BMJ Open

Human papillomavirus vaccine efficacy against invasive, HPV-positive cancers: population-based follow-up of a cluster-randomised trial

Matti Lehtinen,1,2 Camilla Lagheden,1 Tapio Luostarinen,3 Tiina Eriksson,4 Dan Apter,5 Anne Bly,2 Penelope Gray,1 Katja Harjula,6 Kaisa Heikilä,2 Mari Hokkanen,2 Heidi Karttunen,2 Marjo Kuortti,6 Pekka Nieminen,7 Mervi Nummela,2 J Paavonen,8 Johanna Palmroth,9 Tiina Petäjä,10 Eero Pukkala,11 Anna Soderlund-Strand,12 Ulla Veivo,2 Joakim Dillner13

ABSTRACT

Background Human papillomavirus (HPV) vaccination protects against HPV, a necessary risk factor for cervical cancer. We now report results from population-based follow-up of randomised cohorts that vaccination provides HPV-type-specific protection against invasive cancer.

Methods Individually and/or cluster randomised cohorts of HPV-vaccinated and non-vaccinated women were enrolled in 2002–2005. HPV vaxination cohorts comprised originally 16–17-year-old HPV 16/18-vaccinated PATRICIA (NCT00122681) and 012 trial (NCT00169494) participants (2465) and HPV6/11/16/18-vaccinated FUTURE II (NCT00092534) participants (866). Altogether, 3341 vaccines were followed by the Finnish Cancer Registry in the same way as 16256 non-HPV-vaccinated controls. The control cohort stemmed from 15665 originally 18–19-year-old women enrolled in 2003 (6499) or 2005 (9166) and 861 placebo recipients of the vaccine.4 From population-based Finnish Cancer Registry.

Findings During a follow-up time of up to 11 years, we identified 17 HPV-positive invasive cancer cases (14 cervical cancers, 1 vaginal cancer, 1 vulvar cancer and 1 tongue cancer) in the non-HPV-vaccinated cohorts and no cases in the HPV-vaccinated cohorts. HPV typing of diagnostic blocks found HPV16 in nine cervical cancer cases, HPV18, HPV33 and HPV52 each in two cases and HPV45 in one cervical cancer case. The vaginal, vulvar and tongue cancer cases were, respectively, positive for HPV16, HPV52/66 and HPV213. Intention-to-treat vaccine efficacy against all HPV-positive cancers was 100% (95% CI 2 to 100, p<0.05).

Interpretation Vaccination is effective against invasive HPV-positive cancer.

Trial registration number NCT00122681, Post-results; NCT00169494, Post-results; NCT00092534, Post-results.

INTRODUCTION

Human papillomavirus (HPV) vaccines are effective against cervical intraepithelial neoplasia grade 3 (CIN3) 8–14 years postvaccination.1,2 The risk of invasive cervical cancer (ICC) overall is lower among HPV-vaccinated women as compared with non-HPV-vaccinated women.3 From population-based cancer registry follow-up of randomised cohorts, we have previously reported a significant vaccine efficacy (VE) against all invasive HPV-associated cancers.5 We now have extended the follow-up time to 11 years and performed HPV typing of diagnostic tumour blocks. Our objective is to report the first HPV-type-specific VE estimates against invasive cancers in a randomised setting.

METHODS

Enrolment, intervention and follow-up
All the 22412 Finnish women born in annual quartal (Q) 4/1984-Q1/1987 were invited to...
The diagnostic histopathological blocks were requested from the pathology laboratories that had notified the Finnish National Ethical Review Board (ERB, TUKIJA 1150/2002, 1153/2003, 1174/2004) about the incident cancer cases according to a specific permission from the Finnish National Supervisory Authority for Welfare and Health (Valvira) without informing the patients. Before HPV DNA typing, the presence of neoplastic tissue was rereviewed by two experienced pathologists. Blocks were sectioned according to Finnish law. Public involvement in the enrolment was further assured by information lectures given by the study nurses at parental evenings of the secondary high schools and technical schools in the 7 and 18 trial communities.

