer's instructions with *Anyplex™ II HPV HR Detection* (Seegene®). This kit was already validated for use in cervical cancer screening,[25] allowing for simultaneous detection and genotyping of 14 h-HPV subtypes, including HPV-16, −18, −31, −33, −35, −39, −45, −51, −52, −56, −58, −59, −66, and −68, plus an internal control (human beta-globin) in a single reaction. All reactions include positive and negative controls provided in the kit.

TABLE 1 Characterization of cervical cancer screening population (age groups, year, and geographical location)

	Age group	25	30	35	40	45	50	55	60	64
Total, *n*	**462 401**	**40 501**	**47 276**	**54 110**	**64 519**	**69 447**	**67 088**	**62 805**	**51 428**	**5227**
Year										
2016, *n*	16 442	1145	1496	1970	2382	2574	2659	2190	1823	203
2017, *n*	88 280	7034	8856	10 671	13 138	13 574	13 011	11 580	9255	1161
2018, *n*	101 010	8044	10 413	12 224	14 290	15 096	14 848	13 654	11 219	1222
2019, *n*	94 666	8726	9687	11 121	13 205	14 295	13 445	12 846	10 543	798
2020, *n*	55 827	5714	6201	6548	7518	8087	7497	7313	6413	536
2021, *n*	106 176	9838	10 623	11 576	13 986	15 821	15 628	15 222	12 175	1307
Geographical location										
Aveiro, *n*	43 093	3860	4008	4872	5650	6541	6468	6231	5099	364
Braga, *n*	135 013	12 286	13 973	16 093	19 606	20 830	19 416	17 535	13 957	1317
Bragança, *n*	11 858	880	1161	1327	1583	1604	1656	1756	1808	83
Porto, *n*	202 507	17 504	21 368	23 586	27 985	30 387	29 617	27 332	21 777	2951
Viana do Castelo, *n*	33 278	2975	3309	4153	4793	4965	4638	4375	3830	240
Vila Real, *n*	22 975	1821	2159	2548	3158	3213	3289	3429	3202	156
Viseu, *n*	13 629	1170	1291	1527	1740	1899	1996	2140	1750	116

Abbreviation: *n*, number.

2.3 | Cytological evaluation

Hr-HPV positive samples identified are then separated and processed for cytopathological observation, using the ThinPrep™ 5000 Processor (HOLOGIC®, Inc.) and processed in the ThinPrep™ Imaging System (HOLOGIC®, Inc) for cytopathological examination. Samples are classified by dedicated cytotechnicians and cytopathologists according to the Bethesda Classification with the following terminology: Negative for Intraepithelial Lesion or Malignancy (NILM); ASC-US; Atypical Squamous Cells cannot exclude HSIL (ASC-H); Low-Grade Squamous Intraepithelial Lesion (LSIL); High-Grade Squamous Intraepithelial Lesion (HSIL); SCC; Atypical Glandular Cells (AGC) and Adenocarcinomas (AdC).[26]

2.4 | Statistical analysis

Descriptive data (frequencies/prevalence) and table and figure preparation were performed with Microsoft Excel for Mac, Version 16.65 (Microsoft); and the statistical analysis was carried out using the computer software IBM SPSS Statistics for Mac, Version 27.0 (IBM). Patterns of Hr-HPV infection in the population were depicted and correlated with the cytopathological information. The overall Hr-HPVs prevalence data were described by frequencies or percentages. The χ^2 test was used to assess the association between groups and different categorical variables to compute the risk ratio (RR) and respective 95% confidence intervals (CI) using a statistical significance level of 5% ($p < 0.05$).

3 | RESULTS

3.1 | HPV prevalence and genotyping

From the 462 401 cases included in the study, 57 796 (12.50%) were Hr-HPV positive, 32 cases were inconclusive (0.01%), and 358 (0.08%) were considered insufficient for Hr-HPV detection—Table 2. Hr-HPV infection varied according to age, ranging from 20.76% at age 25%–8.32% at age 64. Regarding geographical location, Hr-HPV positivity frequency did not differ significantly among regions, ranging from 10.55% to 13.23%—Table 2. Simultaneous infections by two or more Hr-HPVs genotypes (multiple infections) were detected in 15 687 (26.96%) women, ranging from 18.39% at age 64% to 32.43% at age 25 with no significant variation in geographical origin (range 24.76% –28.03%)—Table 2.

The five most common Hr-HPV genotypes found in our population were HPV-68 (16.09%), HPV-31 (15.30%), HPV-51 (12.96%), HPV-16 (11.06%) and HPV-39 (11.01%)—Figure 1, Supporting Information: Table I. The analysis of Hr-HPV distribution according to age revealed that HPV-31 predominated in women 30–40 years whereas HPV-68 prevailed in women 45–64 years (Figure 2A) and no significant variation was observed according to the different geographic locations (Figure 2B).

