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BRIEF REPORT

Head-to-Head Comparison of Bi- and Nonavalent Human Papillomavirus Vaccine-Induced Antibody Responses

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For head-to-head comparison of human papillomavirus (HPV) antibody levels induced by different vaccines, 25-year-old vaccine-naive women were given either the bivalent (n = 188) or the nonavalent HPV vaccine (n = 184). Six months after vaccination antibodies against pseudovirions from 17 different HPV types (HPV6/11/16/18/31/33/35/39/45/51/52/56/58/59/66/68/73) were measured. Antibodies against HPV16/18 were higher after bivalent HPV vaccination (mean international units [IU] 1140.1 and 170.5 for HPV16 and 18, respectively) than after nonavalent vaccination (265.1 and 22.3 IUs, respectively). The bivalent vaccine commonly induced antibodies against the nonvaccine HPV types 31/33/35/45 or 58. The nonavalent vaccine induced higher antibodies against HPV6/11/31/33/45/52/58 and 35.

Keywords. human papillomavirus; vaccine; nonavalent; bivalent; international units; antibody.

Cervical cancer is the fourth most common form of cancer among women worldwide, claiming annually the lives of 300 000 women [1]. Human papillomavirus (HPV) is a necessary cause for cervical cancer. There are 222 different HPV types (www.hpvcenter.se, accessed 10 February 2022), with 12 of them (HPV16/18/31/33/35/39/45/51/52/56/58/59) classified by the World Health Organization/International Agency for Research on Cancer (WHO/IARC) as human carcinogens (HPV68 is classified as probably carcinogenic) [1].

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Two effective prophylactic vaccines against HPV, the bivalent Cervarix (against HPV16/18) and the quadrivalent Gardasil (HPV6/11/16/18), were licensed and introduced into immunization programs around the world 15 years ago [2]. A nonavalent vaccine, Gardasil 9 (against HPV6/11/16/18/31/33/45/52/58), was introduced in 2014 (www.ema.europa.eu/en/medicines/human/EPAR/gardasil-9, accessed 8 October 2020). All these vaccines induce antibodies against the most carcinogenic HPV types (HPV type 16 and 18, responsible for more than 70% of all HPV-related cancer cases) and have been found to be safe, immunogenic, and efficiently protect against persistent HPV infections, precancerous lesions, and invasive HPV-related cancers [2–6].

While each vaccine is approved for targeting specific genotypes, it is well described that they can also induce cross-protective effect against some nonvaccine types [2, 7, 8]. The bivalent vaccine Cervarix has shown efficacy against nonvaccine HPV types more consistently in both short- and long-term follow-up settings comprising 12 years after vaccination when compared to the quadrivalent vaccine [7, 8], but systematic head-to-head comparisons of the bivalent and the nonavalent vaccines are lacking.

Considering the global aim set by the WHO towards elimination of cervical cancer and a predicted shortage in supply of HPV vaccines, a head-to-head comparison of the immunogenicity of the bivalent and nonavalent vaccines is essential for policy strategies. We therefore compared the HPV type-specific antibody levels induced by HPV vaccination with the bivalent and the nonavalent vaccines.

METHODS

Study Participants

Study participants belonged to the Finnish cohort included in a multinational study (COHEAHR, Comparing Health Services Interventions for the Prevention of HPV-Related Cancer project), conducted in 9 European countries aiming to identify global and regional determinants of HPV vaccination among adult women attending routine cervical cancer screening [9].

We recruited 25-year-old Finnish women attending routine cervical screening in 6 municipalities during 2016–2018. The women were not previously vaccinated against HPV. Noneligibility criteria included current or planned pregnancy within the following months, allergy or hypersensitivity to any vaccine component, history of immune disease, or hysterectomy. HPV vaccines used in the study were the bivalent Cervarix (GlaxoSmithKline Biologicals, targeting HPVs 16 and 18) and the nonavalent Gardasil9 (Sanofi Pasteur MSD, whose current Marketing Authorization Holder is Merck

Sharp and Dohme, targeting HPV6/11/16/18/31/33/45/52/58), which were provided by the respective companies at no cost.

Those accepting vaccination and being eligible were offered 3 doses of HPV vaccination free of charge with either the bivalent vaccine (at 0, 1, and 6 months, n = 207), or the nonavalent vaccine (at 0, 2, and 6 months, n = 235). A total of 188 women were vaccinated with the bivalent Cervarix and 184 women with the nonavalent Gardasil9 (Figure 1).

Serum sampling was performed 6 months after completion of the 3-dose vaccine schedule (0, 1, 6 months and 0, 2, 6 months for the bivalent and nonavalent vaccines, respectively). Participants provided free and written informed consent, and the study adhered to the declaration of Helsinki and was approved by the Regional Ethics Committee of the Expert Responsibility area of Tampere University Hospital (EudraCT 2014-003177-42).