**Laboratory analyses**

The diagnostic histopathological blocks were requested from the pathology laboratories that had notified the FCR about the incident cancer cases according to a specific permission from the Finnish National Supervisory Authority for Welfare and Health (Valvira) without informing the patients. Before HPV DNA typing, the presence of neoplastic tissue was rereviewed by two experienced pathologists. Blocks were sectioned according to...
a contamination-proof manner. Extraction, amplification and typing of the intraluesional HPV DNA were performed as previously described. HPV types that remained unknown were identified by sequencing.

**Statistical analyses**

The enrolled HPV-vaccinated and unvaccinated adolescents provided 80% power for the identification of VE against cervical cancer. The VE was calculated on the intention-to-treat principle including all individuals (regardless of baseline HPV status) who had received at least one vaccine dose in the HPV vaccine arm. Statistical software SAS V.9.4 (SAS Institute, Cary, North Carolina) was used. The 95% CIs were based on exact binomial distribution of number of vaccinated cases conditional on total number of cases.

**RESULTS**

The age-aligned FCR follow-up for incident invasive cancers lasted by cohort for up to 11 years between November 2007 and December 2019. According to Finnish Population Census Register altogether, 10 FUTURE II, 19 PATRICIA and 73 non-HPV-vaccinated participants died during the follow-up (figure 1). It resulted in, respectively, 33,792 and 174,340 follow-up years for the HPV vaccine and non-HPV vaccine cohorts.

No HPV-associated cancer cases were found in the 3341 HPV-vaccinated women through the 33,792 women years of the follow-up (table 1). The follow-up was identified in the 16,526 non-HPV-vaccinated women 15 cases with ICC in the 16,526 non-HPV-vaccinated women during 174,320 women years of follow-up. Four of the ICC cases were adenocarcinomas and 11 were squamous cell carcinomas (SCC). One cervical SCC case, diagnosed late in 2019, was outside the age-aligned time-window of 11 years that guaranteed equal follow-up for the different cohorts. This case was not included in further analyses. The incidence of the 14 eligible ICC cases in the non-HPV-vaccinated women was 8.0 per 100,000 women years (table 1).

The single, invasive vaginal, vulvar and tongue cancer cases were all squamous cell carcinomas. Overall, in the non-HPV-vaccinated women the incidence of the HPV-associated cancers was 9.8 per 100,000 women years. Incidence rates of breast cancer, thyroid cancer and melanoma were essentially similar in all cohorts with highly overlapping 95% confidence intervals (table 1).

HPV DNA was found in all the identified and eligible 17 invasive HPV-associated cancer cases (table 2). HPV16 was present in nine ICC cases. HPV16 was present in one woman with vaginal cancer, who also had HPV16 positive cervical cancer. HPV18, HPV33 and HPV52 were each present in two ICC cases, and HPV45 in one ICC case. HPV66 (a vulvar cancer also positive for HPV52) and HPV213 (a tongue cancer) were present in one case each (table 2). With no identified HPV-associated cancer cases in the HPV-vaccinated women, we found a VE of 100% (95% CI 2 to 100; p<0.05) against all HPV DNA-positive invasive cancers.

**DISCUSSION**

We report the first randomised trial-derived evidence on the significant, 100% HPV-VE against HPV DNA-positive invasive cancers. We followed both the bivalent and the quadrivalent HPV vaccine recipients for 11 years after the clinical trials had ended (up to 17 years postvaccination), and similarly aged non-HPV-vaccinated women, respectively, over approximately 35,000 and 175,000 follow-up years by the population-based, quality-controlled FCR. VE estimates against invasive HPV16/18 positive cancers and against invasive cancer cases positive for the bivalent vaccine cross-protected HPV types 33/45/52 were all 100%, although with very wide CIs.

