**Title: Looking beyond human papillomavirus (HPV) genotype 16 and 18: defining HPV genotype distribution in cervical cancers in Australia prior to vaccination**

**Short title:** Defining HPV genotype distribution in Australian cervical cancers

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Abbreviations: AC: adenocarcinoma; bivalent HPV vaccine: 2vHPV; CI: confidence interval; DNA: deoxyribonucleic acid; FFPE: formalin-fixed paraffin-embedded; H&E: haematoxylin and eosin; HPV: human papillomavirus; HRHPV: “high-risk” human papillomavirus; LCM: laser capture microdissection; LRHPV: “low-risk” human papillomavirus; 9vHPV: nonavalent HPV vaccine; PCR: polymerase chain reaction; PEN: polyethylene naphthalate; 4vHPV: quadrivalent HPV vaccine; SCC: squamous cell carcinoma; WTS: whole-tissue section.

Article category: Cancer epidemiology

Brief description: To help determine the potential impact of cervical cancer vaccines in Australia, we report a baseline analysis of HPV genotypes in 847 Australian cancers using LCM to ensure correct genotype attribution to each lesion. This is the first study to utilize this definitive method for a whole of country study. Compared to previous international studies, we detected higher HPV 16/18 positivity (71.8%) and lower positivity for HPV 31/33/45/52/58 (14.8%), with 7.1% of cancers HPV negative.

**Abstract:**

Australia has implemented a high coverage HPV vaccination program but has not to date established the distribution of HPV types that occur in cervical cancers in Australia. This information is important for determining the potential for cervical cancer prevention with both current and broader spectrum HPV vaccines. We analysed 847 cervical cancers diagnosed 2005-2015 in tertiary centres in the three most populous Australian states with resolution of specimens containing multiple HPV types using laser-capture microdissection. Archived FFPE tissue was reviewed by specialist pathologists, sandwich sectioned, and initially whole tissue sections genotyped for HPV. Samples were first genotyped using SPF10-LiPA25. Negative samples were screened with DNA ELISA kit HPV SPF10, followed by genotyping with SPF+ LiPA if ELISA positive. If still negative, samples were tested on a qPCR assay targeting the E6 region of HPV16,18, 45 and 33. Of the 847 cancers (65.1% squamous, 28.7% adenocarcinoma, 4.3% adenosquamous, 2.0% other), 92.9% had HPV detected. Of the HPV positive cancers, 607/787 (77.1%) contained HPV16 or 18, 125/787 (15.9%) contained HPV31/33/45/52 or 58, and 55 (7.0%) another HPV type. There was a strong correlation between HPV type and age, with younger women most likely to have HPV16/18 detected and least likely HPV negative. Our findings indicate that cervical cancers diagnosed in Australia more frequently contain HPV16/18 than in international series. This could be due to cervical screening in Australia increasing the proportion of adenocarcinomas, in which types 18 and 16 more strongly predominate, due to prevention of squamous cancers.

**Introduction:**

New cases of cervical cancer are detected annually in over half a million women worldwide with approximately 260,000 deaths globally and close to 90% of these occurring in less developed countries (1). The causal role of human papillomavirus (HPV), particularly those defined as the most oncogenic or high risk (HRHPV) genotypes, in the development of cervical cancer is well established (2, 3). Two genotypes, HPV 16 and 18, are detected in approximately 70% of cervical cancers (4), with infection and related disease caused by these two types now effectively preventable by current generation HPV prophylactic vaccines (the bivalent (2vHPV), quadrivalent (4vHPV) and nonavalent (9vHPV) vaccines). The recently registered 9vHPV vaccine provides protection against the four HPV types covered by the 4vHPV vaccine (6,11,16,18) as well as the next most common oncogenic types detected in cervical cancers globally – HPV types 31,33,45,52 and 58. (5) Decisions regarding the potential use of 9vHPV vaccine in each country will rely upon an understanding of the incremental benefits in disease prevention that the vaccine can provide.

In 2007, Australia was the first country to implement a fully government- funded population- based HPV vaccination program. The program initially provided mass catch-up vaccination to females aged 12–26 years using a three-dose course of the 4vHPV vaccine. From 2009 an ongoing program vaccinated females in the first year of high school at age 12-13, with the program extended to include 12–13 year old males in 2013, with a 2-year catch up vaccination program for males aged 14–15 years. Overall, a high vaccine coverage has been achieved, with highest rates achieved within the school based cohorts, with over 70% receiving all three doses (6, 7).

