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Human papillomavirus nonavalent (HPV9) vaccination and risk of immune mediated diseases, myocarditis, pericarditis, and thromboembolic outcomes in Denmark: self-controlled case series study

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► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/bmjmed-2024-000854).

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Cite this as: *BMJMED* 2024;3:e000854. doi:10.1136/bmjmed-2024-000854

Received: 3 January 2024
Accepted: 17 September 2024

ABSTRACT

OBJECTIVE To assess the associations between vaccination with the nonavalent human papillomavirus (HPV9) vaccine and immune mediated diseases, myocarditis, pericarditis, arterial thromboembolism, and venous thromboembolism with or without thrombocytopenia, in adolescent girls and boys in Denmark.

DESIGN Self-controlled case series study.

SETTING Population based study of linked nationwide health registers in Denmark for HPV vaccination and hospital diagnosis data, 1 October 2017 (or age 10 years) to 31 December 2022 or censored. Personal data were obtained from the Central Person Register. Information on dates of HPV vaccination and type of vaccine were obtained from the Danish Vaccination Register. Primary or secondary diagnoses of inpatient or outpatient hospital contact were sourced from the Danish National Patient Register.

PARTICIPANTS Source cohort 854 586. 350 687 individuals aged 10-17 years living in Denmark received at least one dose of HPV9 vaccine. Self-controlled case series analysis of 3354 individuals (1913 girls and 1441 boys) who received at least one dose of HPV9 vaccine and had at least one outcome.

MAIN OUTCOME MEASURES Rate ratios of the study outcomes in a 28 day or 180 day risk period (depending on the type of outcome) after HPV9 vaccination compared with the reference period were calculated. 47 immune mediated diseases, myocarditis, pericarditis, and seven thromboembolic outcomes were assessed. A safety signal for a specific outcome was identified if at least three outcomes were seen in the risk period after vaccination, the rate ratio was significantly increased (lower bound of the 95% confidence interval (CI) for the self-controlled case series rate ratio >1.0), and the false discovery rate adjusted P value was significant (<0.05).

RESULTS 696 776 doses of any HPV vaccine were given during the study period, including 673 530 doses of HPV9 vaccine in 350 687 individuals who received at least one dose. In the self-controlled case series analysis, rate ratios of all immune mediated outcomes combined were 0.99 (95% CI 0.86 to 1.13) and 1.03 (0.89 to 1.20) in girls and boys, respectively, after HPV9 vaccination. Rate ratios for any of the 47 analysed immune mediated outcomes were not increased in the risk periods in girls after vaccination. The only increased rate ratio seen was for Raynaud's disease (rate ratio 2.62, 95% CI 1.07 to 6.40) after HPV9 vaccination in boys, which did not fulfil the criteria of a safety signal. These findings should be interpreted in the light of the study limitations. None of the other 55 outcomes examined showed an association with HPV9 vaccination.

CONCLUSIONS The results of this study did not suggest an association between HPV9 vaccination and the study outcomes in adolescent boys and girls aged 10-17 years. This study contributes to the evidence on the safety of the HPV9 vaccine.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Previous studies found no evidence linking the quadrivalent human papillomavirus (HPV4) vaccine to immune mediated or thromboembolic outcomes in adolescent girls and women

⇒ Research on the newer nonavalent (HPV9) vaccine, particularly among boys, is limited

WHAT THIS STUDY ADDS

⇒ No associations between HPV9 vaccination and immune mediated diseases, myocarditis, pericarditis, or thromboembolic outcomes were found, confirming the safety of the HPV9 vaccine

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

⇒ These findings provide reassurance of the safety of the HPV9 vaccine, answering concerns that contribute to vaccine hesitancy

⇒ This study highlights the importance of promoting HPV vaccination programmes to prevent cervical cancer and other HPV related cancers

Introduction

The first human papillomavirus (HPV) vaccine was licensed in 2006, and has since been introduced into more than 80 countries, as of 2018.[1] Three HPV vaccines are licensed in Denmark and other high income countries: bivalent (HPV2), quadrivalent (HPV4), and nonavalent (HPV9) vaccines.[1] In Denmark, both boys and girls are eligible for HPV vaccination on reaching the age of 12 years under the national child vaccination programme.[2] In the recommended protocol, two doses are given, at least five months apart, for those who receive a vaccine at 12 years. Individuals who are vaccinated at age 15 years, however, are advised to receive three doses.[2]

Despite extensive clinical trials and monitoring of HPV vaccines after licensing, with reassuring results, concerns about safety persist.[3] Safety concerns are cited as a primary reason for the less than enthusiastic uptake of the HPV vaccine, both in Europe and the US.[4] According to the Vaccine Confidence Project,[5] the vaccine is still perceived to be too new, with insufficient evidence to prove its safety and effectiveness. Fear about the potential long term and severe side effects of the vaccine was found to be the second most common concern among European populations.[5]

The World Health Organization has an ambitious target of 90% uptake of the vaccine by 2030,[1] which is challenging even in high income countries. In the US, only 58.6% of adolescents had up-to-date HPV vaccination in a 2020 survey.[6] Continued investigation of the safety of the HPV vaccine is therefore crucial to prevent vaccine hesitancy because of safety concerns and to increase uptake of the vaccine and public trust in vaccines.

