Pneumococcal Polysaccharide Vaccine Associated with Reduced Lengths of Stay for Cardiovascular Events Hospital Admissions

Experience from the Hunter Community Study

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Introduction

Cardiovascular disease (CVD) is the leading cause of death globally and a major burden on healthcare systems.[1] The underlying cause of most CVD is atherosclerosis, the progressive build-up of fatty deposits on the inner wall of arteries.[2] Over the last 20 years, evidence has emerged that the pneumococcal polysaccharide vaccine (PPV) may help to reduce atherosclerosis and related cardiovascular events. Mouse models have demonstrated reductions in atherosclerotic plaque formation after immunization with oxidized low-density lipoprotein (oxLDL) as well as with pneumococcal cell wall preparations.[3,4] It is believed that a phospholipid in the bacterial cell wall, phosphorylcholine, exhibits molecular mimicry with oxLDL.[5-7] Indeed, PPV administration has been observed to induce anti-oxLDL antibodies in humans.[8] Some international observational studies have demonstrated an association between PPV and reduced risk of ischemic events in CVD,[9,10] while other studies have not.[11,12] A meta-analysis of these studies has found a statistically significant reduction of 17% for acute coronary syndrome (ACS) events. However, these observational studies were heterogeneous in terms of sample population, exposures measured, and definition of outcomes.[13]

Influenza vaccination has also been associated with a reduced risk of cardiovascular events,[14] consistent with the suggestion that influenza is a trigger for cardiovascular events. This appears to be a short term effect, with the magnitude of the association between the vaccination episode and the ACS decreasing over weeks and months.[15] Since older people may get both PPV (indicated at age 65 in Australia) and influenza vaccine (indicated annually at age 65 and for those at risk), it is difficult to determine the proportion of risk reduction in cardiovascular events attributable to each vaccine. We therefore examined the effect of PPV on acute coronary events, while adjusting for the influenza vaccine.

The Hunter Community Study (HCS) is a longitudinal population-based cohort of community dwelling residents aged 55 to 85 from the Hunter Region of New South Wales, Australia. Baseline measures were collected between 2004-2008, with postal and clinic follow-ups over the following years.[16] HCS captures a broad and comprehensive range of physical and biological measures, including participants' vaccination status at baseline and hospital admissions.

In the current analysis, we aimed to assess the relationship between exposure to PPV and influenza vaccine and risk of incident CVD. The hypothesis was that participants who had received either PPV or influenza vaccines at baseline would have a reduced risk of incident CVD during follow-up.

Methods

Population

The sample comprised participants from the HCS. Hospital admissions data came from the New South Wales Admitted Patient Data Collection (APDC), and mortality data came from the Registry of Births, Deaths and Marriages (RBDM), both linked to participants via the Centre for Health Record Linkage (CHeReL). Up to 11 years of APDC and mortality data were linked with HCS data, from 1 Dec 2003 to 31 March 2015.

Australia has a universal health insurance system, called Medicare. In a study of patients with suspected or confirmed ACS across Australia and New Zealand, Chew *et al* (2013) showed only 7% in total, and 2% for New South Wales, presented to Private hospitals.[17] Private hospital admission would have mostly related to planned/elective cardiac procedures. Therefore, only public hospital records were considered for this analysis.

For the current study, the following exclusions were applied:

- Those with no consent for data linkage via CHeReL
- Age <65 years at baseline
- History of CVD at baseline, either self-reported or identified using APDC data; this was to remove prevalent cases of CVD at baseline. Primary diagnoses corresponding to the following ICD-10 diagnostic codes were used to define prior hospitalization due to CVD:

    I20 (Angina pectoris),

    I21 (Myocardial infarction),

    I25 (Chronic ischemic heart disease),

    I61 (Intracerebral hemorrhage),

    I63 (Cerebral infarction),

I64 (Stroke, unspecified).

