



Durability of the neutralizing antibody response to vaccine and non-vaccine HPV types 7 years following immunization with either Cervarix[®] or Gardasil[®] vaccine

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ABSTRACT

Bivalent (Cervarix[®]) and quadrivalent (Gardasil[®]) Human Papillomavirus (HPV) vaccines demonstrate remarkable efficacy against the targeted genotypes, HPV16 and HPV18, but also a degree of cross-protection against non-vaccine incorporated genotypes, HPV31 and HPV45. These outcomes seem to be supported by observations that the HPV vaccines induce high titer neutralizing antibodies against vaccine types and lower responses against non-vaccine types. Few data are available on the robustness of the immune response against non-vaccine types. We examined the durability of vaccine and non-vaccine antibody responses in a follow up of a head-to-head study of 12–15 year old girls initially randomized to receive three doses of Cervarix[®] or Gardasil[®] vaccine. Neutralizing antibodies against both vaccine and non-vaccine types remained detectable up to 7 years following initial vaccination and a mixed effects model was used to predict the decline in antibody titers over a 15 year period. The decline in vaccine and non-vaccine type neutralizing antibody titers over the study period was estimated to be 30% every 5–7 years, with Cervarix[®] antibody titers expected to remain 3–4 fold higher than Gardasil[®] antibody titers over the long term. The antibody decline rates in those with an initial response to non-vaccine types were similar to that of vaccine types and are predicted to remain detectable for many years. Empirical data on the breadth, magnitude, specificity and durability of the immune response elicited by the HPV vaccines contribute to improving the evidence base supporting this important public health intervention. Original trial: [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00956553) NCT00956553.

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1. Introduction

The bivalent (Cervarix[®]) and quadrivalent (Gardasil[®]) Human Papillomavirus (HPV) vaccines target the most prevalent oncogenic genotypes (HPV16 and HPV18), while the next generation nonavalent HPV vaccine (Gardasil[®]9) targets an additional 5 oncogenic genotypes (HPV31, HPV33, HPV45, HPV52, and HPV58) [1]. Both quadrivalent and nonavalent vaccines target HPV6 and HPV11, which are associated with the development of genital warts. Cervarix[®] and Gardasil[®] vaccines demonstrate remarkable efficacy

against vaccine types (HPV16 and HPV18) and some degree of efficacy against related non-vaccine types (cross-protection) in clinical trials [2,3]. Vaccine effectiveness studies are beginning to confirm these experimental observations in target populations following introduction of national immunization programmes [4,5].

Neutralizing antibodies against vaccine genotypes can be detected in the serum and genital secretions of vaccinees and passive transfer of neutralizing antibodies can protect animals against papillomavirus challenge, leading to the reasonable assumption that type-specific protection is mediated by neutralizing antibodies [3]. The degree of cross-protection afforded by the HPV vaccines appears to be consistent with the detection of cross-neutralizing antibodies [6] suggesting that such antibodies may be effectors or their detection may be useful as a correlate or surrogate of vaccine-induced cross-protection.

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Empirical data on the breadth, magnitude, specificity and durability of the immune response elicited by the HPV vaccines contribute to improving the evidence base supporting this important public health intervention. We have previously demonstrated that HPV vaccines induce high titer neutralizing antibodies against vaccine types, with lower responses against non-vaccine types, and that Cervarix® responses are typically higher and broader than Gardasil® responses [7]. In this study we examined the durability of vaccine and non-vaccine antibody responses by resampling 12–15 year old girls immunized with three doses of Cervarix® or Gardasil® 7 years post immunization.

2. Methods

2.1. Ethics statement

This follow up study was approved by the NHS Health Research Authority and the London – Hampstead Research Ethics Committee (reference 16/LO/1108). The original study protocol was approved by the UK Medicines and Healthcare products Regulation Agency

(MHRA) and registered on the [ClinicalTrials.gov](https://www.clinicaltrials.gov) website (NCT00956553) [7].

2.2. Study population

The original study protocol and recruitment criteria have been published [7]. Briefly, 12–15 year old girls recruited from two sites in England were randomized to receive three doses of either Cervarix® or Gardasil® vaccine over a six month period. Serum samples were collected at month (M) 0 (prior to vaccination), M2 (one month post second dose), M7 (one month post third dose) and M12 (six months post third dose). Participants from the original study were invited to enrol for this follow up study. For simplicity, the follow up study sample was designated as M84 (84 months following first immunization) (Fig. 1). Following informed consent a single blood sample was collected and shipped to the testing laboratory (Public Health England, London) for serum separation and subsequent storage at -80°C . In the original study, lower genital swab samples were collected at M7 for the detection of vaccine and non-vaccine type antibodies. Vaccine-type

