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Neutralizing and cross-neutralizing antibody titres induced by bivalent and quadrivalent human papillomavirus vaccines in the target population of organized vaccination programmes

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ABSTRACT

Aim of this investigator-initiated study was to evaluate and compare the titres of neutralizing and cross-neutralizing antibodies (NAbs) induced by the bivalent (Cervarix®) and quadrivalent (Gardasil®) HPV vaccines in a cohort of girls aged 11–13 years from organized vaccination programmes. To this aim, HPV16 and HPV18 NAbs were measured by pseudovirion-based neutralization assays in serum collected at 1–6 months after the third vaccine dose in 107 girls vaccinated with Cervarix® and 126 vaccinated with Gardasil®, while HPV31 and HPV45 cross-NAbs were tested in the first 50 consecutive girls of both vaccine groups. The results of this study demonstrated that all vaccinated girls developed HPV16 and HPV18 NAbs, with the exception of two Gardasil® vaccinees with undetectable HPV18 NAbs. Geometric mean titres (GMTs) of both HPV16 and HPV18 NAbs were significantly higher in Cervarix® than in Gardasil® vaccinees [HPV16 NAb GMT 22,136 (95% CI, 18,811–26,073) vs 5092 (4230–6151), respectively; $P < 0.0001$; HPV18 NAb GMT 11,962 (9536–14,363) vs 1804 (1574-2110), respectively; $P < 0.0001$]. Cross-NAbs to HPV31 and HPV45 were detected more frequently Cervarix® (HPV31 NAb positivity rates 92.7% and 36%, respectively; $P < 0.05$) than in Gardasil® vaccinees (HPV45 NAb positivity rates 56% and 6%, respectively; $P < 0.0001$). The titres of cross-NAbs against HPV31 and HPV45 were also significantly higher in Cervarix® than in Gardasil® vaccinees [HPV31 NAb GMT 157.2 (95% CI, 92–269) vs 13.0 (6.5–25.8), respectively; $P < 0.0001$; HPV45 NAb GMT 4.7 (2.1–10.2) vs 1.3 (0.3–3.1), respectively; $P < 0.01$].

In conclusion, in adolescent girls vaccinated within organized vaccination programmes, HPV vaccines drive the generation not only of NAbs to HPV vaccine types, but also of cross-NAbs. The bivalent vaccine induced significantly higher HPV16 and HPV18 NAb titres and more frequently and at higher titre HPV31 and HPV45 cross-NAbs than the quadrivalent vaccine.

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1. Introduction

Persistent infection by high-risk human papillomavirus (HPV) types, mainly HPV16 and HPV18, is the necessary cause of cervical cancer and has been associated with the development of other genital cancers and head and neck squamous cell carcinomas [1,2]. Two prophylactic HPV vaccines, a bivalent and a quadrivalent vaccine, have been licensed in many countries for use in adolescent girls and young women to prevent cervical cancer. The bivalent vaccine (Cervarix®, GlaxoSmithKline Biologicals) protects against HPV16 and HPV18, while the quadrivalent vaccine (Gardasil®, Sanofi Pasteur Merck Sharp & Dohme Ltd.) protects against HPV16, HPV18, and the low-risk HPV6 and HPV11 that are responsible for genital warts [3]. Both vaccines are composed of HPV L1 proteins assembled into virus-like particles (VLPs) but differ in antigen production

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system, antigen dose, antigen structure, adjuvant, and vaccination schedule [3].

Clinical trials have demonstrated that both vaccines are highly effective in the prevention of persistent infection and cervical lesions associated with HPV vaccine types, with approximately 100% efficacy in the prevention of HPV16- and HPV18-related cervical intraepithelial neoplasia grade 3 in young women [4–7]. Protection remained high in follow-up studies up to 8.4 years for Cervarix® [8] and up to 5 years for Gardasil® [9]. Significant cross-protection against non-vaccine HPV types phylogenetically related to HPV16 and HPV18, i.e., HPV31, HPV33, and HPV45, was also demonstrated by clinical trials [10–12].