Immune mediated diseases and haematological disorders have been linked to HPV vaccination, with molecular mimicry and immune hyperactivation in response to the antigen or adjuvant as the suggested mechanisms.[3 7] Results from large observational studies have not supported these claims. Early studies in Denmark and Sweden found no evidence of associations between the HPV4 vaccine and immune mediated or thromboembolic outcomes among adolescent girls and women.[8 9] Large observational studies of the newer HPV9 vaccine are lacking,[3 10] however, and studies in boys, a more recent target group for routine immunisation, are rare. The need for continued vigilance is highlighted by a Norwegian case report of a woman aged 25 years who presented with thrombosis and thrombocytopenia after HPV9 vaccination.[11]

The aim of our study was to provide a comprehensive evaluation of the safety of the HPV9 vaccine in adolescent boys and girls aged 10-17 years from a large nationwide setting in Denmark. We specifically evaluated the association between HPV9 vaccination and immune mediated diseases, myocarditis, pericarditis, arterial thromboembolism, and venous thromboembolism with and without thrombocytopenia.

Methods

Source cohort

In Denmark, all residents are assigned a unique personal identifier used in national demographic and health registries. The study cohort was based on the personal data from the Central Person Register.[12] We included all boys and girls aged 10-17 years in 2017-22, with registered residency in Denmark at least three years before the start of the study. Participants were followed from the study start date (1 October 2017), or their 10th birthday (whichever came latest), until the end of the study (31 December 2022), or until death, emigration, or disappearance from the national register, whichever event occurred first. All individuals with a registered record of the selected adverse events of interest during follow-up were identified and analysed by self-controlled case series analyses.

Vaccination

We analysed data on individuals who received the HPV9 vaccine. The HPV9 vaccine has been used in the Danish national vaccination programme since 2017 (online supplemental figure 1). Receipt of the vaccine was defined by each dose of the vaccination schedule and thus we estimated the risk of each outcome after each dose. Vaccination schedules consist of two or three doses, depending on the age of the individual and the calendar period. Only the first three doses (at most) were considered in this study. If a fourth dose was recorded, follow-up was censored. We considered only homologous vaccine schedules; participants who received a HPV vaccine other than HPV9 were censored at the time of vaccination with the other HPV vaccine. Information on dates of HPV vaccination and the type of vaccine received were obtained from the Danish Vaccination Register.[13] This register includes HPV vaccinations given as part of the national childhood immunisation programme as well as HPV vaccinations privately purchased by individuals who were not eligible for the national programme.

Study outcomes

Study outcomes were defined as primary or secondary diagnoses of inpatient or outpatient hospital contact, obtained from the Danish National Patient Register.[14] In total, 56 unique study outcomes were assessed. Online supplemental table 1 lists all of the study outcomes with their ICD-10 (international classification of diseases, 10th revision) codes. The 56 outcomes were selected based on previous hypothesised links with vaccination,[3 7] well defined outcomes in the context of ICD-10 coding, and a broad representation of immune mediated neurological and cardiovascular outcomes in the 10-17 year age group.

Each outcome was studied independently, and only the first occurrence of each outcome was considered. If an individual had multiple different study outcomes, these were studied in separate analyses. When studying combined outcome groups (eg, immune mediated or venous thromboembolism), only the first instance of any of the contributing outcomes was considered for individuals with several of the contributing outcomes.

