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# Evolution of Cervical Lesions Associated with Human Papillomavirus Infection after the Introduction of Vaccination

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## Abstract

**Background:** The main objective of this study is to analyse the change in the type of lesions developed by HPV-infected patients after the introduction of the vaccine in three different periods; 2002-2006 (years previous to the implementation of the vaccine in Spain), 2009-2011 (shortly after the vaccination) and 2020-2021 (years where the vaccine was well established) at a single hospital. **Methods:** This is an observational, descriptive, retrospective study based on the review of the results of the biopsies of patients with HPV lesions 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, 2020-2021 to try to understand the potential changes in these lesions after vaccine introduction. **Results:** In this time we have reviewed the data from 946 women. In these three periods, a decreasing trend in the rate of squamous cell carcinoma was noted, the rate of adenocarcinoma remains stable, and the rate of cervical intraepithelial neoplasia grades 2 - 3 (CIN 2-3) lesions shows an increasing trend. We have also found a change in the mean ages of the patients with these lesions, as this increased in the three lesions caused by HPV after the implementation of the vaccine. Our study indicates that the identification of other high risk serotypes, apart from 16 and 18, as well as those with indeterminate risk, has undergone a progressive increase, increasing from 24.24% and 14.11% respectively in 2002-2006 to 40.42% and 28.34% in 2020-2021. **Conclusion:** Our study confirms the

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effectiveness of the vaccines developed so far, against the HPV serotypes they contain. This is demonstrated by the evidence, in our population, of a decrease in the incidence of squamous cell carcinoma in uterine cervix. In parallel, an increase in the mean age of diagnosis has been verified, for both squamous cell carcinoma and its CIN 2-3 precursor lesions, as well as a change in the infective trend of HPV serotypes that are not included in the current vaccines.

## Keywords

HPV, Serotypes, Cervix, Vaccine, CIN 2 - 3, Squamous Cell Carcinoma, Women's Health

## 1. Introduction

Human papillomaviruses (HPV) are members of the Papillomaviridae family, with over 200 subtypes identified. These epitheliotropic viruses contain double-stranded circular DNA genomes of approximately 8 kB, encapsulated within non-enveloped particles measuring 52 - 55 nm in diameter [1] [2].

HPV is the most prevalent sexually transmitted infection worldwide, causing both benign and malignant lesions in men and women. All serotypes known up to now have been classified into three risk groups (high, low and indeterminate) based on their association with malignant transformation of cells. Therefore, the associated risk of these benign or malignant conditions varies depending on the HPV serotype [2]. The high prevalence of human papillomavirus (HPV) infection and its relationship with diseases underscores its relevance for public health [2] [3].

The main risk factors associated with the development of cervical cancer include high-risk human papillomavirus (HPV) infection, age, smoking, childbirth, multiparity, oral contraceptive use, diet, and immunosuppressive states (particularly due to HIV). Among these various risk factors, persistent infection with high-risk HPV appears to be the primary driver of cervical carcinoma development, arising from normal cervical epithelium through the progressive development of low- and high-grade cervical intraepithelial neoplasia (CIN). However, the severity of the outcome towards cervical cancer development depends on specific HPV subtypes [3] [4] [5].

The immune system clears most HPV infections within a few months, but some high-risk subtypes, such as HPV 16 and HPV 18, persist and express viral oncogenes E6 and E7, leading to increased genomic instability, accumulation of somatic mutations, and integration of HPV into the host genome resulting in cervical cancer [6]. HPV infection occurs when viral particles reach exposed basal cells, usually through microtrauma in the epithelium. Sites where infection appears to be facilitated include the transitional epithelium of the cervix and anal region, specialised epithelium of salivary glands in the oral cavity, tonsillar

crypts in the oropharynx, hair follicles, eccrine and apocrine glands, as well as the epidermis [3].

The genome of all HPV types contains three functional parts: the early region (E), which encodes proteins (E1-E7) necessary for viral replication; the late region (L), which encodes structural proteins (L1-L2) required for virion assembly; and a mostly non-coding part called the long control region, which contains cis elements necessary for viral DNA replication and transcription. The E1 and E2 proteins initiate viral DNA replication and act as transcriptional activators. The virus expresses early genes (E5, E6, and E7). The E5 protein induces evasion of the immune response and alleviates cellular dependency on growth factors, thus leading to increased cell proliferation. The E6 protein binds to the cellular tumour suppressor protein p53 and induces its degradation. The E7 protein forms a complex with the retinoblastoma protein (pRb) and degrades pRb via the ubiquitin-proteasome pathway [6]. The next step is infecting the suprabasal layer and binding to heparan sulphate proteoglycans of basal cells and the exposed basal membrane, serving as the primary receptor. This binding occurs with the L1 protein, inducing changes in the viral capsid that bind to another, as yet unidentified receptor. Capsid internalisation, which occurs similar to a macropinocytosis mechanism, can take two to four hours. Viral genome entry into the nucleus is mediated by the L2 protein. Subsequently, replication of the circular viral genome initiates, and structural proteins (L1 and L2) are formed [3]. As the virus reaches the upper layers of the epidermis or mucosa, complete viral particles assemble and are released. Capsid viral proteins (L1 and L2) are expressed to assemble virus progeny in differentiated cells, and E4 is also expressed to complete its life cycle when the infected cell enters the upper epithelial layers [6]. Progression from high-grade lesions to carcinoma requires the accumulation of additional epigenetic and genetic alterations, a process that can take 20 to 30 years. Hypermethylation of CpG islands in the promoter regions of tumour suppressor genes has been recognized as a molecular change from HSIL to cervical cancer [5].