In 2013, the Australian government endorsed an HPV Surveillance Plan to monitor the impact of HPV vaccination in Australia (8). The plan outlined key HPV surveillance objectives, including monitoring the impact of vaccination on circulating HPV genotypes and HPV related disease, including cervical cancer. Consequent upon Australia's widely targeted, early and well accepted HPV vaccination program, Australia was the first country to document declines in genital warts (9, 10), cervical abnormalities (11, 12) and HPV prevalence in young women (13, 14). Changes to cervical cancer rates and the associated HPV genotypes present in the cancers will take longer to establish, but may become evident in young women in the next 5 to 10 years.

A global meta-analysis of 30,848 cervical cancer cases found that over 70% of cervical cancers were associated with HPV 16 and 18 and approximately 20% were associated with one or more of the five additional types (31,33,45,52,58) to be covered by the 9vHPV vaccine (15). An Australian meta-analysis of smaller studies, containing 553 specimens in total, demonstrated slightly higher (80%) prevalence of HPV 16 or 18 and significantly lower (approximately 10%) prevalence of the 5 additional types present in 9vHPV compared to the large international meta-analysis (16). Similarly, 170 Australian specimens included in another recent international analysis of 8,977 HPV positive cervical cancers (17) detected lower proportions of the additional five HPV types included in the nine valent vaccine (31,33,45,52,58) (8.8% vs 18.5%) and a higher proportion with HPV16/18 (78.0% vs 70.8%). Although it has been established that a single genotype is the causative agent for individual cervical squamous lesions and cervical cancers (18, 19), simultaneous detection of more than one type occurs in up to 15% of cervical cancers (15). Methods for attributing HPV types in cervical cancers

when more than one type is detected have varied from simply including all types detected (i.e. attributing more than one type to each lesion) or using a variety of ranking or proportional assignment algorithms to weight the likelihood that each genotype caused the lesion (4, 20, 21). A method which can potentially overcome the problem of correct attribution of a type to the underlying lesion, when more than one type is initially detected from the specimen, is laser capture microdissection (LCM), a technique that was developed to isolate lesion-specific tissue from biopsy sections which then undergo HPV genotyping (18, 22).

We aimed to define the genotype distribution in cervical cancers from Australian women prior to any impact of the HPV vaccination program, through a systematic assessment of archived cervical cancer specimens utilising LCM.

## Materials and Methods:

### Participating Sites and Ethics

Cervical cancer specimens were collected from seven tertiary centres in the three most populous States of Australia (Victoria, New South Wales and Queensland), where over three-quarters of the female population live and from where 76.7% (23) of all Australian cervical cancers are diagnosed. Research and ethics approvals were obtained from all participating sites including Austin Hospital and Mercy Health, Heidelberg, Victoria (LNR13Austin170 , R13-60); The Royal Women's Hospital, Parkville, Victoria (13/25); South Eastern Area Laboratory Services, New South Wales Health Pathology, NSW (13/305); Hunter Area Pathology Service, New South Wales Health Pathology, NSW (13/305); Pathology West, Western Sydney Local Health District, NSW

(LNR/13/POW/617); Royal Prince Alfred Hospital, Camperdown, NSW (X13-0445); Royal Brisbane and Women's Hospital, Herston, Queensland (14/QRBW/3).



Recruitment study period

Cases were sequentially selected in reverse chronological order from years 2005-2015 from each site, until adequate precision of estimates was achieved in interim analyses. Archived FFPE tissue specimens and diagnostic histology slides, along with clinical information including diagnosis, date of birth and date of biopsy, were obtained for each case. All cases were reviewed by the specialist gynaecological anatomical pathologists on the research team. Any cases which did not meet the inclusion criteria of invasive cervical cancers upon review, as well as any cases without remaining available tissue in the FFPE specimen(s), were excluded.

Sample size calculation and statistical analysis

Sample size calculations were based on published genotype prevalence data for cervical cancer in an Australian meta-analysis and the IARC 2006-2010 world estimate for HPV16 (60%), HPV18 (20%) and remaining nonavalent HRHPV genotypes HPV31/33/45/52/58 (24%)(24, 25). The sample size was sufficient to determine whether Australian genotype prevalence was significantly different from global prevalence, with 90% power at a level of significance of 5%.

