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Changes in the Rate of Human Papilloma Virus Serotypes after Vaccine Implementation: A Descriptive Study

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Abstract

Background: The main objective of this study is to describe the rate of the different serotypes of HPV in cervical cytologies and biopsies in three different periods: 2002-2006 (prior to the implementation of the vaccination programs in Spain), 2009-2011 (shortly after this implementation) and 2020 (almost 15 years after introduction of the vaccine) at a single hospital. **Methods:** This is an observational, descriptive, retrospective study based on the review of the results of the determination of the HPV serotype using the commercial kit (Genomica®; PharmaMar LTD) in cervical liquid-based cytologies and biopsies at a single large tertiary hospital, Hospital Clínico San Carlos, in Madrid, Spain. We have collected the data from three different time periods: 2002-2006; 2009-2011, and 2020 to try to understand the potential changes associated with the use of the vaccine. **Results:** In these time periods we have reviewed the data from 1420 women. In the three periods the most frequent serotype was HPV 16, followed by HPV 18 or a combination of both. The most frequent low risk serotype was HPV 6 followed by the combination of HPV 6 and 11. It has been verified in our study that the prevalence of the category "others", constituted by the three risk groups, has undergone a progressive increase, beginning with an infection rate of 65.43% in 2002-2006 to finally rise up to 90.92% in the year 2020. **Conclusions:** Our study reveals an

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increase in the number of infections by the HPV serotypes that are not included in the tetravalent vaccine.

Keywords

HPV, Serotypes, Cervix, Vaccine, Women's Health

1. Introduction

The human papillomavirus (HPV) is a member of the Papillomaviridae family and the cause of many lesions, some of them premalignant, that lead to several different types of cancer, mainly in the anogenital and oropharyngeal areas. The advances in the genomic sequencing techniques for virus typing have allowed identification of more than 400 HPV serotypes, 218 of which seem to be related to human infections and at least 45 are recognized as a source of anogenital infections [1]. Denomination for each serotype is established considering as a common criterion the existence of at least 10% discordance between the nitrogenous bases paired with at least a 90% homology [2]. Each serotype is named with a number and is subsequently classified according to their malignant potential in high risk serotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 73), low risk (6, 11, 40, 42, 43, 44, 53, 54, 61, 62, 71, 72, 81, 83, 84 and 89) and indeterminate malignant potential (26, 30, 34, 61, 66, 67, 69, 70, 82, 85 and 97) [3]. As clearly shown, the oncogenic potential of the virus is related to several proteins that are coded in the DNA, the most important being E6 and E7. The virulence of this virus depends on the interaction between these proteins and tumour suppressor genes, p53 and RB, and also between these proteins and the cyclin-dependent kinases inhibitors or the Src family of tyrosine. The former induces chromosomal instability and inhibition of apoptosis in infected cells, while the latter stimulates mitotic activity and proliferation of these cells [2]. This sequence of events eventually leads to many genomic and molecular abnormalities, with chromosomal gains or losses, gene activation or inactivation, methylation, among others [4].

Fluorescent in situ hybridization (FISH) techniques can detect viral DNA in 70% - 90% of precursor lesions and high-grade cervical intraepithelial lesions and also in 20% - 50% of low-grade lesions [5], a fact that confirms the causal relationship between this infection and the development of these lesions. High-risk HPV, specifically HPV 16, has been proposed as the second most frequent carcinogenic agent, only after smoking and is considered responsible for 5% of all human tumours [5] [6]. Despite this clear pathogenetic relationship, in recent years it has been proposed that there are some cervical lesions unrelated to HPV and that they have a bad prognosis [7].

HPV transmission depends on the anatomic region. In all cases, there is a need for micro traumatic lesions in the skin or the mucous membranes that al-

low contact between the virions and the keratinocytes; most of these micro traumatisms are related to sexual intercourse. HPV is the most important sexually-transmitted infection (STI) and it is estimated that 80% of the sexually active population will become infected at least once in their lifetime. Recent data from registries in Spain indicate that 14% of sexually active women are infected by some HPV serotype [8]. This incidence can vary widely in the different age groups, and it is also related to the coexistence of other risk factors, including but not limited to the age of the first intercourse, the number of sexual partners, the history of other STI (mainly Chlamydia trachomatis and human herpes virus 2) or immunosuppression (both primary or secondary) [1] [2] [5] [8] [9]. There is a consensus among the scientific societies that tobacco smoking, contraceptive pills used for more than 5 years and multiparity are important cofactors that influence the carcinogenic potential of HPV [5] [8] [10]. Smoking has been proposed as the most important environmental risk factor that potentiates the progression of neoplastic lesions associated with HPV, although the exact pathogenic mechanism of tobacco has not yet been elucidated [8] [9].