Considering the etiopathogenesis and natural history of cervical cancer and its precursor lesions, particularly after the recognition of HPV as a causal agent, there is clear evidence of the prognostic advantage of early diagnosis through the implementation of screening protocols. [7] [8]. In Spain, a screening protocol has been followed since the 1980s, with the primary objective of early detection and treatment of lesions caused by persistent HPV infection [7] [9]. However, it wasn't until 2018 that the World Health Organization (WHO) declared cervical cancer as the first eradicable cancer, calling for the global implementation of screening and vaccination programs [9]. Implementation of these programs has reduced incidence and mortality of this infection by 70% - 80% [7].

During the early years of sexual activity, the incidence rate of HPV is higher compared to other age intervals. However, most of these infections are transient and do not persist in women. It is after the age of 30 that, although the incidence of infection decreases, the infections tend to be more persistent, increasing the

risk of developing precursor lesions of cancer [7]. Taking this into consideration, the WHO, European Guidelines, and scientific recommendations approved in 2018 state that the target population for these protocols should be women between 25 and 65 years of age [7] [9] [10]. Accordingly, women between 25 and 30 years of age should undergo vaginal cytology every 3 years, while women between 30 and 65 years of age should have cytology every 3 years, HPV testing every 5 years, or co-testing (cytology and HPV testing) every 5 years [7] [10]. Women under 25 years of age and those who have undergone hysterectomies are excluded from the program. Special mention should be made of women with CIN 2 lesions, who should remain under surveillance for 20 years, and immunocompromised individuals, who should undergo annual cytology from the age of 21-years of age and annual co-testing from the age of 30 [7].

In general terms, there are two types of screening protocols. On one hand, there is opportunistic screening, where the patient requests screening during a medical consultation for another reason. This is a non-systematic process, which raises questions about its effectiveness and efficiency [8]. This type of screening hampers optimal coverage and penalises equity, as it focuses more on women with low risk rather than those with high risk. This situation is in line with the different socioeconomic and cultural situations in society, representing a significant limitation of the screening protocol [7]. That is why in Spain, 60% of cervical carcinomas occur in women who have either undergone inadequate screening or have been exempted from it [7] [9]. In contrast, population-based screening offers systematic and active screening to a target population within the healthcare system, allowing continuous evaluation of quality and outcomes [8].

Among the main benefits of this screening program, the following can be highlighted: a higher cure rate after early diagnosis, improved quality of life for the patient after treatment, and the assurance that a woman is not a carrier of precursor lesions or cervical cancer in case of a negative result [7]. However, the greatest achievement is the increased detection capacity for CIN3 lesions and the greater reduction of CIN3 in the interval before the next screening test [7]. As for the harms, noteworthy issues include the risk of overdiagnosis without a benefit in terms of cure, the overdiagnosis of low-grade lesions that would not progress subjecting the patient to unnecessary treatments and anxiety and the impact on mobility and fertility due to falsely positive results, as well as false reassurance and delayed diagnosis in case of a false negative result [7].

The distribution of human papillomavirus (HPV) prior to the implementation of the vaccine has been extensively studied. According to studies conducted by G Voglino *et al.* in the year 2000, the prevalence of high-risk HPV was 73.5% in HSIL, 98.3% in squamous carcinomas, and 100% in cases of adenocarcinoma. HPV 16 was the most represented type in all lesions, with 71.2% of HSIL cases, 73.3% in squamous carcinomas, and 50.6% in adenocarcinomas [11]. On the other hand, a study conducted by Mark F. Evans *et al.* in 2005 on the distribution of HPV serotypes found that HPV-16 was detected in 48.5% of ASC-H samples and 49.0% of HSIL samples [12]. Similarly, in 2005, E. Beerens *et al.*

noted that co-infection with multiple types of HPV was predominantly found in HSIL (35.8%), and the most common types of HPV were 16, 52, 51, and 31, with rare detection of HPV type 18 [13].

However, focusing on the period following the implementation of the vaccination schedule, slight changes have been reported compared to previous data. In 2012, Peng Guan *et al.* [14] conducted a meta-analysis of the cross-sectional distribution of HPV types in 115,789 HPV-positive women. No significant differences were observed in the distribution of HPV types among normal cytology, ASCUS, LSIL, or CIN1. However, HPV16 positivity increased abruptly from normal/ASCUS/LSIL/CIN1 (20% - 28%) to CIN2/HSIL (40/47%) to CIN3/ICC (58/63%). HPV 16, 18, and 45 represented an equal or higher proportion of HPV infections compared to normal cytology and CIN3 [14]. Furthermore, in a study conducted by Kim M *et al.* in 2021, 236 cases with multiple HPV infections were examined and compared with 180 cases with single HPV infection. In the patients infected by multiple HV types, the most prevalent genotype was HPV 53, followed by HPV 16, 58, 52, and 68. HPV 33, 35, 39, 51, 52, 53, 58, and 68 were high-risk HPV genotypes that were more frequently detected in multiple HPV infection compared to single HPV infection, and the association between multiple HPV infection and HSIL was stronger compared to that of infection by a single virus type [15].

