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Multivariate analysis of factors affecting the immunogenicity of trivalent inactivated influenza vaccine in school-age children

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Abstract

We examined factors affecting the immunogenicity of trivalent inactivated influenza vaccination (TIV) in children using the antibody titers of children participating in a Hong Kong community-based study. Antibody titers of strains included in the 2009–10 northern hemisphere TIV (seasonal A(H1N1), seasonal A(H3N2) and B (Victoria lineage)) and those not included in the TIV (2009 pandemic A(H1N1) and B (Yamagata lineage)) were measured by hemagglutination inhibition immediately before and one month after vaccination. Multivariate regression models were fitted in a Bayesian framework to characterize the distribution of changes in antibody titers following vaccination. Statistically significant rises in geometric mean antibody titers were observed against all strains with a range of standard deviations and correlations in rises, with pandemic A(H1N1) showing the least variability and correlation with other titers. The patterns in boosting of antibody titers following vaccination can be taken into account in more detailed models of antibody dynamics in populations.

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COMPETING INTERESTS

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BACKGROUND

Annual vaccination of children with trivalent inactivated influenza vaccination (TIV) is recommended in some countries as a public health measure to reduce the incidence of influenza infections ⁽¹⁾, but there have been few previous studies exploring the variation in immunogenicity of this vaccine in children. Understanding the degree of variation in antibody response, and the extent to which this variation is due to the age of the child, the child's vaccination history, and which influenza virus subtypes are included in the vaccine, could help with assessing vaccine effectiveness ^(2,3) and the cost-effectiveness of vaccination programmes ⁽⁴⁾. Improved information on the trajectories of influenza antibodies following vaccination could also help in improving inferences on influenza incidence in the community in seroepidemiological studies.

In 2008-09 ⁽⁵⁾ and 2009-10 ⁽⁶⁾ we conducted randomized controlled trials of TIV in 119 and 796 children 6-17y of age respectively, in Hong Kong. In previous work we reported on the immunogenicity of repeated administration of TIV vs placebo in 64 children who participated in both studies ⁽²⁾, and further examined the role of age and prior vaccination on immunogenicity of TIV in the 796 participants in the 2009-10 study ⁽³⁾. In the present study, we describe a new multivariate Bayesian model that can be used to quantify the extent, variability and correlations of antibody rises after receipt of TIV in children, and we illustrate the model by reanalysing data from our 2009-10 study.

METHODS

Subjects

A randomized controlled trial of TIV was conducted from August 2009 to December 2010 ⁽⁶⁾. Eligible participants were children 6-17 years of age living in Hong Kong. Participants were randomized to receive TIV or saline placebo (repackaged to maintain blinding) between August 2009 and February 2010 and then followed up until September-December 2010. The TIV used in the study included the strains A/Brisbane/59/2007(H1N1), A/Brisbane/10/2007(H3N2), and B/Brisbane/60/2008 (Victoria lineage). Serum specimens were collected immediately prior to vaccination and again one month after vaccination. The study was approved by the Institutional Review Board of the University of Hong Kong. Proxy written consent from parents or legal guardians was obtained for all participants (who were 6-17 years of age), with additional written assent from those 8 to 17 years of age.

Antibody titers were measured by hemagglutination inhibition (HI) assays against the three strains included in the TIV vaccine, plus the influenza B virus that had prevailed in the preceding season B/Florida/4/2006 (Yamagata lineage) and the pandemic strain A/California/7/2009(H1N1), as detailed previously ^(3,5).

Statistical analysis

We specified a multivariate Bayesian statistical model to describe the changes in antibody titer levels following vaccination. Under the model, the logarithms of the post-vaccination antibody titers of a subject follow a multivariate Normal distribution, with the mean of the distribution equal to the logarithm of the subject's baseline titers plus a vector representing

the average change in antibody titers following vaccination. A variance-covariance matrix of the distribution was also estimated, which reflects how deviations from the average titer rises are correlated between different antibody titers. For example, if subjects with a higher than average baseline titer against the 2009 pandemic influenza virus also have a higher than average titer against the seasonal A(H1N1) influenza subtype, this higher correlation will be reflected in the matrix.

The model can be described with the following equation:

$$\log(X_{i2}) \sim MN_5(\log(X_{i1}) + \delta, \Sigma)$$

where MN_5 represents a 5-dimensional multivariate Normal distribution due to there being five HI assays, X_{it} is the vector of antibody titers before ($t=1$) and after ($t=2$) vaccination for each subject i , δ is the vector of means of the antibody titer changes after vaccination, and Σ is the 5x5 variance-covariance matrix. Using a Bayesian model enables point estimation of the parameters δ and Σ and estimation of their credible intervals. For comparison, we estimated these parameters for children who were randomly allocated to placebo.