In animal models, production of HPV type-specific neutralizing antibodies (NAbs) that transudate to cervicovaginal secretions has been shown to be the mechanism of protection and cross-protection induced by HPV L1 VLP vaccines [13,14].

Following vaccination, NAbs specific for HPV vaccine types are induced in virtually all subjects, with peak titres at one month after the third vaccine dose that are at least 100-fold higher than those generated after natural infection [15–19]. In addition, in line with clinical data of cross-protection, production of cross-NAbs against HPV types related to HPV16 and HPV18 has been demonstrated, especially in Cervarix® vaccine trials [17,20–26].

These data, however, derive mainly from manufacturers' studies and only a few compared the two vaccines. In addition, no data on the immunogenicity of HPV vaccines have been reported so far in the setting of current vaccination programmes. This information would be useful for public health strategies of HPV vaccine implementation and monitoring.

In the present study, the immunogenicity of Cervarix® and Gardasil® was compared in a cohort of girls aged 11–13 years who received HPV vaccination within the organized vaccination programme. In these subjects, NAbs against HPV16 and HPV18 and cross-NAbs against HPV31 and HPV45 were evaluated at 1 to 6 months after the third dose of HPV vaccine.

2. Materials and methods

2.1. Study design

Aim of this investigator-initiated cohort study was to evaluate and compare the immunogenicity of Cervarix® and Gardasil® in the target adolescent population of organized vaccination programmes.

Study subjects were enrolled from December 2011 to December 2012 in two public health districts of the Veneto and Emilia-Romagna regions, where Cervarix® and Gardasil®, respectively, were actively offered to 12 years-old girls by organized vaccination programmes. In these districts, for each vaccine type, a total of 1000 girls aged 11–13 years, who were scheduled for the third HPV vaccine dose, were invited by letter to participate in the study, which consisted in a blood sampling, in the period from 1 to 6 months after the third dose of vaccine, to measure the immune response induced by vaccination. Written informed consent was obtained from each study participant and from a parent or legal representative before enrolment in the study. All 11–13 years-old girls that had completed the third vaccine dose were eligible for enrolment. Exclusion criteria were fever and other symptoms of active infection in the three weeks before blood sampling, presence of chronic inflammatory diseases, immunosuppressive therapy, pregnancy. Blood samples were collected, processed to obtain sera, and stored until testing at the Clinical Microbiology and Virology Units of Padova and Bologna University Hospitals. All neutralization tests were performed at the Clinical Microbiology and Virology Unit of Padova University Hospital by technicians blinded to vaccination groups. Neutralizing antibodies against HPV16 and HPV18 were tested in all study participants, i.e., 126 girls vaccinated with Gardasil® and 107 girls vaccinated with Cervarix™, while cross-NAbs against HPV31 and HPV45 were tested in the first 100 consecutive girls vaccinated with Gardasil® ($n = 50$) and Cervarix® ($n = 50$). Only cross-NAbs against HPV31 and HPV45 were evaluated in this study, since consistent results of cross-protection provided by HPV vaccination have been reported only for these two high-risk HPV types in randomized clinical trials [12].

