Human papillomavirus genotype attribution for HPVs 6, 11, 16, 18, 31, 33, 45, 52 and 58 in female anogenital lesions

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Abstract  Objective: Human papillomavirus (HPV) vaccines can potentially control cervical cancer and help to reduce other HPV-related cancers. We aimed to estimate the relative contribution (RC) of the nine types (HPVs 16/18/31/33/45/52/58/6/11) included in the recently approved 9-valent HPV vaccine in female anogenital cancers and precancerous lesions (cervix, vulva, vagina and anus).

Methods: Estimations were based on an international study designed and coordinated at the Catalan Institute of Oncology (Barcelona-Spain), including information on 10,575 invasive cervical cancer (ICC), 1709 vulvar, 408 vaginal and 329 female anal cancer cases and 587 Vulvar Intraepithelial Neoplasia grade 2/3 (VIN2/3), 189 Vaginal Intraepithelial Neoplasia grade 2/3 (VaIN2/3) and 29 Anal Intraepithelial Neoplasia grade 2/3 (AIN2/3) lesions. Consecutive histologically confirmed paraffin-embedded cases were obtained from hospital pathology archives from 48 countries worldwide. HPV DNA-detection and typing was performed by SPF10-DEIA-LiPA25 system and RC was expressed as the proportion of type-specific cases among HPV positive samples. Multiple infections were added to single infections using a proportional weighting attribution.

Results: HPV DNA prevalence was 84.9%, 28.6%, 74.3% and 90.0% for ICC, vulvar, vaginal and anal cancers, respectively, and 86.7%, 95.8% and 100% for VIN2/3, VaIN2/3 and AIN2/3, respectively. RC of the combined nine HPV types was 89.5% (95% confidence interval (CI): 88.8–90.1)-ICC, 87.1% (83.8–89.9)-vulvar, 85.5% (81.0–89.2)-vaginal, 95.9%...
1. Introduction

Infection with high risk (HR) human papillomavirus (HPV) is recognised as one of the major causes of infection-related cancers worldwide [1,2]. HPV infection is a well-established cause of invasive cervical cancer (ICC) and there is an increasing body of evidence strongly linking HPV DNA with other anogenital cancers (anus, vulva, vagina and penis) and head and neck cancers (particularly the oropharynx, base of tongue and tonsils) [3]. The vast majority of female HPV-related cancers are ICC cases (more than 85%). ICC is the fourth most common female malignancy worldwide, with an estimated 528,000 new cases and 266,000 new deaths in 2012, more than 95% attributable to HPV infection [4–7].

The other female anogenital cancers are less frequent than ICC, but cases HPV-related are also potentially preventable by vaccination. Approximately 88% of invasive anal cancer (IANc) cases, 70% of invasive vaginal cancer (IVuC) cases and 43% of invasive vulvar cancer (IVuC) cases are attributable to HPV infection [1,2]. However, recent data suggest that the HPV contribution in IVuC could be substantially lower, close to 30% [8]. HPV DNA prevalence has also been estimated at 94% of anal intraepithelial neoplasia (AIN) grades 2/3, 91% of vaginal intraepithelial neoplasia (VaIN) grades 2/3 and 85% of vulvar intraepithelial neoplasia (VIN) grades 2/3 lesions [9].

After HPV16, data confirm HPVs 31/33/45/52/58 caused up to 20% of ICC not covered by previous vaccines. The 9-valent HPV vaccine adds protection against five additional HPV types (HPVs 31/33/45/52/58) that caused up to 20% of ICC not covered by previous vaccines. The 9-valent HPV vaccine is as efficacious as quadrivalent HPV vaccine for the prevention of diseases caused by the four shared HPV types (HPVs 16/18/6/11). Several randomised clinical trials have assessed suitable safety, tolerability and immunogenicity profiles of the 9-valent HPV vaccine [14,15]. The 9-valent HPV vaccine would be a cost-effective alternative if it is proven to be highly effective and the additional cost per dose is not excessive compared to current HPV vaccines [16].