HPV infection has low immunogenicity, and this explains why patients are frequently reinfected by the same or a different HPV serotype. Sometimes, the clearance of one serotype is not completed when another HPV infects the same patient and this leads to a chronification of the infection that potentiates the oncogenic capacity of the virus [10].

However, there are also some circumstances that can have a protective role against HPV infection and also against neoplastic transformation. Intrauterine devices (IUD) have been shown to protect against cervical carcinoma, a fact that can be explained by the local inflammatory response against the IUD that helps to clear potential infections by this virus [5]. It is important to highlight that IUD does not protect against other infections. Another preventive factor against HPV infection is male circumcision, as shown by the lower incidence in the countries where circumcision is frequently performed (Israel, Arab countries) [5].

Most infections by HPV have little clinical relevance, are transient and are typically cleared in 1 - 2 years by women and 6 - 12 months by men. However, 3% - 10% of all infected patients do not clear the virus, with chronification of the disease and increased risk of neoplastic transformation [10]. The incidence of cervical carcinoma in Spain was 1972 cases in 2020 [11], with 673 disease-related deaths. 5-year survival reached 65.5% of Spanish patients with cervical carcinoma in the time period 2008-2013, with a clear improvement compared to previous years [8]. This improvement in survival is clearly related to the implementation of widespread screening programs that have led to a 50% reduction in the mortality due to this neoplasm in the last 50 years, and also in the last decade to the vaccination of women against this virus. It is estimated that vaccination of 70% of the world population could prevent more than 340,000 new cases of cervical carcinoma each year and also more than 100,000 deaths due to this tumour [8].

HPV infection and its consequences can be prevented. The improvement in screening programs has led to a reduction in the incidence and mortality of these lesions in developed countries. Nevertheless, the best way to avoid the consequences of infection is primary prevention through population-based vaccination [12]. HPV expresses two late proteins in the capsid, involved in viral replication [7], namely L1 and L2 proteins. L1 protein shows regions that vary in the different serotypes and other regions that are constant and specific for the HPV genome [7]. Vaccines are composed of virus-like particles (VLP) that self-assemble from L1 copies and stimulate an immune system response with antibody production [13]. L2 protein is not useful for vaccine development, as it stimulates little antibody production [7]. As they include no viral DNA, VLP lack oncogenic potential and they are safe for the patients [7] [13].

Three vaccines against HPV are in the market. The Spanish Pediatrics Association recommends vaccination against HPV for all children older than 12 years, although vaccines are approved for administration from 9 years on. **Table 1** summarises the main characteristics of the vaccines [12] [13].

In Spain, the first vaccine went into the market in 2006. The bivalent vaccine has shown cross-immunity against the serotypes 31, 33 and 45, even after only one dose. It can also protect against the anogenital warts caused by the serotypes 6 and 45. Only one dose can reach immunity against infection for an average of 7 years, which increases to 11 years in patients receiving the 3 doses schedule. However, immunity starts to drop after 6 and a half years [7]. The bivalent vaccine is the only one that includes AOS4 as an adjuvant and this doubles the antibody production compared to aluminium hydroxide [13]. The most recently

Table 1. Types of HPV vaccine.

Vaccine	Bivalent	Quadrivalent	Nonavalent
VPL	16/18	6/11/16/18	6/11/16/18/31/33/45/52/58
Adjuvant	ASO4 (detoxified form of lipopolysaccharide and aluminium hydroxide)	Aluminium hydroxide	Aluminium hydroxide
Indications	• Precancerous lesions of the cervix, vulva, vagina, anus • Cancer cervix, anus	• Precancerous lesions of the cervix, vulva, vagina, anus • Cancer cervix, anus • Anogenital warts	• Precancerous lesions of the cervix, vulva, vagina, anus • Cancer cervix, anus • Anogenital warts
Population and dosage	People 9 - 14 years old (inclusive): 2 doses; 0 and 6 months People 15 years or older: 3 doses; 0.1 and 6 months	People 9 - 13 years old (inclusive): two guidelines • 2 doses; 0 and 6 months • 3 doses; 0.2 and 6 months People 14 years and older: 3 doses; 0.2 and 6 months	People 9 - 14 years old (inclusive): two guidelines • 2 doses; 0 and 6 months • 3 doses; 0.2 and 6 months People 15 years or older: 3 doses; 0.2 and 6 months
Coverage	70% cancer of the vulva, cervix, vagina, penis, anus, or opharynx	+90% genital warts	+20% cervical cancer

marketed vaccine is the nonavalent one, that shows the best coverage with an improvement in the rate of preneoplastic lesions prevention. It is also said to confer cross immunity against low risk HPV serotypes [7]. Tetra and nonavalent vaccines also prevent anogenital warts [12] [13].