## 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 of Madrid, Spain. Spanish capital city had a total population registered in the census in the year 2020 of 3,286,662 inhabitants, of which 46.7% were men (1,534,824) and 53.3% women (1,751,838), and most populated district was Carabanchel (255,514 inhabitants).

Hospital Clínico San Carlos provides health coverage to different districts of the capital, including Carabanchel, covering a population of over 400,000 people, which represents 12.17% of the total inhabitants of Madrid, among which there are different nationalities of various continents. The most prevalent of them, and therefore in our sample, are nationalities from Latin countries, followed by North Africa, Eastern Europe, and China. Our hospital is the head of a wide net of primary health centres distributed throughout the different districts it covers, including one health centre which is devoted to sexually transmitted diseases and the Women's Health Institute.

Our study was designed to analyse the change in the type of lesions developed by HPV-infected patients after the introduction of the vaccine in the Spanish Health System, covering a total period that would go from 2002 to 2021. The sample includes women from Hospital Clínico San Carlos and the dependent health centres, that underwent cervical biopsies with lesions caused by HPV and that showed positivity for it with the Genomics® kit for HPV detection. Patients

with HPV-associated lesions in other locations (anus, vagina, vulva, and oropharyngeal region), men, as well as other types of lesions present in the cervix that were not related to HPV, are excluded.

All the data have been obtained from the clinical records and from the data-base of the Surgical Pathology Department, where the study was performed.

For the purpose of this study, lesions in uterine cervix caused by an HPV infection, demonstrated with the Genomica® kit, have been reviewed in three time periods encompassing years prior to the introduction of the vaccine (2002-2006), years when the vaccine had been implemented for a short time (2009-2011) and more recent years where the vaccine has been administered for more than 10 years and therefore more women should be covered by it (2020 and 2021).

All data have been stored in an Excel file and analysed with the SPSS 20.0 for Windows statistical package. We first have performed a descriptive analysis of our cases, with percentages for qualitative variables and mean/median for quantitative ones. Then we performed a Chi-squared test to evaluate the significance of the changes in the type of injuries of high grade caused by HPV in the different periods of time studied.

To fulfil the requirements settled in the Personal Data Protection rules in Spain, all the clinical data were anonymized. This study has been approved by the Institutional Review Board (IRB) of the hospital (approval number 23-011).

As secondary objectives, we have compared the main types of virus involved in high-risk lesions in the periods 2002-2006 vs 2020-2021 and finally we have compared the mean ages of high risk HPV-associated lesions in the different time periods.

## 3. Results

### 3.1. Period and Sample Studied

The number of biopsies with HPV related lesions in the different periods of time studied were; 116 biopsies for the first period (2002-2006), 386 biopsies for the second one (2009-2011) and finally 462 biopsies for the most recent period (2020-2021). Consequently, we have analysed a total of 946 women affected with HPV lesions.

### 3.2. Analysis of the Lesions Caused by HPV in the Different Time Periods

The analysis of the rate of lesions caused by HPV in our centre in the different periods of time is summarised in **Figures 1-3**.

Regarding low-risk lesions, CIN 1, an incidence range that reaches 61.76% in the years 2002-2006, 44.77% in 2009-2011 and 38% in the years 2020-2021 is verified. Considering high-risk lesions, CIN 2-3, its incidence reaches a maximum value of 29.41% in the first interval, 48.25% in the second, and finally 59.24% in the last period of time. On the other hand, the incidence of patients diagnosed