2.2. Pseudovirion-based neutralization assay (PBNA)

HPV type-specific pseudovirions (PsVs) encapsidating a SEAP reporter plasmid were used for the PBNA which was adapted to a 96-microwell plate format according to WHO human papillomavirus laboratory manual [27]. Pseudovirions were obtained by transfection of 293TT cells with equal amounts of p16shell, p18shell, p31shell, or p45shell plasmids and the pYSEAP plasmid, according to WHO protocols [27]. For the PBNA, 293TT cells were seeded in 96-well plates at a concentration of 30,000/well in neutralization buffer (DMEM without phenol red, 10% of FBS, 1% of Glutamax™, 1% of non-essential amino-acids) and incubated for 2–5 h at 37 °C. Meanwhile, HPV PsVs were diluted in neutralization buffer, as well as with sera at 2-fold dilution from 1:40 to 1:163,840. All tests were done in duplicate. The H16.V5 and H18.J4 monoclonal antibodies specific for HPV16 and HPV18, respectively, obtained from the National Institute for Biological Standards and Control in the United Kingdom, were used at 1:20,000 dilution in each assay as positive controls; in addition, an HPV16 neutralizing serum was used at 1:80 dilution as inter-assay reproducibility control. After incubation for 3 h, 80 μl PsVs mixed with different dilution of 20 μl serum or antibody or heparin as positive neutralization controls were cooled on ice for 1 h and then added to the cells. After 72 h, supernatant was tested by using the SEAP Reporter Gene Assay (Roche Applied Science, Penzberg, Germany) according to the manufacturer's recommendations and chemiluminescence was read on a Lumistar Optima luminometer (BMG Labtech GmbH, Ortenberg, Germany). The neutralization titre was determined as the reciprocal of the final dilution of serum that yielded <50% of mean RLU measured with PsVs alone and reported as ED_{50} (effective dose producing 50% response). The limit of quantification of the PBNA was set at 40 ED_{50}. Serum samples with neutralization titres equal or higher than 40 ED_{50} were considered positive. Serum samples with neutralization titres less than 40 ED_{50} were assigned a value of 1 for the purpose of geometric mean titre (GMT) calculation.

2.3. Statistical analysis

The primary objective of this study was to compare prevalence and titres of NAbs against HPV16 and HPV18 between the two vaccine groups. The secondary objective was to compare cross-NAbs against HPV31 and HPV45. The target samples size was set at 100 subjects in each group for the primary endpoint and 50 for the secondary endpoint. Setting type I error probability (α) to .05 (2-sided), power to .90, and assuming a standard deviation of 30% of the mean GMT, these sample sizes allowed to detect differences between groups of about 15% and 20%, respectively.

Continuous variables are reported as mean ± standard deviation, categorical variables are summarized as numbers and percentages. Antibody titres are reported as median and range and as geometric mean and CI 95%. Comparisons between continuous variables was done by two-tailed unpaired *t*-test; comparisons between categorical variables was done by χ^2 test; comparisons of antibody titres were performed in log transformed values by two-tailed unpaired *t*-test and Pearson's correlation. Statistical

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significance was determined by P value of less than 0.05. Stata software version 12 (StatSoft Inc.) was used for statistical analysis.

3. Results

3.1. Comparison of HPV16 and HPV18 neutralization titres

HPV16 and HPV18 PBNAs were performed on serum samples collected from 126 girls vaccinated with Gardasil® (mean age, 11.8 ± 0.2) and 107 girls vaccinated with Cervarix® (mean age, 11.6 ± 0.7 years; $P = 0.2$). Mean time since the third vaccine dose was 87 ± 44 days for girls vaccinated with Gardasil® and 81 ± 39 days for those vaccinated with Cervarix® ($P = 0.2$).

At 1–6 months after the completion of the vaccination cycle, 100% of both Cervarix® and Gardasil® vaccinated girls had NAbs against HPV16 (defined as an antibody titre ≥40 ED_{50}), while 100% of Cervarix® vaccinees and 98.4% of Gardasil® vaccinees had NAbs against HPV18, without statistically significant differences in HPV16 and HPV18 NAb positivity rates between vaccine groups (Table 1).

The GMTs of both HPV16 and HPV18 NAbs were significantly higher in Cervarix® than in Gardasil® vaccinees [i.e., HPV16 NAb GMT, 22,136 (95% CI, 18,811–26,073) vs 5092 (4230–6151), respectively; $P < 0.0001$; HPV18 NAb GMT, 11,962 (9536–14,363) vs 1804 (1.574–2.110), respectively; $P < 0.0001$] (Table 1 and Fig. 1A). The GMT ratios of HPV16 and HPV18 NAbs between vaccine groups were 4.3 and 6.6, respectively.

In both vaccine groups, HPV16 NAb titres were significantly higher than HPV18 NAb titres (Fig. 1A), with GMTs of HPV16 approximately 2-fold higher than HPV18 for Cervarix® ($P < 0.001$) and 5-fold higher for Gardasil® ($P < 0.0001$).