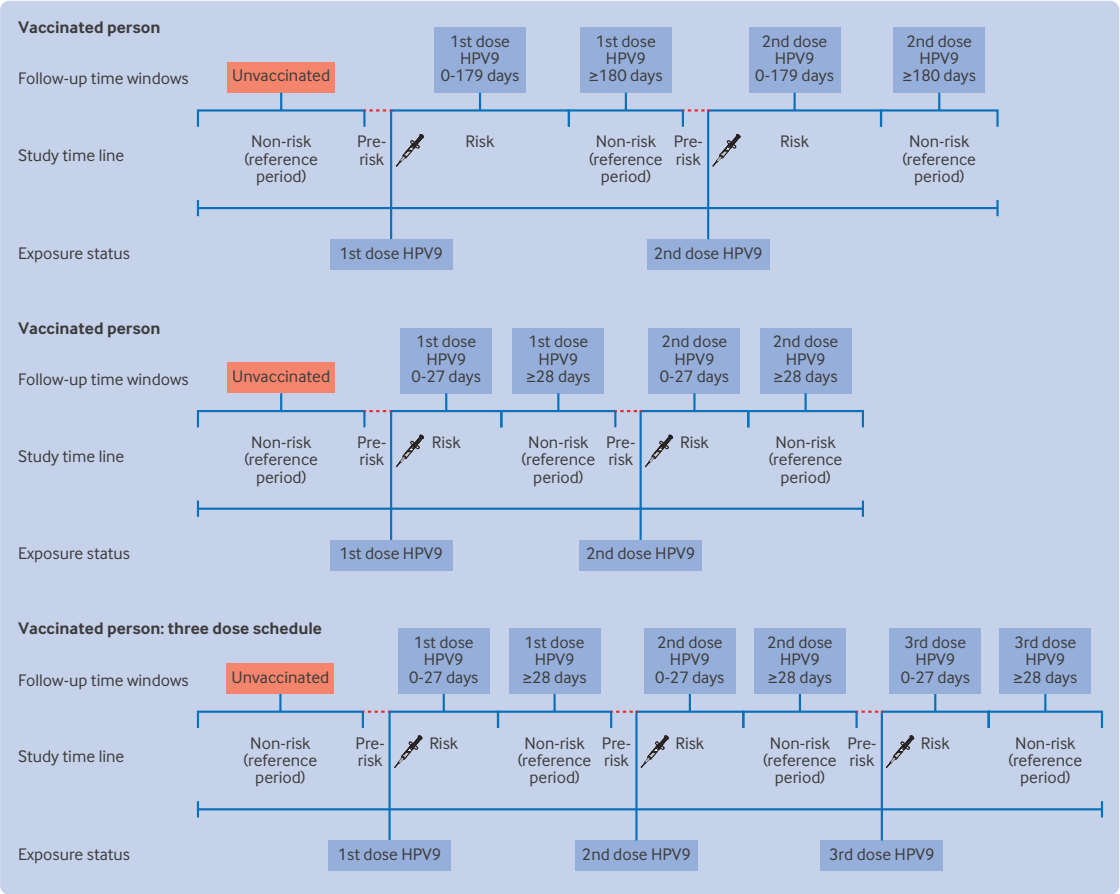

Figure 1 | Schematic illustrations of follow-up periods in the self-controlled case series analysis: risk period of 180 days and 28 days, and three dose schedule. Top panel=example of an individual who was vaccinated with a first dose of nonavalent human papillomavirus (HPV9) vaccine after a 14 day pre-risk period, followed up for 179 days from the first dose, vaccinated with the second dose after a 14 day pre-risk period, and followed up for 179 days after a second dose. Middle panel=example of an individual who was vaccinated with a first dose of HPV9 vaccine after a 14 day pre-risk period, followed up for 27 days from the first dose, vaccinated with the second dose after a 14 day pre-risk period, and followed up for 27 days after a second dose. Bottom panel=example of an individual who was vaccinated with a three dose schedule, with a 28 day risk period

Statistical analysis

With self-controlled case series analyses, we estimated the rate ratio (with 95% confidence interval, CI) of each study outcome, comparing risk versus reference periods in cases only. Cases referred to individuals who had a study outcome during follow-up. The main analysis included risk periods of 180 days after vaccination for most outcomes, whereas a short risk period of 28 days was considered for haematological outcomes, myocarditis, and pericarditis. A pre-risk period of 14 days before vaccination was applied, and all remaining time was considered reference time (figure 1). The long risk period of 180 days was chosen to account for the subtle onset of many of the immune mediated diseases, and the time required for diagnosis. The short risk period of 28 days reflected the likely acute onset and diagnosis of myocarditis, pericarditis, and haematological disorders. The analysis was adjusted for the covariates calendar time (October to December 2017, 2018, 2019, 2020, 2021, and 2022), season (winter, spring, summer, and autumn), and age (10-11, 12-13, 14-15, and 16-17 years).

In the self-controlled case series analysis, only exposed cases directly contributed to estimating the exposure effect. A look back period of either three years before the study or an indefinite look back period was applied to the outcomes, depending on the chronic or acute onset of the outcomes (online supplemental table 1). Only individuals with no previous outcome recorded in the look back period were included.

All data management and analyses were carried out in R, version 4.1.1. Follow-up time was time split with the formatdata function from the self-controlled case series package, version 1.6. Rate ratios were calculated with conditional logistic regression implemented in the clogit function from the stats package, version 4.1.1.

We used the Benjamini-Hochberg method[15] because of the large number of outcomes assessed. The Benjamini-Hochberg method is a sequential

procedure for determining significance while controlling for the expected proportion of false discoveries.[16] This method is in contrast with the overly conservative approach of controlling the family-wise error rate, as in the Bonferroni correction. Each association was evaluated in the context of predefined criteria: clinical relevance: at least three outcomes were seen in the risk period after vaccination; significance: lower bound of 95% CI >1.0; and multiple testing: false discovery rate adjusted P value <0.05.

We also conducted sensitivity analyses which accounted for event dependent exposures, to explore whether the study outcome altered exposure to the vaccine because of, for example, its contraindications to treatment or cancellation of appointments as a direct consequence of illness, reducing the possibility of exposure after the event.[17] Moreover, we conducted analyses specific to the dose of the HPV vaccine, investigated differences in dose specific rate ratios, and assessed alternative risk periods (28 days instead of 180 days and 180 days instead of 28 days, where appropriate). We applied the Benjamini-Hochberg method to the P values of the 56 outcomes for each analysis.

Patient and public involvement

No patients were involved in setting the research question, the outcome measures, or developing plans for design or implementation of the study owing to funding constraints. No patients were asked to advise on interpretation or writing up of results.

Results

Study population

A total of 936 083 people aged 10-17 years lived in Denmark between 2017 and 2022 (figure 2). After excluding individuals who had not resided in Denmark for two years before the study, 854 586 people were included in our source cohort. Online supplemental table 2 describes the characteristics of the individuals analysed in the study according to age and sex. In the overall population, 350 687 individuals aged 10-17 years received at least one dose of the HPV9 vaccine in a homologous schedule. In total, 673 530 HPV9 doses were given, and 3354 individuals received at least one dose of HPV9 vaccine and had at least one outcome: 1913 girls and 1441 boys. Figure 2 illustrates the number of unique individuals for each outcome. Four girls and six boys had multiple outcomes. Only five people from the source population received a vaccine different from HPV9 during the study period and were excluded from the study. Median number of days of follow-up between the first and the second dose was 184 days in girls and 182 days in boys.

Association between HPV9 vaccination and study outcomes

Rate ratios of admissions to hospital with 56 outcomes (immune mediated events, myocarditis or pericarditis, and thromboembolism with and without thrombocytopenia) were assessed. The rate ratios of the study outcomes in the 28 day or 180 day risk period (depending on the type of outcome) after HPV9 vaccination compared with the reference period are presented for adolescent girls aged 10-17 years (figures 3 and 4) and adolescent boys aged 10-17 years (figures 5 and 6).

Rate ratios of all immune mediated outcomes combined were 0.99 (95% CI 0.86 to 1.13) and 1.03 (0.89 to 1.20) in girls and boys, respectively, after HPV9 vaccination. Rate ratios for any of the 47 analysed immune mediated outcomes were not increased in the risk periods in girls after vaccination (figure 3). Among boys who were vaccinated with HPV9, we found an association between the vaccine and Raynaud's disease (rate ratio 2.62, 95% CI 1.07 to 6.40), but the association was not robust to multiple testing adjustment (figure 6).