Exposure

There were two exposure variables of interest: PPV and influenza vaccine. The HCS questions were worded as follows:

1. Have you had a 'flu vaccine in the last 12 months (*fluarix, fluvax, influvac, vaxigrip)?*
2. Have you received the PNEUMOCOCCAL *(pneumovax or prevenar)* vaccine in the last 5 years?

A recent study using a random sample from this cohort (n=116) found similar and overlapping distribution of anti-pneumococcal IgG titer between self-reported vaccination status and vaccination by medical record.[8]

A priori, three main models of interest were fitted to assess individual and joint effects of the exposures:

1. PPV only: this analysis compared participants who had reported PPV only (i.e. no influenza vaccine) with participants who had reported neither vaccine;
2. Influenza vaccine only: this analysis compared participants who had reported influenza vaccine only (i.e. no PPV) with participants who had reported neither vaccine
3. PPV, with or without influenza vaccine: this analysis compared participants who had received PPV with those who had not, irrespective of influenza vaccine status.

Where relevant, we also modeled PPV, adjusted for influenza vaccine; this analysis compared participants who had received PPV with those who had not, adjusted for influenza vaccine status.

Outcomes

Three outcomes were assessed, using hospital admissions or mortality data occurring during follow-up (to 31 March 2015):

1. Time to hospitalization for CVD or death from any cause;

2. Time to hospitalization for CVD, treating death as a competing risk;

3. Total number of hospital bed-days where the primary diagnosis was CVD (ICD-10 diagnostic codes: I20, I21, I25, I61, I63 or I64); and

Total number of hospital bed-days where the primary diagnosis was limb fracture (ICD-10 diagnostic codes: S42, S52, S62, S72, S82 or S92) was used as a control outcome.

Covariates

The following baseline covariates were considered as potential confounders of any association between vaccination and CVD, and were coded for inclusion in multivariate regression models: age, history of diabetes, excessive alcohol intake (defined as participants engaging in hazardous chronic or hazardous binge drinking, based on self-reported alcohol consumption), and smoking status. These were indications to receive the PPV as per Australian National Immunization Program,[18] as well as being risk factors for CVD.

Statistical analyses

Continuous variables were summarized using means with standard deviations, or medians with minima and maxima. Group differences were assessed using t-tests for means or Kruskal-Wallis tests for medians. Categorical variables were summarized using frequencies, with group differences assessed using Chi-square statistics.

Time to hospitalization for CVD or death from any cause: Multivariate association between each binary exposure definition and time to incident CVD or all-cause mortality was assessed using Cox proportional hazards models. All models were adjusted for the covariates specified above. Parameters are expressed as Hazard Ratios (HR) with 95% confidence intervals (CI). The proportional hazards assumption was checked using Martingale and Schoenfeld residuals.

Time to hospitalization for CVD, treating death as a competing risk: Multivariate association between exposures and time to hospitalization for incident CVD was assessed, while accounting for participants who died before experiencing the outcome. These models were fitted using proportional subdistribution hazards models as described by Fine and Gray.[19] Parameters are expressed as subdistribution hazard ratios (SHR), representing the effect of each covariate on the subdistribution hazard. The proportional hazards assumption was checked by testing for lack of significant time variation in each covariate's estimated effect.

Total number of hospital bed-days for CVD and fractures: Multivariate association of exposures with number of CVD bed-days was assessed using zero-inflated negative binomial (ZINB) regression, due to evidence of both overdispersion and excess zero counts. The logarithm of total follow-up time was included as an offset. There are two parts to the ZINB model, corresponding to two outcomes being jointly modelled: the expected count and the probability of having a zero count. This model thus assumes that a zero count can arise from two different processes. Predictors for the count outcome comprised vaccine status and all specified covariates. Predictors for having a zero count comprised the same variables,

excluding vaccination status. Superiority of the ZINB model over Poisson and negative binomial models was confirmed by estimating dispersion parameters and comparing measures of model fit including the Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC). The outcome of fractures was used as a control for any residual or unmeasured confounding.

All statistical analyses were programmed using SAS v9.4 (SAS Institute, Cary, North Carolina, USA).

