Long term follow up of persistence of immunity following quadrivalent Human Papillomavirus (HPV) vaccine in immunocompromised children

C. Raina MacIntyre a, Peter J. Shaw b, Fiona E. Mackie c,d, Christina Boros e, Helen Marshall f, Holly Seale f, Sean E. Kennedy c,d, Aye Moa a, Abrar Ahmad Chughtai f, Mallory Trent a,e, Edward V O’Loughlin g, Michael Stormon g

a Biosecurity Program, Kirby Institute, Faculty of Medicine, University of New South Wales, Kensington, NSW 2052, Australia
b BMT Services, Children’s Hospital at Westmead, Hawkesbury Rd, Westmead, NSW 2145, Australia
c Nephrology, Sydney Children’s Hospital, Randwick, High St, Randwick, NSW 2031, Australia
d School of Women’s & Children’s Health, Faculty of Medicine, University of New South Wales, Kensington, NSW 2052, Australia
e The Women’s and Children’s Hospital and Robinson Research Institute and Adelaide Medical School, The University of Adelaide, 55 King William Road, North Adelaide 5006, Australia
f School of Public Health and Community Medicine, Faculty of Medicine, University of New South Wales, Kensington, NSW 2052, Australia
g Department of Gastroenterology, Children’s Hospital at Westmead, Hawkesbury Rd, Westmead, NSW 2145, Australia

ARTICLE INFO

Article history:
Received 11 March 2019
Received in revised form 17 July 2019
Accepted 22 July 2019
Available online 8 August 2019

Keywords:
Human papillomavirus
Warts
Vaccine
Cancer
Immunisation
Immunodeficiency
Adolescents

ABSTRACT

Background: Human Papillomavirus (HPV) causes significant burden of HPV-related diseases, which are more prevalent in immunosuppressed compared to immunocompetent people. We conducted a multicentre clinical trial to determine the immunogenicity and reactogenicity of HPV vaccine in immunocompromised children. Here we present the immunogenicity results 5 years post vaccination.

Methods: We followed up immunocompromised children (5–18 years) with a range of specified underlying conditions who were previously recruited from three Australian paediatric hospitals. Participants received three doses of quadrivalent HPV vaccine (Gardasil Quadrivalent HPV Types 6, 11, 16, 18) and were followed up between 2007 and 2016 (60 months post-vaccination). The immunogenicity primary outcome was seroconversion and geometric mean titres (GMT) of the quadrivalent HPV vaccine serotypes in the study.

Results: Of the 59 original participants, 37 were followed up at 60 months. The proportion of participants who seroconverted were: 86.5%, 89.2%, 89.2%, 91.9% by competitive Luminex immunoassay (cLIA) and 83.8%, 83.8%, 94.6%, 78.4% by total immunoglobulin G assays (IgG) for serotypes 6, 11, 16 and 18 respectively. GMT values ranged from 118 (95%CI: 79–177) for serotype 11, to 373 (95%CI: 215–649) for serotype 16 by cLIA. For IgG, serotype 16 had the highest GMT of 261 (95%CI: 143–477) and serotype 18 had the lowest value of 37 (95%CI: 21–68). All antibody titres were lower in females compared to males but the difference was not statistically significant except for serotype 16. No serious adverse event was reported during this follow-up period.

Conclusion: Our evidence, although limited by small numbers, is reassuring that a three dose schedule of HPV vaccine remains immunogenic in immunocompromised children to five years post vaccination. Large scale studies are required to determine long term protection in immunocompromised children.

Clinical trial registration: NCT02263703 (ClinicalTrials.gov).
© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Background

Human Papillomavirus (HPV) causes significant burden of HPV-related diseases, which are more prevalent in immunosuppressed children compared to immunocompetent children [1–7]. Vaccination is an effective measure to protect against HPV infection and HPV-related disease and has the potential to reduce HPV disease incidence in immunocompromised patients [8–12]. Based on the 2017 World Health Organisation (WHO) position paper on HPV vaccines, a three-dose schedule (0, 1–2, 6 months) of either bivalent, quadrivalent and nonavalent vaccines is recommended for individuals who are immunocompromised, or over 15 years of age [13].