We found no cases of myocarditis or pericarditis in the risk period after vaccination in girls (figure 4) and hence no analysis was conducted. Rate ratios for myocarditis and pericarditis combined were 0.46 (95% CI 0.06 to 3.40) in boys, consisting of myocarditis only because no cases of pericarditis were identified in the risk period (figure 6). Rate ratios of thromboembolic and thrombocytopenic outcomes combined were 1.48 (0.42 to 5.19) in girls and 2.29 (0.66 to 7.93) in boys, after HPV9 vaccination. No increased rate ratios were found for the individual study outcomes in either boys or girls.

Sensitivity analysis

In the dose specific analysis (online supplemental figures 6 and 7), no safety signals were identified in the risk periods after the first, second, or third doses of HPV9 vaccines for both sexes. Testing for differences in effects between doses gave P values <0.05 for several immune mediated diseases in both boys and girls, indicating that the response to the different doses of the HPV9 vaccine might vary. Many of these effects were based on a small number of observations, however, and none of the individual doses had a significant effect on the outcome.

The modified analysis accounting for event dependent exposures (online supplemental figures 2–5) suggested that a diagnosis of vasculitis might influence a girl's decision to become vaccinated (either at all or in a specific time frame after diagnosis). The vasculitis analysis was based on three individuals with outcomes in the 180 day risk period (rate ratio 31.66, 95% CI 9.36 to 107.17), which was robust to multiple testing adjustment (false discovery rate adjusted P value <0.001). No other increased rate ratio robust to multiple testing adjustment was

Figure 2 | Flow diagram of main analyses

Disease	Reference period Cases	Reference period Person years	Risk period Length	Risk period Cases	Risk period Person years	Rate ratio (95% CI)	Rate ratio (95% CI)	FDR adjusted P value
Immune mediated	1477	6705.7	180	350	1639.2		0.99 (0.86 to 1.13)	0.99
Thyroid	301	1352.3	180	70	331.9		0.88 (0.65 to 1.19)	0.99
Graves' disease	34	151.3	180	7	35.0		0.53 (0.20 to 1.37)	0.99
Hashimoto's thyroiditis	118	509.8	180	20	122.1		0.66 (0.39 to 1.12)	0.99
Other hyperthyroidism	60	267.2	180	13	67.3		1.13 (0.54 to 2.37)	0.99
Hypothyroidism	170	794.2	180	49	195.5		1.01 (0.69 to 1.46)	0.99
Gastrointestinal	432	1914.2	180	97	477.5		0.95 (0.73 to 1.23)	0.99
Coeliac disease	234	1007.7	180	48	253.9		0.84 (0.58 to 1.22)	0.99
Crohn's disease	113	503.4	180	25	125.2		1.09 (0.65 to 1.82)	0.99
Ulcerative colitis	94	430.3	180	22	103.6		0.92 (0.53 to 1.58)	0.99
Pancreatitis	16	69.7	180	3	17.7		1.33 (0.27 to 6.48)	0.99
Primary biliary cirrhosis	0	0.0	180	0	0.0			
Autoimmune hepatitis	4	17.4	180	<3	4.6		2.75 (0.15 to 52.17)	0.99
Musculoskeletal or systemic	340	1590.7	180	90	385.9		0.99 (0.75 to 1.29)	0.99
Ankylosing spondylitis and axial spondyloarthritis	11	50.8	180	3	12.2		1.68 (0.35 to 8.09)	0.99
Behçet's syndrome	<3	19.7	180	3	4.1		15.50 (0.76 to 316.56)	0.99
Henoch-Schönlein purpura	78	344.0	180	18	85.9		1.00 (0.54 to 1.86)	1.00
Juvenile arthritis	203	954.0	180	53	229.1		0.98 (0.69 to 1.38)	0.99
Kawasaki disease	6	34.4	180	3	7.2		3.58 (0.52 to 24.60)	0.99
Myositis	18	80.1	180	5	21.6		0.66 (0.20 to 2.23)	0.99
Polyarteritis nodosa	3	14.3	180	<3	3.2		0.41 (0.02 to 8.07)	0.99
Polymyositis or dermatomyositis	<3	7.1	180	<3	1.9		*	
Reiter's syndrome	0	0.0	180	0	0.0			
Rheumatoid arthritis	6	22.8	180	0	5.5			
Psoriatic arthropathies	25	127.2	180	7	30.7		1.04 (0.38 to 2.84)	0.99
Sjögren's syndrome	<3	8.7	180	0	1.5			
Systemic lupus erythematosus	14	51.6	180	<3	13.8		0.18 (0.01 to 2.43)	0.99
Systemic sclerosis (scleroderma)	3	9.7	180	0	2.9			
Vasculitis unspecified	5	25.8	180	3	5.9		1.80 (0.26 to 12.44)	0.99
Wegener's granulomatosis	4	20.4	180	<3	3.6		0.76 (0.03 to 16.76)	0.99
Haematological	21	91.8	28	0	3.3			
Autoimmune haemolytic anaemia	3	12.5	28	0	0.5			
Idiopathic thrombocytopenic purpura	17	74.5	28	0	2.6			
Pernicious anaemia	<3	4.9	28	0	0.2			

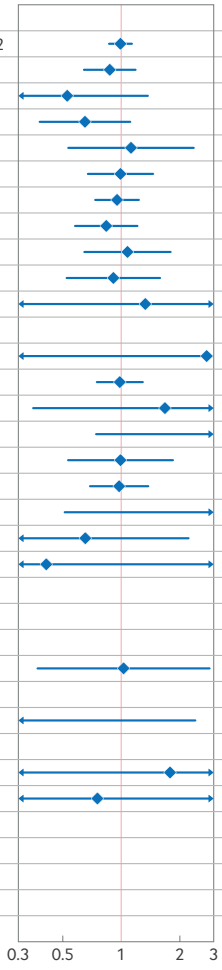

Figure 3 | Self-controlled case series analysis of vaccination with nonavalent human papillomavirus (HPV9) vaccine and rates of admission to hospital for immune mediated thyroid, gastrointestinal, musculoskeletal or systemic, and haematological diseases in the time period immediately after vaccinations versus reference period, in Danish adolescent girls aged 10-17 years. Rate ratios of hospital admissions were adjusted for age (10-11, 12-13, 14-15, and 16-17 years), season (winter, spring, summer, and autumn), and calendar time (October-December 2017, 2018, 2019, 2020, 2021, and 2022). *Not estimable because of lack of data or model convergence. FDR=false discovery rate

identified in the alternative risk periods (28 days instead of 180 days and vice versa) of the sensitivity analysis. We found no qualitatively different estimates in any other analysis compared with the main analysis with the standard self-controlled case series method.