Results

Out of 1074 participants, 91 had a CVD event during follow-up and 983 did not; descriptive data for the two groups are summarized in Table 1. Participants experiencing an incident CVD event during follow-up were more likely to be older, have a history of smoking (past or current) and have excess alcohol intake. For the multivariate analysis, 211 participants had some missing data either for the main exposures or for a covariate, leaving 863 participants with complete data, representing 6,893 person years of observation, 73 CVD events, and 542 CVD bed days. Table 2 shows the number with the PPV or the influenza vaccine, both, and neither, along with the number of CVD events.

Table 1: Descriptive statistics for HCS participants: by CVD event during follow-up

Variable	Statistic/Class	No (N=983)	Yes (N=91)	Total (N=1074
Age	Mean (SD)	72 (5)	72 (6)	72 (5)
CVD bed-days	Mean (SD)	0 (0)	8 (8)	1 (3)
Time to CVD event/death/censoring (y)	Mean (SD)	8.04 (1.68)	4.64 (2.64)	7.76 (2.01)
	Median (min, max)	7.85 (0.11, 10.54)	4.27 (0.10, 9.95)	7.79 (0.10, 10.54)
Age group	65-69	404 (41%)	35 (38%)	439 (41%)
	70-74	288 (29%)	25 (27%)	313 (29%)
	75-79	191 (19%)	17 (19%)	208 (19%)
	80+	100 (10%)	14 (15%)	114 (11%)
Sex	Male	425 (43%)	55 (60%)	480 (45%)
	Female	558 (57%)	36 (40%)	594 (55%)
Education	Did not complete secondary school	232 (26%)	26 (31%)	258 (27%)
	Completed secondary school	234 (27%)	21 (25%)	255 (27%)
	Trade qualifications or TAFE	219 (25%)	22 (26%)	241 (25%)
	University or other tertiary	191 (22%)	15 (18%)	206 (21%)
	Missing	107	7	114
Marital status	Partnered	668 (71%)	62 (72%)	730 (71%)
	Not partnered	279 (29%)	24 (28%)	303 (29%)
	Missing	36	5	41
BMI category	Underweight	9 (1.0%)	1 (1.1%)	10 (1.0%)
	Normal	207 (22%)	8 (8.9%)	215 (21%)
	Overweight	422 (46%)	45 (50%)	467 (46%)
	Obese	287 (31%)	36 (40%)	323 (32%)
	Missing	58	1	59
Smoking category	Never	577 (62%)	40 (47%)	617 (60%)
	Past smoker	318 (34%)	35 (41%)	353 (34%)
	Current smoker	43 (4.6%)	11 (13%)	54 (5.3%)
	Missing	45	5	50
Excess alcohol intake	No	780 (92%)	64 (85%)	844 (91%)

Variable	Statistic/Class	No (N=983)	Yes (N=91)	Total (N=1074
	Yes	68 (8.0%)	11 (15%)	79 (8.6%)
	Missing	135	16	151
Diabetes	No	856 (89%)	69 (77%)	925 (88%)
	Yes	110 (11%)	21 (23%)	131 (12%)
	Missing	17	1	18
Death	No	880 (90%)	73 (80%)	953 (89%)
	Yes	103 (10%)	18 (20%)	121 (11%)

Self–reported PPV	Self-reported influenza vaccine		
	No	Yes	Total
No	19 (14 events	190 (16 events)	386
Yes	26 (3 events)	451 (40 events)	477
Total	222	641	863

Table 2 Self-reported exposure to pneumococcal polysaccharide vaccine (PPV) versus self-reported exposure to influenza vaccine

Time to CVD hospitalization or all-cause mortality

Neither PPV alone, nor influenza vaccine alone, nor PPV regardless of influenza vaccine were associated with time to CVD hospitalization or all-cause mortality (Model 1, Table 3). Factors associated with an increased risk of CVD hospitalization or all-cause mortality in the various models included advancing age, diabetes, excess alcohol intake and current/past smoking.