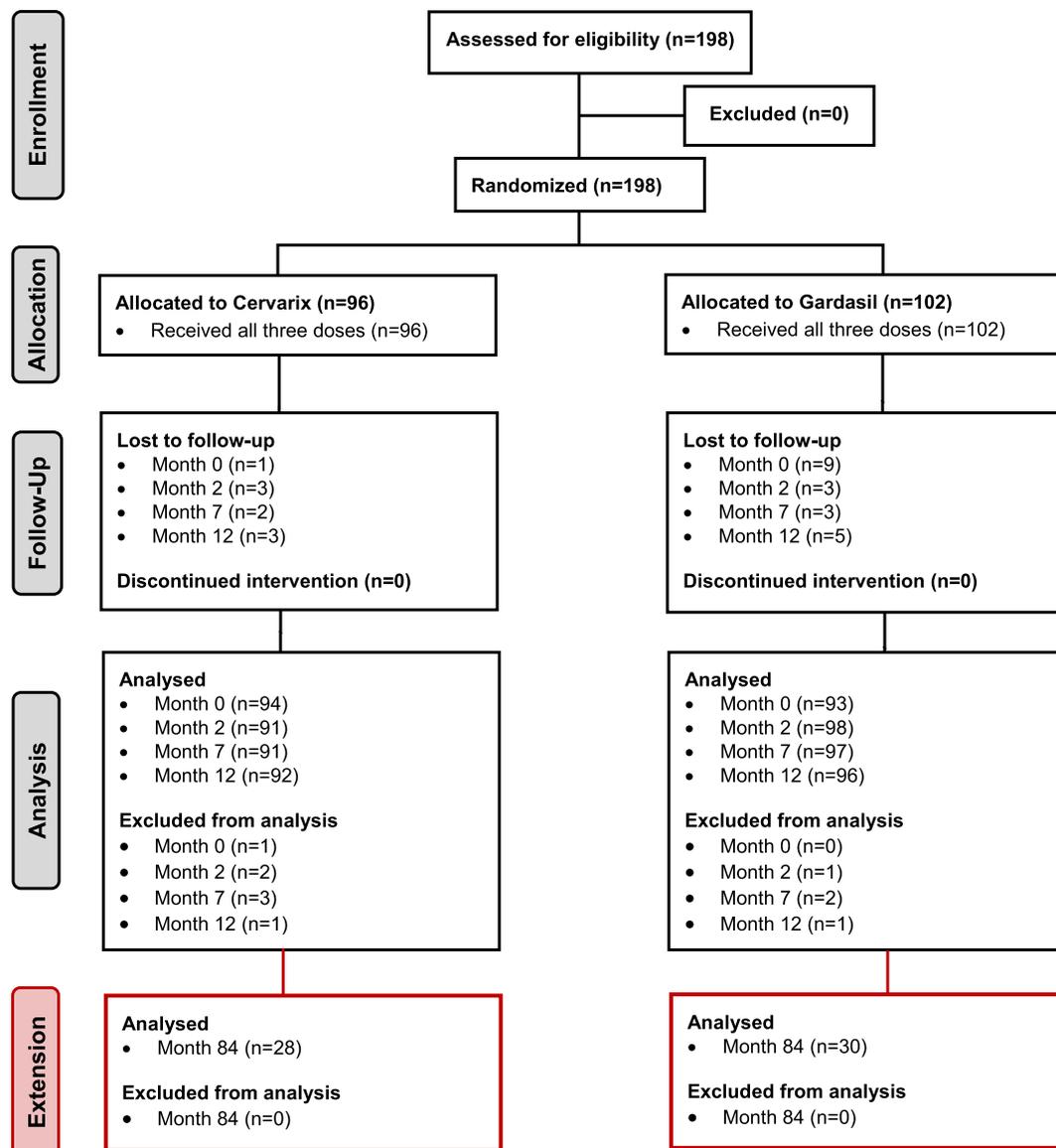


Fig. 1. CONSORT diagram. Allocations, interventions, follow up and analysis of subjects recruited into the comparative HPV vaccine immunogenicity study with the current extension study participants highlighted (red box).

responses were detected in around 100% of samples but non-vaccine type responses in only 4–20% of samples. Given the expected decline in antibody titers between the original study and the follow up study and the expected low enrolment rate, we decided it was unrealistic to expect to be able to detect non-vaccine type antibodies in a sufficient number of genital samples within the present study to conduct any meaningful analysis.

2.3. Laboratory methods

Serum samples (M84) were assessed for the presence of neutralizing and binding antibodies in parallel with the archived individual M12 serum sample from the original study [7]. This paired testing was carried out in order to evaluate and potentially adjust for any differences between the original and repeat M12 data. The pseudovirus (PsV) neutralization assay was carried out as previously described [7] using PsV representing both vaccine (HPV16, HPV18) and non-vaccine (HPV31, HPV45) genotypes. Inter-assay reproducibility was demonstrated by including the High HPV16/18 antibody plasma pool and HPV Negative plasma pool as internal quality controls in every experiment [8]. The median (interquartile range, IQR) titers of the High HPV16/18 plasma pool were as follows: HPV16 (44,351; 32,636–58,979; $n = 12$); HPV18 (18,051; 15,345–25,435), HPV31 (320; 253–369) and HPV45 (42; 39–61). The HPV Negative plasma pool was negative (<40) in all runs.