In order to evaluate its potential impact in the reduction of HPV-related disease burden and to help to formulate recommendations on HPV prevention, we aim to summarise existing HPV type distribution data for the specific nine types: HPVs 16/18/31/33/45/52/58/6/11, targeted by the 9-valent HPV vaccine across world regions.

2. Materials and methods

To estimate the relative contribution (RC) of the nine HPV types included in the recently approved 9-valent HPV vaccine in female anogenital cancer and precancerous lesions we used data from an international project on HPV-related cancers designed and coordinated by the Catalan Institute of Oncology (ICO) (Barcelona-Spain) in collaboration with DDL Diagnostic Laboratory (Rijswijk-Netherlands) [8,11,17,18].

2.1. Study design

The project is a retrospective cross-sectional study to estimate the HPV DNA prevalence and type distribution in women with ICC and other anogenital cancers. Case recruitment protocols were previously described [8,11,17,18]. Briefly, formalin-fixed paraffin-embedded (FFPE) specimens from consecutive cases were obtained from hospital pathology archives in 48 countries (Africa: Algeria, Mali, Mozambique, Nigeria, Senegal, Uganda; Americas: Argentina, Brazil, Chile, Colombia, Ecuador, Guatemala, Honduras, Mexico, Paraguay, Peru, and South America).

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Uruguay, USA, Venezuela; Asia and Oceania: Australia, Bangladesh, China, India, Israel, Japan, Kuwait, Lebanon, New Zealand, Philippines, South Korea, Taiwan, Thailand, Turkey; Europe: Austria, Belarus, Bosnia and Herzegovina, Croatia, Czech Republic, France, Greece, Germany, Italy, Netherlands, Poland, Portugal, Spain, Slovenia, United Kingdom). Information of age at diagnosis, year at diagnosis and original histological diagnosis was also available. The present article uses information from 10,575 ICC, 329 IAnC, 408 IVaC, 1709 IVuC, 29 AIN2/3, 189 VaIN2/3 and 587 VIN2/3 cases. No cases of precancerous cervical lesions were included in the study, so HPV type distribution for these lesions is not provided in this article. General characteristics of cases can be found in Table 1.

2.2. Pathology and laboratory procedures: paraffin block processing, histological evaluation and HPV-DNA detection and genotyping

Paraffin blocks were processed under strict conditions. At least, four paraffin sections were systematically obtained for each block (sandwich method). First and last sections were used for histopathological evaluation after haematoxylin and eosin staining. The intermediate sections were used for HPV DNA testing.

HPV DNA detection was done by polymerase chain reaction (PCR) with SPF-10 broad spectrum primers. The amplified PCR products were tested for the presence of HPV DNA using a DNA enzyme immunoassay (DEIA) that recognised at least 54 mucosal HPV genotypes. Amplimers testing positive for viral DNA by DEIA were genotyped with a reverse hybridisation line probe assay – LiPA25 that detects 25 HR and LR types 6/11/16/18/31/33/34/35/39/40/42/43/44/45/51/52/53/54/56/58/59/66/68/70/74 [19]. Sequence analysis was done to characterise HPV DEIA positive samples with unknown types. If no HPV type could be attributed after DNA sequencing, the HPV was labelled undetermined (Table 1). More detailed descriptions can be found in previous reports [8,11,17,18].

2.3. Statistical analysis

RC of the nine HPV types included in the 9-valent HPV vaccine in female anogenital cancers and precancerous lesions was expressed as the proportion of women positive for a given type among all HPV-positive samples. Type-specific information included information on multiple infections, that were added to single types in accordance with a proportional weighting attribution [20,21].

RCs by region, histology and age were determined. Subjects were classified into four geographical regions (Africa, America, Asia and Oceania and Europe).