The significant 100% VE against all HPV-positive cancers is reassuring for both the bivalent and quadrivalent HPV vaccines, three shots of which were given to, respectively, 26% and 74% of the vaccinees in 2002 and 2004. A recent Swedish study, which was also based on accurate, high-quality cancer registry follow-up, suggested that up to

---

**Table 1** Numbers (n) and incidence rates (/100,000 person years) of human papillomavirus (HPV) associated invasive cancers and other common cancers in cluster-randomised cohorts of altogether 3341 16–17 year-old female HPV16/18 or HPV6/11/16/18 vaccine recipients and 16,526 non-HPV vaccinated, originally 16–19 year-old females followed up* between 2007 and 2019

<table>
<thead>
<tr>
<th>End-point</th>
<th>HPV-vaccinated women (33,792 person years)</th>
<th>Non-HPV-vaccinated women (174,340 person years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Rate (95% CI)</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>All HPV-positive cancers†</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>3</td>
<td>8.9 (2.9 to 28)</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>2</td>
<td>5.9 (1.5 to 24)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>8</td>
<td>23.8 (11.8 to 47)</td>
</tr>
</tbody>
</table>

*For corresponding age-aligned sub-cohorts, up to 11 years of passive follow-up was by the population-based Finnish Cancer Registry. †14 cervical cancers, one vaginal cancer, one vulvar cancer, one tongue cancer.
12% of ICC cases may not be prevented by the quadrivalent vaccine. We have recently shown that long-term cross-neutralising antibody responses induced by these two vaccines differ significantly. Thus, continuation of the cancer registry-based long-term follow-up of the population-based bivalent and the quadrivalent HPV vaccine cohorts versus non-HPV-vaccinated cohorts is warranted to understand if there were a possible difference in their efficacy.

Population-based, age-aligned follow-up of trial cohorts and accurate, histologically confirmed, invasive cancer case definitions of the quality-controlled FCR were pivotal strengths of this study. We could ensure identical cervical screening history of HPV-vaccinated and non-HPV-vaccinated cohorts for the 1983–1988 birth cohorts that were included in this study. The 5-year interval screening at ages 25, 30, 35... is according to local standard of care.

During 2007–2009, entire 1992–1995 birth cohorts of 33 Finnish towns were enrolled in our population-based community-randomised HPV vaccination trial. HPV16/18 vaccine recipients of that trial have since 2014 participated in a rerandomised screening trial at ages 22, 25 and 28 years, while their non-HPV-vaccinated counterparts have not had this opportunity. These sizeable cohorts, >40,000 individuals in total, were not included in this study due to their gradually more and more different screening histories.

Two independent pathologists ensured that neoplastic tissue was present in the sections of the initial diagnostic biopsy blocks that we wanted to use for HPV testing. In three ICC cases, CIN3 was left in the diagnostic block that was used for HPV DNA typing, but the sectioned material was deemed to adequately represent oncogenic HPV types involved. The blocks were found to be positive for HPV16, 33 and 16/52 DNA. Conceivably, due to the lesion size in the diagnostic block, prior sections had removed an ICC before the new sections were made for the HPV typing.

Recruiting and enrolling the different cohorts in a population-based fashion during 2002–2005 was an important prerequisite for the country-wide cancer registry-based follow-up. Due to the follow-up evidence on protection against the most stringent, invasive HPV-associated cancer end points are now emerging more rapidly than the evidence on the efficacy of HBV vaccination against hepatocellular cancer. HPV cancers in young adults may be more rapidly developing but establishing the trial cohorts and concomitant control cohorts, and their follow-up 17 years ago with identical possibilities for organised cervical screening at ages 25, 30 and 35 years has been a strength of this study. At the time of HPV vaccine licensure (2006/2007), the control cohort was above 20 years of age, and opportunistic vaccination was negligible but could not be totally ruled out, which should be listed as a limitation of this study. On the other hand, cross-vaccinating 50% of the FUTURE II placebo arm after age 20 had no material effect on their CIN3 incidence.