Binding antibodies were evaluated in a virus-like particle (VLP) ELISA as previously described [7], except that non-reporter containing L1L2 PsV were used as the target antigens. The median (IQR) titers of the High HPV16/18 plasma pool were as follows: HPV16 (24,798; 24,508–27,348), HPV18 (4832; 4690–5473), HPV31 (634; 519–836) and HPV45 (170; 162–176). The HPV Negative plasma pool was negative (<50) in all runs.

Serum samples were obtained from women (Gynaecology Outpatients Clinic, San Gerardo Hospital, Monza, Italy; ethics committee reference 08/UNIMIB-HPA/HPV1; No. 1191) following a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion [9]. Samples from this cohort of women were used to provide an estimate of the typical type-specific antibody titers generated during natural infection. Samples were selected from women who were HPV DNA negative but HPV seropositive to the genotype in question at the time of sampling and therefore represent women with a cleared infection, though no inference is made about the role of antibodies in infection clearance.

International Standards (IS) for HPV serology are limited to HPV16 (IS16; 05/134; National Institute for Biological Standards and Control, UK) and HPV18 (IS18; 10/140). No IS for HPV31 and HPV45 exist. Although serum neutralization data are usually reported in titers we made use of the IS16 and IS18 to calibrate the High HPV16/18 plasma pool [8] allowing us to also report relative antibody levels (IU/mL) against these two types for study samples. HPV vaccine and natural infection antibody titers against HPV16 or HPV18 were converted to IU/mL by reference to the titer of the calibrated High HPV16/18 plasma pool carried out in the same run. Reporting study data in IU/mL is made to facilitate easier comparisons between studies where such data are also reported in standardized units.

2.4. Statistical analysis

The sample size was limited by the response rate. With an estimated return of 27 per arm (30% return rate) and assuming a standard deviation of 0.68 Log_{10} scale for antibody responses (based on the original M12 data for HPV16, HPV18 and HPV31) and of 0.42 fold changes in geometric mean titers (GMT; based on M7 to

M12 titer decline) the precision (95% CI width) was estimated to be within 1.9 fold for GMT at follow up and within 1.5 fold for fold changes from M12 to M84. Differences of about 3.4 fold between vaccines would be detectable with 80% power at 5% significance. Empirical outcomes were HPV16, HPV18, HPV31 and HPV45 antibody titers from the PsV neutralization assay (cut-off 40) or VLP ELISA (cut-off 50). Values below the indicated assay cut-off were given a censored value of half this limit. The proportion of seropositive samples as well as GMT and fold changes between time points (with 95% confidence intervals) were estimated for each vaccine and non-vaccine type. Proportions were compared by Fisher's exact test. Comparison between study arms was made by the Kruskal-Wallis test. Significance was taken at the 5% level and 95% confidence intervals used. Two-sided significance tests were used. The relationship between time since vaccination and antibody titers was modelled using all post vaccination data (M7, M12 and M84) using a log-time on log-titre mixed effects model with random slope and intercept. For HPV31 and HPV45 this was done within the subset with a response at M7. These models were used to compare decline between vaccines and to extrapolate long term predicted titers to 15 years post vaccination. Stata version 15 (StataCorp, USA) was used for the analyses.

3. Results

3.1. Subject characteristics

A similar number of Cervarix[®] (28/96; 29%) and Gardasil[®] (30/102; 30%) vaccinees took part in the follow up study (Fig. 1). Serum samples were collected a median 7.0 (range 6.7–7.6) years after first immunization. The median age of the vaccinees at initial study entry was 12.9 (12.0–15.9) years and 19.7 (18.2–23.6) years old for the follow up (M84) sample. The median age of participants at initial study entry for those who did not enrol in the follow up study was similar at 12.6 years ($p > 0.05$). The median HPV16, HPV18, HPV31 and HPV45 serum neutralizing antibody titers at M7 for those enrolled in the follow up study were similar to those that did not enrol ($p > 0.05$; Kruskal-Wallis test). These data suggest that the subset of individuals enrolled in the follow up study is representative of the study participants overall.

3.2. Serum neutralization titers

M12 samples ($n = 58$) were retested against all four PsV targets and compared to the original study data for this time point. When combining data across all PsV targets there was a high correlation between original and retest results (Pearson's $r = 0.98$), but there was evidence of a deviation from the line of equivalence at the high end of the titre range with a slope of the regression line (95% CI) of retest vs. original = 0.894 (0.881–0.918). The fitted regression line was therefore used to transform M2 and M7 data for HPV16 and HPV18 to allow appropriate comparisons to be made across the whole study period for the subset of vaccinees taking part in the follow up study.

Seropositivity at M84 was 100% (95% CI 88–100%) against HPV16 for both vaccine arms and against HPV18 for Cervarix[®] vaccinees (Table 1). HPV18 seropositivity for Gardasil[®] vaccinees was 97% (83–100%). HPV31 seropositivity at M84 was 75% (55–89%) for Cervarix[®] vaccinees compared to 50% (31–69%) for Gardasil[®] vaccinees. HPV45 seropositivity at M84 was 25% (11–45%) for Cervarix[®] vaccinees compared to 3% (0–17%) for Gardasil[®] vaccinees.