Histological classification was grouped into the following categories: (1) squamous cell carcinoma (SCC). Subhistologies wary/basaloid only (SCC WB only), non-warty/basaloid only (SCC non-WB only) and variable percentages of warty/basaloid and nonwarty/basaloid features (SCC mixed) were available for anal, vaginal and vulvar cancer; (2) other histologies (including adenocarcinoma, adenosquamous cell carcinoma, undifferentiated, neuroendocrine, not otherwise specified, basal adenoid and cystic adenoid carcinomas).

Prevalence was also calculated for the nine types included in the 9-valent HPV vaccine. Prevalence was expressed as the proportion of women positive for a given type among all tested samples.

The attributable fraction (AF) for the nine HPV types was estimated globally, using statistics on estimated cancer incidence previously calculated by De Martel et al. [1] and Forman et al. [2]. We assumed that presence of oncogenic types is necessary for the development of cervical and anal cancer, so the contribution of HPV types altogether is more than 95% and therefore, we used the RC as AF [6,7,22,23]. For the vagina and vulva, we assume that not all cancer cases are attributable to HPV so we use the presence of HPV DNA in cancer cases as AF.

To evaluate the differences among proportions we used the most suitable test (Chi Square test, Fisher test), adjusting for multiple comparisons (Bonferroni-Holms) when necessary. Evaluations of trends by age were determined by trend test analysis for proportions. Statistically significant p-value was set at 0.05.

3. Results

HPV DNA prevalence was 84.9% in ICC, 90.0% in IAnC, 74.3% in IVaC, 28.6% in IVuC, 100.0% in AIN2/3, 95.8% in VAIN2/3 and 86.7% in VIN2/3. Most cases were SCC from Europe and the Americas. Percentage of multiple infections was higher for precancerous lesions than for cancer (Table 1).

Worldwide, combined RC of the nine HPV types (HPVs 16/18/31/33/45/52/58/6/11) was 89.5% in ICC, 95.9% in IAnC, 85.3% in IVaC, 87.1% in IVuC, 86.2% in AIN2/3, 78.7% in VaIN2/3 and 94.1% in VIN2/3 (Table 2).

The comparison of prevalence versus RC data of the combined nine HPV types showed that the lowest prevalence was observed in cases with IVuC (24.0%), compared to IVaC (63.3%), ICC (76.0%) and IAnC (86.3%). For precancerous lesions, combined nine HPV prevalence was 75.4% in VAIN2/3, 81.6% in VIN2/3 and 86.2% in AIN2/3 (Annexes Fig. A.1).

Relative contribution of HPVs 16/18 was especially prominent in IAnC (87.0%), although it was due to the high contribution of HPV16 (83.4%). Combined RC of HPVs 16/18 was 63.7% in IVaC, 72.6% in IVuC and 70.8% in ICC. Contributions in
<table>
<thead>
<tr>
<th>Region (Region)</th>
<th>Test Cases (N)</th>
<th>HPV+</th>
<th>Total Single Inf.</th>
<th>Multiple Inf.</th>
<th>HPV Undet.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC Eu (22), Am (41), Af (7), AsOc (30) 1940–2009 SCC (90), ADC (7), ADSC (2), Other (1)</td>
<td>10,575</td>
<td>8,338 (92.9)</td>
<td>8977 (84.9)</td>
<td>52 (0.6)</td>
<td></td>
</tr>
<tr>
<td>IAnC (F) Eu (34), Am (54), Af (3), AsOc (9) 1990–2010 SCC WB only (63), SCC non-WB only (29), SCC Mixed (6)</td>
<td>329</td>
<td>284 (95.9)</td>
<td>296 (90.9)</td>
<td>30 (10.3)</td>
<td></td>
</tr>
<tr>
<td>IVaC Eu (37), Am (47), Af (5), AsOc (11) 1940–2011 SCC WB only (2), SCC non-WB only (51), SCC Mixed (4)</td>
<td>408</td>
<td>290 (71.3)</td>
<td>284 (95.9)</td>
<td>30 (7.9)</td>
<td></td>
</tr>
<tr>
<td>IVuC Eu (53), Am (22), Af (1), AsOc (24) 1980–2011 SCC WB only (19), SCC non-WB only (72), SCC Mixed (6)</td>
<td>1709</td>
<td>438 (25.6)</td>
<td>438 (25.6)</td>
<td>30 (17.6)</td>
<td></td>
</tr>
<tr>
<td>AIN2/3 (F) Eu (62), Am (21), Af (0), AsOc (17) 1990–2009</td>
<td>29</td>
<td>24 (82.8)</td>
<td>24 (82.8)</td>
<td>5 (17.2)</td>
<td></td>
</tr>
<tr>
<td>VAIN2/3 Eu (51), Am (42), Af (0), AsOc (7) 1990–2011</td>
<td>189</td>
<td>159 (87.8)</td>
<td>159 (87.8)</td>
<td>20 (11.0)</td>
<td></td>
</tr>
<tr>
<td>VIN2/3 Eu (53), Am (22), Af (1), AsOc (25) 1990–2011</td>
<td>587</td>
<td>509 (86.7)</td>
<td>509 (86.7)</td>
<td>46 (9.0)</td>
<td></td>
</tr>
</tbody>
</table>