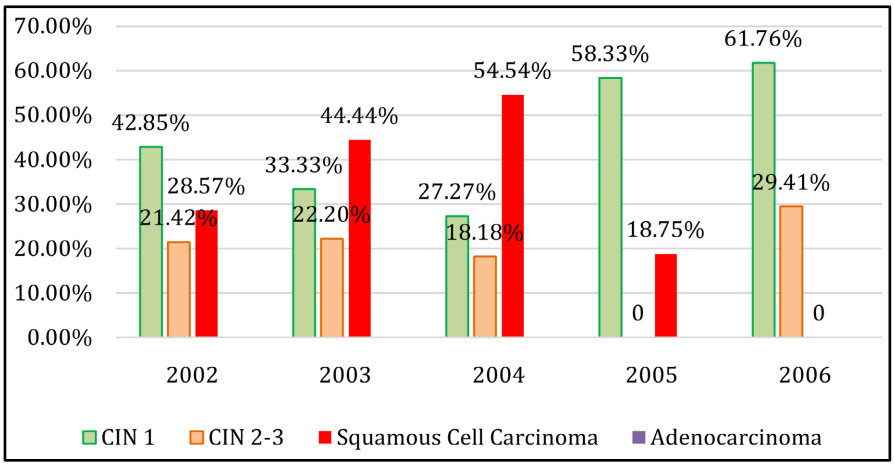


**Figure 1.** Rate of CIN 1, CIN 2-3, Squamous Cell Carcinoma and Adenocarcinoma between 2002 and 2006.

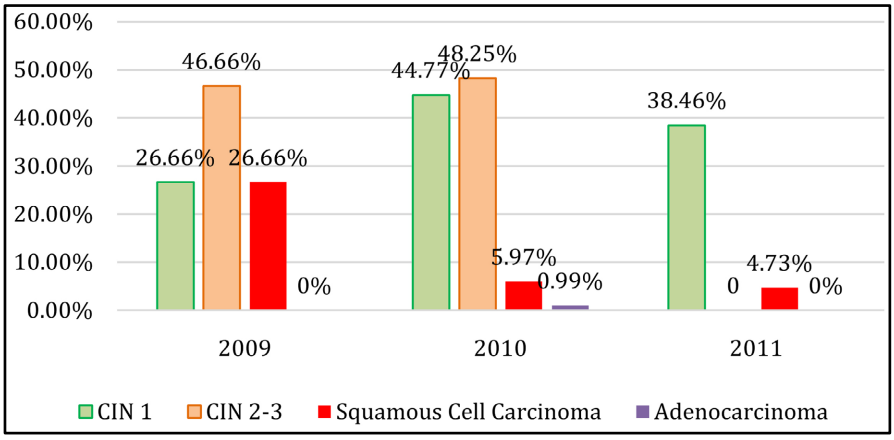


**Figure 2.** Rate of CIN 1, CIN 2-3, Squamous cell carcinoma and adenocarcinoma between 2009 and 2011.

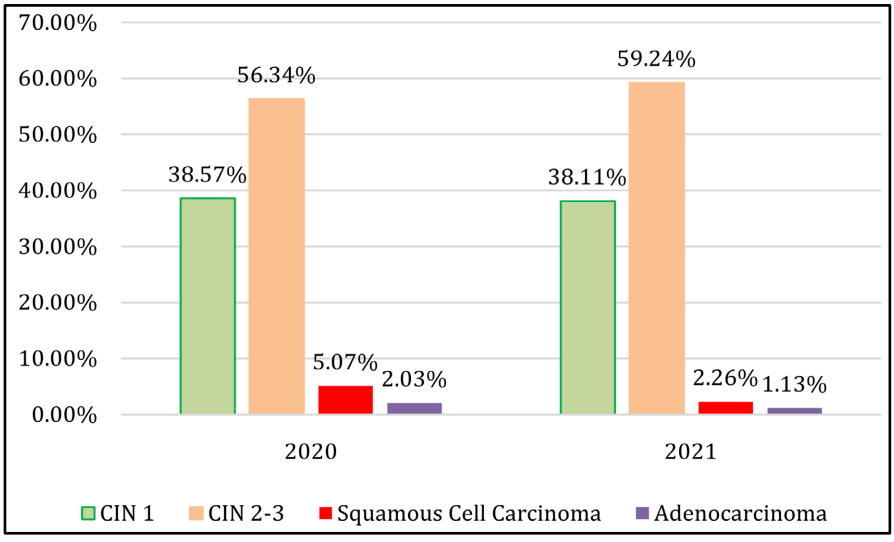


**Figure 3.** Rate of CIN 1, CIN 2-3, Squamous cell carcinoma and adenocarcinoma between 2020 and 2021.

with squamous cell carcinoma reached a maximum value of 54.54% in the years 2002-2006, 26.66% in the years 2009-2011 and 5.07% in the most recent years, 2020 and 2021. It is worth noting the absence of records in our series in the years 2002-2006 of adenocarcinoma and its diagnosis in the years 2009-2011 reaching 0.99% while in the years 2020 and 2021 it reached a maximum of 2.03%.

Chi-Squared test confirms a statistically significant difference in the rate of the different high grade lesions caused by HPV infection between the three time periods ($p < 0.05$).

The evolutionary trend of each type of lesion has been analysed in the different time periods of our study, showing a stable trend for CIN 1 and Adenocarcinoma, an upward trend of high-risk CIN 2-3 lesions and a decrease in the rate of Squamous Cell Carcinoma (**Figure 4**).

### 3.3. HPV Serotypes Determination

On the other hand, the different serotypes that caused lesions in our patients between the first study period prior to the vaccine (2002-2006) and the most current period after its introduction (2020-2021) have been analysed, the results of which are shown in **Figure 5** and **Figure 6**. We make a distinction between the usual high-risk serotypes, 16 and 18, which in the first time interval caused 47.85% of the lesions compared to 24% in the last period analysed, and other high risk serotypes less frequent, whose incidence varied from 20.24% in 2002-2006 to 40.42% in 2020-2021. The undetermined risk category originated 14.11% of the lesions in 2002-2006 and reached 28.34% in 2020-2021, while the low-risk serotypes were responsible for 17.79% of the lesions in the first time interval and 6.73% in the last one.