Overall, HPV-16 and 18 were detected in 8,321 (14.30%) women, ranging from 3.91% at age 25% to 17.50% at age 35; without a significant variation regarding geographical location (range 12.07%–14.97%)—Supporting Information: Table I. Hr-HPVs

TABLE 2 Hr-HPV prevalence according to age groups and geographical location

	Total		HPV positive		HPV multiple infections		HPV-16/−18		HPV-9val	
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
	462 401	100.00	57 796	12.50	15 687	26.96	8321	14.30	32 002	55.00
Age group										
25	40 501	8.76	8408	20.76	2727	32.43	330	3.92	4031	47.94
30	47 276	10.22	8889	18.80	2591	29.15	1385	15.58	5112	57.51
35	54 110	11.70	8009	14.80	2176	27.17	1408	17.58	4762	59.46
40	64 519	13.95	8070	12.51	2029	25.14	1324	16.41	4542	56.28
45	69 447	15.02	7745	11.15	1983	25.60	1350	17.43	4389	56.67
50	67 088	14.51	6444	9.61	1581	24.53	962	14.93	3503	54.36
55	62 805	13.58	5472	8.71	1442	26.35	838	15.31	3027	55.32
60	51 428	11.12	4324	8.41	1079	24.95	674	15.59	2410	55.74
64	5227	9.32	435	8.32	80	18.39	50	11.49	226	51.95
Geographical location										
Aveiro	43 093	9.32	5258	12.20	1474	28.03	764	14.53	2935	55.82
Braga	135 013	29.20	15 954	11.82	4.289	26.88	2320	14.54	8868	55.58
Bragança	11 858	2.56	1489	12.56	397	26.66	197	13.23	825	55.41
Porto	202 507	43.79	26 786	13.23	7.435	27.76	3928	14.66	14 832	55.37
Viana do Castelo	33 278	7.20	4126	12.40	1.054	25.55	562	13.62	2274	55.11
Vila Real	22 975	4.97	2729	11.88	677	24.81	333	12.20	1480	54.23
Viseu	13 629	2.95	1438	10.55	356	24.76	217	15.09	776	53.96

Abbreviation: *n*, number.

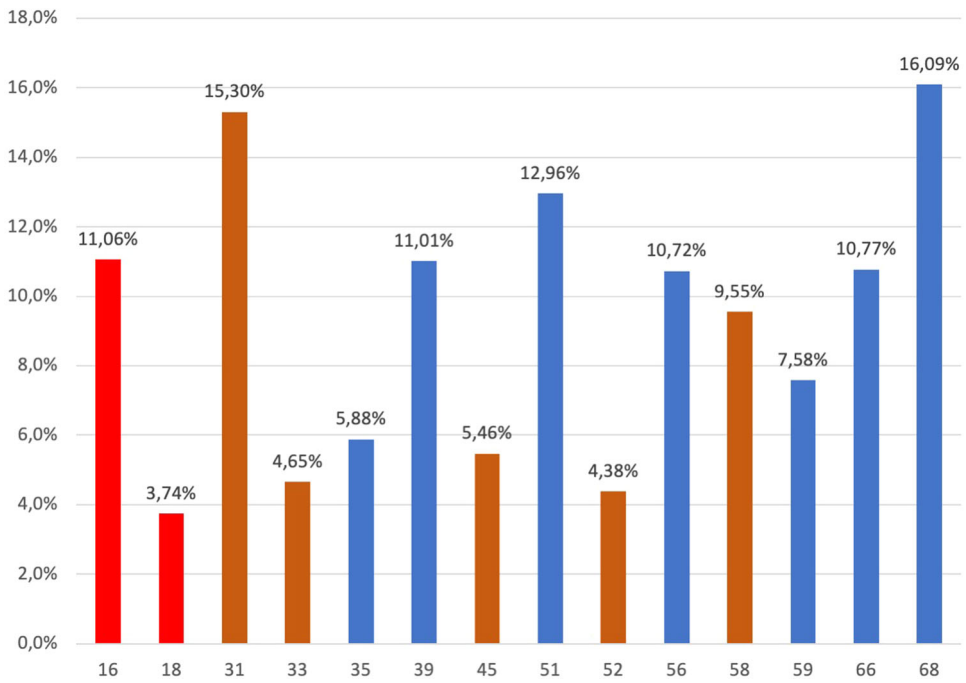

FIGURE 1 High-Risk HPV genotype prevalence in the population. HPV, Human Papillomavirus.