'SD': Standard Deviation; 'N': Number of cases; 'inf': infections; 'undet': undetermined; 'ICC': Invasive Cervical Cancer; 'IAnC': Invasive Anal Cancer; 'IVaC': Invasive Vaginal Cancer; 'IVuC': Invasive Vulvar Cancer; 'F': Female; 'AIN2/3': Anal Intraepithelial Neoplasia grade 2/3; 'VaIN2/3': Vaginal Intraepithelial Neoplasia grade 2/3; 'VIN2/3': Vulvar Intraepithelial Neoplasia grade 2/3; 'Eu': Europe; 'Am': Americas; 'Af': Africa; 'AsOc': Asia and Oceania; 'SCC': Squamous cell carcinoma; 'ADC': Adenocarcinoma; 'ADSC': Adenosquamous cell carcinoma. Additional information: Mixed histology includes SCC with variable proportions of warty/basaloid and non-warty/basaloid features. Available data are from unvaccinated women (pre-vaccination period). No data are available for precancerous cervical lesions. For more details, please see methodological sections from de Sanjose et al., Lancet Oncol 2010; de Sanjose et al., Eur J Cancer 2013; Alemany et al., Int J Cancer 2015; Alemany et al.; Eur J Cancer 2007, 2009, 2013.
Table 2
Worldwide relative contribution of HPVs 16/18/31/33/45/52/58/6 and 11 in female anogenital lesions that tested positive for HPV DNA.

