Statistical Techniques for Comparing Immune Response of Quadrivalent Vaccines with Trivalent Vaccines

Ayca Ozol-Godfrey, PhD[1]
Robert D. Small, PhD[1]

[1] Sanofi Pasteur, 1 Discovery Drive, Swiftwater, PA, 18370

Abstract
A multivalent vaccine is one that has antigens for more than a single virus strain or species. An important question is whether the different antigens can interfere with the human immune response to the other antigens. A trivalent vaccine has three of the antigens of a quadrivalent vaccine. For a flu vaccine the various antigens are chosen from among a number of antigens. A common design then is to compare a quadrivalent vaccine with two trivalent vaccines whose antigens are different subsets of three of the antigens in the quadrivalent vaccine. Usually the various comparisons are made in a univariate manner. This does not use all of the information in the data since the correlations between immune response is significant. We propose multivariate methods that view the trivalent vaccines as a quadrivalent vaccine with missing responses. We use both direct maximum likelihood (ML) methods and missing data methods (multiple imputation-MI) to use all of the information in the data. The result is greater precision with shorter CIs for the comparisons. The methods can be extended to other vaccines than flu and to multivalent vaccines that have occasional missing titer values.

Key Words: vaccine, multivariate, missing data, MI

1. Introduction

In vaccine clinical trials (1), generally different vaccines are compared using their common antigens, but there is no consensus as to how to compare these vaccines for the antigens that they do not all contain. How does one declare non-inferiority of an investigational four-antigen flu vaccine to a control flu vaccine that only has three antigens? (See Table 1) The approach described considers this issue as a missing data (2) problem by viewing the trivalent vaccine as a quadrivalent vaccine with missing responses. This provides a statistically meaningful technique to compare vaccines with different number of antigens.

Commonly the vaccine comparisons are carried out in a univariate fashion for each antigen separately. In this paper, the correlations among the antigens are taken into account to build the imputations. Once the data are filled in, a multivariate technique is used to analyze it.

In this paper, in Section 2, the study design and the competitive methods are presented. Section 3 describes the results. Section 4 is a short discussion.

2. Study Design and Methods

2.1 Study Design

This was a Phase III, randomized, double-blind, active-controlled, multi-center trial to evaluate the immunogenicity and safety of a quadrivalent flu vaccine in adults, 18 to 64 years of age. At enrollment, all eligible subjects were randomized in a 2:1:1 ratio to receive a single injection of either the quadrivalent vaccine or one of the trivalent influenza vaccine formulations containing either the B strain from the primary lineage (Trivalent-1) or the B strain from the alternate lineage (Trivalent-2). Blood samples were randomly taken from 2/3rd of the subjects at Day 0 (pre-vaccination) and Day 28 (post-vaccination). The samples were assayed for antibody response (measured in titers) to the 4 virus strains using the hemagglutination inhibition (HAI) assay. Table 1 shows the per-protocol sample size and the strains each vaccine formulation contains.

Table 1: Per-protocol sample size and the vaccine strains

Quadrivalent vaccine (trt 1) N=1041	**Trivalent-1 (trt 2) N=539**	**Trivalent-2 (trt 3) N=533**
A1	A1	A1
A2	A2	A2
B1	B1	.
B2	.	B2

The primary immunogenicity objective of the study was to demonstrate that the quadrivalent flu vaccine induced an immune response that was non-inferior to the responses induced by the Trivalent-1 and Trivalent-2 flu vaccines for all the 4 vaccine strains.

The logarithm (base 10) of the titer is the endpoint that is analyzed. When the analysis is complete, the antilogarithms are taken and the geometric mean titers (GMTs) are reported. Non-inferiority is demonstrated if the lower bound of the 95% CI of the post-vaccination GMT ratio ($GMR_{Quadrivalent/Trivalent} = GMT_{Quadrivalent} / GMT_{Trivalent}$) is above 2/3 for each strain.

2.2 Methods

In this paper, we compared three statistical methods. We used the per-protocol analysis set for all methods. The primary protocol method to compare the vaccine groups was the traditional independent samples t-test for each strain. This method assumes that the vaccine strains are independent of each other, which is a common assumption used in vaccine trials. It is denoted as the ORIGINAL method in this paper.

The second method is the multivariate mixed effects model which is denoted as the MIXED method in this paper. It takes into account the correlations between strains and covariates like age, gender, race, previous year influenza status and pre-vaccination titers are added to the model. For the purposes of this paper, we used the unstructured covariance-variance matrix.