Variable response to vaccination was observed among vaccinees, with NAb titres ranging from the lower limit of detection of the assay to 20,480 ED_{50} for Gardasil® and from 640 to 163,840 ED_{50} for Cervarix® (Table 1). Subjects with high HPV16 NAb titres had generally also high HPV18 NAb titres, while those with low HPV16 NAb titres had also a low response against HPV18. In particular, the sera from the two girls vaccinated with Gardasil® with negative HPV18 NAbs results (i.e., HPV18 NAb titres <40 ED_{50}) were tested at lower dilutions and demonstrated to have HPV18 NAb titres of 10 and <5, respectively. These two sera had also low HPV16 NAb titres (i.e., 40 and 160, respectively), indicating poor immune response to vaccination in these subjects. This trend was confirmed by Pearson correlation analysis that demonstrated a linear relationship between HPV16 and HPV18 NAb titres in both Cervarix® and Gardasil® vaccinees (Fig. 2).

Stratification of subjects according to time since vaccination showed a trend towards a decrease of HPV16 and HPV18 NAb GMTs in Gardasil® vaccinees from month 1 to month 6 after completion of vaccination, while NAb titres remained stable in Cervarix® vaccinees (Fig. 3).

3.2. Comparison of HPV31 and HPV45 neutralization titres

PBNAs for HPV31 and HPV45 were performed on a sub-set of 50 subjects from each vaccination group.

At 1–6 months after the third dose of vaccine, Cervarix® vaccinees had cross-NAbs against HPV31 significantly more frequently (92.7% vs 56%, $P < 0.05$) and at higher titre (12-fold higher) than Gardasil® vaccinees (i.e., HPV31 NAb GMT, 157.2 [95% CI, 92–269] vs 13.0 [95% CI, 6.5–25.8], respectively; $P < 0.0001$), as detailed in Table 1 and Fig. 1B. Likewise, cross-NAbs against HPV45 were detected more frequently (i.e., 36% vs 6%, $P < 0.0001$) and at higher titre in Cervarix® than in Gardasil® vaccinees (i.e., HPV45 NAb GMT 4.7 [95% CI, 2.1–10.2] vs 1.3 [0.3–3.1], respectively; $P < 0.01$; Table 1 and Fig. 1B).