Since HPV vaccination began, several million vaccines have been administered all over the world. The safety profile of the vaccine has been confirmed in post commercialization analysis. In Spain, 3.5 million patients have been vaccinated so far, with a steady but slow tendency to increase vaccination. It is estimated that 80% of the target population has been vaccinated so far with a good acceptance. The main reason to avoid vaccination has been the parental fear of potential side effects and also the doubts about the need of vaccination, mainly due to the lack of knowledge about the virus and its consequences on health. Several informative campaigns aimed to improve compliance with vaccination have been implemented, together with efforts from the health professionals to increase the vaccination rate with great success [14] [15] [16] [17].

The objective of this study is to analyse the change in the rate of HPV serotypes in the uterine cervix samples of women at the Hospital Clínico San Carlos in Madrid after the introduction of the quadrivalent vaccine (against high-risk serotypes 16 and 18, and low risk serotypes 6 and 11).

2. Materials and Methods

This is an observational, descriptive study based on the results of the usual clinical practice and performed at Hospital Clínico San Carlos, a large tertiary hospital that gives medical care to over 400,000 people living in Madrid (Spain). It has 49 medical and surgical specialties, and also three specialty centres, one of which is devoted to sexually transmitted diseases, four health centres and various areas of specialisation, including the Women's Health Institute, making it the 5th most valued hospital in Spain in the year 2022. In order to contextualise the data obtained, the reader is provided with the most relevant sociodemographic data of the population from which the sample of study was chosen (**Table 2**).

Our study was designed to compare the evolution of HPV infections after the start of vaccination, covering a total period that would go from 2002 to 2020. The sample includes women from the Hospital Clínico San Carlos that underwent either cervical cytologies or biopsies and with a positive diagnosis for HPV infection, thus excluding men, lesions in other locations (vulva, vagina, anus)

Table 2. Sociodemographic information of the population covered by our Hospital.

Population Madrid (City)	Population Moncloa-Aravaca District	Total Women		Mean Age	Socioeconomic Level
		65.708 (54%)			
3.334.730	121.683 (3.65%)	**Spanish Women**	**Foreign Women***	44.1 years old	Medium/High
		57.836 (47.53%)	7.871 (6.46%)		

*Most prevalent nationalities: Latin American, North African, Eastern European and Chinese countries.

and those patients who presented a lesion in the cervix without HPV. The final sample included 1420 patients.

All the data have been obtained from the clinical records and from the database of the Surgical Pathology Department, where the study has been performed.

Since 1996 the Surgical Pathology Department has determined the serotype of HPV in cytological and biopsy samples with the kit by Genomica®. For the aim of the present study, we have reviewed the results of the HPV determination in three different time periods: 2002-2006 (to sample women with no vaccination); 2009-2011 (to sample women shortly after vaccination started) and 2020 (to sample a period in which most women should already have been vaccinated). Liquid-based cytology was not routinely used in our department before 2009 and for this reason, most determinations in the first period were performed in biopsies, as opposed to the other two, which correspond mainly to cytologies. We routinely perform HPV determination in all cytologies with diagnosis of atypia of undetermined significance in squamous cells (ASC-US) or potential high grade (ASC-H) and in all the cases with preneoplastic o neoplastic lesions (all grades of dysplasia). We also perform HPV determination if required by the clinicians in patients with no cytological abnormalities.

All the data have been stored in an Excel file and subsequently been analyzed with the SPSS 20.0 for Windows statistical package. First we have performed a descriptive analysis of our cases, with percentages for qualitative variables and mean/median for quantitative ones. Consequently we have performed a chi-squared test to evaluate the significance of the differences between the three most frequent serotypes (16, 18 and 6) as well as those included in the other category, in the different periods of time analysed.

To fulfill the requirements settled in the Personal Data Protection rules in Spain, all the clinical data anonymized. This study has been approved by the Institutional Review Board (IRB) of the hospital (approval number 22-016).