Discussion

Principal findings

In this study, we investigated the association between HPV9 vaccination and 56 unique outcomes (eg, immune mediated diseases, myocarditis, pericarditis, and thromboembolism and thrombocytopenia) in adolescent boys and girls aged 10-17 years living in Denmark. We found no significant associations in our self-controlled case series analyses, nested within a large nationwide cohort of 854 586 individuals given 696 776 doses of the HPV vaccine (673 530 HPV9 doses), including data on 3354 individuals with outcomes. Although an association between the HPV9 vaccine and Raynaud's disease in boys aged 10-17 years was seen, the association was not robust to adjustment for multiple testing (false discovery rate adjusted P value=0.85).

Comparison with other studies

Evidence of the safety of the HPV9 vaccine is limited, but the few studies conducted in the US based on the Vaccine Safety Datalink[18] did not find any safety concerns after HPV9 vaccination. In a study by Sundaram et al, comparing 1.8 million doses of HPV9 with comparator vaccinations in US individuals aged 9-26 years, no increased risk of Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, or stroke were detected.[19] In another safety surveillance study of the HPV9 vaccine and

Disease	Reference period Cases	Reference period Person years	Risk period Length	Risk period Cases	Risk period Person years	Rate ratio (95% CI)	FDR adjusted P value
Dermatological	186	866.3	180	44	211.2	1.02 (0.70 to 1.48)	0.99
Erythema nodosum	56	246.7	180	10	61.7	1.10 (0.49 to 2.49)	0.99
Localised lupus erythematosus	6	20.5	180	0	5.0		
Localised scleroderma	20	91.9	180	5	23.3	0.74 (0.24 to 2.28)	0.99
Pemphigoid	<3	6.0	180	<3	2.0	*	
Pemphigus foliaceus	0	0.0	180	0	0.0		
Pemphigus vulgaris	0	0.0	180	0	0.0		
Psoriasis	70	328.7	180	17	79.8	1.12 (0.61 to 2.07)	0.99
Vitiligo	18	95.9	180	7	23.2	1.03 (0.35 to 3.00)	0.99
Dermatological vasculitis	17	84.6	180	4	18.1	1.48 (0.42 to 5.27)	0.99
Neurological	30	130.9	180	6	30.8	0.77 (0.28 to 2.16)	0.99
Guillain-Barré syndrome	5	22.4	28	0	0.7		
Multiple sclerosis	11	56.7	180	4	12.6	2.21 (0.47 to 10.51)	0.99
Other demyelinating disorders	7	35.6	180	<3	7.9	2.11 (0.15 to 29.64)	0.99
Narcolepsy	10	40.6	180	<3	10.1	0.25 (0.03 to 1.98)	0.99
Miscellaneous	294	1333.1	180	72	322.5	1.05 (0.77 to 1.43)	0.99
Acute rheumatic fever	4	16.4	180	0	3.9		
Addison's disease	4	15.5	180	0	3.4		
Raynaud's disease	47	222.9	180	11	52.6	1.09 (0.49 to 2.43)	0.99
Sarcoidosis	<3	12.5	180	<3	4.0	0.34 (0.00 to 44.22)	0.99
Type 1 diabetes	237	1065.8	180	60	258.7	1.12 (0.80 to 1.58)	0.99
Myocarditis or pericarditis	17	78.9	28	0	2.6		
Myocarditis	8	38.0	28	0	1.3		
Pericarditis	11	50.2	28	0	1.7		
Thromboembolism and thrombocytopenia	45	205.3	28	3	7.6	1.48 (0.42 to 5.19)	0.99
Thrombosis with thrombocytopenia syndrome	0	0.0	28	0	0.0		
Venous thromboembolism	38	173.9	28	3	6.6	1.92 (0.54 to 6.86)	0.99
Deep vein thrombosis	23	105.7	28	<3	4.0	2.19 (0.45 to 10.61)	0.99
Pulmonary embolism	10	52.8	28	<3	2.0	5.67 (0.92 to 34.94)	0.99
Cerebral venous sinus thrombosis	<3	4.9	28	0	0.2		
Hepatic-portal-renal vein thrombosis	4	15.3	28	0	0.6		
Arterial thromboembolism	7	31.4	28	0	1.0		
Ischaemic stroke	7	31.4	28	0	1.0		
Myocardial infarction	0	0.0	28	0	0.0		