https://doi.org/10.1016/j.vaccine.2019.07.072
0264-410X/© 2019 The Authors. Published by Elsevier Ltd.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
Persistence of immune protection is crucial for overall effectiveness of the vaccine, as the potential risk of HPV exposure remains present throughout an individual’s lifetime. Several studies have investigated long-term immunogenicity of HPV vaccine. Einstein et al. [14] compared immunogenicity among healthy women aged 18–45 years up to 60 months post vaccination with bivalent or quadrivalent vaccines and found adequate seroconversion rates for HPV-16. However, for HPV-18, whilst there were high seropositivity rates among the bivalent vaccine group (98.1–100%), decreasing seropositivity rates were reported among the quadrivalent vaccine group (61.1–76.9%). A randomised, partially blind study evaluated the immunogenicity of bivalent HPV vaccine given in two doses to girls 9–14 years of age and in three doses to women 15–25 years of age. All participants who were seronegative at baseline were seropositive for anti-HPV16 and –18 after five years, and statistical models predicted that antibody levels would remain higher than natural-infection levels for at least 21 years in 95% of subjects [15]. A long-term follow up study measuring the immunogenicity of bivalent HPV vaccine in women aged 15–55 found that ten years after vaccination, seropositivity to anti-HPV-16 among women aged 15–25 years was 100% and to anti-HPV-18 was 99.2%. Immunity was predicted to remain above natural infection levels for at least 30 years [16].

In a double-blind, randomized, placebo-controlled study evaluating the long-term efficacy of bivalent HPV vaccine among young women aged 15–25 years, vaccine efficacy was found to remain high (95.6%) at 9.4 years [17]. A double-blind, placebo-controlled study evaluating the immunogenicity of quadrivalent HPV vaccine among 9- to 15-year-old boys and girls found no persistent infections among the participants 8 years post vaccination [18].

The immunogenicity of HPV vaccine among immunosuppressed paediatric populations has been evaluated in a handful of studies, however few studies investigated long-term (>2 years) persistence of immunogenicity [19–24]. Weinberg et al. [25] measured antibody levels of 97 HIV-positive children four to five years after receiving three or four doses of quadrivalent HPV vaccine in a double-blind, placebo-controlled study. This study found that T-cell responses to HPV-16 and -18 persisted over the follow up period for both study groups, but there were notable decreases in B-cell responses and T-cell function [25].

Our previous study, a multicentre clinical trial in Sydney and Adelaide, Australia showed a robust immune response in immunocompromised children after 7 and 24 months post vaccination with quadrivalent HPV vaccines [24]. The seroconversion rates were 93.3%, 100%, 100% and 88.9% for serotypes 6, 11, 16 and 18 respectively. The corresponding rates at 24 months follow up were 82.2%, 91.1%, 91.1% and 68.9% [24]. The present study evaluated the long-term persistence of immunogenicity by means of seroconversion and geometric mean titres (GMT) in participants at 5 years post vaccination with the quadrivalent HPV vaccine.

2. Methods

2.1. Participants and study design

The patient characteristics, eligibility criteria, study methods, and 7- and 24-month results of the clinical trial have previously been published [24]. A prospective, multicentre clinical trial was conducted between November 2007 and October 2012 at three paediatric hospitals in Sydney and Adelaide, Australia to determine the immunogenicity and reactogenicity of HPV vaccine in immunocompromised children. Participants included unvaccinated patients aged between 5 and 18 years, diagnosed with either solid organ transplantation (liver (LT) or kidney (KT)), haematological stem cell transplantation (HSCT), or an autoimmune disorder (Juvenile Idiopathic Arthritis (JIA) or inflammatory bowel disease, IBD). A total of 59 participants were enrolled (13 LT, 16 KT, 20 HSCT, 7 JIA and 3 IBD).

As reported previously, participants were given three doses of quadrivalent HPV vaccine (Gardasil Quadrivalent HPV Types 6, 11, 16, 18). Participants were followed for up to 5-years post vaccination. Serum samples for serologic analysis were collected at baseline (before first dose), 7, 24 and 60 months. Serum antibody levels to serotypes 6, 11, 16 and 18 were quantified by a IgG Luminex immunoassay [26–28] and a Luminex immunoassay in a competitive format (cLIA), in which type-specific, phycoerythrin-labelled, neutralising antibodies compete with patient serum antibodies for binding to conformationally sensitive, neutralising epitopes on the VLPs [24,26,29,30]. Additional details on serum collection and assay methods have been published previously [24]. Serologic analysis was conducted by Merck Laboratories. Seroconversion and geometric mean titres (GMT) for serotypes 6, 11, 16 and 18 were calculated for each serotype at respective time points. Titres were compared over time for each serotype and between different groups (age, gender and use of immunosuppressive drugs) using students t-test with lognormal distribution. Here we present the analysis at month 60, as well as the analysis at months 7 and 24 among the participants that completed the five year follow up.

2.2. Ethics approval and consent

Ethics approval was obtained from the Human Research Ethics Committee (HREC) of the SESLHD/Northern Hospital Network (no – 07/280), Children, Youth and Women’s Health Service (CYWHS) Research Ethics Committee (no – REC2016/12/10) and the Children’s Hospital at Westmead Ethics committee (no – 2007/028). This trial was registered at ClinicalTrials.gov (identifier: NCT02263703). Informed consent was obtained from the parent of the participants prior to vaccination.