Analysis was primarily descriptive with proportions of each HPV type detected in the cancers and 95% confidence intervals (CIs), the primary outcomes of interest. HPV genotypes were grouped into 16/18, 31/33/45/52/58 and "Other" HPV types for further analysis. For analysis of specimens with multiple types detected, these were assigned to a

group hierarchically with those containing 16 or 18 grouped as 16/18, then those containing one of 31/33/45/52/58 assigned as within this multiple, or otherwise as "Other". Proportions were compared with world estimates using Pearson's chi squared test. The association between age and HPV positivity and type was also investigated using a simple binary regression model given that, using a likelihood ratio test, there was no evidence of non-linearity of age.

Tissue sectioning and histological review

FFPE samples were sandwich sectioned as previously described (26, 27). Briefly, all biopsy blocks were serially sectioned with an initial 3µm section which was mounted and stained with H&E for histopathological review, followed by one 9µm section mounted onto an Arcturus PEN membrane glass slide (Applied Biosystems, Foster City, CA) for LCM, two unmounted 9µm WTS and another 3µm H&E section for histopathological review. To minimise potentially contaminating HPV DNA carryover from the mucosal layer, where possible, sections were cut from the basal side towards the superficial epithelial side (27). To avoid cross-contamination from other specimens, the microtome stage and forceps were cleaned with Para-Kleaner (United Biosciences, Carindale, Australia) or xylene followed by ethanol, and a fresh blade and a new container of water for floating sections was used for each paraffin block. All eligible cases underwent genotyping of the WTS as described below.

DNA extraction and LCM isolation of lesions

WTS were deparaffinised and digested as previously described with minor modifications (22). Briefly 800µl of histolene was added to WTS in 1.5 ml polypropylene

tubes and inverted 3-4 times. Subsequently, 400µl of absolute ethanol was added and then inverted 3-4 times. The WTS was then pelleted at 17,000×g for 4 min, washed in 1ml of absolute ethanol, air dried at 55°C for 10min and digested with 200µl of digestion buffer [160µl Tissue Lysis Buffer (Roche Molecular Systems, Alameda, CA) and 40µl Proteinase K 10mg/ml (Roche Molecular Systems)] at 55°C for 1h-2h followed by overnight incubation at 37°C. The digested WTS was then extracted on the MagNA Pure 96 (Roche Diagnostics GmbH, Penzberg, Germany) using the MagNA Pure 96 DNA and Viral NA small volume kit (Universal Pathogen 200 protocol) according to the manufacturer's instructions with a final volume of 100µl eluted DNA.

Cases where HPV was not detected on WTS, or where multiple HPV genotypes or only LRHPV types were detected, were re-reviewed by MS. Cases which did not contain areas of cervical cancer after sectioning were excluded from the study. Confirmed cancers were annotated and regions of cervical cancer were excised by LCM as follows.

Lesion-specific tissue was isolated from the PEN membrane slide as previously described (22, 27). Briefly, PEN membrane slides were deparaffinised using two washes of 100% xylene, followed by two washes of absolute ethanol. Slides were dried at 37°C for an hour. LCM was performed on the Veritas 704 Laser Capture Microdissecting System (Arcturus Bioscience, Mountain View CA, USA) and tissue was captured onto CapSure Macro LCM Caps (Applied Biosystems). Where possible, viable nests of invasive carcinoma were captured and superficial mucosa and necrotic tissue were avoided, to minimise contamination with incidental HPV DNA. The Arcturus PicoPure DNA extraction kit (Applied Biosystems) was used to extract DNA from each cap as per

manufacturer's instructions. Subsequently the digested cells were subjected to PCR. Lesions still positive for multiple HPV genotypes following initial LCM underwent additional LCM sectioning, to attempt to determine the single causative genotype of each lesion (18, 27).