Antibody Binding Assay

Antibody detection was performed by subjecting serum samples to a previously described and validated HPV serology method based on Luminex technology, using pseudovirions from all HPV types included in the nonavalent vaccine (HPV types 6/11/16/18/31/33/45/52/58) as well as another 8 HPV types that are either oncogenic (HPV types 35/39/51/56/59), probably oncogenic (HPV68), or possibly oncogenic (HPV66 and 73) [7, 10].

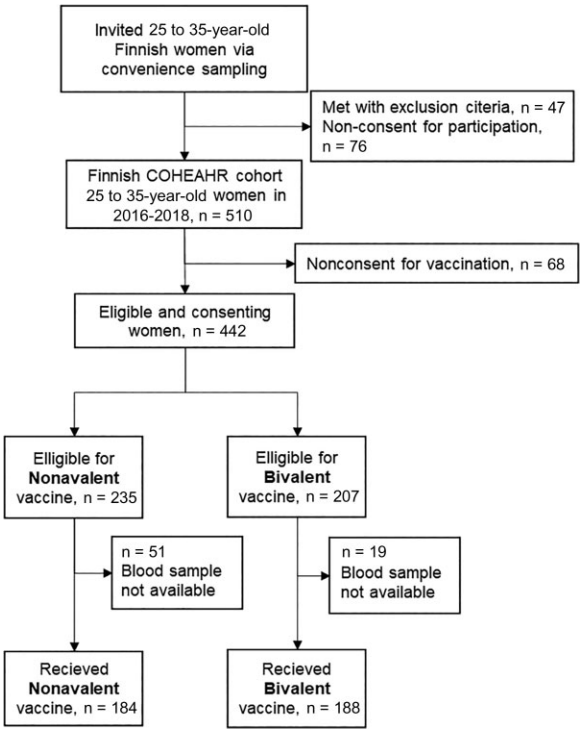

Figure 1. Flowchart of the study enrollment and participation.

Cutoff Values and International Units

Cutoff values to define seropositivity were calculated independently for each HPV type by analyzing the mean fluorescence intensity unit (MFI) values obtained from 192 children ≤12 years old (average age 4.8 years). For each HPV type, cutoff values were assigned as described in the WHO HPV laboratory manual (mean MFI value of a negative control serum panel plus 3 standard deviations) [11]. If this cutoff value was unreasonably low (<250 MFI), we used 250 MFI as cutoff to obtain a sensitivity and specificity similar to the classical enzyme-linked immunosorbent assay (ELISA) [12].

MFI values were transformed into units using the PLL (parallel line) method, as described previously [12]. For antibody responses to HPV16 and 18, data were converted to international units (IU)/mL, traceable to the International Standards for antibodies to HPV16 and 18 [13]. For the other HPV types, we established an in-house reference standard and transformed the MFI values to in-house units (IHUs) using the PLL (parallel line) method, in the same manner.

Statistics

Differences in antibody levels across the vaccines were evaluated by comparing median antibody levels with nonparametric Wilcoxon rank-sum test and relative proportions of antibody positives among the bivalent and nonavalent vaccine recipients were analyzed using a 2 proportion Z test and its associated *P* value.

RESULTS

Serum samples were collected 6 months after completion of the vaccine schedule from 91% (188/207) of women having received the bivalent vaccine and from 78% (184/235) of women having received the nonavalent vaccine. Median antibody levels and relative proportion of seropositive recipients for all HPV types tested are shown in Table 1. Median anti-HPV16 and 18 antibody levels were significantly higher with the bivalent vaccine recipients (1140.1 IU and 170.5 IU for HPV16 and 18, respectively) compared to the nonavalent vaccine recipients (265.1 and 22.3 IU, for HPV16 and 18, respectively). The majority of bivalent vaccine recipients had antibodies against HPV31 and 45, although the median antibody levels were significantly lower than those detected against the same types from the nonavalent vaccine recipients. The bivalent vaccine also induced cross-reactive antibodies in a notable minority of subjects for HPV6, HPV33, and HPV58 (between 43% and 48% of subjects) and against HPV35 (in 35% of subjects) (Table 1).