3. Results

3.1. Characteristics of study participants

Of the 59 original participants, 37 were followed up at 60 months (Fig. 1). Of these 37 participants, 34 were successfully followed up at 7 and 24 months. Patient characteristics are summarized in Table 1. Participant age at the 60 months follow up ranged from 10 years to 23 years, with a mean and median of 17.0 years. Nineteen (51.4%) were female, and 18 (48.6%) were male. Most participants (64.9%) in the follow up study were enrolled from Hospital 1. Fifteen (40.5%) participants had undergone HSCT at the time of recruitment, and 14 (37.8%) underwent a solid organ transplant at the time of recruitment. Six participants (16.2%) were on one immunosuppressive agent at the time of recruitment, and 14 participants (37.8%) were on more than one immunosuppressive agent. The remaining participants were not on an immunosuppressive agent at baseline (n = 16, 43.2%) or the data were missing (n = 1, 2.7%).

Participants in the follow up study did not differ significantly from the full study population in terms of age, gender, underlying condition, or use of immunosuppressive agents. However, hospital of origin did differ significantly between the follow up group and the full study population, as a much greater proportion of patients were followed up from hospital 1 compared to hospitals 2 and 3.

3.2. Immunogenicity

The proportion of participants who seroconverted were: 86.5%, 89.2%, 89.2%, 91.9% by competitive Luminex immunoassay (cLIA)
and 83.8%, 83.8%, 94.6%, 78.4% by total immunoglobulin G assays (IgG) for serotypes 6, 11, 16 and 18 respectively. GMT values obtained by cLIA at 60 months, ranged from 118 (95% CI: 79–177) for serotype 11, to 373 (95% CI: 215–649) for serotype 16. GMTs were higher at 60 months, compared to baseline. GMTs of serotypes 6 and 18 were higher at 60 months compared to 24 months, but the difference was not significant. Serotype 16 had the highest IgG GMT of 261 (95% CI: 143–477) and serotype 18 had the lowest value of 37 (95% CI: 21–68) (Table 2).

All antibody titres were lower in females compared to males at 60 months, but the difference was not statistically significant except for serotype 16. Antibody titers at 60 months were not significantly different between <12 and ≥12 years age groups nor between patients taking immunosuppressive drugs or not (Table 3). No serious adverse event was reported during this follow up period.

GMT values did not differ significantly between those that completed the follow up and those that did not for any time point or serotype. Results obtained from the follow up sample did not differ from the full sample, except when comparing GMT’s by age. GMT’s were lower among the older age group compared to the younger age group for all serotypes at all timepoints, but this difference

---

1Haematological stem cell transplantation
2Liver transplant
3Kidney transplant
4Inflammatory bowel disease
5Juvenile idiopathic arthritis
6Indicates number of subjects that received each of three doses of quadrivalent HPV vaccine (Dose 1, Dose 2, Dose 3).
7Indicates number of subjects that had serum samples tested for antibody titres at each of four time points (Test 1 = baseline pre-vaccination, Test 2 = 7 months post-vaccination, Test 3 = 24 months post-vaccination, Test 4 = 60 months post-vaccination).

Fig. 1. Recruitment. 1Haematological stem cell transplantation. 2Liver transplant. 3Kidney transplant. 4Inflammatory bowel disease. 5Juvenile idiopathic arthritis. 6Indicates number of subjects that received each of three doses of quadrivalent HPV vaccine (Dose 1, Dose 2, Dose 3). 7Indicates number of subjects that had serum samples tested for antibody titres at each of four time points (Test 1 = baseline pre-vaccination, Test 2 = 7 months post-vaccination, Test 3 = 24 months post-vaccination, Test 4 = 60 months post-vaccination).
was not significant in the full sample. However, in the smaller follow up sample, the difference was significant for serotype 11 at 24 months.

4. Discussion

This follow-up study examined long-term immunogenicity of HPV vaccine among immunocompromised children. In this heterogeneous group of immunosuppressed children, there was an adequate immunogenic response to HPV vaccine irrespective of age or the cause of immunosuppression. The HPV vaccine was found to be immunogenic and all antibody titres remained higher than baseline at the end of 5 years follow-up in immunocompromised children 60 months after completion of a 3-dose schedule. Although antibody titres are the most common endpoints in HPV vaccine studies, it is important to note that there are no clearly defined correlates of protection against HPV-related outcomes [31]. Although the proportion of seroconverted children at 5 years post HPV vaccination was lower than 7 months post-vaccination, immunity was higher for serotypes 6 and 18 compared to 24 months. Among the four serotypes present in the vaccine, serotypes 16 and 18 are associated with cervical cancer. The majority (7/8) of participants lost to follow up between 24 months and 5 years were seronegative for serotype 18 at 24 months, and half (4/8) were seronegative for serotype 6 at 24 months. Therefore, immunity at 5 years for these two serotypes may be biased towards higher results in the study participants who completed the 5 years follow-up. Increasing GMTs could also reflect natural exposure or undocumented re-vaccination. Increased exposure to HPV in the children is likely, given many were in their late teens or older at 60 months of follow up. Vaccination is provided as a school-based program in high school in Australia, so it is possible some children were revaccinated although this question was asked at follow-up study visits. Ferris et al reported higher GMTs for all serotypes in their follow-up study of immunocompetent patients, and showed GMTs for serotype 6, 11, 16 and 18 were higher at 72 months compared to 60 months [18].