### 3.4. Mean Ages of the Patients with HPV Related Lesions

Likewise, **Figures 7-9** show the mean ages of the patients at the time of diagnosis

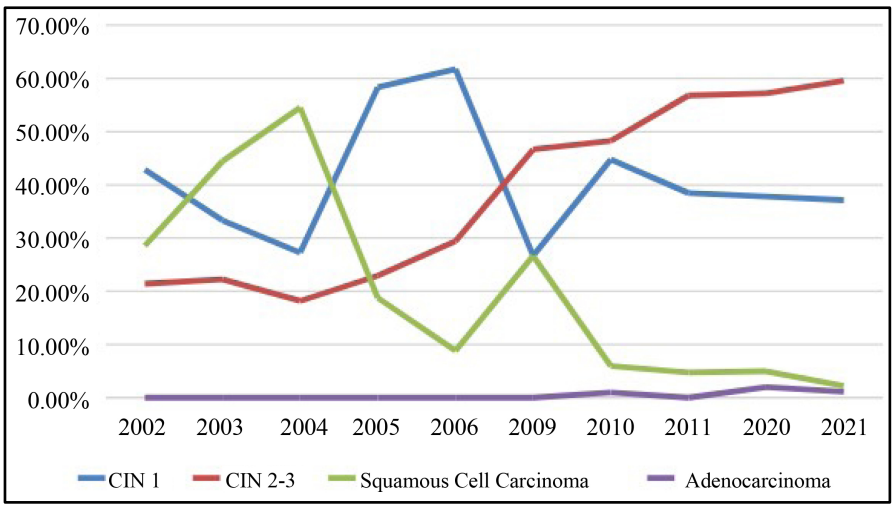


**Figure 4.** Evolution of CIN 1, CIN 2-3 lesions, Squamous cell carcinoma and adenocarcinoma over the time in our Hospital.