Time to CVD hospitalization, treating death as a competing risk

Neither PPV alone, nor influenza vaccine alone, nor PPV regardless of influenza vaccine were associated with time to hospitalization for a CVD event (Model 2, Table 3), using death as a competing risk. Factors associated with an increased risk of CVD event in the various models included diabetes, excess alcohol intake and current/past smoking.

CVD hospital bed-days

Neither PPV alone nor influenza vaccine alone were associated with CVD bed-days. However, PPV regardless of influenza vaccine (n=863) was associated with a reduction in bed days; the unadjusted IRR was 0.72 (95% CI 0.45-1.13) and this became statistically significant after adjustment for confounding (IRR=0.65, 95% CI 0.45-0.94) (Model 3, Table 3). Results were unchanged by additionally adjusting this last model for influenza vaccine (IRR=0.64, 95% CI: 0.43-0.96).

In a *post hoc* analysis, influenza vaccine regardless of PPV (n=864) was not significantly associated with CVD bed-days. (Model 3, Table3)

	Model 1 (CVD and death)		Model 2 (CVD, treating death as competing risk)		Model 3 (CVD bed-days)	
	HR (95% CI)	p-value	HR (95% CI)	p-value	IRR (95% CI)	p-value
PPV only	1.0 (0.37-2.5)	0.94	1.3 (0.34-5.1)	0.69	1.3 (0.67-2.7)	0.40
Flu Vac only	0.74 (0.43-1.3)	0.29	1.0 (0.46-2.2)	0.99	1.2 (0.67-2.1)	0.55
PPV (+/- flu vac)	1.2 (0.85-1.7)	0.30	1.1 (0.69-1.8)	0.66	**0.65 (0.45-0.94**	**0.02**
Flu vac (+/- PPV)					0.86 (0.54-1.35)	0.51

Table 3: Results of statistical models for effect of pneumococcal polysaccharide vaccine (PPV) alone, influenza vaccine (flu vac) alone, PPV regardless of influenza vaccine, and

influenza vaccine regardless of PPV. Model 1 is a Cox regression model for CVD events and all-cause mortality; Model 2 is a Cox regression model for CVD events, with mortality as a competing risk; Model 3 is a zero-inflated negative binomial model for CVD bed-days. All models are adjusted for age, smoking, diabetes and alcohol status.

Limb fracture hospital bed-days

62 participants had a hospital stay of 1 day or more for a limb fracture, with a total of 641 bed days. Both PPV (regardless of influenza vaccine) and influenza vaccine (regardless of PPV) were associated with reduction in fracture bed-days. However, PPV was no longer associated with reduction in fracture bed-days when adjusted for influenza vaccine. (Table 4)

	Model 4 (Limb fracture bed-days)	
	IRR (95% CI)	p-value
PPV (+/- flu vac)	0.37 (0.17-0.80)	0.01
Flu vac (+/- PPV)	0.28 (0.12-0.64)	<0.01
PPV (adjusted for flu vac)	**0.75 (0.28-2.0)**	**0.56**

Table 4: Results of statistical model for effect of pneumococcal polysaccharide vaccine (PPV) regardless of influenza vaccine (flu vac), influenza vaccine regardless of PPV, and PPV adjusted for influenza vaccine. Model 4 is a zero-inflated negative binomial model for CVD bed-days. All models are adjusted for age, smoking, diabetes and alcohol status.

Discussion

This study found no association between PPV or influenza vaccine and time to CVD event or all-cause mortality. We previously reported a 17% reduction in CVD events from a meta-analysis of observational studies. It is ***not*** surprising that we could not confirm this effect here given the small number of people with CVD events (n=91). A *post hoc* power calculation for our primary outcome (time to CVD event or all-cause mortality), with an effect size consistent with that previously shown in meta-analysis and a sample size of 863 of whom 386 were PPV-unexposed (consistent with allocation ratios observed in our sample) showed our sample had only 18% power.