GMTs for Cervarix[®] vaccinees were typically higher than Gardasil[®] vaccinees throughout the study. For example, the GMT (95% CI) of HPV16 neutralizing antibodies at M84 was 11,661 (8272–16,439) and 2898 (1854–4530) for Cervarix[®] and Gardasil[®]

Table 1
Seropositivity and geometric mean neutralizing antibody titers against vaccine and non-vaccine type HPV pseudoviruses.

HPV	Timing	Percentage seropositive			Geometric mean titers		
		Cervarix	Gardasil	p value	Cervarix	Gardasil	p value
16	M0	0/28 0% (0–12%)	1/27 4% (0–19%)	1.000	20 (20–20)	21 (19–24)	0.308
	M2	28/28 100% (88–100%)	28/28 100% (88–100%)	1.000	4589 (3357–6274)	1885 (1188–2992)	0.002
	M7	26/26 100% (87–100%)	29/29 100% (88–100%)	1.000	78,951 (57715–107999)	25,153 (16566–38191)	<0.001
	M12	28/28 100% (88–100%)	30/30 100% (88–100%)	1.000	32,089 (22522–45720)	11,803 (8017–17376)	<0.001
	M84	28/28 100% (88–100%)	30/30 100% (88–100%)	1.000	11,661 (8272–16439)	2898 (1854–4530)	<0.001
	<i>Fold change</i>				<i>0.36 (0.28–0.48)</i>	<i>0.25 (0.19–0.31)</i>	<i>0.090</i>
18	M0	0/28 0% (0–12%)	0/27 0% (0–13%)	N/A	20 (20–20)	20 (20–20)	1.000
	M2	28/28 100% (88–100%)	28/28 100% (88–100%)	1.000	2694 (1818–3993)	611 (400–932)	0.001
	M7	26/26 100% (87–100%)	29/29 100% (88–100%)	1.000	45,395 (27547–74808)	10,660 (6618–17170)	0.002
	M12	28/28 100% (88–100%)	30/30 100% (88–100%)	1.000	10,489 (6617–16626)	3011 (1897–4778)	0.003
	M84	28/28 100% (88–100%)	29/30 97% (83–100%)	1.000	3201 (2149–4768)	799 (494–1292)	0.002
	<i>Fold change</i>				<i>0.31 (0.23–0.41)</i>	<i>0.27 (0.22–0.33)</i>	<i>0.418</i>
31	M0	1/28 4% (0–18%)	0/27 0% (0–13%)	1.000	22 (19–25)	20 (20–20)	0.326
	M2	7/28 25% (11–45%)	3/28 11% (2–28%)	0.314	33 (22–49)	24 (19–31)	0.166
	M7	23/26 88% (70–98%)	20/29 69% (49–85%)	0.684	396 (192–817)	121 (65–223)	0.019
	M12	23/28 82% (63–94%)	21/30 70% (51–85%)	0.842	196 (105–368)	100 (58–172)	0.089
	M84	21/28 75% (55–89%)	15/30 50% (31–69%)	0.399	110 (65–185)	56 (37–85)	0.069
	<i>Fold change</i>				<i>0.56 (0.4–0.79)</i>	<i>0.56 (0.44–0.72)</i>	<i>0.434</i>
45	M0	0/28 0% (0–12%)	0/27 0% (0–13%)	N/A	20 (20–20)	20 (20–20)	1.000
	M2	0/28 0% (0–12%)	1/28 4% (0–18%)	1.000	20 (20–20)	21 (19–22)	0.317
	M7	10/26 38% (20–59%)	1/29 3% (0–18%)	0.009	39 (26–59)	21 (19–23)	0.001
	M12	7/28 25% (11–45%)	1/30 3% (0–17%)	0.058	32 (23–46)	21 (19–22)	0.015
	M84	7/28 25% (11–45%)	1/30 3% (0–17%)	0.058	26 (21–32)	21 (19–22)	0.018
	<i>Fold change</i>				<i>0.82 (0.64–1.05)</i>	<i>1 (0.9–1.1)</i>	<i>0.306</i>

p values < 0.05 highlighted in bold type. N/A, not applicable. Fold change refers to the difference between M84 and M12 neutralizing antibody titers. Titers < 40 were assigned a censored value of 20 for analysis purposes.

vaccinees, respectively. HPV16 and HPV18 antibody titers were maintained above the GMT typical of natural infection up to 7 years following initial immunization (Fig. 2). The overall fold change in GMT (95% CI) between M12 and M84 combined across both vaccinees was 0.30 (0.25–0.36) for HPV16 and 0.28 (0.24–0.34) for HPV18.