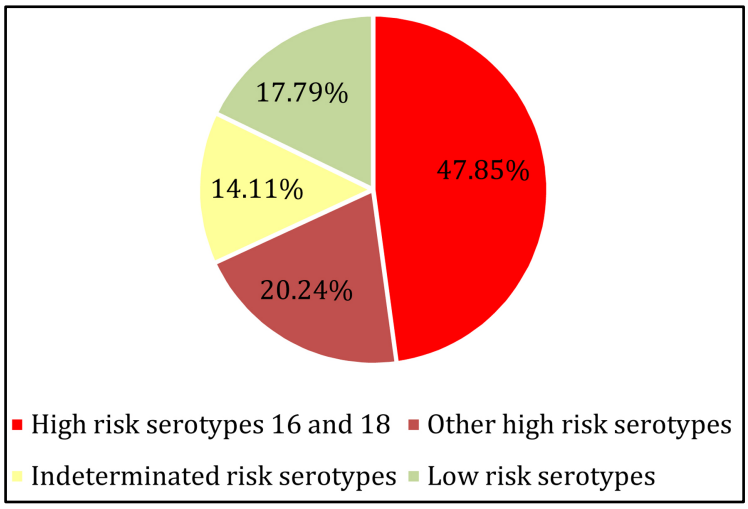


**Figure 5.** Incidence of different serotypes in 2002-2006.

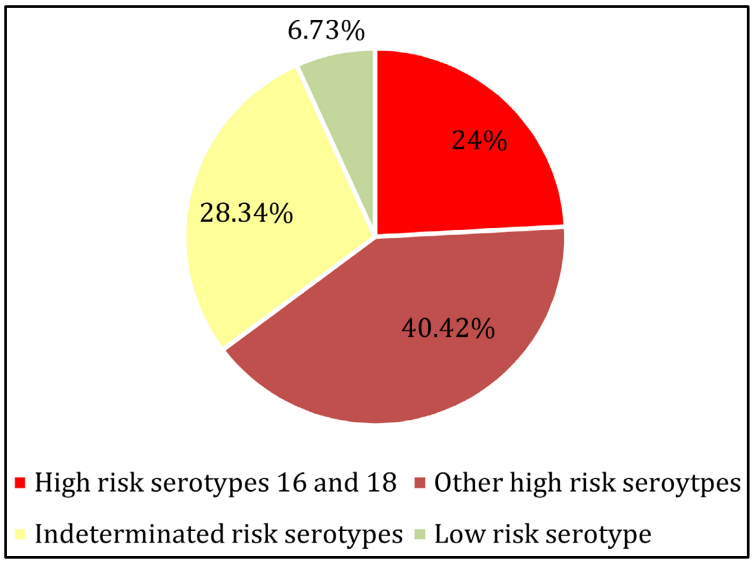


**Figure 6.** Incidence of different serotypes in 2020 and 2021.

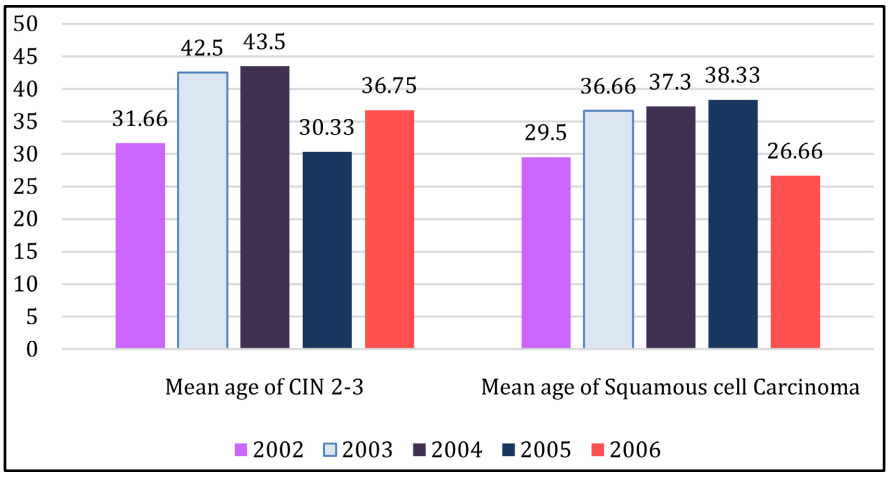


**Figure 7.** Mean ages of CIN 2-3 and squamous cell carcinoma between 2002-2006.

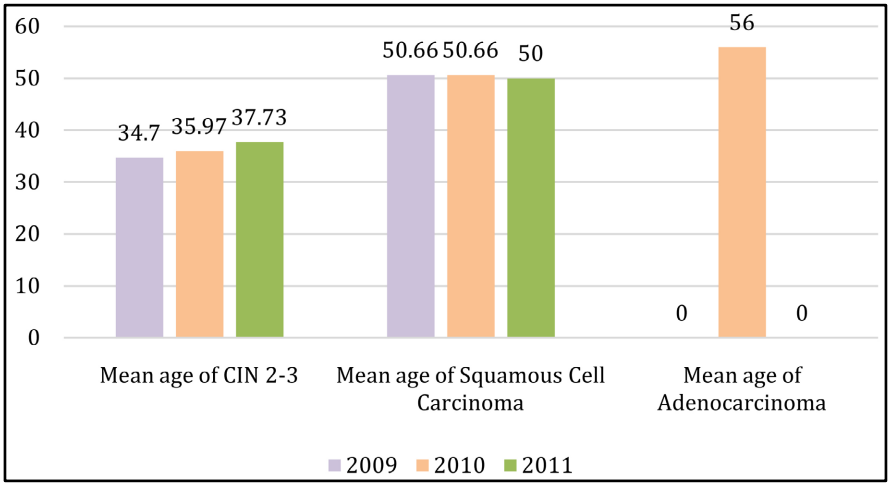


**Figure 8.** Mean ages of CIN 2-3, Squamous cell carcinoma, and adenocarcinoma between 2009-2011.

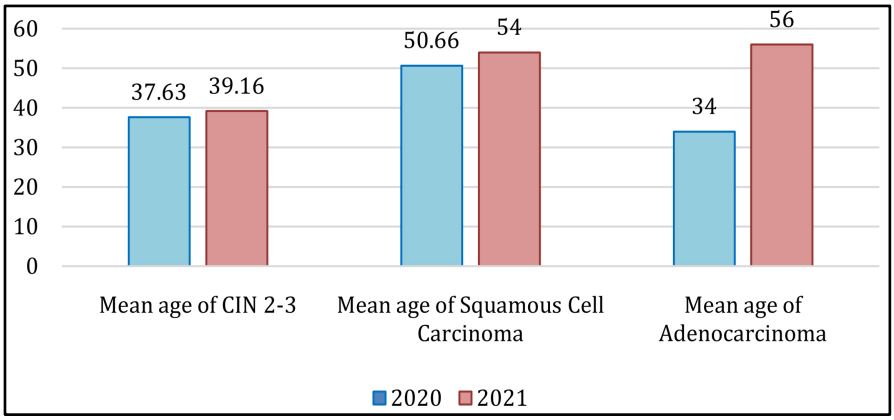


**Figure 9.** Mean ages of CIN 2-3, Squamous cell carcinoma, and adenocarcinoma between 2020-2021.

of high-grade lesions and cervical carcinoma were studied. It should be noted that in the first years studied, the mean age of diagnosis of CIN 2-3 is in women between 30.33 - 43.5 years, in 2009-2011 is 34.7 - 37.73 years, while in the years 2020-2021 the mean age is between 37.63 - 39.16 years. Squamous Cell Carcinoma was diagnosed between the ages of 26.66 and 38.33 in 2002-2006, remaining at approximately 50 years of age between 2009 and 2011 and fluctuating between 50.66 and 54 years of age in 2020-2021. Finally, no record of any Adenocarcinoma was obtained in the first time interval, however, in the years 2009-2011, those diagnosed correspond to patients with a mean age of 56.34 years in 2020 and 56 years in 2021.

## 4. Discussion

In the present study, an upward trend has been observed in recent years in the rate of uterine cervix carcinoma precursor lesions, CIN 2-3. This may be concerning as a negative trend would be expected following the introduction of the

vaccine in the population. However, it is important to consider characteristics and socio-demographic factors that influence HPV infection. In recent years in the Spanish population, there has been a shift in sexual behaviour, with sexual activity starting at earlier ages and an increase in the number of both stable and casual sexual partners [16] [17] [18]. Regarding this fact, it is particularly noteworthy the lack of information and knowledge about this infection among men, both regarding their own risk and the risk of transmitting the infection to their female partners, as well as the absence of HPV vaccination programs for males in the years analysed in the present study in Spain.