As secondary objectives, we compare the mean and median ages at the first registration of HPV infection 16 and 18 in all the intervals studied with the Student's t-test; and finally, those serotypes of high, low and indeterminate risk included in the category of "other serotypes" that currently predominate after vaccination are highlighted.

3. Results

3.1. Period and Sample Studied

In the first period (2002-2006) we found 269 samples with HPV, 150 of which were biopsies and 119 cytologies. In the second period (2009-2011), we found 204 samples, 192 cytologies and only 12 biopsies. In the third period (2020), we collected 947 samples, all of them cytologies.

3.2. Age Analysis

Figure 1 summarises the results of the age analysis in our series. The age of the

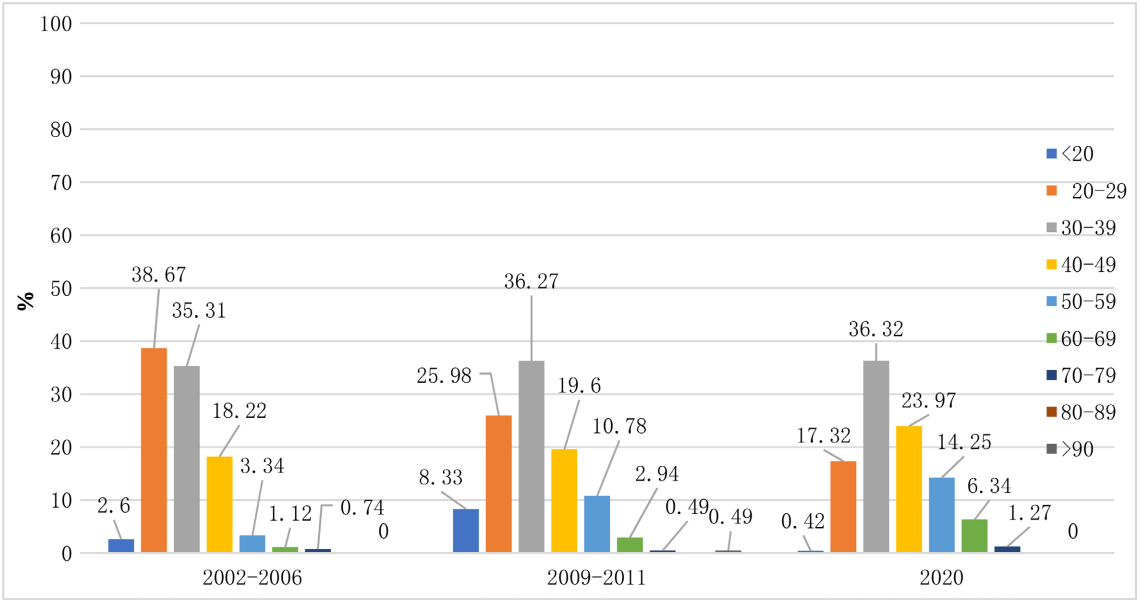

Figure 1. Distribution of the frequency of HPV positivity by age intervals, adjusted to the sample size of each study period.

269 women found between 2002-2006 ranged from 17 to 79 years, with a mean of 32.92 and a median of 32 years. In the second period, the age ranged from 18 to 98 years, with a mean age of 37.38 and a median of 34 years. Last, in 2020 the age ranged from 18 to 80 years, with a mean of 40.83 years and a median of 39 years. Although we have noted a progressive increase in the age at the moment of diagnosis, which peaked in 2020, the differences did not reach statistical significance.

3.3. HPV Serotype Determination

Table 3 summarises the results of the HPV serotype determination in the three periods of the study. In all of them, the most frequent high-risk serotype was HPV-16, but the rate has decreased from 15.62% in 2002-2006 to 6.65% in 2020. Our results indicate that the rate of the low-risk virus included in the vaccines has also been reduced, from 14.5% of cases with HPV-6 in the first period to 1.27% in 2020. As expected, we have noted an increase in the number of other viruses not included in the vaccine and collectively denominated others in Table 3. They represented 65.43% of the cases in 2002-2006 but reached 86.77% in 2009-2011 and 90.92% in 2020. Chi-squared test confirms a statistically significant difference in the rate of the different serotypes between the three time periods ($p = 0.000$ for HPV 16; $p = 0.01$ for HPV 18; $p = 0.000$ for HPV 6; and $p = 0.000$ for other serotypes).