HPV DNA genotyping

WTS and LCM DNA were amplified and HPV genotyped according to a modified version of a previously described algorithm (22). Briefly, DNA was HPV genotyped on the RHA kit HPV SPF10-LiPA25, V.1 (Labo Bio-medical Products BV, Rijswijk, The Netherlands) according to the manufacturer's instructions. To discriminate between HPV68 and 73, samples that were positive for HPV 68/73 by the SPF10-LiPA25 assay were tested on HPV68 and 73 type-specific qPCR assays targeting the E6 region, as previously described (22, 26). Samples that were SPF10-LiPA negative were tested for HPV DNA on the DNA ELISA kit HPV SPF10, V.1 (LaboBio-medical Products BV) and ELISA-positive samples were then genotyped on the RHA Kit HPV SPF+ (Labo Bio-medical Products BV), as per manufacturer recommendation. ELISA-negative samples were tested on a qPCR assay targeting the E6 region of HPV16, 18 and 45 as previously described (22, 26), in addition to HPV33 (forward primer 5'-TCAAGACACTGAGGAAAAACCA-3'; reverse primer 5'-AAATCTGCAAATGCAAAATCA-3'; probe 5'-LC610-AACATTGCATGATTTGTGCCAAGCA-BHQ2-3'), HPV31 (forward primer 5'-TGCAGAAAGACCTCGGAAAT-3'; reverse primer 5'-TTTCTGTTAACTGACCTTTGCAG-3'; probe 5'-FAM-

CCAATGCCGAGCTTAGTTCATGCA-BHQ1-3') and HPV52 (forward primer 5'-TGAGGTGCTGGAAGAATCG-3'; reverse primer 5'-AAAGCGTAGGCACATAATACACAC-3'; probe 5'-LC610-TGCACTGCACACACTGCAGCC-BHQ2-3'), using the same amplification conditions as previously described (22, 26). A 110 bp region of the human β-globin gene, utilized as internal control, was detected by quantitative real-time PCR to confirm successful DNA extraction of all HPV negative samples, as previously described (26). Any sample that was negative for HPV on all tests, and also β-globin negative, was considered unassessable. In the event that all available LCM samples from a case were unassessable, then that case was omitted from analysis. Samples that were positive for β-globin, but negative for all HPV tests were denoted 'HPV not detected'. Samples that were positive for HPV DNA ELISA, but not able to be genotyped were denoted 'HPV X'. Cases that were negative for HPV were re-extracted on the next serial WTS section and testing was repeated, as described above to confirm results.

**Results:**

Overall 941 available blocks were identified from the seven centres. Ninety four were excluded either because there was no cancer tissue remaining in the block, they were microinvasive only (n=4) or they were duplicates (for example cone biopsies following punch biopsies on the same patient). The 847 cases in this study were from women with a median age of 48 years (range 20-98, mean 50.35, standard deviation 16.4). Overall 53.1% (n=450), 34.0% (n=288) and 12.9% (n=109) of specimens came from New South Wales, Victoria and Queensland respectively. The specimens were grouped into four pathological classifications, consistent with Australian national reporting standards: squamous cell carcinoma (65.1% (n=551); median age 50, range 21-98), adenocarcinomas (28.7% (n=243) ; median age 45, range 20-87), adenosquamous carcinoma (4.3% (n=36) ; median age 42, range 24-80) and other (2.0% (n=17) ; median age 50, range 20-91). The 'other' category consisted of 16 cases of neuroendocrine tumours and one sarcoma.

Initial results from the 847 blocks tested resulted in 703 (83%) positive for a single HRHPV, 65 positive for multiple types (7.7%), 14 positive for a single LRHPV and 65 negative for HPV. LCM was performed on 146 blocks with multiple genotypes, only LRHPV, no HPV DNA or unexpected positive HPV (one case of a patient with adenoma malignum arising in the context of known Peutz-Jeghers syndrome). Following LCM, 760 (89.7.0%) were positive for a single HRHPV, 20 (2.4%) positive for a single LRHPV and 7 (0.8%) still positive for multiple HPV genotypes. Sixty (7.1%) remained negative for HPV after repeat testing using L1 and E6 gene targets, to rule out the

possibility of a false negative result due, for example, to a disruption in the L1 target region caused by integration of the HPV genome. Two cancers that were negative by L1 were positive by E6 and two cancers that were negative on WTS were positive by LCM. Overall distribution of genotypes is given in Table 1. The seven specimens with unresolved multiple types were two with HPV 16 and 18 (an adenocarcinoma and an adenosquamous carcinoma), one with HPV 16/18/39 (SCC), one with HPV 16/39 (SCC) and three with Other HR genotypes (all SCCs).