The High HPV16/18 plasma pool control [8] was calibrated to the International Standards for HPV16 and HPV18 antibodies allowing the HPV16 and HPV18 titers to be reported in IU/mL. The geometric mean concentration (GMC; 95% CI) of neutralizing antibodies against HPV16 declined from 2433 (1755–3372) IU/mL to 884 (664–1177) IU/mL between M12 and M84 for Cervarix[®] vaccinees (n = 28) and from 839 (551–1277) IU/mL to 206 (128–331) IU/mL between M12 and M84 for Gardasil[®] vaccinees (n = 30). By comparison, the GMC of neutralizing antibodies following natural infection in women who were HPV16 antibody positive but HPV16 DNA negative at the time of sampling was 14 (9–22; n = 29) IU/mL. The GMC of neutralizing antibodies against HPV18 declined from 753 (471–1206) IU/mL to 230 (152–347) IU/mL between M12 and M84 for Cervarix[®] vaccinees (n = 28) and from 214 (133–346) IU/mL to 57 (35–94) IU/mL between M12 and M84 for Gardasil[®] vaccinees (n = 30). By comparison, the GMC of neutralizing antibodies following natural infection in women who were HPV18 antibody positive but HPV18 DNA negative at the time of sampling was 12 (7–20; n = 17) IU/mL.

Non-vaccine type titers were substantially lower than vaccine type titers (Table 1). For example, the GMT (95% CI) for HPV31 neutralizing antibodies at M84 was 110 (65–185) and 56 (37–85) for Cervarix[®] and Gardasil[®] vaccinees, respectively. The overall fold change in GMT (95% CI) between M12 and M84 was 0.56 (0.46–0.69) for HPV31 and 0.91 (0.80–1.03) for HPV45. Both HPV31 and HPV45 antibody responses were lower than the GMT typical of natural infection (Fig. 2). There are no International Standards for HPV31 or HPV45 antibodies so antibody levels could not be formally benchmarked.

A VLP ELISA was employed to corroborate the seropositivity and antibody decline rates seen in the neutralization assay. Seropositivity at M84 was 100% (95% CI 88–100%) against HPV16 for both vaccine arms and against HPV18 for Cervarix[®] vaccinees. HPV18 seropositivity for Gardasil[®] vaccinees was 90% (73–98%). HPV31 seropositivity at M84 was 75% (55–89%) for Cervarix[®] vaccinees compared to 53% (34–72%) for Gardasil[®] vaccinees. HPV45 seropositivity at M84 was 25% (11–45%) for Cervarix[®] vaccinees compared to 3% (0–17%) for Gardasil[®] vaccinees. The overall fold change in GMT (95% CI) between M12 and M84 was 0.32 (0.27–0.39) for HPV16, 0.28 (0.24–0.33) for HPV18, 0.37 (0.31–0.45) for HPV31 and 0.66 (0.53–0.82) for HPV45. Seropositivity at M84 and the fold change in binding antibodies between M12 and M84 were generally similar to those derived from the pseudovirus neutralization assay.

3.3. Model prediction

The decline models for each vaccine for HPV16, HPV18, HPV31 and HPV45 showed an approximate 20–35% decline per doubling of time (Fig. 3). The rate of decline for HPV16 titers was 0.74 (95% CI 0.71–0.78) fold per doubling of time compared to 0.66 (0.63–0.70) and 0.79 (0.75–0.83) for HPV18 and HPV31, respectively. The rate of decline for HPV45 in the Cervarix[®] arm was 0.82 (0.76–0.89). The decline rates were similar for both vaccinees (p > 0.1) but Cervarix[®] antibody titers were predicted to remain 3–4 fold higher than Gardasil[®] over the long term. Extrapolating these declines out to 15 years following the third vaccine dose gave estimated geometric mean HPV16 titers of 7080 (4820–10,380) for Cervarix[®] and 2260 (1560–3280) for Gardasil[®]. For HPV18 the titers at 15 years post vaccination are estimated as 1820 (1180–2820) for Cervarix[®] and 460 (300–700) for Gardasil[®]. For HPV31 within those who initially responded to vaccination, Cervarix[®] and Gardasil[®] titers are predicted to decline to an average of 110 (65–187) and 67 (38–118) respectively. For HPV 45,