Distribution of Serotypes in High, Low and Indeterminate-Risk Categories

The clear preponderance of other viruses in 2020 has led us to specifically analyse the distribution of serotypes in high, low and indeterminate-risk categories (Figures 2-4). As shown in these Figures, the most prevalent high-risk serotypes

Table 3. Frequency of HPV serotypes.

Year	Number of samples	Virus serotype
2002-2006	Cytologies: 119	Type 16: 42 (15.62%)
	Biopsies: 150	Type 18: 9 (3.34%)
	Total: 269	Types 16 + 18: 1 (0.37%)
		Type 6: 39 (14.5%)
		Types 6 + 11: 2 (0.74%)
		Others: 176 (65.43%)
2009-2011	Cytologies: 12	Type 16: 14 (6.86%)
	Biopsies: 192	Type 18: 2 (0.98%)
	Total: 204	Types 16 + 18: 1 (0.49%)
		Type 6: 9 (4.41%)
		Types 6 + 11: 1 (0.49%)
		Others: 177 (86.77%)
2020	Cytologies: 947	Type 16: 63 (6.65%)
	Biopsies: 0	Type 18: 10 (1.05%)
	Total: 947	Types 16 + 18: 1 (0.11%)
		Type 6: 12 (1.27%)
		Types 6 + 11: 0 (0%)
		Others: 861 (90.92%)

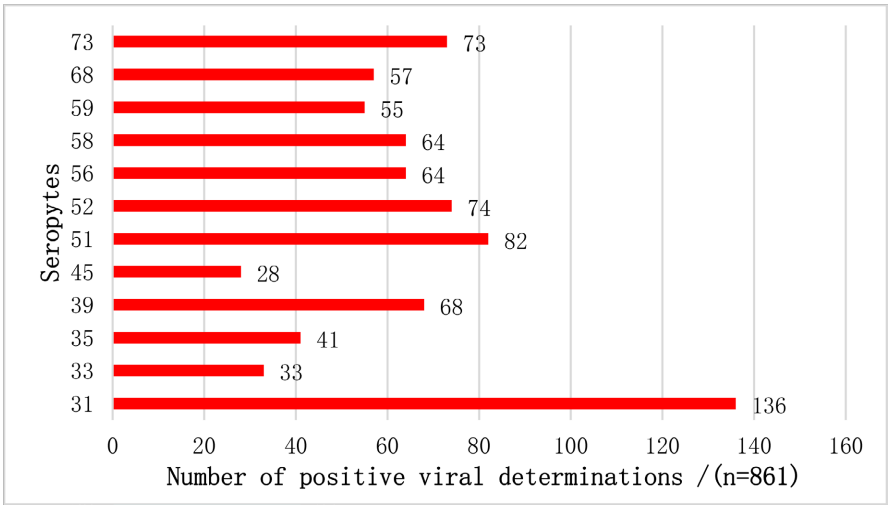

Figure 2. Frequency in our series of other high-risk serotypes.

have been HPV-31 (136 cases), HPV-51 (82 cases) and HPV-52 (74 cases); the most prevalent low-risk ones have been HPV-53 (114 cases), HPV-54 (107 cases) and HPV-42 (84 cases) and in the indeterminate-risk categories we must note the serotypes HPV-61 and HPV-66, followed by the HPV-70.

Figure 5 compares the relative frequency of the different types of virus according to the risk in the others category in 2020. It is clearly shown that high-risk

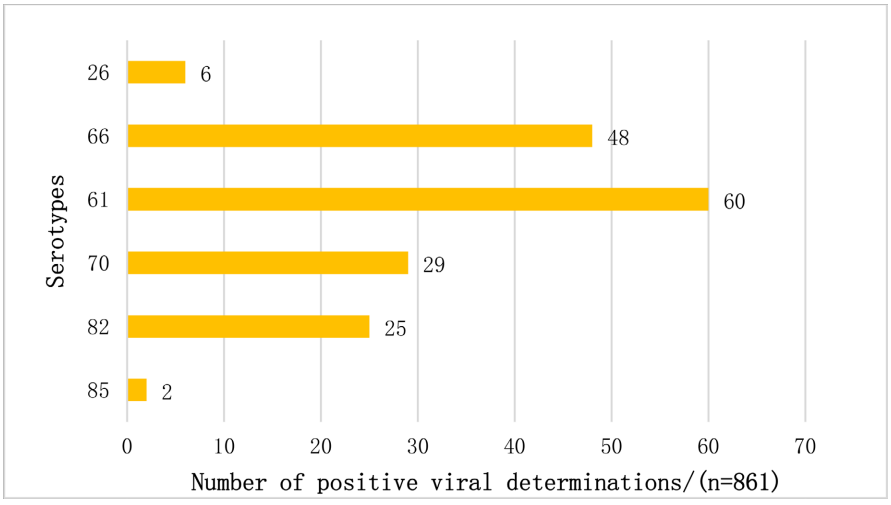

Figure 3. Frequency of other indeterminate risk serotypes.