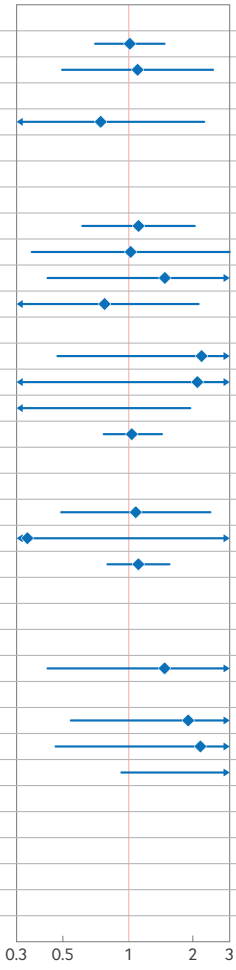

Figure 4 | Self-controlled case series analysis of vaccination with nonavalent human papillomavirus (HPV9) vaccine and rates of admission to hospital for immune mediated dermatological and neurological diseases, myocarditis or pericarditis, thrombosis with thrombocytopenia syndrome, venous and arterial thromboembolism, and other miscellaneous diseases in the time period immediately after vaccinations versus reference period, in Danish adolescent girls aged 10-17 years. Rate ratios of hospital admissions were adjusted for age (10-11, 12-13, 14-15, and 16-17 years), season (winter, spring, summer, and autumn), and calendar time (October-December 2017, 2018, 2019, 2020, 2021, and 2022). *Not estimable because of lack of data or model convergence. FDR=false discovery rate

12 prespecified outcomes, no safety concerns were identified after assessing adverse events, such as Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, pancreatitis, stroke, or venous thromboembolism, supporting the results of our study.[20] A recent report from Norway described a young woman who presented with thrombosis with thrombocytopenia syndrome after HPV9 vaccination. This patient was not directly comparable with the target group for this vaccine, however, because of underlying factors, such as older age and infection.[11] Our findings do not support an association between the HPV9 vaccine and thrombosis with thrombocytopenia syndrome.

A US study of the HPV4 and HPV9 vaccines in individuals of both sexes aged 11-26 years found no increased risk of type 1 diabetes mellitus.[21] Currently, no evidence exists to suggest that HPV vaccination, including the HPV2, HPV4, and HPV9 vaccines, increases the risk of Raynaud's disease. Previous studies in Denmark and Sweden have evaluated the safety of HPV4 vaccines in adolescent boys and girls aged 10-17 years. These large cohort studies did not find an increased risk of immune mediated or thromboembolic outcomes, similar to the results of our study.[8 22] In contrast with a Danish study in adult women that identified an association between HPV4 vaccination and coeliac disease (rate ratio 1.56, 95% CI 1.29 to 1.89), after multiple testing adjustment,[9] our analysis did not confirm this association in younger girls with the newer vaccine.

Several large scale studies have evaluated the association between HPV vaccination and Guillain-Barré syndrome.[23–26] A study from Spain

Disease	Reference period Cases	Reference period Person years	Risk period Length	Risk period Cases	Risk period Person years	Rate ratio (95% CI)	Rate ratio (95% CI)	FDR adjusted P value
Immune mediated	1067	4878.5	180	281	1159.8		1.03 (0.89 to 1.20)	0.92
Thyroid	88	375.9	180	19	88.7		0.74 (0.43 to 1.28)	0.89
Graves' disease	7	29.9	180	<3	8.0		0.79 (0.09 to 7.20)	0.97
Hashimoto's thyroiditis	22	98.9	180	5	22.6		0.67 (0.22 to 2.02)	0.89
Other hyperthyroidism	18	68.3	180	<3	15.6		0.34 (0.07 to 1.63)	0.89
Hypothyroidism	53	235.0	180	14	56.4		1.05 (0.54 to 2.03)	0.97
Gastrointestinal	312	1476.8	180	89	344.7		1.09 (0.83 to 1.41)	0.91
Coeliac disease	125	555.4	180	29	133.7		1.03 (0.65 to 1.64)	0.97
Crohn's disease	109	548.5	180	35	123.8		1.22 (0.79 to 1.86)	0.89
Ulcerative colitis	80	374.5	180	22	86.7		0.89 (0.52 to 1.52)	0.92
Pancreatitis	14	74.5	180	6	17.5		0.99 (0.33 to 2.94)	1.00
Primary biliary cirrhosis	0	0.0	180	0	0.0			
Autoimmune hepatitis	3	20.5	180	<3	3.9		*	
Musculoskeletal or systemic	257	1185.1	180	59	281.7		0.94 (0.68 to 1.30)	0.95
Ankylosing spondylitis and axial spondyloarthritis	8	39.4	180	3	10.1		1.48 (0.24 to 9.30)	0.92
Behçet's syndrome	3	16.5	180	<3	3.3		*	
Henoch-Schönlein purpura	89	393.6	180	17	96.3		0.87 (0.48 to 1.57)	0.92
Juvenile arthritis	130	618.5	180	34	142.8		1.00 (0.65 to 1.53)	1.00
Kawasaki disease	7	35.9	180	<3	8.3		2.34 (0.21 to 26.58)	0.89
Myositis	9	49.4	180	3	12.1		3.47 (0.52 to 23.08)	0.89
Polyarteritis nodosa	<3	4.0	180	0	1.0			
Polymyositis or dermatomyositis	<3	7.8	180	0	1.9			
Reiter's syndrome	0	0.0	180	0	0.0			
Rheumatoid arthritis	6	19.9	180	0	5.4			
Psoriatic arthropathies	15	56.4	180	<3	12.5		0.39 (0.04 to 3.80)	0.89
Sjögren's syndrome	0	0.0	180	0	0.0			
Systemic lupus erythematosus	<3	7.7	180	<3	1.8		*	
Systemic sclerosis (scleroderma)	0	0.0	180	0	0.0			
Vasculitis unspecified	3	9.0	180	0	2.6			
Wegener's granulomatosis	0	0.0	180	0	0.0			
Haematological	23	99.9	28	<3	3.5		1.28 (0.16 to 10.18)	0.97
Autoimmune haemolytic anaemia	6	24.9	28	0	0.9			
Idiopathic thrombocytopenic purpura	17	75.0	28	<3	2.7		2.04 (0.23 to 18.12)	0.89
Pernicious anaemia	0	0.0	28	0	0.0			