The nonavalent vaccine consistently (in >95% of subjects) induced antibodies against all the 9 HPV types targeted by this vaccine and also induced antibodies cross-reactive with HPV35 in 58% of subjects, which was significantly more

Table 1. Anti-Human Papillomavirus Antibody Levels

	Bivalent HPV Vaccine (n = 188)				Nonavalent HPV Vaccine (n = 184)				Lowest Detectable Antibody Level Bivalent/ Nonavalent	Unit	*P* Value, Difference in Antibody Median Level	*P* Value, Difference in Proportion Seropositive
	Median	Minimum	Maximum	No. (%) positive	Median	Minimum	Maximum	No. (%) positive				
HPV6	0	0	815.5	85 (45.21)	32.28	0	11064.14	181 (98.37)	0.12/1.29	IHU	<.0001	<.0001
HPV11	0	0	216.21	26 (13.83)	14.18	0	376.58	182 (98.91)	0.09/0.36	IHU	<.0001	<.0001
HPV16	1140.11	6.86	109027.4	188 (100)	265.08	2.69	15163.39	184 (100)	6.86/2.69	IU	<.0001	NA
HPV18	170.54	0	29999.78	187 (99.47)	22.28	0	9418.97	183 (99.46)	1.06/0.31	IU	<.0001	1
HPV31	1.85	0	1791.49	178 (94.68)	21.66	0.11	1937.33	184 (100)	0.08/0.11	IHU	<.0001	.0044
HPV33	0	0	501.15	80 (42.55)	7.97	0	629.61	181 (98.37)	0.04/0.07	IHU	<.0001	<.0001
HPV35	0	0	2493.39	66 (35.11)	31.17	0	5288.8	107 (58.15)	7.38/10.76	IHU	<.0001	<.0001
HPV39	0	0	1550.5	7 (3.72)	0	0	540	7 (3.80)	252.00/275.00	MFI	.98	1
HPV45	2.24	0	6929.66	152 (80.85)	45.8	0	1426.97	182 (98.91)	0.24/0.28	IHU	<.0001	<.0001
HPV51	0	0	1521	14 (7.45)	0	0	1099	17 (9.24)	281.00/279.00	MFI	.748	.6616
HPV52	0	0	52.15	30 (15.96)	31.75	0	6422.03	175 (95.11)	0.78/1.31	IHU	<.0001	<.0001
HPV56	0	0	340.5	3 (1.60)	0	0	328	1 (0.54)	278.5/328.00	MFI	.861	.6304
HPV58	0	0	34787.6	90 (47.87)	17.01	0	463.49	182 (98.91)	0.02/0.09	IHU	<.0001	<.0001
HPV59	0	0	684	7 (3.72)	0	0	324	2 (1.09)	261.50/300.50	MFI	.658	.1878
HPV66	0	0	272	1 (0.53)	0	0	0	0 (0.00)	272.00/NA	MFI	.929	1
HPV68	0	0	672288	35 (18.62)	0	0	7514.07	41 (22.28)	37.36/49.14	IHU	.497	.4544
HPV73	0	0	2359.5	34 (18.09)	0	0	1749	39 (21.20)	269.00/253.00	MFI	.597	.5322

Anti-HPV antibody levels are given in IU for the HPV types where an international standard has been established by the World Health Organization (for HPV16 and 18) or in IHU for the other types. For some HPV types, the seroreactivity was so low that antibody levels in units could not be calculated and the results for these types are therefore presented as the crude MFI obtained when testing sera in a 1:50 dilution. Differences in antibody levels across the vaccines were assessed with nonparametric Wilcoxon rank-sum test and relative proportions using 2 proportion Z test and its associated *P* value.

Abbreviation: HPV, human papillomavirus; IHU, in-house units; IU, international units; MFI, mean fluorescence intensity; NA, not applicable.

compared to the bivalent vaccine recipients ($P < .0001$; Table 1). There was a strong correlation between the individual-level antibodies against HPV35 and HPV58 among Gardasil9 recipients (correlation coefficient 0.33, data not shown).

We also tested for several additional genital HPV types not included in any of the vaccines (HPV39, 51, 56, 59, 66, 68, and 73), but very low levels of antibodies were detected against these types. For HPV types 39, 51, 56, 59, 66, and 73, the reactivity of the standard serum (secondary standard) used for the calculation of units was too low and the reactivity of the samples was low as well (except for a few samples). It was not possible to calculate an antibody level in units and therefore the crude MFI is displayed instead for these HPV types. For HPV68, seroreactivity was detected in only about 20% of subjects and the median IHU was zero for both vaccines (Table 1).

DISCUSSION

This is the first study to report a head-to-head comparison of the antibody levels against the major genital HPV types that are present after vaccination with the bivalent and nonavalent HPV vaccines. The major findings are that (1) both vaccines regularly induce high anti-HPV antibody levels against all the HPV types that they contain, (2) the bivalent vaccine elicits a higher median antibody level for both HPV16 and HPV18 compared to the nonavalent vaccine, and (3) cross-reactivity against nonvaccine types is seen somewhat irregularly, and found in a majority of subjects only against HPV31 and 45 in the case of the bivalent vaccine and for HPV35 with the nonavalent vaccine.