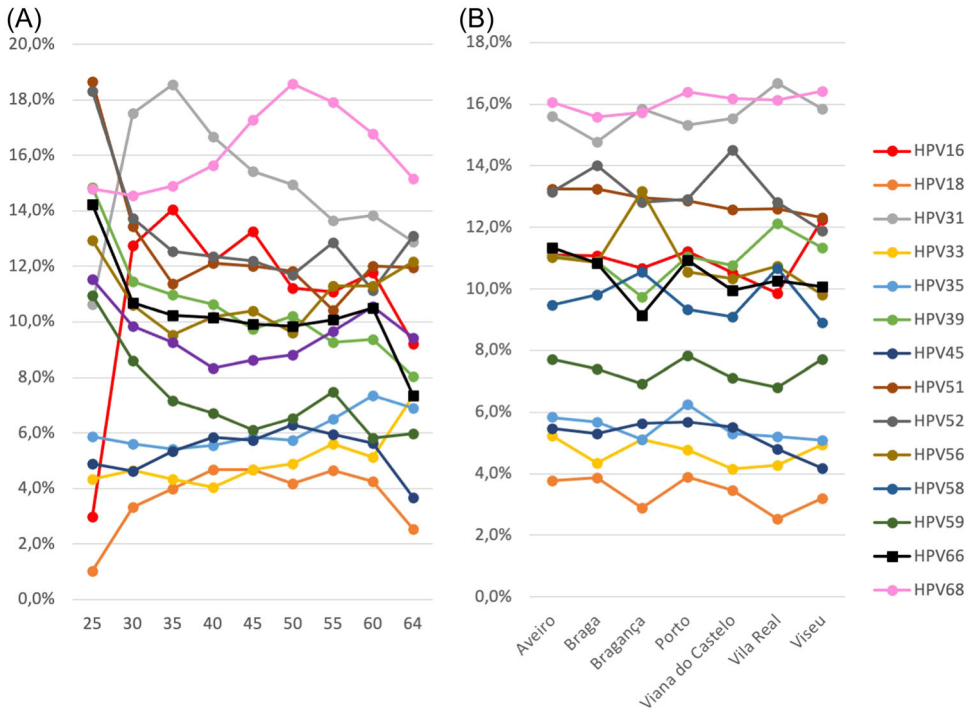

FIGURE 2 HPV genotype distribution according to age (A) and according to geographic location (B). HPV, Human Papillomavirus.

genotypes included in the 9-valent vaccine (16, 18, 31, 33, 45, 52, and 58) were detected in 32 002 (55.00%) Hr-HPV positive women, ranging from 47.78% to 59.18% across age groups, with no significant variation observed in terms of geographical location (range 53.52%–55.27%)—Supporting Information: Table I.

3.2 | Cytological distribution and HPV genotyping

Cervical cytology results were available for 57 100 h-HPV positive cases of which 37 506 (65.68%) were NILM, 11 893 (20.83%) ASC-US, 5056 (8.85%) LSIL, 943 (1.65%) HSIL, 1,630 (2.85%) ASC-H, 53 (0.09%) AGC, 14 (0.02%) AdC, and 10 (0.02%) SCC—detailed data in Supporting Information: Table II.

Taking age into consideration, frequencies of NILM ranged from 62.78% at 35% to 77.47% at 64 years old; while ASC-US ranged from 17.47% at 64% to 22.08% at 40 years old; LSIL ranged from 0.92% at 64% to 11.27% at 25 years old; HSIL ranged from 0.70% at 25% to 2.40% at 35 years old; and ASC-H ranged from 1.50% at 25% to 4.05% at 35 years old. SCC cases were detected at 40 ($n = 2$), 45 ($n = 1$), 50 ($n = 2$), 55 ($n = 2$), 60 ($n = 2$), and 64 ($n = 1$) years of age, whereas AdC were at 30 ($n = 2$), 35 ($n = 7$), 40 ($n = 3$), 45 ($n = 1$), and 55 ($n = 1$); and AGC at 25 ($n = 1$), 30 ($n = 7$), 35 ($n = 10$), 40 ($n = 10$), 45 ($n = 11$), 50 ($n = 3$), 55 ($n = 6$), and 60 ($n = 3$)—Figure 3, Supporting Information: Table II.

The analysis of the prevalence of the different genotypes in NILM versus all cervical abnormalities showed changes in the prevalence of HR-HPVs with the exception of HPV-45 and HPV-68. Indeed, HPV-68, the most prevalent in our population, was revealed to be more prevalent in NILM than any other cytological abnormality (17.1% vs. 14.0, respectively)—Figure 4 (Supporting Information: Table II).