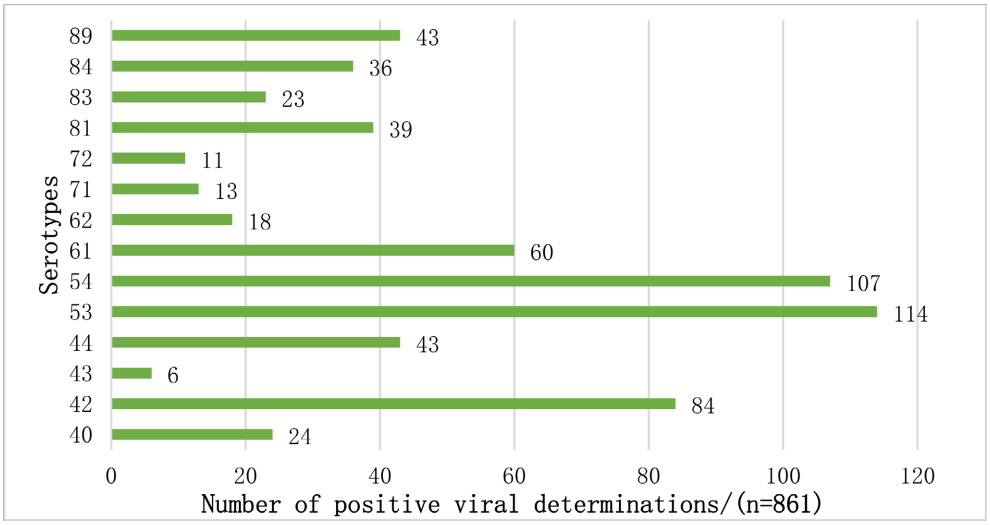

Figure 4. Frequency of other low-risk serotypes.

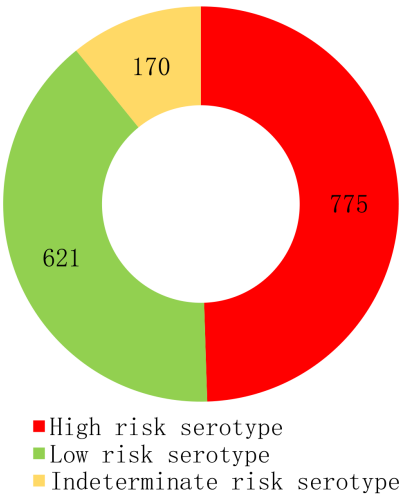

Figure 5. Frequency of positive viral determinations of each risk group in the category "others" in 2020.

serotypes are more prevalent than indeterminate and low-risk ones.

Finally, Figure 6 shows a comparison of the frequency of infection of the high-risk serotypes, between the different periods of time analysed. Despite the fact that the sample size in each period analyzed is not alike, the figure shows the comparison of the infective trend between the usual high-risk serotypes (16, 18) with respect to the high-risk viruses within the category "others", which previously were not so common. As can be seen, a striking upward trend is shown over time.

4. Discussion

Our results show a change in the rate of the different HPV serotypes over the years, which can be explained, at least partially, by the population-based vaccination against the most frequent serotypes of this virus (high-risk serotypes 16 and 18 and low-risk ones 6 and 11). Despite these 4 serotypes remaining as the most frequent ones in our population, our study shows a clear decrease in the frequency of all these serotypes after vaccination. However, this reduction has been paralleled by an increase in the rate of other high-risk serotypes that were less frequent previously. This change in serotypes could maintain the infectivity of HPV despite vaccination and dampen the preventive effect and efficiency of the tetravalent vaccines.

Another interesting finding is the increase in the mean age of the patients infected by HPV in our area. It seems that the group of patients that were not vaccinated when they started their sexual activity remains at higher risk of being infected than the younger age group, as vaccines are supposed to give cross-protection between different viral serotypes.