Antibody response to serotype 11 and 16 were reduced at 60 months in our study, compared to 24 months, although not significantly. This is a potential concern as serotype 16 is the most common cause of cervical cancer [32]. Einstein et al reported a reduction in GMTs for serotypes 16 and 18 over time in immunocompetent patients. In their study GMTs peaked at 7 months post-vaccination and declined to a plateau at 18–24 months through to 60 months [14]. Plateau antibody levels of serotype 16 and 18 induced by the quadrivalent vaccine and bivalent vaccine were above the levels induced by natural infection, although plateau levels induced by the bivalent vaccine were higher than that of the quadrivalent vaccine [14]. Naud, Roteli-Martins [17] similarly reported a decline in serotype 16 and 18 IgG antibodies over time in immunocompetent subjects. Whilst all vaccinated study participants remained seropositive to serotypes 16 and 18 and up to 113 months post-vaccination, plateaus were reached at approximately 18 months after the first vaccine dose [17].

Ferris et al conducted a long-term study of quadrivalent HPV vaccine administered at 0, 2, and 6 months in healthy children and adolescents similar in age to our study [18,33]. A plateau effect was observed in this study as well, which persisted throughout the ten year follow up [33]. Interestingly, the GMT levels at month 60 were lower for all four serotypes compared to our study. Thus, although GMT levels decreased slightly between 24 and 60 months in our study, they may remain fairly stable over time.

The GMT levels obtained through the cLIA were higher compared to the IgG assays, although the clinical significance of this

| Table 1 | Patient characteristics at 60 months follow up (n = 37). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variable             | Number (%)       |-----------------|-----------------|-----------------|-----------------|-----------------|
| Age               | Mean (range)    | 17.0 (10–23)    |-----------------|-----------------|-----------------|-----------------|
| Gender            | Male            | 18 (48.6)       |-----------------|-----------------|-----------------|-----------------|
|                  | Female          | 19 (51.4)       |-----------------|-----------------|-----------------|-----------------|
| Hospital of origin | Hospital 1     | 24 (64.9)       |-----------------|-----------------|-----------------|-----------------|
|                  | Hospital 2     | 7 (18.9)        |-----------------|-----------------|-----------------|-----------------|
|                  | Hospital 3     | 6 (16.2)        |-----------------|-----------------|-----------------|-----------------|
| Underlying condition | Haematological  | 24 months       |-----------------|-----------------|-----------------|-----------------|
|                  | stem cell transplantation (HSCT) | cLIA * |-----------------|-----------------|-----------------|-----------------|
|                  | Liver transplantation | cLIA * | 15 (40.5)       |-----------------|-----------------|-----------------|
|                  | Kidney transplantation | cLIA * | 8 (21.6)        |-----------------|-----------------|-----------------|
|                  | Juvenile Idiopathic Arthritis | cLIA * | 16 (43.2)       |-----------------|-----------------|-----------------|
|                  | Inflammatory bowel disease | cLIA * | 6 (16.2)        |-----------------|-----------------|-----------------|
|                  | Missing data   | 1 (2.7)         |-----------------|-----------------|-----------------|-----------------|

| Table 2 | Proportion of seroconversion and Geometric mean titres (GMT) at three time points. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Serotype (seropositive/total) | 7 months cLIA % (seropositive/total) | 24 months cLIA % (seropositive/total) | 60 months cLIA % (seropositive/total) | 24 months IgG % (seropositive/total) | 60 months IgG % (seropositive/total) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 6               | 0% (0/36)       | 91.2% (31/34)   | 88.2% (30/34)   | 86.3% (32/37)   | 97.3% (36/37)   | 83.8% (31/37)   |
| 11              | 0% (0/36)       | 100% (34/34)    | 94.1% (32/34)   | 89.2% (33/37)   | 100% (37/37)    | 83.8% (31/37)   |
| 16              | 0% (0/36)       | 100% (34/34)    | 94.1% (32/34)   | 89.2% (33/37)   | 97.3% (36/37)   | 94.6% (35/37)   |
| 18              | 0% (0/36)       | 91.2% (31/34)   | 79.4% (27/34)   | 91.9% (34/37)   | 100% (37/37)    | 78.4% (29/37)   |