Despite small changes, the pattern of Hr-HPV genotypes was similar for NILM, ASC-US, and LSIL, while it showed significant differences for all other cytological abnormalities: HPV-68 was more frequent amongst NILM (17.25%) and ASC-US (15.61%); HPV-31 was frequent in all groups, especially in ASC-H (24.66%); HPV-16 was significantly associated with HSIL (48.04%), ASC-H (33.44%) and SCC (90.0%); and HPV-18 was overrepresented in AGC (22.64%) and AdC (46.15%). The results showed that all SCCs were associated with HPV-16 (90%) and HPV-31 (10%). Furthermore, multiple Hr-HPV infections were responsible for 22.44% of NILM, 33.36% of ASC-US, 44.52% of LSIL, 35.10% of HSIL, 34.85% of ASC-H, 22.64% of AGC, and 46.15% of AdC; HPV-16/18 infections were responsible for 11.40% of NILM, 16.68% of ASC-US, 15.72% of LSIL, 51.96% of HSIL, 38.04% of ASC-H, 90.00% of SCC, 43.40% of AGC, and 76.92% of AdC; and Hr-HPVs included in the HPV-9val vaccine accounted for 52.13% of NILM, 59.21% of ASC-US, 55.06% of LSIL, 90.14% of HSIL, 83.50% of ASC-H, 100.00% of SCC, 83.02% of AGC, and 84.62% of AdC—Figure 5 (Supporting Information: Table II).

3.3 | Risk analysis

Based on the prevalence of the different HR-HPV genotypes, we calculated the relative risk of the development of cytological abnormalities (detailed data in Supporting Information: III).

The risk of development of any cytological abnormality when compared to NILM, depending on the Hr-HPV genotype, revealed