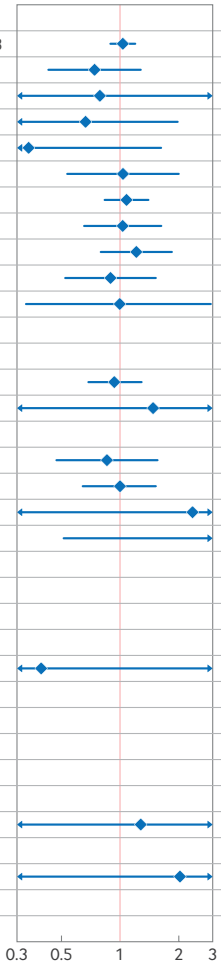

Figure 5 | Self-controlled case series analysis of vaccination with nonavalent human papillomavirus (HPV9) vaccine and rates of admission to hospital for immune mediated thyroid, gastrointestinal, musculoskeletal or systemic, and haematological diseases in the time period immediately after vaccinations versus reference period, in Danish adolescent boys aged 10-17 years. Rate ratios of hospital admissions were adjusted for age (10-11, 12-13, 14-15, and 16-17 years), season (winter, spring, summer, and autumn), and calendar time (October-December 2017, 2018, 2019, 2020, 2021, and 2022). *Not estimable because of lack of data or model convergence. FDR=false discovery rate

of 388 849 girls compared the incidence of the syndrome between vaccinated and unvaccinated groups and concluded that the adjusted risk of Guillain-Barré syndrome after HPV vaccination did not differ between the two groups.[25] Another study in the US of 2 773 185 HPV4 doses received by individuals aged 9-26 years found no evidence of an increased risk of Guillain-Barré syndrome,[26] corroborating our results across all analyses. A recent systematic review and meta-analysis of 22 relevant studies that evaluated HPV post-licensure safety studies and summarised risk estimates for immune mediated diseases, showed an overall absence of association between both HPV2 and HPV4 vaccines and Guillain-Barré syndrome.[27] Although the studies used different methodological approaches, they found a reduced risk of paralysis, immune thrombocytopenia purpura, and chronic fatigue syndrome, and increased risk of Hashimoto's and Raynaud's diseases among 35 outcomes evaluated. The authors also emphasised the need for systematic evaluations of different types of HPV vaccines.

Strengths and limitations of this study

This study had several strengths, including the use of a self-controlled case series approach, which by design, eliminates time invariant confounding. Also, the study used nationwide data from a free and universal healthcare system, and allowed the inclusion of all HPV9 vaccinations given to adolescent boys and girls aged 10-17 years living in Denmark up to the end of 2022.

We used the Benjamini-Hochberg method to effectively control the false discovery rate and improve the validity of associations between vaccination

Disease	Reference period Cases	Reference period Person years	Risk period Length	Risk period Cases	Risk period Person years	Rate ratio (95% CI)	FDR adjusted P value
Dermatological	137	575.6	180	24	141.5	0.72 (0.45 to 1.17)	0.89
Erythema nodosum	30	133.9	180	5	31.2	0.77 (0.25 to 2.33)	0.92
Localised lupus erythematosus	0	0.0	180	0	0.0		
Localised scleroderma	6	33.7	180	4	9.4	3.43 (0.51 to 23.00)	0.89
Pemphigoid	0	0.0	180	0	0.0		
Pemphigus foliaceus	<3	1.7	180	0	0.7		
Pemphigus vulgaris	0	0.0	180	0	0.0		
Psoriasis	65	274.2	180	11	66.5	0.72 (0.35 to 1.48)	0.89
Vitiligo	25	87.3	180	<3	22.7	0.23 (0.05 to 1.19)	0.89
Dermatological vasculitis	13	57.1	180	3	14.1	0.66 (0.14 to 3.03)	0.92
Neurological	26	115.1	180	5	25.9	0.48 (0.16 to 1.42)	0.89
Guillain-Barré syndrome	4	23.9	28	<3	0.8	*	
Multiple sclerosis	3	12.9	180	<3	2.5	*	
Other demyelinating disorders	11	41.6	180	<3	7.5	0.28 (0.03 to 2.71)	0.89
Narcolepsy	10	47.4	180	<3	11.8	0.76 (0.12 to 4.71)	0.97
Miscellaneous	284	1337.5	180	95	321.7	1.31 (1.00 to 1.71)	0.85
Acute rheumatic fever	<3	6.4	180	0	2.0		
Addison's disease	<3	13.7	180	<3	3.4	*	
Raynaud's disease	11	101.9	180	15	23.8	2.62 (1.07 to 6.40)	0.85
Sarcoidosis	<3	4.1	180	0	1.0		
Type 1 diabetes	269	1222.3	180	80	294.3	1.18 (0.88 to 1.58)	0.89
Myocarditis or pericarditis	47	210.2	28	<3	7.5	0.46 (0.06 to 3.40)	0.89
Myocarditis	23	102.4	28	<3	3.6	1.10 (0.14 to 8.77)	0.97
Pericarditis	30	134.0	28	0	5.0		
Thromboembolism and thrombocytopenia	33	156.7	28	3	5.4	2.29 (0.66 to 7.93)	0.89
Thrombosis with thrombocytopenia syndrome	0	0.0	28	0	0.0		
Venous thromboembolism	25	119.6	28	<3	3.8	1.15 (0.14 to 9.20)	0.97
Deep vein thrombosis	16	78.1	28	<3	2.5	1.66 (0.19 to 14.95)	0.92
Pulmonary embolism	7	33.8	28	0	1.0		
Cerebral venous sinus thrombosis	<3	4.5	28	0	0.2		
Hepatic-portal-renal vein thrombosis	4	17.5	28	0	0.4		
Arterial thromboembolism	9	41.6	28	<3	1.7	4.05 (0.66 to 24.89)	0.89
Ischaemic stroke	7	34.7	28	<3	1.4	4.47 (0.72 to 27.68)	0.89
Myocardial infarction	<3	6.9	28	0	0.3		