| Geometric mean titres (GMT) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 6               | 11.2 (10.8–11.5) | 458.2 (263–797) | 137 (89–210)    | 149 (102–219)   | 117 (69–199)    | 60 (35–102)     |
| 11              | 8.0 (8.0–8.0)   | 833 (525–1322)  | 156 (101–240)   | 118 (79–177)    | 97 (59–161)     | 45 (27–75)      |
| 16              | 11 (11.0–11.0)  | 3122 (1780–5476)| 543 (316–932)   | 373 (215–649)   | 532 (301–938)   | 261 (143–477)   |
| 18              | 10.0 (10.0–10.0)| 548 (290–1033)  | 88 (52–150)     | 141 (100–198)   | 88 (52–150)     | 37 (21–68)      |

* cLIA – competitive Luminex immunoassay.
1 IgG – Total immunoglobulin G assays.
1 GMT in milli-Merck units (mMU) per millilitre and 95% confidence interval.
60 month titre levels were available for 37 participants – Male (18), female (19), <12 years (17) and missing data (1).

24 month titre levels were available for 34 participants – Male (17), female (17), <12 years (15) and missing data (1).

Vaccine responses are not fully understood [37–40]. Vaccine responses have been well documented, though the specific mechanisms for immune responses to vaccinations between males and females have not been clearly established. Differences in gender differences. In boys, post vaccination antibody levels for both serotype 16 and 18 were observed to be up to threefold higher compared to women and girls [34–36]. Differences in immune responses to vaccinations between males and females have been well documented, though the specific mechanisms for this are not fully understood [37–40]. Vaccine responses are thought to be modulated by sex-differential factors such as sex hormones and sex-linked immune response genes [39,40].

Limitations of this study include the small sample size and a heterogeneous patient group ranging from bone marrow and solid organ transplant to autoimmune diseases. We did not have adequate statistical power to compare different patient groups. We had a significantly greater proportion of patients lost to follow up from hospitals 2 and 3 compared to hospital 1, which could have resulted in some selection bias if the three hospitals served significantly different populations. While we did not observe any differences in GMT levels at 7 and 24 months between those that were followed up at 60 months and those that were lost to follow up, we may have lacked sufficient statistical power to detect these differences. Further, at the time of the study, Australia recommended a 2-dose schedule of 9-valent HPV vaccine for children 9–14 years in 2018 [41–45] with a three dose schedule still being recommended for medically at risk children.

Whilst this study provides immunological evidence of persisting immunity, further studies are needed to determine long term clinical protection in immunocompromised children.