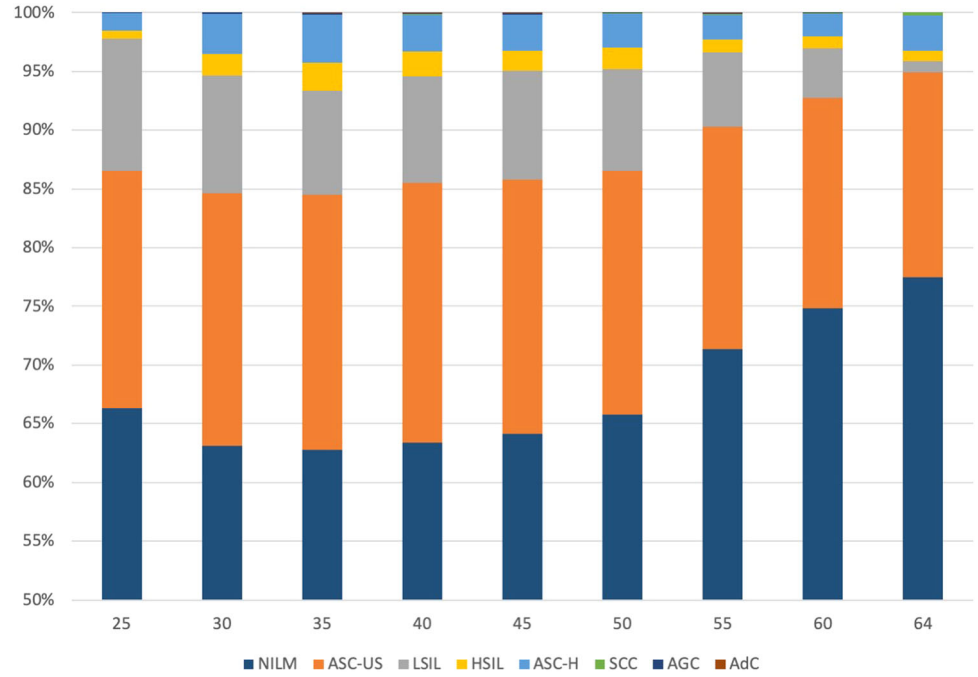

FIGURE 3 Cytology evaluation distribution according to age

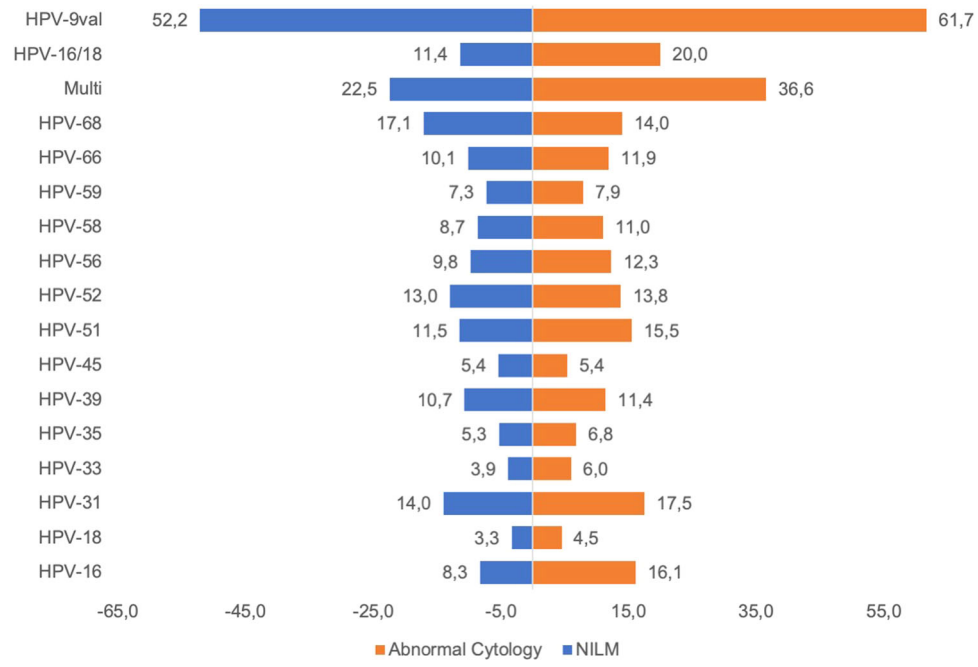

FIGURE 4 Hr-HPVs genotypes prevalence (%) and presence of cytological abnormality. Hr-HPVs, High-Risk-Human Papillomavirus.

statistically significant differences ($p < 0.001$) for multiple Hr-HPV infections (RR = 1.95; 95% CI 1.88–2.02), HPV-16/18 (RR = 1.92; 95% CI 1.84–2.02) and HPV-9val (RR = 1.46; 95% CI 1.41–1.58)—Figure 6A. Moreover, most of the Hr-HPV genotypes were also associated with an increased risk of development of cervical abnormalities, with HPV-16 having a higher risk (RR = 2.06; 95% CI 1.96–2.17). On the opposite side, we found that HPV-68 was associated with a reduced risk ($p < 0.001$; RR = 0.79; 95% CI 0.75–0.83)—Figure 6A.

The risk of development of cervical lesions ≥ HSIL (includes HSIL, ASC-H, and SCC) revealed statistically significant differences ($p < 0.001$) for multiple Hr-HPV infections (RR = 1.46; 95% CI 1.34–1.58), HPV-16/18 (RR = 5.16; 95% CI 4.75–5.93) and HPV-9val (RR = 5.23; 95% CI 4.68–5.85)—Figure 6B. The analysis also

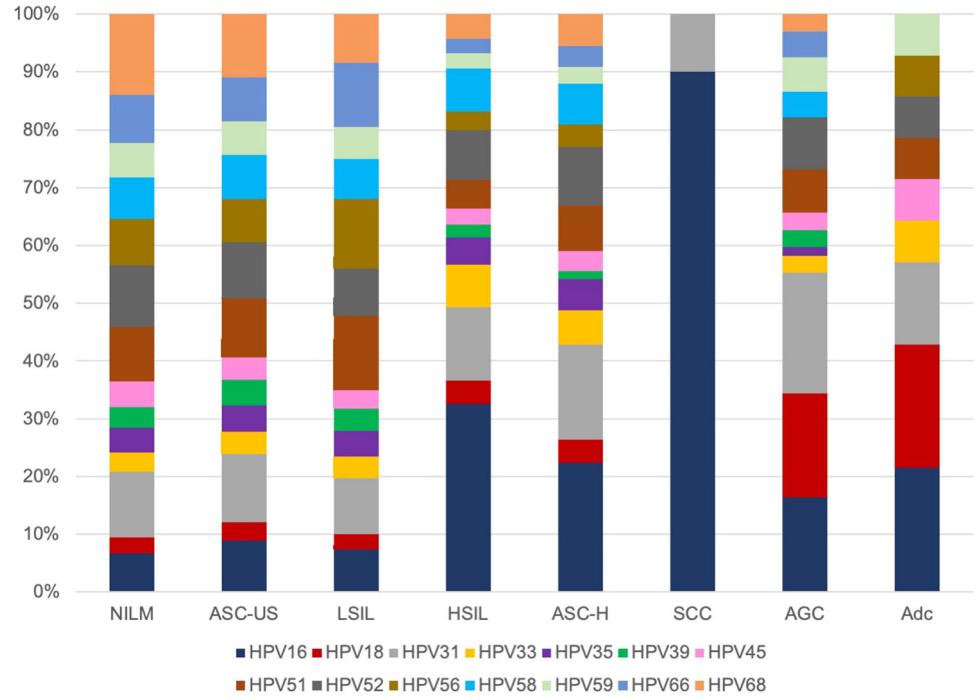

FIGURE 5 HPV genotype distribution according to cytology. HPV, Human Papillomavirus.

revealed that in addition to HPV-16 (RR = 5.96), only six other Hr-HPVs were associated with increased risk, namely HPV-33 (RR = 2.29), HPV-18 (RR = 1.77), HPV-31 (RR = 1.66), HPV-35 (RR = 1.34), HPV-58 (RR = 1.14) and HPV-52 (RR = 1.07)—Figure 6B.

