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BRIEF REPORT

Pneumonia Following Symptomatic Influenza Infection Among Nicaraguan Children Before and After Introduction of the Pneumococcal Conjugate Vaccine

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Influenza is associated with primary viral and secondary bacterial pneumonias; however, the dynamics of this relationship in populations with varied levels of pneumococcal vaccination remain unclear. We conducted nested matched case-control studies in 2 prospective cohorts of Nicaraguan children aged 2–14 years: 1 before pneumococcal conjugate vaccine introduction (2008–2010) and 1 following introduction and near universal adoption (2011–2018). The association between influenza and pneumonia was similar in both cohorts. Participants with influenza (across types/subtypes) had higher odds of developing pneumonia in the month following influenza infection. These findings underscore the importance of considering influenza in interventions to reduce global pneumonia burden.

Keywords. influenza; pneumonia; child health; global health; Nicaragua.

Influenza is an important cause of pneumonia, with evidence suggesting that this occurs both directly via primary viral pneumonia and indirectly via secondary bacterial pneumonias caused by pathogens including *Streptococcus pneumoniae* and *Staphylococcus aureus* [1–4]. Globally, the introduction of pneumococcal conjugate vaccines has been associated with decreased pneumonia morbidity and mortality [5, 6]. However, the effect this might have on influenza-associated pneumonias requires examination.

The pneumococcal conjugate vaccine (PCV13) was introduced to Nicaragua in December 2010 and, by the end of 2012, approximately 100% of children were appropriately vaccinated for their age [5]. In this analysis, we conducted a nested matched case-control study within a cohort of Nicaraguan children aged 2–14 years followed from June 2007 to December 2010 to assess the risk period for influenza-associated pneumonias in a population largely unvaccinated for pneumococcus. We repeated these analyses within another pediatric cohort in the same community from January 2011 to December 2018 (where PCV13 coverage was approximately 100% for children in targeted age groups) to describe the association between influenza and pneumonia before and after PCV13 was introduced. This analysis builds on previous examination of the risk period of pneumonia following influenza within a larger cohort of Nicaraguan children largely vaccinated for pneumococcus.

METHODS

Ethics Statement

This study is a collaboration between the Sustainable Sciences Institute, the Nicaraguan Ministry of Health, the University of California, Berkeley (UCB), and the University of Michigan (UM). It was approved by the institutional review boards of the Nicaraguan Ministry of Health, UCB, and UM. Written informed consent was obtained from a parent/guardian of all participants. Verbal assent was obtained from children aged ≥6 years.

Study Population and Sample Collection

This analysis used data from 2 prospective cohort studies of Nicaraguan children aged 2–14 years [7, 8]. The first, conducted from June 2007 through 2010, is hereafter referred to as the pre-PCV cohort and the second, conducted from January 2011 through 2018, is hereafter referred to as the post-PCV cohort. The methods of both studies have been described in detail previously [7, 8]. Briefly, participants were enrolled from District II of Managua, Nicaragua, at Health Center Sócrates Flores Vivas (HCSFV). A detailed sociodemographic survey was collected upon enrollment and yearly thereafter for the duration of the child's participation. A clinical history was also collected on enrollment and updated any time the child came to the HCSFV. Healthcare was provided to all study participants and study nurses and physicians were available at the HCSFV 24 hours/day, 365 days/year. Parents agreed to bring their child to the clinic when they were sick, particularly when they had a recorded fever or were "feverish."

Upon presenting at the study clinic, participants were assessed for influenza-like illness (ILI) defined as fever or reported fever and rhinorrhea, cough, or sore throat. In the pre-PCV cohort, respiratory samples for influenza testing were obtained from a random sample of 25% of participants presenting with ILI using nasal and oropharyngeal polyester-tipped plastic swabs

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[8]. In the post-PCV cohort, samples were obtained from all participants with ILI [7]. Episodes of clinical pneumonia were diagnosed by study physicians using guidelines based on the Integrated Management of Childhood Illness [9]. Pneumonia onset was considered to be the date of diagnosis, while the start of symptoms reported with an influenza-positive episode was considered to be the date of influenza onset.

Laboratory Methods

The QIAamp Viral RNA Mini Kit (Qiagen) was used to extract RNA from swabs, which was then tested for influenza A and B by reverse transcription polymerase chain reaction (RT-PCR) following the Centers for Disease Control and Prevention protocol. Samples positive for influenza A were subtyped (H3N2, H1N1, or H1N1pdm09). Samples were not routinely tested for other pathogens.

Statistical Analysis

Cases of clinical pneumonia were matched on age (months) and week of study to a maximum of 4 controls. Participants with no episodes of clinical pneumonia in the previous 45 days were eligible to serve as controls.

Conditional logistic regression models were fit to assess the relative odds of clinical pneumonia in the 30 days post influenza. Separate models were fit to examine the relationship between influenza subtype and subsequent pneumonia (model 1), and the time-lag (0–6 days, 7–13 days, and 14–29 days) between influenza and pneumonia (model 2) to ensure sufficient power.