Table 3

| Antibody type | Month | GMT (mMU per ml) | GMT ratio (mMU per ml) |
|--------------|-------|------------------|------------------------|
| Gender       |       | 95% confidence interval | 95% CI               |
| Male         | 6     | 868 (523–1440) | 242 (94–620) | 3.59 (1.29–10.02) |
|              | 24    | 203 (134–308)  | 92 (43–196)  | 2.21 (0.97–5.05)  |
|              | 60    | 191 (118–207)  | 118 (63–220)  | 1.61 (0.75–3.46)  |
|              | 11    | 1355 (903–2033)| 513 (228–1153)| 2.64 (1.11–6.31) |
|              | 24    | 225 (134–379)  | 107 (53–218)  | 2.10 (0.90–4.89)  |
|              | 60    | 144 (84–247)   | 98 (53–184)   | 1.46 (0.65–3.26)  |
| Female       | 6     | 6125 (4000–9378)| 1591 (592–4277)| 3.64 (1.37–10.83) |
|              | 24    | 1083 (607–1932)| 272 (118–630) | 3.98 (1.49–10.60) |
|              | 60    | 695 (347–1389) | 208 (91–472)  | 3.35 (1.18–9.51)  |
|              | 18    | 1064 (558–2026)| 282 (97–820)  | 3.77 (1.14–12.49) |
|              | 24    | 150 (81–282)   | 51 (22–119)   | 2.93 (1.07–8.02)  |
|              | 60    | 169 (103–277)  | 118 (71–197)  | 1.43 (0.72–2.85)  |
| Age <12 years| 6     | 740 (308–1782) | 314 (151–653) | 2.36 (0.79–7.02)  |
|              | 24    | 217 (115–410)  | 95 (53–170)   | 2.28 (0.99–5.22)  |
|              | 60    | 191 (101–361)  | 121 (74–198)  | 1.58 (0.73–3.39)  |
|              | 11    | 1020 (479–2172)| 711 (379–1332)| 1.43 (0.56–3.66)  |
|              | 24    | 270 (161–453)  | 101 (53–190)  | 2.68 (1.18–6.07)  |
|              | 60    | 161 (90–269)   | 91 (51–161)   | 2.12 (0.80–3.92)  |
| 16 years     | 7     | 3467 (1354–8878)| 2874 (1342–6155)| 1.20 (0.38–3.81) |
|              | 24    | 770 (408–1453) | 412 (174–974) | 1.87 (0.63–5.53)  |
|              | 60    | 527 (240–1159) | 279 (123–630) | 1.89 (0.63–5.72)  |
| Immunosuppressive drugs | 6     | 625 (246–1586) | 494 (192–1268)| 1.26 (0.35–4.62) |
|              | 24    | 114 (53–245)   | 72 (33–157)   | 1.58 (0.54–4.61)  |
|              | 60    | 158 (97–257)   | 128 (76–214)  | 1.24 (0.62–2.48)  |
| Yes          | 6     | 380 (142–1019) | 582 (304–1114) | 1.53 (0.49–4.83) |
|              | 24    | 126 (64–250)   | 141 (75–264)  | 1.12 (0.46–2.74)  |
|              | 60    | 154 (88–270)   | 136 (74–251)  | 0.88 (0.40–1.96)  |
| No           | 7     | 561 (245–1284) | 1158 (755–1776)| 2.06 (0.83–5.14) |
|              | 24    | 143 (69-297)   | 157 (89-279)  | 1.10 (0.45-2.71)  |
|              | 60    | 137 (77–241)   | 93 (49–177)   | 0.68 (0.30–1.55)  |
| 16 years     | 7     | 2016 (712–5711)| 4569 (2804–7446)| 2.26 (0.73–7.00) |
|              | 24    | 425 (177–1019) | 647 (306–1367)| 1.52 (0.50–4.62) |
|              | 60    | 369 (170–803)  | 347 (138–868) | 0.94 (0.30–2.97)  |
| 18 years     | 7     | 310 (102–943)  | 647 (469–1532)| 2.73 (0.80–9.36)  |
|              | 24    | 69 (32–147)    | 98 (44–219)   | 1.43 (0.50–4.11)  |
|              | 60    | 133 (82–216)   | 135 (80–226)  | 1.02 (0.51–2.02)  |

24 month titre levels were available for 34 participants – Male (17), female (17), <12 years (15) and ≥ 12 years (19). Participants on immunosuppressive drugs (17), no drugs (16), missing data (1).

60 month titre levels were available for 37 participants – Male (18), female (19), <12 years (17) and ≥ 12 years (20). Participants on immunosuppressive drugs (20), no drugs (16), missing data (1).

7 month titre levels were available for 34 participants – Male (17), female (17), <12 years (15) and ≥ 12 years (19). Participants on immunosuppressive drugs (17), no drugs (16), missing data (1).

Females, <12 years, and those on immunosuppressive drugs had lower responses to the vaccine. Similarly, Einstein et al reported a significantly higher number of participants in younger age groups remained seropositive for serotype 18 antibodies compared with the older age groups [14]. Other studies found similar gender differences. In boys, post vaccination antibody levels for both serotype 16 and 18 were observed to be up to threefold higher compared to women and girls [34–36]. Differences in immune responses to vaccinations between males and females have been well documented, though the specific mechanisms for this are not fully understood [37–40]. Vaccine responses are thought to be modulated by sex-differential factors such as sex hormones and sex-linked immune response genes [39,40].
Acknowledgement

The study was in part supported by a CARG grant (Roche, 2008) received by Fiona Mackie, Sean Kennedy and Raina Macintyre. Raina Macintyre is supported by a NHMRC Principal Research Fellowship (1137582). Helen Marshall acknowledges support from the National Health and Medical Research Council (1084951). Laboratory testing was done by Merck and supported by a Merck Investigator Sponsored Proposal.

Declaration of Competing Interest

CRM: C. Raina Macintyre has received in-kind support and funding for investigator-driven research from GlaxoSmithKline and Merck and has sat on advisory boards for Merck and GlaxoSmithKline. HM is an investigator on vaccine studies sponsored by Industry. Her institution has received grants from GSK, Sanofi Pasteur, BioCSL and Pfizer for Investigator led research. HM has not received any personal payments from industry. The remaining authors declare that they have no competing interests and have no non-financial interests that may be relevant to the submitted work.