4 | DISCUSSION

Hr-HPV infection is the leading cause of cervical cancer, particularly HPV-16 and—18 which account for nearly 70% of all cervical cancers.[27,28] The current strategy for cervical cancer secondary prevention is based on screening using frontline Hr-HPV testing (higher sensitivity) followed by a triage of Hr-HPV positive results through cytology (higher specificity).[20–22,29–31] Literature shows that Hr-HPV DNA testing is more sensitive for identifying women with CIN 2+, compared with cytology, regardless of having a lower specificity.[16,32–34] Indeed, previous studies show that, in women aged 30–69 years, the sensitivity of the Hr-HPV test is approximately 95.0%, in contrast with 55.0% for cytology.[30]

Portugal holds a high incidence of cervical cancer compared to most European countries.[35] In 2008, the Portuguese government initiated a vaccination program and more effective screening strategies, and since September 2017, cervical cancer screening was recommended to be performed using the Hr-HPV test as the primary screening method in a 5-year interval period. Although there are numerous methods for HPV testing, only a few have been clinically validated for use in a screening context.[17] Furthermore, broad Hr-HPV genotyping has been employed more frequently because it provides valuable epidemiological data.[34,36–39] In this study, we assessed the prevalence of Hr-HPV genotypes detected in samples from participants in cervical cancer screening from the Northern Region of Portugal over 5 years (a complete screening round) and correlated these data with cytological findings in Hr-HPV positive cases.

Overall, a total of 462 401 individual samples were analyzed, making this, to the best of our knowledge, the largest study on Hr-HPV genotyping reported to date. We found an overall prevalence of Hr-HPV of 12.5% which is slightly higher than the previously reported data from our population,[24] but significantly dissimilar from the values reported in the CLEOPATRE study (19.4%) [40] or in the Catalan Institute of Oncology (ICO)/IARC Information Center on HPV and Cancer for Portugal.[41] Nonetheless, this prevalence is comparable to that of most European countries,[41] and given our sample size, it must be considered the closest to real-world conditions. The data we formerly reported between August 2016 and December 2017 disclosed a prevalence of 10.2% (among a total of 105 458 cases) ranging from 17.1% at age 25% to 6.2% at age 64, and with simultaneous infections by two or more Hr-HPVs representing 25.7% (31.0% at age of 25%–16.5% at age of 64).[24] Currently, by analyzing data from a complete cycle of cervical cancer screening (5 years), results differ in magnitude: Hr-HPV prevalence of 12.5%, varying according to age from 20.8% at age 25% to 8.3% at age 64. Despite the differences, our data corroborate published evidence that Hr-HPV infections are considerably more frequent among young women and tend to substantially decrease after the age of 45.[41]

Regarding the prevalence of the different Hr-HPV genotypes, the five most common Hr-HPV genotypes in our population were HPV-68 (16.09%), HPV-31 (15.3%), HPV-52 (13.33%), HPV-51