Because only a random subsample of pre-PCV cohort participants were tested for influenza, some influenza-positive participants may have been misclassified as influenza negative. However, restricting to those tested would have introduced selection bias (Supplementary Material and Supplementary Figure 1). Therefore, to achieve unbiased estimates, we repeated the previously described models with 100 complete datasets using multiple imputation methods described by Keogh et al [10]. Influenza type/subtype was imputed within each matched set for those who met the testing criteria and were not sampled. Imputation models were fit using logistic or multinomial regression (if >1 subtype circulated) with sex and ILI as predictors. Age and seasonality were accounted for by conducting the imputation within each matched set. All analyses were conducted using R version 3.6.3. Additional information regarding the statistical methods can be found in the Supplementary Material.

RESULTS

A total of 4517 and 2731 children participated in the pre-PCV and post-PCV cohorts, respectively. No participants were vaccinated for pneumococcus in the pre-PCV cohort, while nearly 100% of participants enrolled before their second birthday into the post-PCV cohort were vaccinated with PCV13. Influenza vaccination also occurred in the post-PCV cohort but at very low levels (<1% per year).

There were 1117 cases of clinical pneumonia that occurred in the pre-PCV cohort and 314 in the post-PCV cohort (Supplementary Tables 1 and 2 and Supplementary Figure 2). This disparity remained after standardizing the age distributions for comparison, with the incidence rate of all-cause pneumonia in the pre-PCV cohort 3.4 times that of the post-PCV cohort (Supplementary Table 3). A greater proportion of all-cause pneumonias in the post-PCV cohort required hospitalization compared to the pre-PCV cohort (13.1% vs 1.7%, $P = <.0001$). However, the opposite was true for pneumonias occurring within 30 days of laboratory-confirmed influenza.

Of the 1117 episodes of clinical pneumonia in the pre-PCV cohort, 39 (3.5%) were classified as influenza associated. There were 3 influenza A subtypes that circulated in the population during the study period (H3N2, H1N1, and H1N1pdm09), along with influenza B. H1N1pdm09 was most frequently associated with clinical pneumonia with 15 episodes (38.5%), followed by influenza B (12 episodes, 30.8%), H3N2 (9 episodes, 23.1%), and H1N1 (3 episodes, 7.7%). There were 21 (6.7%) episodes of influenza-associated pneumonia in the post-PCV cohort. Influenza B was the most commonly observed with 9 (42.9%) episodes, followed by H3N2 and H1N1pdm09, each with 6 (28.6%) episodes.

In the pre- and post-PCV cohorts, participants with symptomatic influenza infections displayed higher odds of pneumonia (pre-PCV matched odds ratio [mOR], 6.3, 95% confidence interval [CI], 5.4–7.3; and post-PCV mOR, 8.5, 95% CI, 3.9–18.9) in the 30 days following influenza onset compared to participants without influenza illness (Table 1). Odds of subsequent pneumonia associated with specific influenza types/subtypes were similar in both the pre- and post-PCV cohorts. The importance of male sex differed between the cohorts, as it was associated with 30% higher odds of developing pneumonia in the post-PCV cohort but showed no difference in the pre-PCV cohort.

We also examined how the odds of pneumonia following symptomatic influenza changed over time, specifically in the periods 0–6, 7–13, and 14–29 days following influenza onset. In the pre- and post-PCV cohorts, we observed the greatest odds of pneumonia in the first 0–6 days after influenza (Table 2). The odds that participants with any symptomatic influenza infection would develop pneumonia in the first 0–6 days were 11.3 times (95% CI, 5.0–25.4) and 63.8 times (95% CI, 8.2–498.5) that of participants without influenza in the pre- and post-PCV cohorts, respectively. We observed similarly elevated odds of pneumonia in the 7–13 days following influenza in both cohorts, specifically 4.1 times (95% CI, 1.3–13.1) and 9.6 times (95% CI, 1.2–75.1) that of participants without influenza in the pre- and post-PCV cohorts. Finally, for the period 14–29 days post influenza, which we hypothesize to be the risk period for

Table 1. Matched Odds Ratios for Developing Pneumonia in the 30 Days Following Symptomatic Influenza Infection Among Nicaraguan Children Aged 2–14 Years

	Pre-PCV[a]					
	Crude Analysis		Imputation Analysis		Post-PCV	
Characteristic	**mOR**	**95% CI**	**mOR**	**95% CI**	**mOR**	**95% CI**
Male	1.0	.8–1.1	1.0	.98–1.0	1.3	1.0–1.7
Influenza (overall)	4.2	2.6–6.7	6.3	5.4–7.3	8.5	3.9–18.9
Influenza A	3.5	2.0–6.1	5.0	4.1–6.1	8.1	2.8–23.7
A/H3N2	7.3	1.8–29.6	9.3	3.4–34.0	23.6	2.7–204.7
A/H1N1pdm09	5.3	2.4–11.5	6.4	5.2–13.5	5.2	1.5–18.1
A/H1N1	1.0	.3–3.7	1.3	.6–2.7	…[b]	…[b]
Influenza B	5.3	2.2–12.7	11.7	6.7–20.6	9.0	2.8–29.4

Abbreviations: CI, confidence interval; mOR, matched odds ratio; PCV, pneumococcal conjugate vaccine.