The study was powered originally to ensure adequate precision in the estimates individually for the five additional HPV types covered by the nine valent vaccine. The final estimates, with exact binomial 95%CIs for these types and 16 and 18, using all cancers as the denominator (including HPV negative cancers and including that type for cases where there was more than one type detected) are given in Table 2 below. Results from the international study by Li et al (15) for the most recent time period, and the recent US study of 777 cervical cancers by Saraiya et al (28), are also presented for comparison. As shown in Table 3, when comparing our HPV positive cancers with grouped type distribution of 8,977 HPV positive cancers internationally (17), our data indicate that a higher proportion of HPV positive cervical cancers in Australia contain one of the seven oncogenic types covered by the 9vHPV vaccine than international data (93.0% (95%CI 91.0-94.7%) vs 89.4% (95%CI 88.8-90.1)), with a higher proportion due to HPV types 16/18.

As shown in Table 4, HPV16/18 were the most common types detected across all morphological subtypes, being detected in 68.6% of SCC, 76.5% of adenocarcinomas,

80.6% of adenosquamous cancers and 82.4% of other cancer types. The next most common HPV grouping result for SCC was a nine valent type in 18.7% of specimens. Also shown in Table 4, HPV 16/18 cancers are less likely to occur with advancing age and conversely the proportion of cancers positive for other HPV types increases with age (p trend <0.001). A clear relationship between age and HPV type distribution was evident. Results of binary logistic regression analyses, using age in years as a predictor of HPV group, demonstrated a-decrease in odds of positivity for HPV16 or 18 for every year increase in age (HPV16/18 OR 0.966 (95%CI 0.957-.0975) p<0.001) and an increase in odds of HPV31/33/45/52/58 and Other types with every year increase in age (HPV31,33,45,52,58 OR 1.017 (95%CI 1.006-1.029) p=0.003; Other HPV OR 1.024 (95%CI 1.008-1.041) p=0.003).

Overall, HPV DNA was not detected in 7.1% of cancers. The greatest proportion by type that were HPV negative were neuroendocrine cervical cancers (18.8%), then adenocarcinomas (13.2%), adenosquamous (11.1%) and squamous cancers (3.8%) (Table 4). There was a strong relationship between age and HPV positivity, with older women less likely to have HPV positive cancers. In a binary logistic regression analysis, age was a significant predictor of a cervical cancer being HPV negative (OR 1.05 (95%CI 1.03-1.07);p<0.001 i.e. for every year increase in age, the odds of HPV negative status increases by 1.05).

**Discussion:**

Our data indicate that, contrary to the previously existing literature that suggested Australia had relatively less to gain (17, 29), Australia has a higher proportion of

cervical cancer attributable to nine valent types than other countries. Of all cervical cancers analysed, HPV16/18/31/33/45/52 or 58 were detected in 86.4% and, when restricted to those cancers in which HPV was detected, 93.0% had one of the seven oncogenic types covered by the 9vHPV vaccine detected. This is significantly higher than the global proportion of 89.4% of HPV positive cancers (17). We found that HPV16/18 were the major contributors to cervical cancer across all age groups and all cervical cancer types, but that these types dominated more strongly amongst younger women. This finding is highly consistent with recent similar studies internationally, which indicate dominance of HPV16/18 in cervical cancers at younger ages (28, 30, 31). We also identified that, according to available data, Australia has a significantly lower burden of cervical cancer due to type 58 than globally. However, different methods of accounting for multiple infections when estimating type specific prevalence, and the over representation of Asian countries (where type 58 is more common) in global meta-analyses (15), mean that this finding should be interpreted with caution.

It is notable that the higher overall proportion of cervical cancers positive for the 9vHPV vaccine types is driven by a higher proportion of cancers containing HPV 16 or 18 (notably HPV18 in comparison to international studies, Table 2). This suggests that the incremental benefit between quadrivalent or bivalent HPV vaccine and the 9-valent HPV vaccine may be lower in Australia than in other countries. It is likely that the success of the national cervical screening program in Australia has directly resulted in a higher proportion of cervical cancers being attributable to HPV16/18. This could be for two main reasons. Firstly, slower developing cancers due to the less oncogenic HPV types are

more likely to be prevented by detection and treatment of pre-cursor lesions during repeated screening episodes over time, resulting in a bias towards development of more aggressive cancers due to the more oncogenic types 16/18. Secondly, cytology based screening programs are far less successful at preventing adenocarcinomas, which are predominantly caused by HPV18 and 16 (32). In countries with successful cytology based screening programs, the ratio of adenocarcinoma: SCC is higher than in those without screening, where SCCs predominate (33). The increase over time in this ratio in Australia is marked; from 1:7 in 1982 to 1:3 in 2012 (34). The ratio in the US series is similar to the Australian data (28, 35). By contrast, in the international analysis of Serrano et al, only 5% of cancers were adenocarcinomas giving a ratio of 1:18 (17). Future studies should consider the importance of reporting HPV typing results by cancer type and age. This will allow an assessment of whether apparent differences in global distribution of HPV types causing cervical cancers might in fact reflect differences screening, in the proportion of samples by histological type and age group, and differences in HPV detection methods (15).