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Female anogenital lesions</th>
<th>High grade precancerous lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cancer</td>
<td>Vulva (N = 488)</td>
</tr>
<tr>
<td></td>
<td>Cervix (N = 8,977)</td>
<td>RC% (95%CI)</td>
</tr>
<tr>
<td></td>
<td>Anus (N = 296)</td>
<td>RC% (95%CI)</td>
</tr>
<tr>
<td></td>
<td>Vagina (N = 303)</td>
<td>RC% (95%CI)</td>
</tr>
<tr>
<td></td>
<td>Vulva (N = 488)</td>
<td>RC% (95%CI)</td>
</tr>
<tr>
<td>Combined nine HPV types*</td>
<td>89.5 (88.8–90.1)</td>
<td>86.2 (68.3–96.1)</td>
</tr>
<tr>
<td></td>
<td>HPVs 16/18</td>
<td>95.9 (93.0–97.9)</td>
</tr>
<tr>
<td></td>
<td>70.8 (69.9–71.7)</td>
<td>70.4 (66.9–73.9)</td>
</tr>
<tr>
<td></td>
<td>HPVs 31/33/45/52/58</td>
<td>18.5 (17.7–19.4)</td>
</tr>
<tr>
<td></td>
<td>HPV 16</td>
<td>60.6 (59.6–61.6)</td>
</tr>
<tr>
<td></td>
<td>10.2 (9.6–10.9)</td>
<td>10.6 (9.9–11.2)</td>
</tr>
<tr>
<td></td>
<td>HPV 18</td>
<td>3.7 (3.3–4.1)</td>
</tr>
<tr>
<td></td>
<td>3.0 (2.6–3.4)</td>
<td>3.0 (2.5–3.5)</td>
</tr>
<tr>
<td></td>
<td>HPV 31</td>
<td>3.8 (3.5–4.3)</td>
</tr>
<tr>
<td></td>
<td>3.1 (2.8–3.5)</td>
<td>3.1 (2.8–3.5)</td>
</tr>
<tr>
<td></td>
<td>HPV 33</td>
<td>5.9 (5.6–6.4)</td>
</tr>
<tr>
<td></td>
<td>5.0 (4.6–5.4)</td>
<td>5.0 (4.6–5.4)</td>
</tr>
<tr>
<td></td>
<td>HPV 45</td>
<td>2.8 (2.5–3.2)</td>
</tr>
<tr>
<td></td>
<td>2.0 (1.7–2.4)</td>
<td>2.0 (1.7–2.4)</td>
</tr>
<tr>
<td></td>
<td>HPV 52</td>
<td>2.3 (2.0–2.6)</td>
</tr>
<tr>
<td></td>
<td>1.0 (0.7–1.4)</td>
<td>1.0 (0.7–1.4)</td>
</tr>
<tr>
<td></td>
<td>HPV 6</td>
<td>0.1 (0.1–0.2)</td>
</tr>
<tr>
<td></td>
<td>0.3 (0.0–1.1)</td>
<td>0.3 (0.0–1.1)</td>
</tr>
<tr>
<td></td>
<td>HPV 11</td>
<td>0.0 (0.0–0.1)</td>
</tr>
<tr>
<td></td>
<td>0.2 (0.1–0.3)</td>
<td>0.2 (0.1–0.3)</td>
</tr>
<tr>
<td></td>
<td>Other HPV types</td>
<td>10.5 (9.9–11.2)</td>
</tr>
<tr>
<td></td>
<td>4.1 (2.1–7.0)</td>
<td>4.1 (2.1–7.0)</td>
</tr>
</tbody>
</table>
|                   | RC: Relative Contribution; '95%CI': 95% Confidence Interval (one-sided, 97.5%CI calculated when appropriate); 'N': number of cases that tested positive for HPV DNA; 'HPV': Human Papillomavirus; 'nine HPV types' includes the ones in 9-valent HPV vaccine; HPVs 16/18/31/33/45/52/58/6/11. Additional information: Available data are from unvaccinated women (pre-vaccination period). No data are available for precancerous cervical lesions. Type specific RC estimations: Numerator = single infections + proportional attribution of multiple types; Denominator = HPV/DNA positive cases (for more details, please see methodological section from de Sanjó et al., Lancet Oncol 2010; de Sanjó et al., Eur J Cancer 2013;Alemany et al., Int J Cancer 2015; Alemany et al., Eur J Cancer 2014 [8,11,17,18]).
pre-cancerous lesions were 82.8%, 63.8% and 79.8% in AIN2/3, VaIN2/3 and VIN2/3, respectively, with HPV16 also being the dominant type (Table 2).

Worldwide, the additional contribution of HPVs 31/33/45/52/58 was higher in ICC (18.5%) and IVaC (20.3%) than in IAnC (7.9%) and IVuC (13.0%). The additional contribution in precancerous lesions was 3.4% in AIN2/3, 13.5% in VaIN2/3 and 13.0% in VIN2/3 (Fig. 1).