However, the study did find a significant association between PPV and total CVD bed-days, with an estimated 35% relative reduction in CVD bed-days. Influenza vaccine as the control exposure in this study, did not demonstrate a significant reduction in CVD bed-days. Since there was a large overlap of PPV and influenza vaccination, we further adjusted PPV for influenza vaccine and obtained similar results, confirming the protective association of PPV on CVD bed-days.

It is possible that residual confounding exists, such as the healthy-user effect, whereby those who received PPV were healthier than those who did not, and therefore more likely to experience shorter hospital admissions. To test this effect, we used limb fractures as the control outcome, as there is no plausible biological link between PPV and limb fractures. Interestingly, both PPV (regardless of influenza vaccine) and influenza vaccine (regardless of PPV) were significantly associated with reduced fracture bed-days, indicating a similar degree of residual confounding (i.e. vaccination in general can be a marker for healthy habits). However, when PPV was adjusted for influenza vaccine, it was no longer associated with fracture bed-days. This finding strengthens the explanation that reduction in CVD bed-days is more likely due to PPV, than other factors, and the association is also robust to residual confounding.

Despite small numbers of CVD events, this study was able to detect a significant difference in total bed-days, which may be an indicator of severity of CVD events or complications during admission. Reducing hospital burden is vitally important to the healthcare system, given its finite resources and the ageing population of the nation. The cumulative risk

reduction across all potential patients internationally, and the accumulated savings on healthcare systems worldwide would be of even greater consequence. Cost savings produced from reduced lengths of stay as well as cost avoided by using an already commercially available vaccine, are worth considering when making public health decisions about vaccination programs.

The suggested mechanism of effect is from the phosphorylcholine lipid antigens in the *S. pneumoniae* cell wall that induce the production of antibodies that cross-react with oxLDL, a component of atherosclerotic plaques. These antibodies may bind to and facilitate the regression of the plaques. In fact, pneumococcal vaccination has been shown to induce anti-oxLDL antibodies.[3] A significant association has also been observed between pneumococcal IgG and anti-oxLDL titers.[8] It is therefore postulated that the PPV, by inducing the production of anti-phosphorylcholine and anti-oxLDL antibodies that block the uptake of LDL by macrophages, may have a protective effect on cardiovascular disease in humans.[3]

A recent meta-analysis of six RCTs involving 6735 patients, with mean follow up time of 7.9 months, demonstrated that **influenza vaccination** was also associated with a significantly lower risk of major adverse cardiovascular events (risk ratio = 0.64, 95% CI: 0.48 – 0.86).[14] It has been suggested that the reduced short-term risk of cardiovascular events offered by this vaccination is due to a reduction in infection, which can be a trigger in the inflammatory cascade that leads to the progression of atherosclerosis.[9,20] The protective effect of the influenza vaccine however seems to wane by 9-12 months. In contrast, most of studies included in our PPV meta-analysis [13] demonstrated a protective association of PPV over

several years, suggesting a different mechanism that takes longer to develop, consistent with the proposed anti-oxLDL pathway.

Limitations of this study include a modest number of participants, a small number of clinical events and use of some self-reported data on exposure, resulting in potential misclassification. We also did not capture which pneumococcal vaccine (polysaccharide vs protein conjugate) was being reported; however, in Australia the protein conjugate vaccine (Prevenar) is mainly used for children, whereas the polysaccharide vaccine (Pneumovax23) is on the national immunization schedule for those aged 65 and over. Strengths of the study include an older cohort that was prospectively followed for 7-11 years, use of data linkage to obtain unbiased endpoints, and the ability to tease out the independent effects of the pneumococcal and influenza vaccines.

The multiple models and outcomes explored in this paper do open the possibility of inflated type I error, i.e. false positive p-value due to multiple comparisons, so the significant association with CVD bed days needs to be further studied in a large RCT, which may be able to confirm the mechanism of action for this association. Such a trial is already underway in Australia, known as the Australian Study for the Prevention through Immunization of Cardiovascular Events (AUSPICE) (Trial registration: ACTRN12615000536561).[21] The results of a sub-study are due in 2019, which will hopefully provide some preliminary indication of the efficacy of the vaccine using surrogate markers of CVD.

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