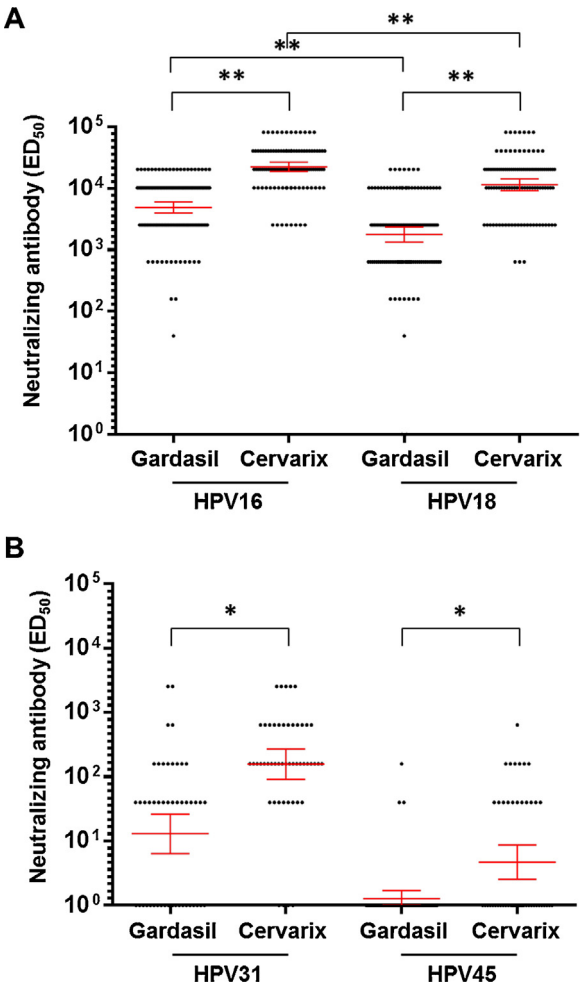

Fig. 1. HPV type-specific neutralizing antibodies (NAbs) measured by the PBNA and reported as ED_{50} values in Cervarix® and Gardasil® vaccinees at 1–6 months after the third dose of vaccine. Geometric mean titres (GMTs) and 95% confidence interval of ED_{50} values of vaccine groups are indicated with red bars, while NAbs titres of each case are represented by black dots. (A) NAbs to HPV16 and HPV18 evaluated in Cervarix® ($n = 107$) and in Gardasil® ($n = 126$) vaccinees; (B) NAbs to HPV31 and HPV45 evaluated in Cervarix® ($n = 50$) and in Gardasil® ($n = 50$) vaccinees. * $P < 0.0001$ and ** $P < 0.05$ as calculated by two-tailed unpaired t-test in log transformed values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In subjects with detectable NAbs, titres were markedly lower than those against HPV16 and HPV18, i.e., up to 2560 ED_{50} for HPV31 in both vaccine groups and up to 640 and 160 ED_{50} for HPV45 in Cervarix® and Gardasil® vaccinees, respectively (Table 1 and Fig. 1).

Although correlations between HPV16 and HPV31 NAb titres and between HPV18 and HPV45 NAb titres were not statistically significant, in both vaccine groups subjects with high HPV16 and HPV18 NAb titres had more frequently cross-NAbs against HPV31 and HPV45, respectively.

4. Discussion

This study for the first time evaluated and compared NAb and cross-NAb titres induced by Gardasil® and Cervarix® vaccines in a cohort of girls aged 11–13 years who were vaccinated within organized universal HPV vaccination programmes. The results of this study demonstrated that both HPV vaccines drove the generation

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Table 1
Comparison of positivity rates, median titres, and geometric mean titres of HPV16 and HPV18 neutralizing antibodies and HPV31 and HPV45 cross-neutralizing antibodies in Gardasil® and Cervarix® vaccinated girls at 1 to 6 months after the third vaccine dose.

HPV type	Cervarix® group	Gardasil® group	*P* value
NAb positivity rate (percentage, 95% CI)			
HPV16	107/107 (100%; 100, 100%)	126/126 (100%; 100, 100%)	NS
HPV18	107/107 (100%; 100, 100%)	124/126 (98.4%; 96.2, 100%)	NS
HPV31	46/50 (92.7%; 84.5, 99.5%)	28/50 (56%; 42.2, 69.7%)	<0.05
HPV45	18/50 (36%; 22.7, 49.3%)	3/50 (6%; −0.6, 12.6%)	<0.0001
NAb ED_{50} median (range)			
HPV16	20,480 (2560–163,840)	10,240 (40–20,480)	–
HPV18	20,480 (640–81,920)	2560 (<40–20,480)	–
HPV31	160 (<40–2560)	40 (<40–2560)	–
HPV45	<40 (<40–640)	<40 (<40–160)	–
NAb GMT ED_{50} (95% CI)			
HPV16	22,136 (18,811; 26,073)	5092 (4230; 6151)	<0.0001
HPV18	11,962 (9536; 14,363)	1804 (1.574; 2.110)	<0.0001
HPV31	157.2 (92; 269)	13.0 (6.5; 25.8)	<0.0001
HPV45	4.7 (2.1; 10.2)	1.3 (0.3; 3.1)	<0.01

NAb: neutralizing antibody measured by pseudovirion-based neutralization assay; GMT: geometric mean titre; ED_{50}: effective dose producing 50% response. *P* values were determined by χ^2 test and two-tailed unpaired *t*-test.