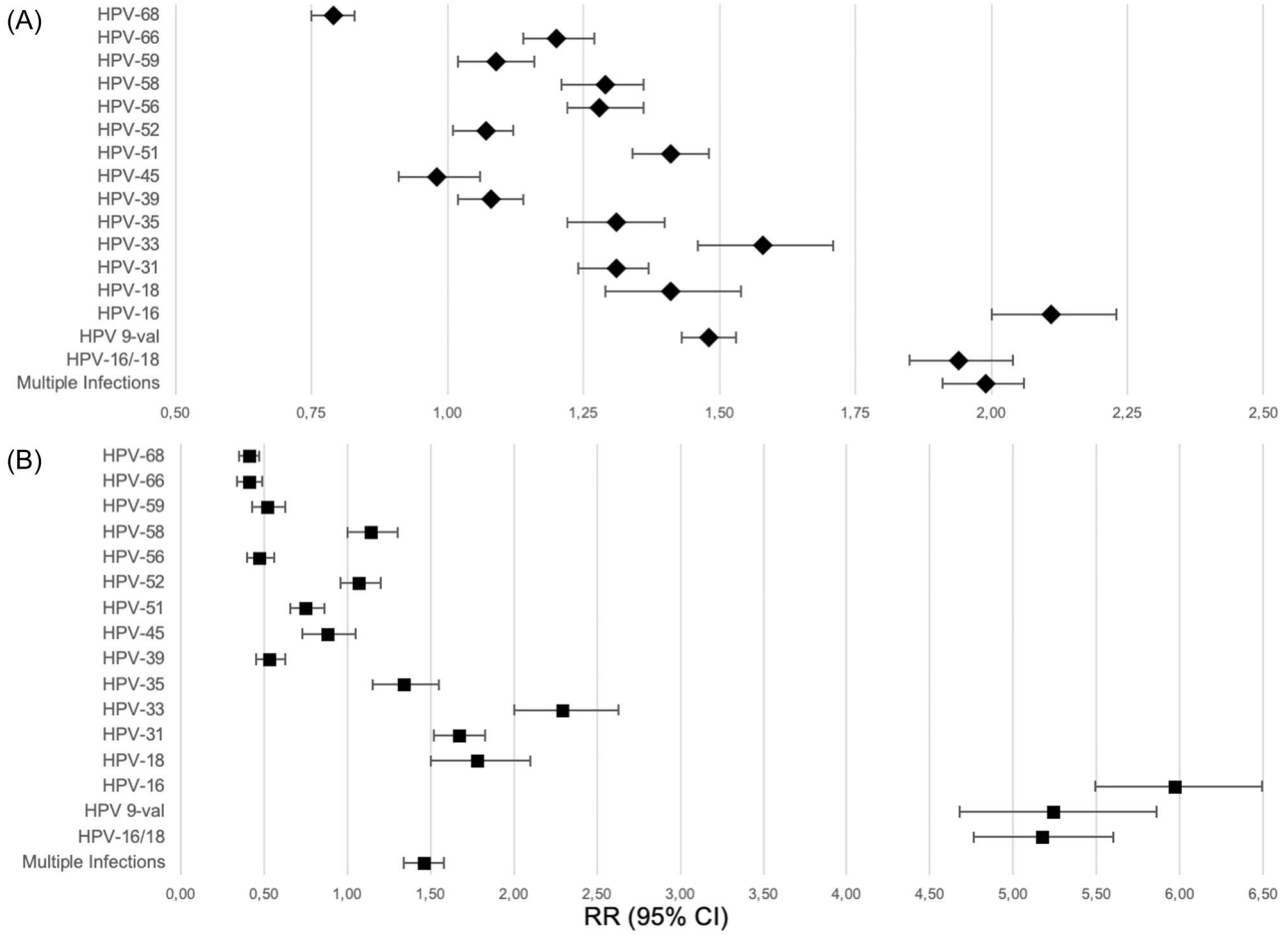

FIGURE 6 Risk of development of cervical abnormalities (a) and cervical lesions equal to or worse than HSIL (includes HSIL, ASC-H or carcinomas) (b) according to the different Hr-HPVs genotypes. AdC, Adenocarcinomas; AGC, Atypical Glandular Cells; ASC-H, Atypical Squamous Cells cannot exclude HSIL; ASC-US, Atypical Squamous Cells of Undetermined Significance; HSIL, High-Grade Squamous Intraepithelial Lesions; LSIL, Low-Grade Squamous Intraepithelial Lesion; NILM, Negative for Intraepithelial Lesion or Malignancy; SCC, Squamous Cervical Cancer.

(12.96%) and HPV-16 (11.06%) in contrast to previous data that showed that the most prevalent were HPV-16 (17.5%), HPV-39 (16.7%), HPV-31 (15.0%), HPV-68 (13.2%), HPV-52 (10.7%) and HPV-51 (10.6%).[24] We believe that differences between our previous report and the ones presented herein are most likely due to sample size and population variability through years.[24] Worldwide, the most prevalent genotypes are HPV-16, followed by HPV-18, HPV-52, HPV-31, and HPV-58.[41] Interestingly, in our population, HPV-18 is the most infrequent (3.72%), whereas HPV-68 displays the highest frequency. In the study by Felix et al. which analyzed Hr-HPVs associated with cervical cancer in Portugal, between 1928 and 2005, the most common Hr-HPVs in SCC cases were HPV-16 (58.2%), HPV-18 (9.2%), HPV-33 (6.2%), HPV-45 (4.7%) and HPV-31 (4.4%).[28] These data, however, are not directly comparable with our study, as they refer to the distribution of genotypes among cancer cases, whereas our study analyzed the prevalence of Hr-HPV in a screening population.

Although worldwide HPV-16 has been reported to be prevalent in women under the age of 25,[7,42,43] in our study, it predominated in women between the ages of 30 and 45. This may be explained in part by the inclusion of the first vaccinated women (in the cohort of 25 years old) in the last two years of the screening round since the vaccination began in 2008 for girls aged 12 years. Indeed, we observed that in this cohort, HPV-16 and HPV-18 are now becoming infrequent. Interestingly, the Hr-HPV multiple infection rate was 27.2%, with no significant differences based on age or geographic location, implying the need for further research into the biological behavior of multiple infections and their outcomes. Moreover, Hr-HPVs included in the HPV-9val accounted for 55.00% of all cases (ranging from 47.78% to 59.18% among age groups), clearly supporting the nonavalent vaccine. Indeed, different reports have reported similar findings.[44,45] In Portugal, the vaccination strategy started in 2008 based on Gardasil ® (quadrivalent HPV, Merck & Co.) for girls from 12 to 17 years old, hence is expected that a proportion of the women now with 25 years old are already vaccinated. Since 2017 Gardasil® 9 (nonavalent HPV, Merck & Co.) has been provided for girls aged 10 years old, and more recently, the vaccination program includes boys of the same age. Hence, it would be important

to have access to data on vaccination so we could compare the prevalence of both Hr-HPV and cytological abnormalities and predict the exact impact of the vaccination strategies on this disease. The fact that the HR-HPVs not covered by the vaccine represents 45% of all cases reinforces the importance of sustaining cervical cancer screening in the population, even in the vaccinated women, probably by developing a new program based on a risk approach.