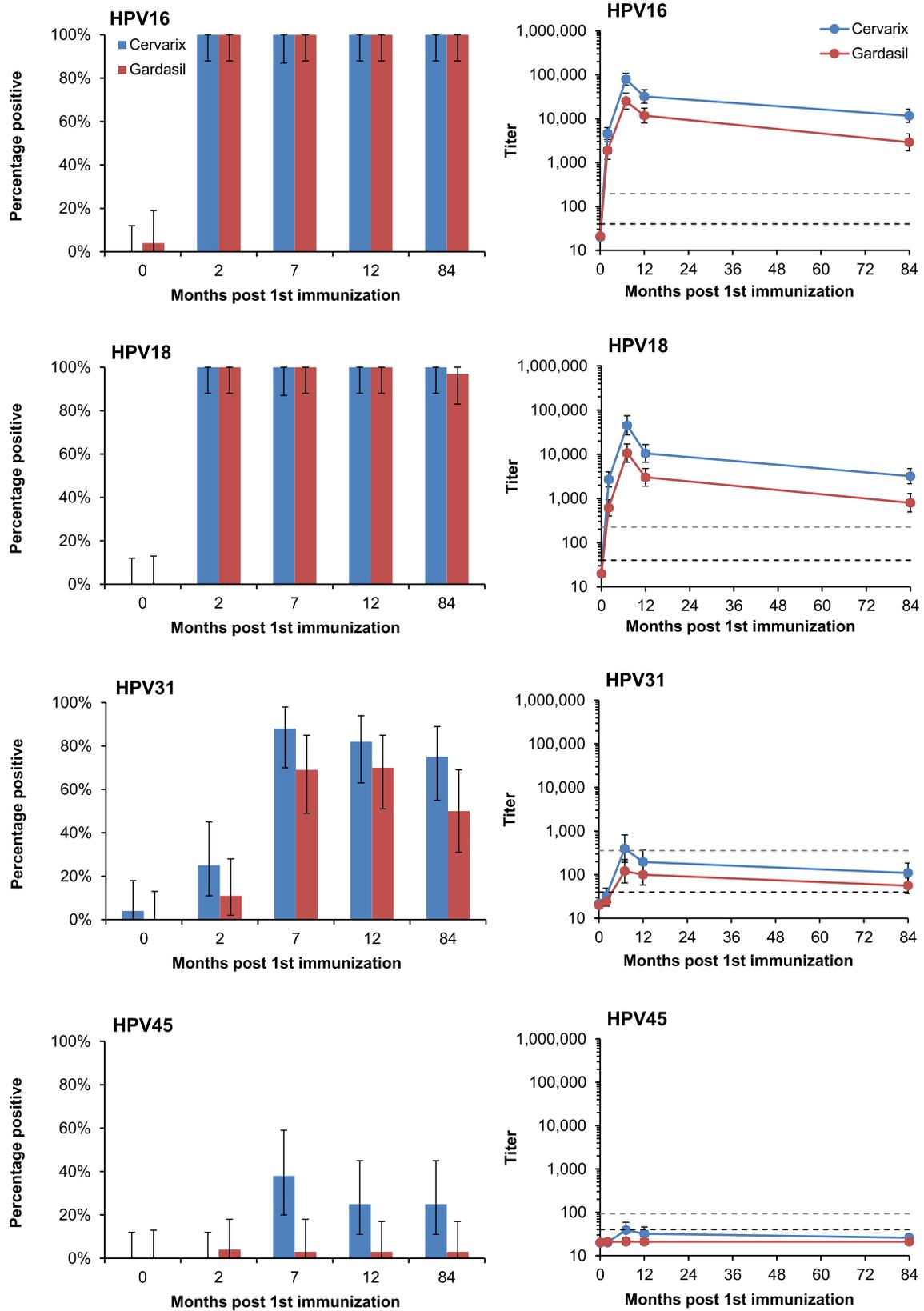


Fig. 2. Seropositivity and neutralizing antibody titers. Left panels: Percentage seropositive and 95% confidence intervals. Right panels: Error bars denote 95% confidence intervals of geometric mean titers (GMT). Antibody titers are presented on a Log₁₀ scale. Black dashed line, limit of detection (LOD) of the assay. Titers < 40 were assigned a censored value of 20 for analysis purposes. Grey dashed line, GMT of neutralizing antibody titers in women from a separate study who were, at the time of sampling, HPV seropositive and HPV DNA negative for the genotype under study and represent typical antibody titers induced by natural infection for: HPV16 (196; 125–309; n = 29), HPV18 (225; 134–378; n = 17), HPV31 (356; 213–594; n = 20), and HPV45 (93; 65–133; n = 13).