Regional variations were observed. HPV16 was the dominant type in female anogenital cancer and precancerous lesions in all geographical regions. Within ICC, RC of HPV16 ranged from 47.7% in Africa to 65.5% in Europe; in IAnC from 33.3% in Africa to 92.0% in Asia and Oceania; in IVaC from 46.2% in Africa to 66.6% in Europe; and in IVuC from 63.9% in the Americas to 82.4% in Africa. HPV16 was the most frequently detected type after HPV16 in cervical and anal cancer lesions (10.2% and 3.6% respectively), with the highest contributions in Africa (22.6% in ICC and 16.7% in IAnC). HPV31 was the second most frequently detected type in vaginal cancer (5.3%), with the highest contributions observed in Africa (7.7%) and the lowest in Europe (2.8%). Finally, within vulvar cancer lesions, HPV33 was the second most common type, ranging from 0.0% in Africa to 6.9% in America (Annexes Table A.1).

Although cumulative RC seemed to vary by region in all female anogenital lesions, differences were not significant when comparing regions with world data (p > 0.05). The only exception was observed for the combined RC of the nine HPV types in ICC in Asia and Oceania (p < 0.05) (Fig. 1).

Regarding HPV contribution by histology; HPV16 was the prominent type in all histological categories in all cancer sites. In ICC, the combined RC of the nine HPV types was significantly higher (p < 0.05) in histologies different to SCC, 89.1% versus 94.0% (more detailed histological data in De Sanjose et al. [11]. For other anogenital cancers variations in the contribution were observed by histology, ranging from 60.0% in vaginal cancer with SCC mixed histology, to 100.0% in anal cancer for the same histology (Annexes Tables A.3 and A.4). Differences were not statistically significant, with the exception of vulvar cancer, where the combined RC of the nine types and the combined RC of HPVs 16/18 was higher for SCC WB only than for SCC non-WB (p < 0.05).

Global RC of the nine HPV types by cancer lesion and age is summarised in Fig. 2. No specific age trends were observed, except in ICC, where the RC of the nine types altogether decreased with age (p-value <0.001); mainly explained by the decrease of HPVs 16/18/45 in older ages (more detailed data in Serrano et al. [24]. When prevalence data were analysed instead of RC data, it was observed that the prevalence of the combined nine HPV types altogether decreased with age in all female anogenital lesions, although only significant
in ICC (p-value < 0.001), IAnC (p-value = 0.050) and IVuC (p-value < 0.001) (Annexes Fig. A.2). Specifically, in IVuC we observed a changing trend of the prevalence of the nine HPV types altogether, being flat from younger ages until 45-49 years (p-value = 0.500) and decreasing significantly in ages older than 50 years (p-value < 0.001).

If we assume that 100% of cervical and anal cancer cases are attributable to HPV infection, but not all vaginal and vulvar ones are attributable to HPV, the 9-valent HPV vaccine could prevent close to 470,000 cervical, 12,400 anal, 5700 vaginal and 3000 vulvar cancer cases, accounting for 87.4% of female anogenital cancers [1] (Table 3).

4. Discussion

We estimated the contribution of the nine types included in the recently approved 9-valent HPV vaccine across HPV-positive female anogenital lesions, by geographical region, histology and age. The inclusion of five additional HR types, HPVs 31/33/45/52/58, to those covered by current licenced HPV vaccines, would increase the protection to almost 90% of the infections responsible for cervical, 96% for anal, 85% for vaginal and 87% for vulvar cancers and also a high percentage of precancerosis lesions (86%, 79% and 94% for AIN2/3, VaIN2/3 and VIN2/3 respectively). The incremental HPV-related preventable fraction added by the 9-valent HPV vaccine ranged from 7.9% in anal cancer to 20.3% in vaginal cancer.

The fact that HPV-related anal, vaginal and vulvar cancers share similar risk factors and aetiology to ICC, make them potentially preventable by vaccination [1]. Moreover, the incidence of some of these diseases, especially anal and cervical cancers, seems to have increased during the last decades probably linked to several risk factors such as changes in sexual behaviour and changes in cervical cancer screening policies. For vaginal and vulvar cancer, it is difficult to estimate changes in incidence trends due to the small number of cases involved [2,25-28].