We also fitted regression models to investigate how the rises of each titer differed by each age, sex and vaccination history, so that

$$\log(X_{i2}) \sim MN_J(\log(X_{i1}) + \delta_{a,s,v}, \Sigma)$$

where $\delta_{a,s,d}$ is the vector of titer rises for each combination of age (being 6-8 years old, or 9 years old or older), sex, and seasons of previous influenza vaccination (not being vaccinated during the previous two years, or being vaccinated for the 2007-08 season, for the 2008-09 season, or for both of these years). This was modeled as both a main effects model

$$\delta_{a,s,v} = \delta_a + \delta_s + \delta_v$$

and a saturated model where a separate $\delta_{a,s,v}$ was estimated for each combination of age, sex, and vaccination history. The posterior probability of each model was used to determine which model to select. When the value of a covariate for a subject was missing, it was simulated in the analysis in proportion to its observed frequency.

For each parameter, we specified uninformative prior distributions across the range of possible values. The posterior distributions were estimated with a Hamiltonian Monte Carlo sampler using the package “rstan”^(7,8) in R (R Foundation for Statistical Computing, Vienna, Austria). For each model and dataset, two chains of 60,000 iterations each were simulated to assess convergence, with the first 10,000 iterations of each chain used for burn-in, followed by 50,000 iterations used for analysis. Convergence was assessed using the potential scale reduction statistic⁽⁹⁾. We validated this model by plotting the difference between observed and predicted titer rises and assessing model fit.

RESULTS

In total, 479 participants were randomized to receive TIV, and all participants received the vaccine. Pre-vaccination sera could not be collected for 5 participants and in addition post-vaccination sera were not collected for 10 further individuals, and these 15 participants were excluded from further analysis. Table 1 shows the characteristics of the 464 subjects included in the analyses here, of whom 52% were male, and 35% were aged 6-8y with the remainder aged 9-17y. Vaccination status was available for all but 12 of the subjects, with the majority reporting no receipt of TIV during the previous two years.

Figure 1 shows the geometric mean estimates and 95% credibility intervals for the multiplicative rises in the various titers following vaccination, i.e. $\exp(\delta)$ using the notation in the Methods section. The strongest rises are for the antibody titers against two seasonal influenza A viruses, with those against seasonal A(H1N1) rising on average 10.1-fold (95% CI: 8.4-12.2) and those against the seasonal A(H3N2) variety rising 13.4-fold (95% CI: 11.0-16.0). The geometric mean titer rise against the B/Brisbane (Victoria lineage) strain included in the TIV was 7.9-fold (95% CI: 6.7-9.3) while that against the previous season's prevalent B strain B/Florida (Yamagata lineage) was 4.2-fold (95% CI: 3.6-4.8). The geometric mean fold rise in titers against pandemic A(H1N1) was 1.5 (95% CI: 1.3-1.7).

Figure 2 shows the estimated geometric standard deviations of the titer rises, along with their 95% credibility intervals. There was less variability in titer rises against the 2009 pandemic A(H1N1) virus compared to the titer rises against seasonal A(H1N1) and A(H3N2) viruses. The antibody titer rises against the B/Brisbane (Victoria lineage) virus included in the TIV varied more than those against B/Florida (Yamagata lineage).

The correlations between the titer rises are shown in Figure 3. The most closely correlated titer rises are those for the antibodies against the two B lineages, followed by the antibodies against the two seasonal A viruses. The antibody rise against pandemic A(H1N1) had very low correlation with any of the other antibodies.

There was some indication of different antibody dynamics following vaccination between children with different ages, sexes and vaccination histories. The simpler model, where the titer rises were estimated as a sum of age, sex, and vaccination history effects, had a higher posterior probability than the saturated model where the mean titer rises were modelled independently for each combination of the factors. The posterior geometric mean titer rise ratios for each factor and virus subtype are shown in Figure 4. The most significant differences in antibody titer behaviour were in the seasonal A virus titers between those who were vaccinated in previous seasons and those who were not, with the former rising by only around a quarter as much as the latter, with smaller rises for more recent vaccinations. There was also a statistically significant difference in the titers against the B/Brisbane (Victoria lineage) included in the TIV between those of different sexes and ages, with boys and older children exhibiting higher rises. There were also significant or near-significant effects of sex on B/Florida (Yamagata-lineage) and pandemic A(H1N1) titers, with boys experiencing higher titer rises. Significant effects of vaccination history were also observed for the B/Florida (Yamagata lineage) antibody titers, with children previously vaccinated during the

2008-09 season, when the TIV included this strain, having higher titer rises against this subtype than children who were either vaccinated only during the 2007-08 season or both the 2007-08 and 2008-09 seasons.

Figure 5 shows the distribution of differences between the predicted logarithms of the antibody rises for each study participant and their observed antibody titers rises. The normal distribution of the errors around zero indicates that the model is well-specified.

There was a low risk of natural influenza infection during the month between the two titer measurements, according to analysis of children in the placebo arm of the study. Figure 6 shows the geometric mean fold antibody rises for each titer. All titers showed minimal rises, but only pandemic A(H1N1) titers showed a significant geometric mean fold rise of 1.3 (95% CI: 1.1-1.5).