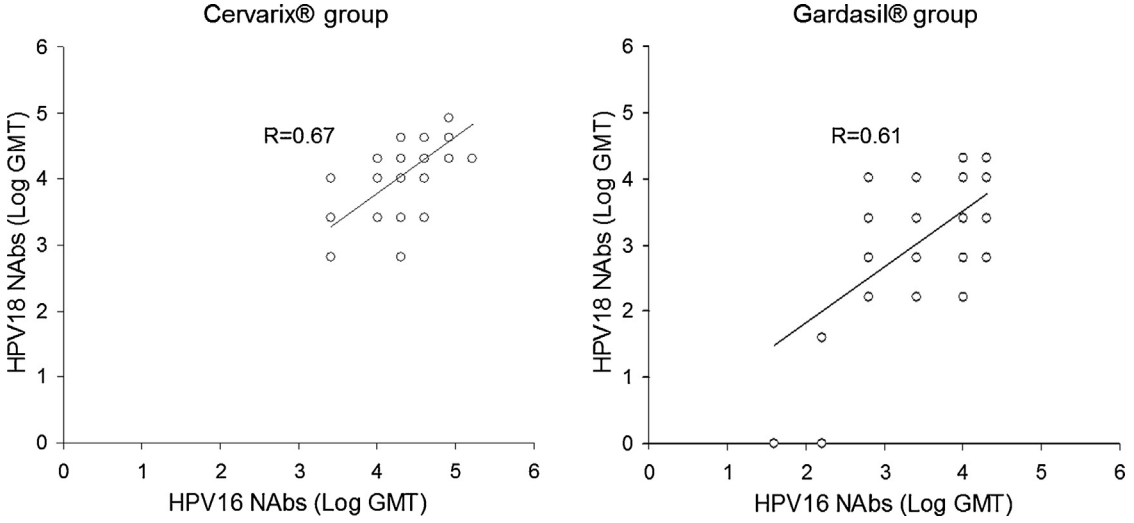

Fig. 2. Relationship between HPV16 and HPV18 neutralizing antibodies (NAbs) measured by the PBNA and reported as log transformed ED_{50} values in Cervarix® ($n = 107$) and Gardasil® ($n = 126$) vaccinees at 1–6 months after the third dose of vaccine. Overall Pearson's (R) coefficients are given.