In the period of analysis our hospital began to use the LBC more frequently over the classical Pap smear. In fact, many reports in literature have compared

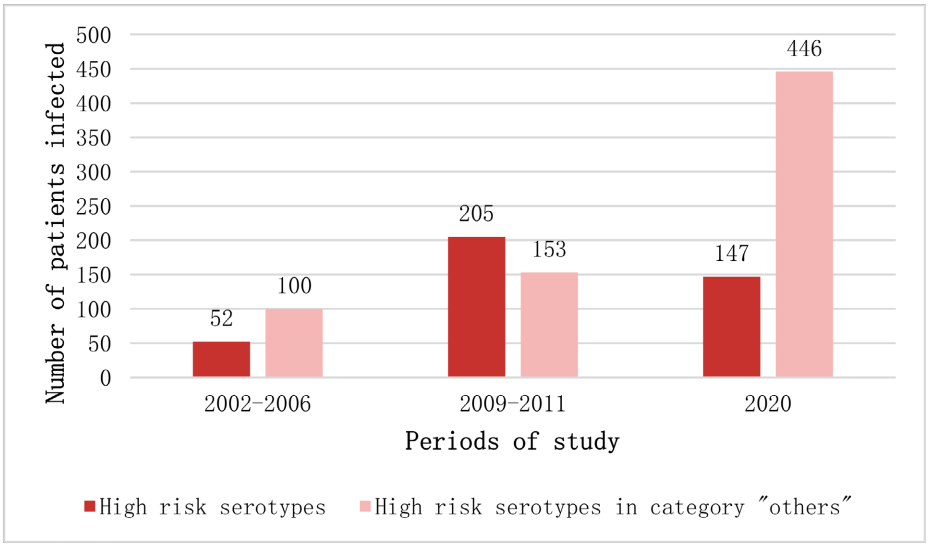

Figure 6. Distribution of infected patients with high risk and other high risk serotypes in each period of study.

the cost-effectivity of Pap smears and LBC, showing that LBC renders less unsatisfactory samples and improves reliability in the diagnosis of morphological abnormalities after a period of training. This is mainly due to the clearance of blood, debris and mucus, which hinder diagnosis of dysplasia in Pap smears. This is why our hospital decided after demonstrating these improvements to implement this type of diagnostic test in screening, which explains the dramatic increase in the number of HPV determinations in the last time period of the study. The increase in the number of determinations in 2020 can also be attributed to the introduction of the clinical record in our Hospital, which allows an easier obtention of the clinical data.

Taking into account the advantages of LBC, it is remarkable that it is much more expensive than Pap [18], and this increase in the cost has slowed the introduction of this technology in some areas. In developed countries, LBC has been implemented in most laboratories and it offers the great advantage of allowing HPV detection. It is well known that most neoplastic cervical lesions are associated with this virus and LBC can better classify patients with undetermined cytological lesions like ASC-US or ASC-H and avoid patients' unnecessary fear and further investigations [19]. It has been shown that a repeat cytology is less cost-effective than HPV identification, and this translates in a benefit for women's health.

Vaccine acceptance has evolved over time in Spain. Shortly after vaccine introduction, many parents had doubts regarding its security and efficacy and there was a clear social concern about potential side effects [15]. These fears were partially fueled by the social media that highlighted the controversies among scientists and health professionals about the need of this vaccine. The health professionals in Spain then started informative campaigns in the hospitals and outpatient clinics to inform parents about the benefits of vaccination [16]. Several studies in Spain have shown that information by the paediatricians was considered more reliable by parents and increased the vaccination rate, despite social media ongoing controversies. The highest vaccination rate was found in families with married couples (as opposed to single parents), mothers of Spanish ancestry (as compared with immigrant population) and most importantly in families in which mothers underwent regular screening against cervical carcinoma themselves [16]. The main reasons to avoid vaccination were lack of information or medical advice. Recent surveys have shown that most parents are aware of the protective effect of vaccination against cervical carcinoma, but not of the protective effect against penile carcinoma or anogenital warts. In fact, in Spain, vaccination had been restricted to girls, 12 years or older and only recently a change in vaccination policies has also incorporated boys of the same age. In some regions the vaccination program has been implemented by schools rather than health centres, with a higher compliance [17]. Despite these ongoing efforts, the vaccination coverage in Spain remains lower than in other developed countries [20].