| Table 2 | Numbers (n) and incidence rates (/100,000 person years) of human papillomavirus (HPV) positive invasive cancers by HPV type in cluster-randomised cohorts of altogether 3341 16–17-year-old female HPV16/18 or HPV6/11/16/18 vaccine recipients and 16526 non-HPV vaccinated, originally 16–19-year-old females followed up* during 2007–2019 |
|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| End-point                  | HPV-vaccinated women          | Non-HPV-vaccinated women      |                             |
|                            | (33792 person years)          | (174340 person years)         |                             |
|                            | n | Rate (95% CI) | n | Rate (95% CI) |
| Cervical cancer            |   |              |   |              |
| HPV16                      | 0 | –             | 9 | 5.2 (2.7 to 9.9) |
| HPV18                      | 0 | –             | 2 | 1.1 (0.3 to 4.6) |
| HPV16/18                   | 0 | –             | 11| 6.3 (3.5 to 11.4) |
| HPV33                      | 0 | –             | 2 | 1.1 (0.3 to 4.6) |
| HPV45                      | 0 | –             | 1 | 0.6 (0.1 to 4.1) |
| HPV52†                     | 0 | –             | 1 | 0.6 (0.1 to 4.1) |
| Any HPV                    | 0 | –             | 14| 8.0 (4.8 to 13.6) |
| Vaginal cancer             |   |              |   |              |
| HPV16                      | 0 | –             | 1 | 0.6 (0.1 to 4.1) |
| Vulvar cancer              |   |              |   |              |
| HPV52‡                     | 0 | –             | 1 | 0.6 (0.1 to 4.1) |
| Tongue cancer              |   |              |   |              |
| HPV213                     | 0 | –             | 1 | 0.6 (0.1 to 4.1) |

*For corresponding age-aligned sub-cohorts, up to 11 years of passive follow-up was by the population-based Finnish Cancer Registry. †Positive for both HPV16 and HPV52. ‡Positive for both HPV52 and HPV66.
The excellent VE against HPV-positive cancers now documented from a randomised study setting, it is an important evidence of the long-term impact of HPV vaccination. It indicates that prevention of a sexually transmitted infection and associated cancer by prophylactic vaccination are doable and paves the way for the WHO’s initiative on the elimination of cervical cancer.24

Acknowledgements
The review of diagnostic histopathological blocks for HPV DNA analysis by Drs Mensur Dzabic and Ralf Butzow is gratefully acknowledged.

Contributors
The corresponding author was the guarantor for the overall content and attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. ML, TL, DA, JD, TE, JPaa and EP were involved in the conception and/or the design of the study. ML, DA, AB, TE, KHa, KHe, MH, HK, MK, TL, PN, MN, JPal, TL, PN, MN, JPal, TP, AS-S and UV participated in the collection and generation of the study data. TE, PG, CL and TL performed the analyses. ML, DA, TL, JPaa and EP were involved in the analyses and/or interpretation of the data. All authors contributed to development of this manuscript, had full access to the data and gave final approval before submission.

Funding
Finnish Cancer Society, Swedish Cancer Society, Nordic Cancer Union, and GlaxoSmithKline Biologicals SA (study identifier 115006) supported the study, which discloses in the publication all the data on invasive cancers collected. GlaxoSmithKline Biologicals SA was provided the opportunity to review a preliminary version of this manuscript for factual accuracy, but the authors are solely responsible for final content and interpretation. GSK Biologicals SA (HPV-027, 115006) and Finnish Cancer Foundation (MS/790) supported the study financially.

Competing interests
We declare that ML, DA, JD and JPaa have received grants from Merck & Co. Inc. and/or from the GSK group of companies through their respective employers. GSK Biologicals SA was provided the opportunity to review this manuscript but the authors are solely responsible for final content and interpretation.

Patient and public involvement
Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication
Not applicable.

Provenance and peer review
Not commissioned; externally peer reviewed.

Data availability statement
Data are available upon reasonable request. Data is available upon reasonable request from corresponding author.

Open access
This is an open access article distributed in accordance with the Creative Commons Attribution 4.0 Unported (CC BY 4.0) license, which permits others to copy, redistribute, remix, transform and build upon this work for any purpose, provided the original work is properly cited, a link to the licence is given, and indication of whether changes were made. See: https://creativecommons.org/licenses/by/4.0/.

ORCID iDs
Matti Lehtinen http://orcid.org/0000-0002-9481-0535
Tapio Luostarinen http://orcid.org/0000-0003-3231-8550
Joakim Dillner http://orcid.org/0000-0001-8588-6506

REFERENCES