DISCUSSION

We have described the development and application of a Bayesian model for antibody titer rises following vaccination. We used the model to identify substantial variation and correlation in antibody titer rises against different strains following TIV vaccination in children, and explored how factors such as age, sex, vaccination history and the subtypes included in the vaccine affect the behaviour of post-vaccination antibody titer rises (^{12,13}). This will help in future work that relies on the immunogenicity of TIV in a population of children, such as evaluations of annual vaccination of schoolchildren, as well as increasing our understanding of antibody titer dynamics following vaccination which is relevant for seroepidemiological studies.

There are some limitations in our study. First, we could not investigate the extent to which other factors affected antibody dynamics, particularly pre- or post-vaccination infection by influenza or other respiratory diseases, as all RT-PCR-confirmed infections in the study occurred after both antibody titer measurements. The effect of infection on post-vaccination antibody titers is known to be different from that on titers where no vaccination had taken place that season (¹⁴), but the exact nature of the difference is still not known. Second, the assays used for this study determined antibody levels by the standard method of doubling the dilution of serum until hemagglutination is detected. The exact titer levels are therefore not available and the titer levels used in the analysis must be considered to be approximations. Additionally, measurement error could not be estimated in this study due to lack of repeated measurements (¹⁵). We identified a low risk of natural influenza virus infections during the period between administration of TIV and collection of post-vaccination sera (Figure 6). Comparison of the antibody responses of the TIV recipients (Figure 1) with the placebo recipients (Figure 6) is consistent with little to no cross-reactivity of TIV with 2009 pandemic influenza A(H1N1), as has been reported elsewhere (¹⁰).

The results obtained here are consistent with the previous analysis reported by Ng et al (³), and discussion of the potential explanations for the effects of age and prior vaccination are not repeated here. Our model allowed us to further assess the effect of sex on antibody response. We found higher antibody rises in boys compared to girls for antibodies to the two

influenza B viruses tested as well as pandemic A(H1N1), but not the two seasonal influenza A viruses. Re-analysis of the data revealed that boys both over and under 9 had significantly higher or non-significantly different rises than girls across all subtypes tested, these rises were significantly lower in older boys for the subtypes included in the TIV. We found no previous discussion of sex differences in humoral antibody responses to influenza vaccination in children. It is well known that there are sex differences in both innate and adaptive immune responses⁽¹⁶⁾. A literature review of sex differences in vaccine response⁽¹⁷⁾ found 6 studies where adult females have a stronger humoral response to TIV. It is possible that female adults have a stronger antibody response to vaccination while in children boys have the higher titer rises, as seems to be the case for measles vaccination⁽¹⁸⁾. Further investigation of this phenomenon is warranted, for example how sex-specific hormonal changes after puberty might affect immunity and vaccine responses.

Even though the recorded antibody titers analysed in this paper could only take one of several discrete values, we modeled the logarithms of the antibody titer rises as following a multivariate normal distribution, which is a continuous probability distribution⁽¹⁹⁾. This reflects that the underlying titer levels are on a continuous scale, and is the approach used by other modelers of antibody titers⁽²⁰⁾. Plotting the distribution of the difference between the observed and predicted antibody titer rises (Figure 5) shows that this is a reasonable model.

The model presented here will form a component of more comprehensive modeling framework for antibody titer kinetics that can improve inferences on infection status and incidence of influenza using longitudinal serological measurements. The approach developed here can be extended to incorporate as many virus types and assays as desired, such as new assays that were not available for this study, for example protein microarrays⁽²¹⁾. Future work will include understanding the behaviour of humoral antibody titers after influenza infection, with and without vaccination, and the rate of waning after infection or vaccination.

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Figure 1.

The geometric mean (with 95% credibility intervals) fold rises following vaccination of all assays. The means and intervals are shown on a logarithmic scale. The three strains included in the TIV are indicated with asterisks.

Figure 2.

The geometric standard deviation (with 95% credibility intervals) of each titer's rise after vaccination. These represent the diagonal elements of Σ , the variance-covariance matrix of antibody rises. The three strains included in the TIV are indicated with asterisks.

Figure 3.

The correlations between the antibody titer rises. The darker squares indicate higher correlation. The three strains included in the TIV are indicated with asterisks.

Figure 4.

The posterior geometric mean ratio in rises between complementary subpopulations for each assay. The three strains included in the TIV are indicated with asterisks.

Figure 5.

The distributions of the differences between observed and predicted logarithms of titer rises for each subtype. The distributions appear to follow normal distributions, consistent with the model specification. The three strains included in the TIV are indicated with asterisks.

Figure 6.

The geometric mean (with 95% credibility intervals) fold rises of all titers for subjects in placebo arm. The means and intervals are shown on a logarithmic scale. The three strains included in the TIV are indicated with asterisks.

Table 1

The characteristics of 452 subjects who received trivalent inactivated influenza vaccination

Characteristic	Number (<i>percent</i>)
Male	241 (52%)
Female	223 (48%)
Age 6-8 years	163 (35%)
Age 9 years or older	301 (65%)
Recent vaccination history against seasonal influenza	
Not vaccinated before	304 (66%)
Vaccinated during 2007-08 season only	37 (8%)
Vaccinated during 2008-09 season only	79 (17%)
Vaccinated during both 2007-08 and 2008-09 seasons	32 (7%)
Vaccination history missing	12 (3%)