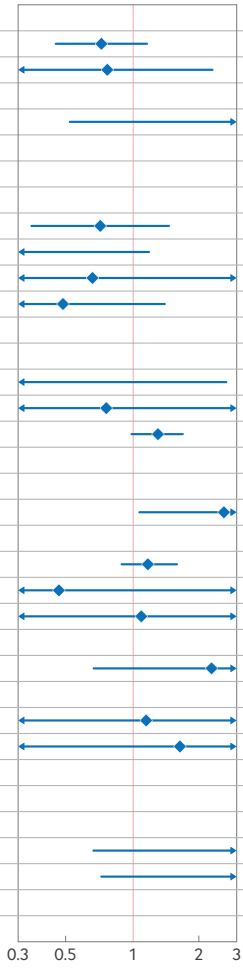

Figure 6 | Self-controlled case series analysis of vaccination with nonavalent human papillomavirus (HPV9) vaccine and rates of admission to hospital for immune mediated dermatological and neurological diseases, myocarditis or pericarditis, thrombosis with thrombocytopenia syndrome, venous and arterial thromboembolism, and other miscellaneous diseases in the time period immediately after vaccinations versus reference period, in Danish adolescent boys aged 10-17 years. Rate ratios of hospital admissions were adjusted for age (10-11, 12-13, 14-15, and 16-17 years), season (winter, spring, summer, and autumn), and calendar time (October-December 2017, 2018, 2019, 2020, 2021, and 2022). *Not estimable because of lack of data or model convergence. FDR=false discovery rate

and the study outcomes, reported as safety signals. Because we studied a wide range of mostly unrelated outcomes where only a few people contributed to multiple analyses, the tests were treated as independent, in line with the assumptions of the Benjamini-Hochberg method. This approach reduced the risk of false positive findings.

Our study did not suggest an association between HPV9 vaccination and the outcomes studied in boys and girls aged 10-17 years, but several limitations should be considered. Because of the rarity of many of the outcomes, statistical precision was lacking for some analyses. The total number of boys aged 10-17 years with outcomes who received the HPV vaccine was relatively smaller (472 fewer boys than girls) because of their later inclusion in the HPV national vaccination programme in 2019. Restrictions in statistical precision limited any firm conclusions about rarer outcomes that occurred in only a few individuals during the follow-up period after HPV9 vaccination.

We defined the occurrence of outcomes by using the dates of diagnoses. A delay between symptom onset and diagnosis is possible, however, especially for immune mediated conditions with a more insidious onset. In vaccine safety research, an unmasking phenomenon has been identified.[28] This phenomenon suggests that individuals who receive vaccines might be more likely to have certain disorders diagnosed or diagnosed earlier, because the vaccination visit provides an opportunity to evaluate symptoms that might have otherwise gone unnoticed. Consequently, this finding could bias the results towards an increased risk attributed to vaccination.

The unmasking phenomenon is less likely to occur for diseases with clear and recognisable symptoms, such as type 1 diabetes, but is more plausible for diseases that present with vague or mild symptoms, such as Raynaud's disease. Also, our reliance on hospital diagnoses to define outcomes might underestimate the number of outcomes. Individuals with some disorders, such as dermatological outcomes, often seek care from private dermatologists who might not report diagnostic codes to the registers. Consequently, the number of individuals for these outcomes might be underestimated. The findings of this study might be applicable to similar age groups but cannot be directly extrapolated to adults. Finally, because of the rarity of many of the outcomes, statistical precision was lacking for some study outcomes.

Conclusions

In this nationwide, self-controlled case series analysis, we found no associations between HPV9 vaccination and immune mediated diseases, myocarditis, pericarditis, or thromboembolic outcomes, that would constitute a safety signal. These findings support the overall safety of the HPV9 vaccine. Interpreting these results in the context of statistical precision is important, however, which was lacking for some of the rarer study outcomes. Our findings contribute to the evidence on the safety of the HPV9 vaccine that is important in preventing vaccine hesitancy based on safety concerns, a major contributor to the less than enthusiastic update of the HPV vaccine seen in many countries. The benefits and safety of HPV vaccination in preventing cervical cancer, and other HPV related cancers, are well established, and efforts should be made to increase public support for HPV vaccination programmes worldwide.

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Contributors All authors fulfilled the following authorship criteria: substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published. AH and ADL had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors were involved in the study concept and design, and acquisition, analysis, and interpretation of the data. KF drafted the manuscript. AH contributed to critical revision of the manuscript for important intellectual content, obtained the funding, and supervised the study. ADL performed the statistical analysis. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. KF and AH are the guarantors. Transparency: The guarantors affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Funding This study was supported by the Lundbeck Foundation (R313-2019-68). The funder had no role in considering the study design or in the collection, analysis, interpretation of the data, writing of the report, or decision to submit the article for publication.

Competing interests All authors have completed the ICMJE uniform disclosure form at www.icmje.org/disclosure-of-interest/ and declare: support from the Lundbeck Foundation for the submitted work; AH holds unrelated grants from the Novo Nordisk Foundation and Independent Research Fund Denmark; AH is a scientific board member of VAC4EU; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethics approval The study was reviewed and approved (j.nr. 22/03940) by the Department of Data Protection and Information Security at Statens Serum Institut, which oversees data protection and privacy matters. This internal review ensured compliance with applicable data protection regulations and ethical standards for research. According to Danish law, ethics approval is exempt for register based research.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No data are available. Owing to data privacy regulations, the raw data cannot be shared.

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► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/bmjmed-2024-000854).