Table 3
Burden of female anogenital cancer cases attributable to HPV infection by the nine types included in the 9-valent HPV vaccine.

<table>
<thead>
<tr>
<th>Cancer site</th>
<th>HPV AF (%)</th>
<th>Nine HPV types AF (%)</th>
<th>Burden</th>
<th>Cases attributable to the nine HPV types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervix</td>
<td>100.0</td>
<td>89.5</td>
<td>528,000</td>
<td>472,560</td>
</tr>
<tr>
<td>Anus</td>
<td>100.0</td>
<td>95.9</td>
<td>13,000</td>
<td>12,467</td>
</tr>
<tr>
<td>Vagina</td>
<td>74.3</td>
<td>63.3</td>
<td>9000</td>
<td>5697</td>
</tr>
<tr>
<td>Vulva</td>
<td>28.6</td>
<td>24.9</td>
<td>12,000</td>
<td>2988</td>
</tr>
</tbody>
</table>

AF: Attributable Fraction; ‘HPVs’: Human Papillomavirus.

Additional information: For cervix and anus, we assume that almost 100% of cancer cases are caused by HPV infection so we used the relative contribution as AF. For vagina and vulva, we assume that not all cancer cases are attributable to HPV so we use the prevalence of HPV infection as AF. Estimated numbers of new cancer cases are based on Globocan 2012 for cervix and on Forman et al., Vaccine 2012, for anal, vaginal and vulva cancers [2,4].
Similar to previous data, overall HPV prevalence was lower for vulvar and vaginal cancers, with only 28.6% and 74.3% of IVuC and IVaC positive for HPV DNA [1,9,29]. Therefore, the 9-valent HPV vaccine could prevent 24.9% and 63.3% of vulvar and vaginal cancers cases respectively. By contrast, the presence of oncogenic HPV types is necessary for the development of cervical and anal cancers, so the contribution of HPV types altogether is almost 100% and the 9-valent HPV vaccine is expected to prevent more than 90% of the cases of both cancers [14]. For precancerous lesions, a high prevalence of HPV is detected for VaIN2/3 (95.8%), VIN2/3 (86.7%) and AIN2/3 (100.0%). Results are similar to previous studies [1,9,30].

Data confirm the major contribution of HPV16. Other HPV types, such as HPVs 18/31/33/45, usually involved in cervical cancer, seem to also be important in non-cervical female anogenital neoplasias, although at a lesser extent. The lowest contribution is registered for anal cancer, with less than 17% of the cases related to non-HPV16 types. The increased contribution of HPV16 in IAnC compared to other sites may reflect a differential tropism of HPV16 towards anal mucosa, and a higher capacity to lead to malignant transformation [9,17,27]. HPV45 was enriched in cancer lesions compared to high-grade ones. Similar to ICC, it might be the result of the rapid progression and integration of HPV45 [11,31].

We observed a higher contribution of HPVs 31/33/45/52/58 in Africa compared to other regions in anal and vaginal cancers, but the differences were not statistically significant ($p > 0.05$) and based on a small number of cases. A higher contribution of these types was observed in the Americas and Asia and Oceania in cancers of the cervix and vulva, but differences were only significant for the comparison of ICC in Asia and Oceania compared to global estimates. Further research is needed to provide solid estimations by region especially among non-cervical female anogenital lesions.