References

[1] Garland SM, Brotherton JML, Moscicki AB, Kaufmann AM, Stanley M, Bhatla N, et al. HPV vaccination of immunocompromised hosts. Papillomavirus Res 2017;4:35–8.
[2] Klumb EM, Araujo Jr ML, Jesus GR, Santos DB, Oliveira AV, Albuquerque EM, et al. Is higher prevalence of cervical intraepithelial neoplasia in women with lupon due to immunosuppression? J Clin Rheumatol: Pract Rev Rheum Musculoskeletal Dis 2010;16(4):153–7.
[3] Conley LJ, Efferbrock TV, Bush TJ, Chasson MA, Sawo D, Wright TC. HIV-1 infection and risk of vulvovaginal and perianal condyloma acuminata and intraepithelial neoplasia: a prospective cohort study. Lancet (Lond, Eng) 2002;359(9301):108–13.
[4] Frisch M, Biggar RJ, Goedert MY. Human papillomavirus-associated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome. J Natl Cancer Inst 2000;92(18):1500–10.
[5] Wang J, Alldabagh B, Yu J, Arron ST. Role of human papillomavirus in cutaneous squamous cell carcinoma: a meta-analysis. J Am Acad Dermatol 2014;70(4):621–9.
[6] Feldman CH, Liu J, Feldman S, Solomon DH, Kim SC. Risk of high-grade cervical dysplasia and cervical cancer in women with systemic lupus erythematosus receiving immunosuppressive drugs. Lupus 2017;26(7):882–9.
[7] Kim SC, Glynn RJ, Giovannucci E, Hernandez-Diaz S, Liu J, Feldman S, et al. Risk of high-grade cervical dysplasia and cervical cancer in women with systemic inflammatory diseases: a population-based cohort study. Ann Rheum Dis 2015;74(4):1360–7.
[8] Steben M, Tan Thompson M, Rodier C, Mallette N, Racovita P, DeAngelis F, et al. A review of the impact and effectiveness of the quadrivalent human papillomavirus vaccine: 10 years of clinical experience in Canada. J Obstet Gynaecol Can: JOCG = Journal d’obstétrique et gynécologie du Canada J OGC 2018, 12:379–91.
[9] Wei L, Xie X, Liu J, Zhao Y, Chen W, Zhao C, et al. Efficacy of quadrivalent human papillomavirus vaccine against persistent infection and genital disease in Chinese women: a randomized, placebo-controlled trial with 78-month follow-up. Vaccine 2019;37(27):3617–24.
[10] Arbyn M, Xu L, Simoons C, Martin-Hirsch PP. Prophylactic vaccination against human papillomaviruses to prevent cervical cancer and its precursors. Cochr Datab Syst Rev 2018;5:CD009069.
[11] Garland SM, Kjaer SK, Muñoz N, Block SL, Brown DR, DiNubile MJ, et al. Impact of HPV-16/18 AS04-adjuvanted vaccine on females vaccinated at 15–55 years of age. Can Med 2017;6(11):2723–31.
[12] Van NC, Roteli-Martins CM, De Carvalho NS, Teixeira JC, de Borba PC, Sanchez N, et al. Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine: final analysis of a long-term follow-up study up to 9.4 years post-vaccination. Hum Vaccin Immunother 2014;10(4):2147–62.
[13] Mok CC, Ho LY, Fong LS, To CH. Immunogenicity and safety of the quadrivalent human papillomavirus vaccines (types 6, 11, 16, and 18) vaccine in HPV-infected children 7–12 years old. J Acad Inmun Def Synd (1999) 2010;55(2):197–204.
[14] Weberberg A, Song LY, Saah A, Brown M, Moscicki AB, Meyer 3rd WA, et al. Humoral, mucosal, and cell-mediated immunity against vaccine and nonvaccine genotypes after administration of quadrivalent human papillomavirus vaccine to HIV-infected children. J Infect Dis 2012;206(8):1309–18.
[15] Heijstek MW, Scherpenserie M, Groot N, Wulffraat NM, Van Der Klis FR. Immunogenicity and immunogenicity of the quadrivalent human papillomavirus-like particle vaccine in girls and young women with inflammatory bowel disease. Inflamm Bowel Dis 2013;19(7):1441–9.
[16] Mok CC, Ho LY, Fong LS, To CH. Immunogenicity and safety of a quadrivalent human papillomavirus vaccine in patients with systemic lupus erythematosus: a case-control study. Ann Rheum Dis 2013;72(5):659–64.
[17] Macintyre CR, Shaw P, Mackie FE, Boros C, Marshall H, Barnes M, et al. Immunogenicity and persistence of immunity of a quadrivalent Human Papillomavirus (HPV) vaccine in immunocompromised children. Vaccine 2016;34(36):4343–50.
[18] Weinberg A, Huang S, Moscicki A-B, Saah A, Levin MJ, Team FHTTP. Persistence of memory B-cell and T-cell responses to the quadrivalent HPV vaccine in HIV-infected children. AIDS 2018;32(7):851–60.