Our study sample appears representative of the underlying distribution of cervical cancers diagnosed in Australia. The mean age of women at the date the specimen was collected (year 2005-2015) was 50.4. In comparison, mean age at cervical cancer diagnosis nationally in 2007 was 51.2 and in 2011 was 48.7 (36, 37). Overall distribution by histological type was similar to the distribution of cancer types in 2011 national data (SCC 65.1 vs 67.0%, adenocarcinoma 28.7 vs 24.2%, adenosquamous 4.3 vs 3.8%; p

all>0.05), with the only significant difference noted for cancers designated as 'Other' which were less common in our sample (2% (17/847) vs 5% (34/682);p=0.001) (34).

Strengths of our study include the large sample size, use of contemporary HPV typing methods, and the use of LCM to determine the type in the lesion when more than one type was detected at whole tissue section. In specimens with multiple types detected, where we could not identify a single type in the tumour (n=7), the most likely explanation remains DNA carryover when sectioning. Use of LCM avoids the problem of potential misattribution of causal type in cases of multiple type detection. A potential limitation of our study is that we only collected specimens from the eastern seaboard of Australia, where the majority of the Australian population live. There are no data to indicate whether type distribution in cervical cancers may vary in other regions of Australia, although we view this as unlikely given the universal predominance of HPV16/18 demonstrated across the country pre-vaccination and the similar rates of cervical screening seen across the country (34, 38).

The overall fraction of cervical cancers in which we could not detect HPV is similar to previous reports (15), with HPV negative cancers relatively more frequent in older women (39, 340). We aimed to minimise false negative results by utilizing several methods including additional E6 targeted PCR, and confirming each result on at least two WTS and LCM excised tissue sections from pathologist confirmed cancer cases. Proposed explanations for why some cervical specimens are HPV negative include misdiagnosed uterine cancers, failure to detect integrated HPV DNA, failed detection due to assay or specimen issues, or true negative cervical cancers (28). True negative cervical cancers can rarely occur, particularly some unusual types of adenocarcinomas, such as

gastric type adenocarcinomas (32, 41). The possibility of a "hit and run" scenario for HPV (where HPV was once present and facilitated accumulated mutations sufficient to cause cancer but was then cleared) cannot be ruled out (42). One potential way of ascertaining this would be to examine tissue from previous high grade lesions from the same patients, to determine if the precursors of the cancers were HPV positive. Further analysis of the HPV negative cancers detected in this series is being undertaken, including formal review of histology, history and further typing studies.

We also note that we detected a single LR type in 2.4% (20/847 Table 1) of the cancers, consistent with previous series (15, 25, 43). Most of these cases had a type detected which is in fact classified as a possible HR type (types 26, 53, 66, 67, 70, 73, and 82) by the International Agency for Research on Cancer. A recent analysis found molecular evidence of carcinogenicity of these types in a series of cervical cancer specimens (44). Of interest the high risk assays eligible for use in primary cervical screening in the new HPV based screening program in Australia will detect the 12 high risk types, the probable high risk type 68 and possible HR type 66. If the cancers in our series had been subjected to these screening tests (despite the fact that screening is designed to detect pre-cancers and not cancers and it is unknown how many of the cancers in our series were symptomatic vs screen detected), we would have detected 5 of the 20 cancers we eventually classified as single LR infected – one with type 66 and the others due to the concurrent presence of HR types in the specimen.

In summary, we found that cervical cancers in Australia, compared to international data, had slightly higher proportions of HPV 16/18 and slightly lower rates of HPV31/33/45/52/58 detected. The lower prevalence of the latter was primarily due to

significantly less HPV58. We hypothesise that cervical screening programs, which alter the relative distribution of SCCs and adenocarcinomas in a population, may have an important influence on the relative proportion of cervical cancers due to varying HPV types. Our data can assist in determining the potential incremental benefits of 9vHPV vaccine and thus potential cost-effectiveness in the Australian setting. This study also strongly suggests that routine cervical cancer genotyping, using LCM where necessary, is feasible and should be implemented in Australia as part of the assessment of the HPV vaccination program and for monitoring of the effectiveness of the renewed HPV based cervical screening program.