RC by age groups was evaluated for the nine HPV types altogether. Analysis for individual types was not possible. The lower contribution of the nine HPV types by age is mainly due to the lower contribution of HPV16. HPV16 related tumors, but also HPV18 and 45, are more aggressive and cause tumors or precancerous lesions that are detected at an earlier age, while some other carcinogenic HPV genotypes might develop tumors at later ages [32]. Although in our study, overall RC decreased with age in all female anogenital cancers, the low number of cases included for anogenital sites other than cervix can lead to instability in the estimates. Only a clear decreasing trend with age can be assessed for cervical cancer driven by the decrease in older ages of HPV 16/18/45 [24]. Regarding prevalence instead of RC, the combined prevalence of the nine HPV types decreased with age in all female anogenital lesions, although it was only significant in cervical cancers, vulvar cancers from women older than 50 years and slightly significant in anal cancers. Although data on time trends of vulvar cancer are scarce, it should be remarked that the majority of vulvar cancer cases diagnosed in late adulthood are keratinising SCC that are largely HPV unrelated, while cases diagnosed in younger women, generally SCC WB, are associated with HPV DNA detection (75–100%) [3,8,9,27].

This is the largest study on HPV contribution in female anogenital cancers and precancerous lesions worldwide, using standardised protocols and centralised HPV testing technology. The good concordance of HPV estimations with previous reports suggests the representativeness of the data [9,29]. Assessment of multiple infections was possible, adding multiple infections to single types in accordance with a proportional weighting attribution [20,21]. A decrease of multiple infections with neoplastic disease progression was observed [9,33]. It may be explained by the selection of the most carcinogenic types and the clearance of those that are less carcinogenic during the tumourigenic process. In the study, 15% of ICC and 10% of anal cancer lesions were HPV negative. After several verifications, it was assumed that HPV negativity could be attributable not only to technical artefacts, but also to the quality of the biological specimen. However, we cannot safely ignore the possibility that a small proportion of cases of invasive cervical cancer, perhaps in the group with adenocarcinomas, might arise independent of HPV exposure [11].

Finally, in a subset of invasive cases in the study, the highly sensitive HPV DNA detection and genotyping system (SPF-10/DEIA/LiPA25) was used together with $p16^{INK4a}$ expression evaluation, a cellular surrogate marker for HPV associated transformation that is detected in HPV associated tumors but nearly absent in HPV unrelated ones. An over expression of $p16^{INK4a}$ was observed in 95% of HPV positive IAnC, 98% ICC, 88% IVuC and 87% IVaC. It strongly points towards an aetiological implication of the virus in the oncogenic process, highlighting the utility of the HPV vaccines in the prevention of female anogenital lesions [8,17,18,34].

In conclusion, our results suggest that an effective HPV vaccination programme with the 9-valent HPV vaccine could substantially reduce the incidence rates of female cervical cancer, but also a significant proportion of anal and vaginal cancers, approximately a quarter of vulvar cancers and a large part of high grade precancerous lesions (AIN2/3, VaIN2/3 and VIN2/3).

Conflict of interest statement

The following facts may be considered as potential conflicts of interest. BQ and ST: Institutional support:
HPV vaccine trials and epidemiological studies sponsored by GlaxoSmithKline, Merck and Sanofi Pasteur MSD. BS and LA: Institutional support: HPV vaccine trials and epidemiological studies sponsored by GlaxoSmithKline, Merck and Sanofi Pasteur MSD. Personal support: Travel grants to conferences occasionally granted by Merck and Sanofi Pasteur MSD. NM: member of the Merck HPV Global Advisory Board. XB: Institutional support: HPV vaccine trials and epidemiological studies sponsored by GlaxoSmithKline, Merck and Sanofi Pasteur MSD, Qiagen and Roche. Personal support: Travel grants to conferences/symposia/meetings are occasionally granted by GlaxoSmithKline, Merck, Sanofi Pasteur MSD, Roche or Qiagen. SS: Institutional support: HPV vaccine trials and epidemiological studies sponsored by GlaxoSmithKline, Merck, Roche and Sanofi Pasteur MSD. Personal support: Travel grants to conferences/symposia/meetings are occasionally granted by GlaxoSmithKline, Merck, Sanofi Pasteur MSD and Qiagen.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejca.2015.06.001.

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