[19] Brown D, Müller M, Sehr P, Pavlita M, Seitz H, Rubio I, et al. Concordance assessment between a multiplexed competitive Lumines imunoassay, a multiplexed IgG ELISA, a competitive Lumines immunoassay, and a pseudovirion-based neutralization assay for detection of human papillomavirus types 16 and 18 Vaccine 2014;32(44):5880–7.
[20] Opalka D, Matsy K, Bojczuk P, Green T, Gesser R, Saah A, et al. Multiplexed serologic assay for nine anogenital human papillomavirus types. Clin Vaccine Immunol 2010;17(5):818–27.
[21] Brown DR, Garland SM, Ferris DG, Joura EA, Steben M, James M, et al. The humoral response to Gardasil over four years as defined by total IgG and competitive Lumines immunoassay. JAMA 2005;313(11):772–80.
[22] Dias D, Van Doren J, Schlottmann S, Kelly S, Puchalski D, Ruiz W, et al. Optimization and validation of a multiplexed Lumines assay to quantify antibodies to neutralizing epitopes on human papillomaviruses 6, 11, 16, and 18. Clin Diagn Lab Immunol 2012;19(12):1288–95.
[23] Opalka D, Lachman CE, MacMullen SA, Jansen KU, Smith JS, Chirmule N, et al. Human papillomavirus type 16 and 18 neutralizing antibody titers in children and adults. J Virol Methods 2014;201:108–15.
[24] Harper DM, DeMars LR. HPV vaccines – a review of the first decade. Gynecol Oncol 2017;146(1):196–204.
[25] Baseman JG, Koutsly LA. The epidemiology of human papillomavirus infections. J Clin Virol: Offic Publ Pan Am Soc Clin Virol 2005;32(Suppl 1):356–24.
[26] Pedersen C, Petaja T, Strauss G, Ramcke H, Poder A, Richardus JH, et al. Immunization of early adolescent females with human papillomavirus type 16 and 18 L1 virus-like particle vaccine containing AS04 adjuvant. J Adol Health: Offic Publ Soc Adol Med 2017;61(3):564–7.
[27] Petaja T, Keranen H, Karpia T, Kawa A, Lantela S, Siitari-Mattila M, et al. Immunogenicity and safety of human papillomavirus (HPV16/18) adjuvanted vaccine in healthy boys aged 10–18 years. J Adol Health: Offic Publ Soc Adol Med 2017;61(3):568–79.
[28] De Vincenzo R, Conte C, Ricci C, Sambia G, Capelli G. Long-term efficacy and effectiveness of the quadrivalent human papillomavirus vaccine in females: a two-dose schedule in adolescent girls: five-year clinical data and modelling predictions from a randomized study. Hum Vaccin Immunother 2016;12(1):20–9.
[29] Schwarz TF, Galaj A, Spaczynski M, Wysocki J, Kaufmann AM, Poncelet S, et al. Three-year immune response patterns and safety of the HPV-16/18 AS04-adjuvanted vaccine in females vaccinated at 15–55 years of age. Can Med 2017;6(11):2723–31.
[30] Van NC, Roteli-Martins CM, De Carvalho NS, Teixeira JC, de Borba PC, Sanchez N, et al. Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine: final analysis of a long-term follow-up study up to 9.4 years post-vaccination. Hum Vaccin Immunother 2014;10(4):2147–62.
[31] Van NC, Roteli-Martins CM, De Carvalho NS, Teixeira JC, de Borba PC, Sanchez N, et al. Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine: final analysis of a long-term follow-up study up to 9.4 years post-vaccination. Hum Vaccin Immunother 2014;10(4):2147–62.
[41] National Centre for Immunisation Research & Surveillance. Significant events in human papillomavirus (HPV) vaccination practice in Australia; 2018. <http://www.ncirs.org.au/sites/default/files/2018-11/Human-papillomavirus-history-July-2018.pdf>.

[42] Australian Technical Advisory Group on Immunisation (ATAGI). Australian immunisation handbook. Canberra: Australian government department of health; 2018. <immunisationhandbook.health.gov.au>.

[43] Leung TF, Liu AP, Lim FS, Thollot F, Oh HM, Lee BW, et al. Comparative immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and HPV-6/11/16/18 vaccine administered according to 2- and 3-dose schedules in girls aged 9–14 years: results to month 12 from a randomized trial. Hum Vaccin Immunother 2015;11(7):1689–702.

[44] Romanowski B, Schwarz TF, Ferguson LM, Peters K, Dionne M, Schulze K, et al. Immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine administered as a 2-dose schedule compared with the licensed 3-dose schedule: results from a randomized study. Human Vaccin 2011;7(12):1374–86.

[45] Iversen OE, Miranda MJ, Ulied A, Soerdal T, Lazarus E, Chokephaibulkit K, et al. Immunogenicity of the 9-Valent HPV vaccine using 2-dose regimens in girls and boys vs a 3-dose regimen in women. JAMA 2016;316(22):2411–21.