[a]Data from 2007 excluded from analysis, see Supplementary Material.

[b]The prepandemic (2009) H1N1 strain has not circulated in Nicaragua beyond 2009.

bacterial pneumonia, the mOR in each cohort were similar, and slightly but nonsignificantly greater than 1 (Table 2).

DISCUSSION

In this study, we described the risk period for developing clinical pneumonia following symptomatic influenza infection within cohorts of Nicaraguan children before and after the introduction of PCV. Notably, we did not observe a difference in the odds of developing pneumonia in the 30 days post influenza following the introduction of PCV13. There were, however, substantially more episodes of pneumonia in the pre-PCV cohort.

The literature exploring the risk period for pneumonia following influenza at an individual level remains limited. A 2018 review by Li et al found only 2 individual-level studies exploring pneumococcal disease following influenza, and both had substantial limitations [11–13]. A matched case-control study from 2000 reported that patients hospitalized with severe pneumonia were more likely to have positive convalescent serology (titer ≥ 1:40) for H1N1 than non-ill controls (mOR, 3.7; 95% CI, 1.0–18.1) [12]. While we did not see a significant association between H1N1 and subsequent pneumonia, this difference may be attributable to our use of RT-PCR for influenza diagnosis, a more reliable indicator of acute infection than convalescent serology.

We recently published an analysis assessing the risk period for pneumonia following influenza in a broader cohort of Nicaraguan children from which the post-PCV cohort in this analysis was drawn [14]. The only difference is that in this analysis, children aged <2 years were excluded to make the pre- and post-PCV cohorts comparable. In that analysis, we similarly observed that influenza, regardless of type/subtype, was associated with increased risk of developing pneumonia in the next 30 days. There were some (nonsignificant) differences in which subtype was associated with the highest risk of pneumonia, and in the apparent time trend of pneumonia risk. This suggests that the risk period may differ for children aged <2 years, as most influenza-pneumonia cases in our previous analysis occurred in this age group. However, the wide and overlapping confidence intervals also indicate we are underpowered to conclusively identify such differences.

This study has several strengths. First, as a case-control study nested within a prospective cohort, we were better able to ensure temporality between exposure and outcome. Second, while power limitations remained a challenge given

Table 2. Matched Odds Ratios Assessing the Risk Period for Pneumonia in the 30 Days Following Symptomatic Influenza Illness Among Children Aged 2–14 Years

	Pre-PCV[a]					
	Crude Analysis		Imputation Analysis		Post-PCV	
Characteristic	**mOR**	**95% CI**	**mOR**	**95% CI**	**mOR**	**95% CI**
Male	1.0	.9–1.2	1.0	.99–1.01	1.4	1.0–1.7
0–6 d	11.3	5.0–25.4	13.6	10.0–18.4	63.8	8.2–498.5
7–13 d	4.1	1.3–13.1	5.9	3.2–11.1	9.6	1.2–75.1
14–29 d	1.5	.7–3.4	1.2	.7–1.9	1.9	.5–6.6

Abbreviations: CI, confidence interval; mOR, matched odds ratio; PCV, pneumococcal conjugate vaccine.

[a]Data from 2007 excluded from analysis, see Supplementary Material.

the rarity of the outcome, this analysis had a larger sample size than much of the literature exploring the risk period for pneumonia following influenza, particularly at an individual level. Third, both cohorts were enrolled from the same Nicaraguan community (ie, base population). This provided a unique opportunity to describe the nature of the influenza/pneumonia association both before and after PCV was introduced. Fourth, our use of multiple imputation to account for incomplete exposure ascertainment provides a reasonable approximation of the unbiased association between influenza and pneumonia in the pre-PCV cohort.

This study also has some limitations. First, in the pre-PCV cohort, not all participants presenting to the study clinic and meeting the sampling criteria were tested for influenza. Instead, a 25% random sample was obtained. However, through multiple imputation and additional bias analysis, we were able to confirm that our imputed values fall within the bounds of what we would expect for the unbiased estimate (Supplementary Table 4). Second, as pneumonia is a rare outcome, sparse data limited power and the covariates we were able to include in our models. Third, our analysis did not include children aged <2 years, the pediatric age group with the greatest burden of pneumonia. Finally, use of symptom-based criteria for clinical pneumonia to define cases limited our ability to make definitive distinctions between viral and bacterial etiologies. However, this is a common problem in pneumonia diagnosis, even with diagnostics like chest radiography or PCR [2, 15].

In this analysis, we observed that influenza substantially increased the odds of pneumonia in the subsequent 30 days and that the risk period for pneumonia following symptomatic influenza infection among children was similar before and after PCV13 was introduced to Nicaragua. This further underscores the importance of influenza in considering how best to reduce the global burden of pneumonia.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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