Sexual behaviour has dramatically changed in the last decades. The mean age of the first sexual intercourse in Spain is 15 years, much lower than in the past [21] [22] [23]. Despite this earlier initiation in sexual activity, most young people do not receive an adequate sexual education at schools or at home and many rely on peers or the Internet to learn about sex. The surveys about contraceptive methods in the young population reveal that girls prefer contraceptive pills or the withdrawal method, while boys prefer condoms [22].

The last data about sexual behaviour in Spain reveal that up to 57.7% of women aged 18 - 25 have had sex for compromise and almost 64% believe that sex is not to be related to emotional or sentimental implications, a fact that might increase the number of sexual partners [23] and the subsequent risk of contracting sexually transmitted infections (STI). The risk of STI is also related to socioeconomic factors (poverty, gender inequity), cultural factors, biological factors (age, gender) and sexual behaviour (multiple sexual partners, type of sexual practices, drugs or alcohol consumption) [24]. Diagnosis of STI can be delayed due to the lack of timely consultation and also to the use of antibiotics, which can modify the disease progression and also lead to microbial resistances. Besides, young people seem to be less afraid of STI, including HIV [25] [26]. All these psychological and social factors explain why each day there are around 1 million new cases of STI, mainly gonococcus, chlamydia, syphilis and trichomonas, in people aged between 15 and 49. It is estimated that 417 million people are carriers of human herpes virus type 2. In Spain, there are five notifiable STI, namely syphilis, congenital syphilis, gonococcal infection and, since 2015, also infections by Chlamydia trachomatis and lymphogranuloma venereum [24].

HPV infection is the most frequent STI and it is estimated that at least 80% of the sexually active population will become infected during their lives. The prevalence of HPV in women ranges from 5% - 10% in developed countries to 15% in developing ones [27]. HPV infections, are not only related to cancer, but can also have other consequences on the patients' health. It is not clear whether HPV infection influences fertility and literature shows discrepant results [28]. HPV has been linked to an increase in the number of spontaneous foetal loss and also of premature rupture of membranes. HPV has been found in the sperm and has been linked to a higher risk of abortion, as infected blastocysts have a reduced potential for normal implantation. It is not clear whether vaccination can prevent these health problems, but it is clearly shown it can help prevent not only cervical, but also anal and oropharyngeal cancer [5] [29] [30].

There is still debate whether HPV vaccination programs should include males. It is clear that men can develop malignant diseases related to this virus, although malignant disease is more frequent in women. Male vaccination could help prevent transmission and also reduce the incidence of penile, anal and oropharyngeal carcinoma, and should be recommended [13] [31] [32].

The cost-efficiency of vaccination programs could improve if both genders were vaccinated and also if nonavalent vs. tetravalent vaccines were employed, as

these vaccines widen the coverage of other virus serotypes that seem to be occupying the niche left by the control of the four HPV serotypes included in the tetravalent vaccines [31].

The Spanish Association of Pediatrics recently published a new set of recommendations for HPV vaccination stating that nonavalent vaccines should be favoured if possible and that males should also be vaccinated [33]. The Spanish government has accepted these recommendations and the new schedules include for the first time males.

The population where the Hospital is located in an area of the capital which has a medium-high socioeconomic level, equivalent to other European capitals. However, it is administered by the Spanish Health System, making it unnecessary to have medical insurance in private entities to receive their services. In this way, the data that we expose in our work are applicable to cities with similar socioeconomic characteristics. Nevertheless, taking into account the sociocultural differences and educational level of the different populations, not only at a European level but worldwide, the results could not be accurately extrapolated.

5. Conclusion

Our study confirms that the tetravalent vaccine has modified the rate of the different HPV serotypes and increased the prevalence of those not covered in the tetravalent vaccine, mainly of high-risk type. These results speak for the convenience of incorporating new vaccines with a higher coverage, namely the nonavalent, but also of the need to control which serotypes are gaining prevalence in each country to incorporate them in future designs of the vaccines. HPV neoplastic disease is an important public health problem that can be prevented and the health professionals should make all possible efforts to increase the vaccination rate and also to analyse the evolution of HPV frequencies after vaccination to improve the vaccines and reduce the morbidity related to this virus.

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Institutional Review Board Statement

The study was approved by the Institutional Review Board (or Ethics Committee) of Hospital Clínico San Carlos (protocol code 22-016 and date of approval: 17/10/2022).

Informed Consent Statement

Patient consent was waived due to the fact that we do not use data that allows the recognition of any of the study participants.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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