It is equally important to consider the immigration of patients from countries where adequate screening methods, sexual education, and social awareness may be lacking, as well as the implementation of vaccination in their healthcare systems, which allows equal access for women regardless of their economic conditions. On the contrary, the presence of high-risk lesions in patients who have received a complete and correct vaccination schedule may be due to infections previous to the vaccination, or infections caused by other high-risk serotypes, nowadays more prevalent than serotypes 16 and 18, as we demonstrate an increased trend in these virus infectivity rate. Similarly, the presence of lesions in these patients can be justified by the change in the infective trend of HPV serotypes, which, as observed in this study, experiences an approximate 50% decrease for the better-studied high-risk serotypes protected by both vaccines [16] [18], while, on the other hand, it increases by approximately 50% for other high-risk serotypes different from 16 and 18, as well as for those previously considered indeterminate risk. Both factors (socio-demographic and infective serotype changes) demonstrate the protective efficacy of well-established vaccines and highlight the need not only to develop vaccination strategies that include newly prevalent serotypes but also to conduct more studies to categorise with greater criteria and rigour those serotypes with indeterminate risk as either high or low risk.

Regarding the detection of low-risk serotypes in patients with precursor lesions of squamous cell carcinoma of the cervix, we understand that these are cases of co-infection with other serotypes, as no exclusive cases caused by low-risk serotypes were observed in our series of CIN 2-3 lesions. Likewise, a decrease in their infective rate has been observed, possibly due to the protection provided by the vaccine.

With Squamous cell carcinoma, we witnessed a decline both in its incidence and in the average age of diagnosis among patients over the past years. Increased population screening provides greater and early detection of precursor lesions of squamous cell carcinoma, through both cytology and subsequent histological studies, enabling earlier therapeutic management, either through surgical procedures such as conization or with topical medication in less severe cases. This would justify the decrease in its incidence in recent years. On the other hand, the average age of diagnosis in our series has increased from an average of 29.5 years in 2002 to 54 years in 2021, which can be attributed to various factors. These

factors include being women who, due to their age, did not enter the vaccination program implemented by the Spanish healthcare system and did not receive vaccinations on their own, not following a proper screening program, or again, being women of foreign nationalities.

As for Adenocarcinoma, the present research detects an incidence clearly lower than squamous cell carcinoma in all the years of study. However, it is necessary to consider that the pathogenesis of this type of neoplastic lesion is less related to HPV infection and may even be independent of it [19]. Additionally, the most well-known serotypes implicated in its aetiology are 16 and 18, serotypes that, once again, are better covered by the vaccines [20]. Regarding the average age of diagnosis for these lesions, a stable trend around 56 years has been observed in the analysed time periods. It is important to note the smaller number of patients with this type of lesion in our sample, with no cases recorded in the 2002-2006 interval, so this data may not be entirely representative of the rest of the population.

Another important point to consider is that we must highlight those patients in our series who had developed HPV-related lesions but tested negative for the implicated serotypes during molecular analysis at the time of diagnosis. In this regard, there are different hypotheses proposed in various studies.

HPV infection has been considered the primary cause of cervical cancer development, and different lines of research mainly focus on the involvement of this virus in its pathogenic mechanism. However, it has been highlighted in the literature that between 5% and 30% of patients with cervical lesions have a negative molecular HPV test result [21] [22]. In our sample, the percentage of patients with high-grade lesions and HPV-negative squamous cell carcinomas was 7.61%.

Several authors have suggested that these negative results are actually false negatives in high-risk HPV detection tests performed in Pap smears for squamous cell carcinomas. They justify this by hypothesising that it could be infections caused by virus genotypes not currently included in standard testing platforms, as well as variability in sampling, low viral load, decreased transcriptional activity, technical errors, or rapidly progressive cancers that develop between cervical cancer screening intervals [22]. A novel approach that could improve screening is to use viral load as a biomarker for triage, as there is a significant correlation between increased viral load and the severity of cervical lesions. However, further research is needed to determine optimal cutoff points and evaluate the feasibility and effectiveness of this strategy in different populations and clinical settings [23] [24]. In very few cases, squamous cell carcinoma in the cervix has been reported with truly negative high-risk HPV detection [22].

In 2022, Regauer S. *et al.* presented the first histological description of high-grade cervical lesions negative for HPV that were not associated with invasive squamous cell carcinoma. Initially, HPV negativity was defined as the absence of DNA from 32 HPV serotypes, absence of mRNA from E6/E7 of 14 HPV serotypes, and the absence of HPV sequences in Wide Genome Sequencing readings.

It was found that all these lesions occurred within the metaplastic squamous epithelium of the transformation zone, with an abrupt transition between the precursor lesion and the squamous epithelium of the exocervix. The majority resembled HPV-induced high-grade squamous intraepithelial lesions (HSIL) according to the WHO definition (undifferentiated basaloid proliferations), but some precursor lesions were highly keratinized proliferations similar to differentiated vulvar intraepithelial neoplasia, showing diffuse p16 staining.