As expected, in our population, NILM was the most prevalent cytological finding, while ASC-US represents only 2.57 of the total cohort. The proportion of ASC-US/LSIL was 2.3:1, whereas for ASC-H/HSIL was 1.7:1, which is within the expected range of cytopathology performance supporting the current screening strategy. For the first time in our population, we were able to correlate Hr-HPV genotypes with cervical cytology findings: multiple Hr-HPV infections accounted for between 22.44% of NILM and 44.52% of LSIL cases; HPV-16/−18 infections represented 11.40% of NILM in contrast with 51.96% of HSIL and 90.00% of SCC; and HPV-9val represented over 50% of all cases in different cytological abnormalities (90.14% HSIL, 83.50% ASC-H, and 100.00% SCC). The most prevalent Hr-HPVs amongst the cytological findings were HPV-68 (17.35%) in NILM, HPV-31 (17.13%) in ASC-US, HPV-51 (20.83%) in LSIL, and HPV-16 in HSIL (48.04%), ASC-H (33.44%) and SCC (90.00%). Despite these relevant data, it would be helpful if we could confirm either the Hr-HPV genotype or cytological abnormalities in histological samples from all women who underwent colposcopy. Nevertheless, these data demonstrate that HPV-9val genotypes, and HPV-16 in particular, are the major culprits in most high-risk lesions (invasive tumors included), emphasizing the importance of vaccination programs.

Risk analysis showed that HPV-16, −33, −18, −31, −35, −58, and −52 are associated with an increased risk of developing HSIL or worse. Although HPV-33 (RR = 2.29) is not a frequent genotype in the overall population (4.65% of Hr-HPVs) it seems to be significantly associated with HSIL or cancer, surpassing HPV-18. These data reinforce the suggestions made in the literature that women infected by HPV-33 would likely benefit from early referral to colposcopy.[18,30,46] Similar results were found for HPV-31 (RR = 1.66), the second most frequent Hr-HPV in our population (15.3%). These findings have been previously demonstrated by other studies but were never shown in our population nor in the specific context of cervical cancer screening.[30,46] On the opposite side, we found that despite HPV-68 is the most prevalent genotype, it is more common in NILM than in cases with cervical abnormalities (RR = 0.78) and more evident if we consider only HSIL or worse (RR = 0.41). This is consistent with the most recent descriptions regarding the classification of Hr-HPV that show that HPV-68 is now a Group 2A agent considered probably carcinogenic (probable HR-HPV).[16,47,48] The inclusion of an expanded Hr-HPV genotype testing in our screening program allowed us to confirm that HPV-16/−18 (RR = 5.16) or HPV-9val genotypes (RR = 5.23) and multiple Hr-HPV infections (RR = 1.46) are important markers for HSIL or worse. The relevance of multiple Hr-HPV infections remains unclear.[10,49]

In summary, the characterization of a large population-based cervical cancer screening cohort confirmed a relatively high prevalence of Hr-HPV infection, especially among young women. Furthermore, Hr-HPV genotypes present in the nonavalent vaccine are responsible for the vast majority of cervical lesions and cancers, emphasizing the potential effectiveness of this vaccine in the strategy for cervical cancer eradication in our population. Our findings also indicate that the vaccine will progressively change the prevalence of Hr-HPV genotypes in the population, and thus the data generated by extended genotyping provides invaluable information about the relative frequencies and dynamics of specific Hr-HPV infection in the target population. The strategy of specifically identifying Hr-HPV genotypes is, in our viewpoint, clearly advantageous since it enables improved assessment of the actual and future efficacy of vaccination programs and forecasts changes in infection patterns. Finally, changes in the management of cervical cancer screening should take into account the impact of the genotypes present in the nonavalent vaccine and the relative risk of disease development.

AUTHOR CONTRIBUTIONS

Andreia Rosário and Hugo Sousa performed the data collection, analysis and wrote the manuscript. Andreia Rosário, Ana Sousa, Joana Marinho-Dias, and Hugo Sousa were responsible for the HR-HPV test data collection. Cláudia Lob, Luís Leça, Nuno Coimbra, and Paula Monteiro are the clinicians responsible for the cytopathological data collection. Fernando Tavares is the responsible for the cervical cancer screening plan in the North Region of Portugal. Paula Monteiro, Inês Baldaque, and Gabriela Martins provided clinical support to the data. Rui Medeiros verified the analytical methods and supervise the project. Rui Medeiros and Rui Henrique provided scientific support to the project/manuscript. Hugo Sousa conceived the original idea and coordinated the project. All authors discussed the results and contributed to the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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