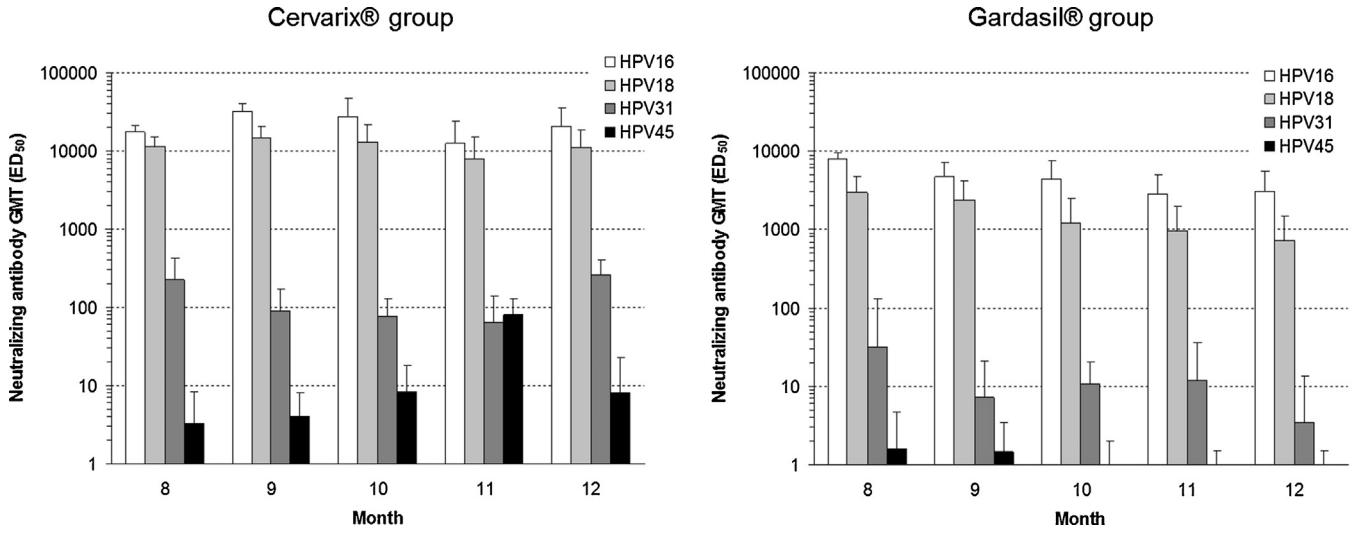

Fig. 3. Neutralizing antibodies (NAbs) measured by the PBNA and reported as geometric mean titre (GMTs) and 95% confidence interval of ED_{50} values in Cervarix® and Gardasil® vaccinees stratified according to months of vaccination (month 8 correspond to the second month after the third vaccine dose). NAbs to HPV16 and HPV18 were evaluated in 107 girls vaccinated with Cervarix® and 126 vaccinated with Gardasil®; NAbs to HPV31 and HPV45 were evaluated in 50 girls vaccinated with Cervarix® and in 50 vaccinated with Gardasil®. Groups for each measure were as follows: HPV16/18 NAbs, Cervarix® M8 ($n = 35$), M9 ($n = 34$), M10 ($n = 15$), M11 ($n = 11$), M12 ($n = 12$); Gardasil® M8 ($n = 47$), M9 ($n = 30$), M10 ($n = 16$), M11 ($n = 18$), M12 ($n = 15$); HPV31/45 NAbs, Cervarix® M8 ($n = 17$), M9 ($n = 15$), M10 ($n = 7$), M11 ($n = 5$), M12 ($n = 6$); Gardasil® M8 ($n = 18$), M9 ($n = 10$), M10 ($n = 8$), M11 ($n = 8$), M12 ($n = 6$).

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not only of high titre NAbs to HPV vaccine types, but also of NAbs to other HPVs related to the vaccine types (i.e., HPV31 and HPV45). A secondary finding of this study was the demonstration of the higher immunogenicity of Cervarix® than Gardasil®. In fact, at 1–6 months after the third vaccine dose, Cervarix® vaccinees had significantly higher HPV16 and HPV18 NAbs titres than Gardasil® vaccinees and serum HPV31 and HPV45 cross-NAbs significantly more frequently and at higher titre than Gardasil® vaccines, in agreement with previous reports in older age groups [17–19,28].

In vaccinated subjects, HPV16 and HPV18 NAbs titres fell within a range comparable to previous studies [15–19] and were markedly higher than those measured in women with natural HPV infection that ranged from below the limit of detection of the PBNA to 640 ED_{50} [Squarzon L. and Barzon L., unpublished data]. However, three subjects vaccinated with Gardasil® had low NAb titres, with HPV16 NAbs ranging from 40 to 160 ED_{50} and HPV18 NAbs ranging from undetectable to 40 ED_{50}.

The levels of cross-NAbs to HPV31 and HPV45 measured in vaccinated girls were lower than HPV16 and HPV18 NAbs. However, these low level NAbs are probably relevant for cross-protection, since the positivity rates in the two vaccine groups detected in the present study are in line with the results on cross-protection against non-vaccine HPV persistent infection and related disease from randomized trials [10–12]. In particular, in vaccine efficacy trials, Cervarix® showed 77.1% and 79.0% cross-protection against 6-month persistent infection by HPV31 and HPV45, respectively [10], while Gardasil® had significant efficacy against persistent HPV31 infection only, with 46.2% reduction of infections compared to controls [11]. Accordingly, in our study, cross-NAbs against HPV31 and HPV45 were detected in 93% and 36%, respectively, of Cervarix® vaccinees and in 56% and 6% of Gardasil® vaccines and the GMTs of HPV31 and HPV45 NAbs were significantly higher in Cervarix® than Gardasil® vaccinees.

The higher and sustained immunogenicity of the Cervarix® vaccine has been ascribed to the MLP component of the AS04 adjuvant, which binds and activates the toll-like receptor 4, an important player in innate and adaptive immune responses [29]. Differences in conformation and exposure of L1 VLPs epitopes due to antigen structure and production process might also account for the different immunogenicity of the two vaccines [30].

The results of vaccine efficacy trails also suggest that cross-protection might decrease during follow-up [12], but these trials were performed in women aged 15–26 years, older that the target population of organized HPV vaccination programmes for whom efficacy data are not yet available. It cannot be excluded that the high peak antibody levels post vaccination observed in young subjects could led to long-term cross-protection.