In cases of keratinized invasive squamous cell carcinoma of the cervix negative for HPV infection, a surprisingly vascularized stroma was observed, along with a dense inflammatory infiltrate that included numerous stromal and intratumoral eosinophils. All these cases had clearly identifiable intraepithelial precursor lesions and lacked p16 staining.

On the other hand, Hanahan and Weinberg have proposed that the microenvironment and immune factors in vaginal and cervical secretions play a crucial role in the development of lesions in patients with negative high-risk CIN and HPV. Cervical neoplasms are complex biological behaviours, and their development is actually a process in which tumour cells interact with the body's environment [21]. The cervical immune microenvironment includes:

- Vaginal microecology, with its main components being Lactobacillus vaginalis and the microbial flora. Under normal circumstances, the vaginal microecology maintains a dynamic balance, but when this balance is disrupted, the number of lactobacilli decreases or their function is inhibited. The acidic vaginal environment is destroyed, hypoxia occurs, local immune function is reduced, the anti-tumor effect weakens, and cervical lesions are prone to occur. [21].
- The local vaginal immune system, which includes key players such as IL-2 and IL-10, representing cellular immunity, Th1 and Th2 cells that can enhance cellular immunity and suppress immune response, respectively, and secretory immunoglobulin A (SIgA) and immunoglobulin G (IgG), which are the main components of humoral immunity. It has been observed that the expression of IL-2 is decreased in cervical cancer and precancerous lesions, there is an increase in the level of IL-10, and a decrease in SIgA and IgG in vaginal infections. As the lesion progresses, IL-2 decreases, IL-10 increases, the Th1/Th2 ratio decreases, Th1 shifts to Th2, immunosuppression occurs, and cervical lesions develop [21].

However, due to the small number of precancerous lesions and HPV-negative invasive squamous cell carcinomas, many questions remain unanswered regarding their cellular pathogenesis, as well as the triggers and drivers of this abnormal proliferation and the natural history of HPV-negative squamous cell carcinoma [25]. Considering the potential role of the vaginal microenvironment, there is a need to work collaboratively and in parallel with the Microbiology services of hospitals to elucidate these associations, as well as the need to implement therapeutic measures that promote a healthy vaginal microenvironment in patients infected or with HPV-induced lesions.

Regarding our study, we consider that the lack of viral genome detection in samples with high-grade lesions may be due to technical errors, such as inadequate material, as well as all the aforementioned factors, without being able to confidently confirm any of these assumptions.

As limitations of the present study, we highlight that in the early years analysed, we found a considerably lower number of patients compared to subsequent years. This is due to the lack of computerization in our hospital during the years 2002-2006, its gradual implementation in the following years, and the incomplete transfer of medical records to the intranet of our Hospital. Additionally, during the same intervals of years, there were cases where we had no record of the HPV test results as they were conducted in other healthcare facilities, and therefore, we did not have access to them due to the lack of interconnectivity between centres at that time.

Lastly, it is noteworthy that this study includes the most recent data from the years 2020 and 2021, a period when Spain and the rest of the world were immersed in the global pandemic caused by COVID-19. This fact may have led to a decrease in the number of registered cases during those years, especially in 2020, as well as a delay in the diagnosis and follow-up of patients. However, since this study is conducted in 2023, it is not considered that enough time has passed for patients who were undiagnosed or not properly followed up in 2020 to have developed high-grade lesions or squamous cell carcinoma at present. However, surgical interventions of patients diagnosed with CIN 2-3 lesions in years prior to 2020 but after 2011 may have been delayed, but it would not affect the results of the present study.

As strengths, we would highlight the large sample size and extended study period, which have allowed us to draw statistically significant conclusions that support the effectiveness of current vaccines against different serotypes of human papillomavirus.

## 5. Conclusions

Our study confirms the effectiveness of the vaccines developed so far, against some of the HPV serotypes included in these, by verifying a decrease in the incidence of Squamous Cell Carcinomas in uterine cervix in our population, with an increase in the mean age of the same, as well as a change in the infective trend of HPV serotypes, as those high-risk viruses not covered by currently marketed vaccines are now more prevalent. Taking into account the impact of HPV infection in terms of Health and Public Health, not only in women, but also in men, and the carcinogenic risk that this infection entails, we emphasise the need to carry out more studies with the intention of correctly categorising those serotypes for which we still do not know their potential risk, as well as the importance of promoting new lines of research towards the creation of vaccines for the new serotypes that are currently most prevalent.

Another important point to highlight is that the increase in the incidence of

premalignant lesions (CIN 2-3) may be mainly due to two factors. On the one hand, an update and improvement of the population screening criteria and improvement in the diagnostic techniques used (liquid cytology). On the other hand, better knowledge about HPV infection and greater awareness on the part of the female population leads to an increase in the participation in population screening and detection of lesions before they reach the degree of carcinoma.

## Institutional Review Board Statement

The study was approved by the Institutional Review Board (or Ethics Committee) of Hospital Clínico San Carlos (protocol code 23-011 and date of approval: 28/2/2023).

## Informed Consent Statement

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

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## Conflicts of Interest

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

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