Strengths of the study include a population-based enrolment of homogeneous groups of vaccine recipients that allows a direct head-to-head comparison and that we, as far as possible, have used the optimally reproducible and comparable manner to report serology results (in IU). The laboratory procedures have been extensively evaluated, including in international collaborative proficiency studies, and have systematically measured sensitivity and specificity in longitudinal cohort studies that have used an independent measure of HPV infection (HPV DNA detectability) as the reference comparator [11, 12]. We also employed a wide panel of genital HPV types, including nonvaccine types to also assess cross-reactive antibodies.

Weaknesses include the age of the vaccine recipients (25 years old), who may have been previously infected with a number of high-risk HPV types. A national survey in the United States found 43.1% of 14 to 26-year-old women were sexually active before vaccination [14]. Antibody responses in our study may therefore also have been induced by natural infections. However, vaccine-induced antibodies are typically several logarithms higher compared to those induced by natural infection.

We measured the levels of antibodies that bind to pseudovirions, not specifically neutralizing antibodies. The neutralizing vaccine-induced antibodies are considered the primary mechanism of protection [8] However, the antibody levels to pseudovirions or virus-like particles (VLPs) are known to correlate strongly with the neutralizing activity [7, 8].

Previous studies have compared antibody levels for HPV16 and HPV18 between the bivalent Cervarix and the quadrivalent vaccine Gardasil [7, 8, 12]. However, as the amount of VLPs included in the nonavalent vaccine (Gardasil9) is not the same as the amount of VLPs included in quadrivalent vaccine (Gardasil), the present study provides a novel comparison.

Although the minimum antibody level required for protection is not known for certain, a regular induction of high levels of anti-HPV antibodies is presumably desirable for optimal protection. We found that for ensuring a regular response in >95% of vaccinees, including the actual HPV type in the vaccine is essential. However, it should be noted that HPV16 and 18 are the major cancer-causing HPV types, responsible for >70% of cervical cancers worldwide and that the proportion of cervical cancers caused by HPV16/18 is even higher in young women. The protection against invasive cervical cancer afforded by HPV vaccines containing HPV16/18 has indeed been consistently reported to be >80% [5, 6]. Therefore, adequate protection against HPV16/18 may be the most important feature of HPV vaccines. Both vaccines in this study induced high antibody levels at month 12 postvaccination against HPV16/18 (in 100% of subjects for HPV16 and in 99.5% of subjects for HPV18).

Evidence of cross-protection against persistent infection has been shown in several studies [2, 8]. We found that the bivalent vaccine induced cross-reactive antibodies in a notable proportion of subjects for HPV31 and 45 (between 80% and 94% of subjects) while the proportion was lower for HPV33 and 58 (42%–47%), and very low for HPV52 (15%). No studies so far have been performed previously on cross-reactive antibodies with the nonavalent vaccine. We detected cross-reactive antibodies against HPV35 in a majority of the nonavalent vaccine recipients, perhaps because HPV35 is closely related to 5 HPV types that also belong to the A9 species of HPV and are included in the nonavalent vaccine, in line with the correlation of antibody levels found for HPV35 and HPV58.

Although the cross-reactivity seen was somewhat irregular, it is likely to be of public health importance if found in a majority of subjects. It is thus promising that the HPV types for which the bivalent vaccine consistently induced antibodies among the 25-year-old vaccine recipients (HPV45 and 31) are the third and fourth most important HPV types when it comes to causing cervical cancer. Similarly, the induction of cross-reactive antibodies against HPV35 by the nonavalent vaccine is likely to be of importance for global equity in cervical cancer prevention as HPV35 is the nonvaccine type that contributes most to the cervical cancer burden and is overrepresented among women with cervical neoplasia with origin from sub-Saharan Africa [15].

In conclusion, direct evidence from systematic head-to-head comparison of antibody levels after vaccination with major HPV vaccines is likely to be useful for the continued effort towards global elimination of HPV and cervical cancer.

Notes

Author contributions. M. L. and J. D. contributed conceptualization, supervision, and project administration. C. E. and C. L. contributed methodology and formal analysis. C. E., C. L., and L. S. A. M. performed validation and data curation. C. E., C. L., L. S. A. M., and V. P. performed investigations. M. L., J. D., T. E., P. G., and V.P. contributed resources. C. E., C. L., L. S. A. M., and J. D. contributed visualization. L. S. A. M. wrote the original draft, and C. E., C. L., M. L., J. D., T. E., P. G, and V. P. contributed to review/editing. All authors participated in writing and critical revision of the manuscript for important intellectual content. All the authors have read and approved the final manuscript.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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