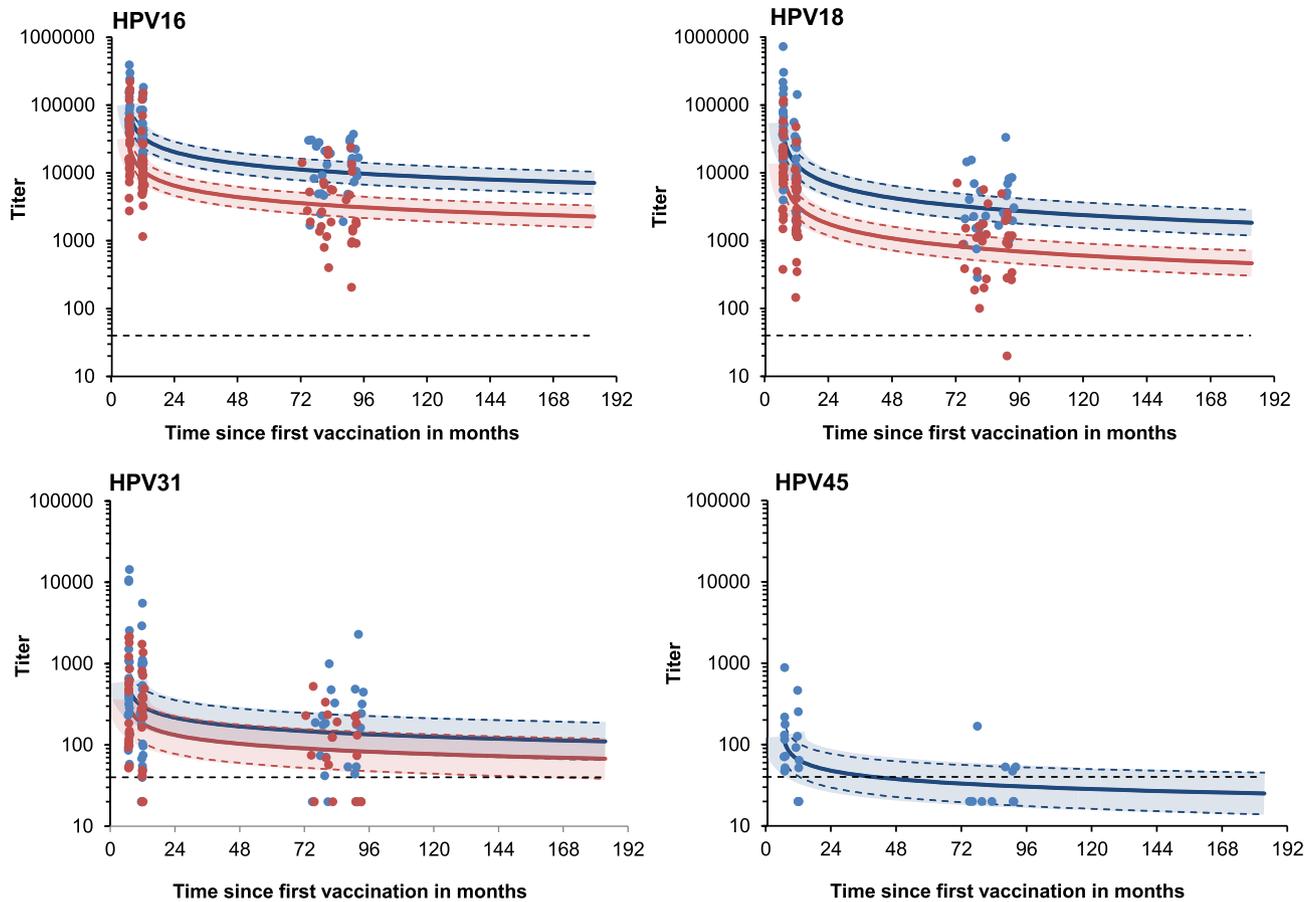


Fig. 3. Modelling antibody declines by time since vaccination using a log-titre log-time relationship. The relationship between time since vaccination and antibody titers was modelled using all post vaccination data (M7, M12 and M84) using a log-time on log-titre mixed effects model with random slope and intercept. For HPV31 and HPV45 this was done within the subset with a response at M7. Antibody titers are presented on a Log_{10} scale. Filled circles represent individual point data for Cervarix[®] (Blue) and Gardasil[®] (Red) vaccinees. Titers < 40 were assigned a censored value of 20 for analysis purposes. Solid lines represent model estimate for Cervarix[®] (Blue) and Gardasil[®] (Red) vaccines with dashed lines showing 95% confidence intervals on the modelled declines. Black dashed line, limit of detection (LOD) of the assay. Antibody declines by time since first vaccination extrapolated to 15 years (184 months). For plotting, data were modelled by time since 3rd vaccination dose then shifted by 6 months to show as time since first vaccination.

Cervarix[®] titers are predicted to decline to 25 (14–45) over the long term.

4. Discussion

This study assessed the durability of the functional antibody response against vaccine and non-vaccine HPV genotypes elicited by the Cervarix[®] and Gardasil[®] HPV vaccines when administered to 12–15 year old girls in a three dose regimen. Although there was a decline over the examination period, neutralizing antibodies against both vaccine and non-vaccine types remained detectable up to 7 years following initial vaccination.

All participants remained seropositive to HPV16 following immunization with either vaccine and against HPV18 following immunization with Cervarix[®], but seropositivity to HPV18 in the recipients who received the Gardasil[®] vaccine was lower than 100% after 7 years of follow up. These data are consistent with antibody binding data from 8 to 9 years of follow up of adolescents [10] or 16–23 year old women [11] immunized with three doses of Gardasil[®], a 10 year follow up study of 15–25 year old women immunized with three doses of Cervarix[®] [12] and long term follow up (ca. 12 year) of women enrolled in the Finnish cohorts of the Phase 3 licensure trials of both Cervarix[®] and Gardasil[®] vaccines [13]. A lower vaccine-type seropositivity rate for the neutralizing antibody response in Gardasil[®] vaccinees was also reported at

5 years in a head to head study of both vaccines in 18–26 year old women [14]. Lower HPV18 seropositivity rates in Gardasil[®] vaccinees were evident in both the neutralizing and binding antibody responses reported here but these were higher than the rates reported from other studies [11,12,14] likely due to the younger age of the vaccine recipients in the present study.