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Table 1: Distribution of HPV genotypes in 847 invasive cervical cancer specimens, Australia 2005-2015

| | Number | (%) | Cumulative Frequency (%) |
|---|---|---|---|
| **Single HR HPV genotype** | **760** | **89.7%** | |
| 16 | 437 | 51.6% | 51.6% |
| 18 | 166 | 19.6% | 71.2% |
| 45 | 42 | 5.0% | 76.2% |
| 33 | 36 | 4.3% | 80.5% |
| 31 | 21 | 2.5% | 82.9% |
| 52 | 20 | 2.4% | 85.2% |
| 56 | 9 | 1.1% | 86.3% |
| 35 | 8 | 0.9% | 87.2% |
| 39 | 8 | 0.9% | 88.1% |
| 58 | 5 | 0.6% | 88.7% |
| 68* | 4 | 0.5% | 89.2% |
| 59 | 3 | 0.4% | 89.6% |
| 51 | 1 | 0.1% | 89.7% |
| **Single LR HPV genotype** | **20** | **2.4%** | |
| 73** | 5 | 0.6% | 0.6% |
| 6 | 2 | 0.2% | 0.8% |
| 40 | 2 | 0.2% | 1.1% |
| 53** | 2 | 0.2% | 1.3% |
| 67** | 2 | 0.2% | 1.5% |
| 26** | 1 | 0.1% | 1.6% |
| 30 | 1 | 0.1% | 1.8% |
| 61 | 1 | 0.1% | 1.9% |
| 66** | 1 | 0.1% | 2.0% |
| 69 | 1 | 0.1% | 2.1% |
| 70** | 1 | 0.1% | 2.2% |
| 82** | 1 | 0.1% | 2.4% |
| **Multiple HPV genotypes** | **7** | **0.8%** | |
| 16, 18 | 2 | 0.2% | 0.2% |
| 16, 39 | 1 | 0.1% | 0.4% |
| 35, 59 | 1 | 0.1% | 0.5% |
| 39, 45 | 1 | 0.1% | 0.6% |
| 51, 82 | 1 | 0.1% | 0.7% |
| 16, 18, 39 | 1 | 0.1% | 0.8% |

| | | |
|---|---|---|
| **HPV not detected** | **60** | **7.1%** |
| **Total** | **847** | **100%** |

*defined as probably carcinogenic by IARC[2], ** defined as possibly carcinogenic by IARC[2]

Table 2 Single type HPV prevalence in 847 Australian cervical cancers specimens compared to US and international data

| HPV type | Prevalence (95%CI) | Saraiya USA (2015)* (95%CI) | Li world 2006-2010 (95%CI) |
|---|---|---|---|
| 16 | 51.6% (48.2-55.0%) | 50.1% (46.6-53.6%) | 60.0% (56.2-63.7%) |
| 18 | 19.6% (17.0-22.4%) | 16.1% (13.7-18.8%) | 15.8% (13.9- 17.8%) |
| 31 | 2.5% (1.5-3.8%) | 2.1% (1.3- 3.3%) | 3.8% (3.1- 4.6%) |
| 33 | 4.3% (3.0-5.8%) | 3.5% (2.4- 5.0%) | 4.7% (3.5- 6.4%) |
| 45 | 5.0% (3.6-6.6%) | 5.5% (4.1- 7.3%) | 4.9% (3.8-6.2%) |
| 52 | 2.4% (1.4-3.6%) | 1.8% (1.1- 3.1%) | 4.2% (2.9-6.1%) |
| 58 | 0.6% (0.2-1.4%) | 1.8% (1.1- 3.0%) | 6.1% (3.3-10.9 %) |
| Neg | 7.1% | 9.4% | 7.1% |

* Estimates with 95% Wilson CI kindly provided by Mona Saraiya. These data use proportional distribution to allocate attribution to types where more than one type was detected. This occurred in 8.2% of specimens (vs 0.8% in our series using LCM). This method results in the lowering of prevalence for each type compared to the crude results. Note the Li analysis has NOT accounted for multiple type detection in a lesion meaning that the prevalence for each type will be higher and that prevalence of a type does not necessarily equate to attribution of type to the lesion.