Stratification of vaccinated subjects on the basis of time since the last HPV vaccine dose showed that HPV16, HPV18, HPV31, and HPV45 NAb titres remained stably high after 6 months in Cervarix® vaccinees, while a decrease of NAb titres was observed in Gardasil® vaccinees. Also these results are in line with data from vaccine trials in older age groups, which reported the persistence of high and sustained levels of antibodies for up to 8.4 years after vaccination with Cervarix® [8,15,31] and a marked reduction of antibodies in the first year post-vaccination with Gardasil®, followed by a further a decline with undetectable antibodies to HPV18 in 40–50% of vaccinees at 4-year follow-up [5,32]. Protection against HPV18 persistent infection and related disease was however maintained, suggesting the presence of vaccine-induced immune memory [5,32]. In this context, the presence of sustained HPV16- and HPV18-NAb titres in Cervarix® vaccinees for longer times than in Gardasil® vaccinees correlates with the significantly higher rate of specific memory B cells detectable in the peripheral blood of Cervarix® than Gardasil® vaccinees [[18,19] and unpublished data by Caputo and Mantelli].

These findings indicate that monitoring NAb titres to HPV vaccine types and cross-NAbs to related HPV types in vaccinated cohorts could be useful to estimate the degree and duration of protection and cross-protection, especially in individuals who did not correctly complete their vaccination schedule, and to provide information useful for the design and implementation of the most appropriate cancer prevention strategies. In addition, evaluation of the immune response to HPV vaccines could allow to determine the immune correlates of protection. Actually, the minimum level of antibodies required for protection (i.e., the immune correlate of protection against HPV infection and related disease) has not been identified yet. A study performed in mice suggests that the titre of NAb that is still protective against HPV infection is probably very low [33]. In fact, mice with serum levels of antibodies against HPV VLPs 500-fold lower than the limit of detection of in vitro neutralization assays were protected from cervicovaginal challenge with HPV16 PsVs [33]. Anyway, high levels NAbs and cross-NAbs in serum and hence in cervicovaginal secretions are reasonably desirable, since high and sustained antibody responses might be required to maintain vaccine efficacy for a long time without boosting, to provide wider cross-protection to different HPV types, and to reduce the number of vaccine doses [23,34].

In this regard, a two-dose vaccination schedule has been recently approved for both HPV vaccines, based on the non-inferiority of the antibody response to that of the standard three-dose schedule. In particular, analysis of data from the phase III trial of Cervarix® in young women in Costa Rica demonstrated that the efficacy of two or even a single dose of vaccine was similar to that of three doses in preventing persistent HPV16 and HPV18 infection over 4 years [35]. In the same trial, at the end of the 4 years of follow-up, antibody responses even after a single dose were higher than those following natural infection [23]. In addition, the antibody response after the two-dose vaccination schedule (i.e., at months 0 and 6) with Cervarix® was non-inferior to the standard three doses schedule at 4 years after vaccination in a randomized partially-blind study in healthy females aged 9–25 years [36] and, at 21 months of follow-up, in an open-label nonrandomized clinical trial conducted in adolescent girls and young women in Mexico [37]. As for the Gardasil® vaccine, a randomized phase III, postlicensure, immunogenicity study in Canada demonstrated, at 3 years from vaccination, the non-inferiority of the antibody responses in girls who received the two-dose schedule to those among young women who received three-doses, while, at 2 and 3 years, the antibody response to HPV18 was lower in girls who received two doses than girls who received three doses [34]. Thus, considering the relevance of the duration of protection and cross-protection, evaluation and comparison of antibody responses within organized HPV vaccination programmes will be useful also in subjects vaccinated according to new two-dose schedule.

In conclusion, this study in adolescent girls vaccinated within organized vaccination programmes demonstrated that HPV vaccines drove the generation not only of NAbs to HPV vaccine types, but also of cross-NAbs. In additions, this study demonstrated that the bivalent vaccine induced significantly higher HPV16 and HPV18 NAb titres and more frequently and at higher titre HPV31 and HPV45 cross-NAbs than the quadrivalent vaccine.

Ethical approvals

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The study protocol (Protocol no. 2413P) and the informed consent and assent forms were approved by the Ethics Committees of Padova University Hospital and Bologna University Hospital.

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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