Vaccine-type neutralizing antibody titers remained high and above the GMT observed in a study of natural infection for both HPV16 and HPV18. Cervarix[®] titers continued to be higher than Gardasil[®] titers. When calibrated against the International Standards for HPV16 and HPV18 antibodies the GMC for HPV16 and HPV18 antibodies remained 1–2 orders of magnitude higher than the natural infection antibody level. The decline in vaccine-type neutralizing antibody titers over the study period was estimated to be 30% every 5–7 years and would be expected to remain above the level of natural infection at least 15 years following three doses of HPV vaccine in keeping with studies that have attempted to model the durability of the vaccine type neutralizing antibody response [14]. The robust immune memory induced by the HPV vaccines, exemplified by the durability of the antibody response, is supported by studies demonstrating a strong anamnestic response to a booster dose 5–7 years following initial immunization [15,16].

Neutralizing antibody seropositivity to non-vaccine types was lower than for vaccine types. Nevertheless, responses against both HPV31 and HPV45 were detectable after 7 years, with Cervarix[®]

vaccinees exhibiting higher rates of seropositivity than Gardasil[®] vaccinees. There are few studies examining the durability of the neutralizing antibody response against non-vaccine types. The rates seen here are higher than those found in a head to head study of 18–26 year old women 2 years after immunization [17] and for HPV31 higher than that reported from a study of 18–25 year old women 4 years after initial immunization with Cervarix[®] [18]. Non-vaccine type titers were substantially lower than vaccine type titers following initial immunization and remained so during follow up. There are no International Standards for HPV31 or HPV45 antibodies so it was not possible to benchmark the titers observed here although they were clearly lower than the typical GMT of antibodies elicited following natural infection. The antibody decline rate within those with an initial response was similar to that estimated for vaccine-type antibodies and it is estimated that low levels of antibodies against non-vaccine types would be detectable for many years following vaccination in some individuals.

Following recommendation by the World Health Organization [19], many countries have implemented a two dose HPV vaccination schedule for young girls. Limited non-vaccine type serological data derived from reduced dosing schedules are available but anecdotal data from a trial of older women vaccinated with Cervarix[®] does suggest [18] a reduced seropositivity to the non-vaccine type, HPV31, although vaccine efficacy against this type remains high [20].

There are no defined correlates of protection for HPV vaccination. Empirical studies have suggested that the amount of vaccine-type antibody required to protect mice challenged with HPV PsV *in vivo* is substantially lower than that required to neutralize the same PsV *in vitro* [21]. Although the level of natural infection antibody has been used as a benchmark for gauging vaccine immunity in vaccine efficacy studies, little is known about the specificity of antibodies elicited during natural infection although there are suggestions that they can be modestly protective [22]. A study of B cell clones suggested that the specificity of antibodies elicited by vaccination, while overlapping with those derived from natural infection in some cases, were largely distinct in their specificity [23]. In addition, cross-reactive HPV31 antibodies elicited by the HPV vaccines appear to target antigenic domains distinct from those targeted by type-specific HPV31 antibodies [24]. Vaccine efficacy [2] and impact surveillance [5] studies suggest that vaccine effectiveness against HPV31 and HPV45 genotypes is significant, despite having relatively lower immunity against these genotypes. Indeed, in reduced dose settings vaccine efficacy against these genotypes remained robust [20]. Taken together these data might suggest that, despite the low levels of non-vaccine type antibodies elicited by the HPV vaccines, it is their specificity that distinguishes them from type-specific antibodies elicited by natural infection and contributes to the vaccine effectiveness against non-vaccine types observed in vaccine trials and impact studies [2,5]. A recent review [25] and post-hoc analysis of vaccine efficacy data [26] have attempted to delineate possible mechanism(s) behind the potency of the HPV vaccines, including the activation of additional immune pathways and uncertainty about the levels and site of action of these immune effectors. The role that neutralizing antibodies play in the long-term protection against infection and subsequent disease is uncertain and the utility of such antibodies as a potential correlate or surrogate of such protection is unclear.

This study has several strengths including the evaluation of the durability of the immune response to both vaccines in participants at the target age for vaccination in national immunization programmes; evaluation in the context of a randomized head-to-head trial; the reporting of vaccine and natural infection antibody levels in International Units for the vaccine types; and the report-

ing of data to 7 years derived from the neutralization assay which is considered to be the gold standard for *in vitro* serology testing. Shortcomings in this study include the relatively low number of responders to the follow up study which inevitably impacts on the precision of some of the estimates. Nevertheless, these data provide additional empirical support for the observed effectiveness of the HPV vaccines, particularly against non-vaccine genotypes.

The nonavalent Gardasil[®]9 vaccine has demonstrated broad efficacy in a three dose schedule [27] and will likely be adopted by many national immunization programmes in time. However, tens of millions of adolescent girls have been vaccinated with the bivalent or quadrivalent vaccine [28], and a better understanding of vaccine immunity, particularly the breadth, magnitude and durability of the antibody responses, is warranted.

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Author contributions

Study design: AG KP NA JS PT EM SB. Data generation: AG KP MH. Data handling and analysis: KP NA SB. Material contribution: CEC EM SB. Manuscript preparation: AG KP MH CEC NA JS PT EM SB.

Declarations of interest

None.

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