Table 3: Distribution of genotypes placed in 4vHPV/2vHPV and 9vHPV targeted groups, 847 Australian cervical cancers, compared with results from Serrano et al 2012.

| HPV type group | Number | % of total cases n=847 (95% CI) | % of HPV positive n=787 (95% CI) | % of HPV positive n=8977 Serrano et al global data (95%CI) |
|---|---|---|---|---|
| 16,18 | 607 | 71.8% (68.5-74.7%) | 77.1% (74.0-80.0%) | 70.8% (69.8-71.7%) |
| 31,33,45,52,58 | 125 | 14.8% (12.4-17.3%) | 15.9% (13.4-18.6%) | 18.5% (17.7-19.3%) |
| Any 9vHPV | 732 | 86.4% (83.9-88.7%) | 93.0% (91.0-94.7%) | 89.4% (88.8-90.1) |
| Other HPV | 55 | 6.5% (4.9-8.4) | 7.0% (5.3-9.0%) | 10.6% (9.9-11.2%) |
| Negative | 60 | 7.1 (5.4-9.0%) | NA | NA |

NA= not applicable

| Classification | | | HPV group | | | | Total |
|---|---|---|---|---|---|---|---|
| | | | 16,18 | 31,33,45,52, 58 | Other | Negative | |
| | | | N (%) | N (%) | N (%) | N (%) | N (%) |
| Adenocarcinoma | Age groups (years) | 20 to 39 | 73 (88.0%) | 7 (8.4%) | 0 | 3 (3.6%) | 83 (100%) |
| | | 40 to 59 | 88 (77.9%) | 9 (8.0%) | 3 (2.7%) | 13 (11.5%) | 113 (100%) |
| | | 60+ | 25 (53.2%) | 4 (8.5%) | 2 (4.3%) | 16 (34.0%) | 47 (100%) |
| | **Total** | | **186 (76.5%)** | **20 (8.2%)** | **5 (2.1%)** | **32 (13.2%)** | **243 (100%)** |
| Adenosquamous | Age groups (years) | 20 to 39 | 14 (100%) | 0 | 0 | 0 | 14 (100%) |
| | | 40 to 59 | 12 (85.7%) | 2 (14.3%) | 0 | 0 | 14 (100%) |
| | | 60+ | 3 (37.5%) | 0 | 1 (12.5%) | 4 (50.0%) | 8 (100%) |
| | **Total** | | **29 (80.6%)** | **2 (5.6%)** | **1 (2.8%)** | **4 (11.1%)** | **36 (100%)** |
| Other* | Age groups (years) | 20 to 39 | 7 (100%) | | | 0 | 7 (100%) |
| | | 40 to 59 | 5 (83.3%) | | | 1 (20.0%) | 6 (100%) |
| | | 60+ | 2 (50.0%) | | | 2 (50.0%) | 4 (100%) |
| | **Total** | | **14 (82.4%)** | | | **3 (17.6%)** | **17 (100%)** |
| Squamous cell carcinoma | Age groups (years) | 20 to 39 | 122 (83.0%) | 15 (10.2%) | 10 (6.8%) | 0 | 147 (100%) |
| | | 40 to 59 | 156 (67.5%) | 45 (19.5%) | 23 (10.0%) | 7 (3.0%) | 231 (100%) |
| | | 60+ | 100 (57.8%) | 43 (24.9%) | 16 (9.2%) | 14 (8.1%) | 173 (100%) |
| | **Total** | | **378 (68.6%)** | **103 (18.7%)** | **49 (8.9%)** | **21 (3.8%)** | **551 (100%)** |
| All cervical cancers | Age groups (years) | 20 to 39 | 216 (86.1%) | 22 (8.8%) | 10 (4.0%) | 3 (1.2%) | 251 (100%) |
| | | 40 to 59 | 261 (71.7%) | 56 (15.4%) | 26 (7.1%) | 21 (5.8%) | 364 (100%) |
| | | 60+ | 130 (56.0%) | 47 (20.3%) | 19 (8.2%) | 36 (15.5%) | 232 (100%) |
| | **Total** | | **607 (71.7%)** | **125 (14.8%)** | **55 (6.5%)** | **60 (7.1%)** | **847 (100%)** |

Table 4: HPV genotype distribution stratified by age group and histological classification, 847 Australian cervical cancers

* Other refers to 16 neuroendocrine cancers and one sarcoma

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