The third method is called the MI-MIXED method. Multiple imputation (MI) is done using the fully conditional specification (FCS) imputation method (3). FCS is a two-phase imputation technique: fill-in and the imputation phase (4). In the fill-in phase, missing values are filled in sequentially with preceding variables used as covariates. During each iteration of the imputation phase, a specified model is fitted for each variable with missing values, by using observed observations for that variable, which might also include observations with imputed values for other variables. With the new resulting model, a new model is drawn and then used to impute missing values for the imputed variable. Several iterations of the process are repeated long enough to reliably simulate approximately independent draws of the missing values for an imputed dataset. With FCS, one can impute both continuous and categorical variables. In addition to the B2 of Trivalent-1 and B1 of Trivalent-2 being missing, 1.4% of the covariates mentioned above had missing data. Once the imputed datasets (m=100 datasets in this case) are obtained, each dataset is analyzed separately using proc mixed, and the estimates and the standard deviations are combined (3) to obtain the final GMT ratios.

We have used SAS Proc MI-FCS option to do our imputations with the covariates from the MIXED model included in the imputation model here, PROC MIXED to do the multivariate analysis modelling and then PROC MIANALYZE to combine the results (4).

3. Results

In this section we compare the three methods based on the 95% CI of the GMR. Depending on the antigen of interest, the comparisons will differ.

Table 2 shows the GMT ratios along with their CIs obtained using the ORIGINAL method for each strain.

Table 2: ORIGINAL method GMTs and GMT ratios (95% CI)

	Quadrivalent vax		**Pooled trivalent formulations 1+2**				**GMT**	
Strain	**GMT**	**(95% CI)**	**GMT**		**(95% CI)**		**Ratio**	**(95% CI)**
A1	**589**	**(546, 636)**	**680**		**(629,724)**		**0.866**	**(0.777,0.966)**
A2	**368**	**(342, 397)**	**430**		**(397, 464)**		**0.857**	**(0.770,0.955)**
			Trivalent-1		**Trivalent-2**		**GMT**	**(95% CI)**
			GMT	**(95% CI)**	**GMT**	**(95% CI)**	**Ratio**	
B1	**105**	**(99.1, 112)**	93.5	(85.9,102)	N/A	N/A	1.13	(1.02, 1.25)
B2	**136**	**(128, 145)**	N/A	N/A	130	(118,143)	1.05	0.939, 1.16)

For A1 and A2, the quadrivalent vaccine is compared to the pooled trivalent vaccines. For B1, the quadrivalent vaccine is compared to the Trivalent-1 vaccine; and for B2, the quadrivalent vaccine is compared to the Trivalent-2 vaccine. These comparisons will be used as a basis when the MIXED and the MI-MIXED methods will displayed.

For all 4 independent comparisons the non-inferiority objective is achieved. However, these comparisons are made assuming the 4 strain data are uncorrelated. Table 3 shows the variances and the correlations between the post-dose titers of the 4 strains for each vaccine group separately.

Table 3: Correlations between strains for each vaccine group					
Vaccine	**Strain**	**A1**	**A2**	**B1**	**B2**
Quadrivalent	**A1**	1	0.30	0.32	0.33
	A2	0.30	1	0.34	0.31
	B1	0.32	0.34	1	0.57
	B2	0.33	0.31	0.57	1
Trivalent-1	**A1**	1	0.36	0.34	.
	A2	0.36	1	0.38	.
	B1	0.34	0.38	1	.
	B2	.	.	.	.
Trivalent-2	**A1**	1	0.40	.	0.35
	A2	0.40	1	.	0.32
	B1	.	.	.	.
	B2	0.35	0.32	.	1

As seen from Table 3, the significant correlations between A1, A2, and B1 are around 0.3-0.4 within each vaccine, and the correlation between B1 and B2 is 0.57 for the quadrivalent vaccine. Therefore, we should take these correlations into account in our analysis.

The results show that when the correlations between strains are taken into account, the CIs are narrower. Figure 1 shows the results of the three methods for each strain. The number labels show the CI widths. In the figure, for strains A1 and A2, quadrivalent vaccine is compared to the pooled trivalent vaccines. For the B1 strain, the quadrivalent vaccine is compared to the trivalent vaccine that contains the B1 strain (Trivalent-1 or trt 2). For the B2 strain, the quadrivalent vaccine is compared to the trivalent vaccine that contains the B2 strain (Trivalent-2 or trt 3). For all 4 strains, the CI width is the widest using the per-protocol independent t-test. The MIXED model method and the MI-MIXED model method have shorter CIs. The MI-MIXED model method has a slightly larger CI width compared to the MIXED model method which may be due to the uncertainty that comes with the multiple imputation.

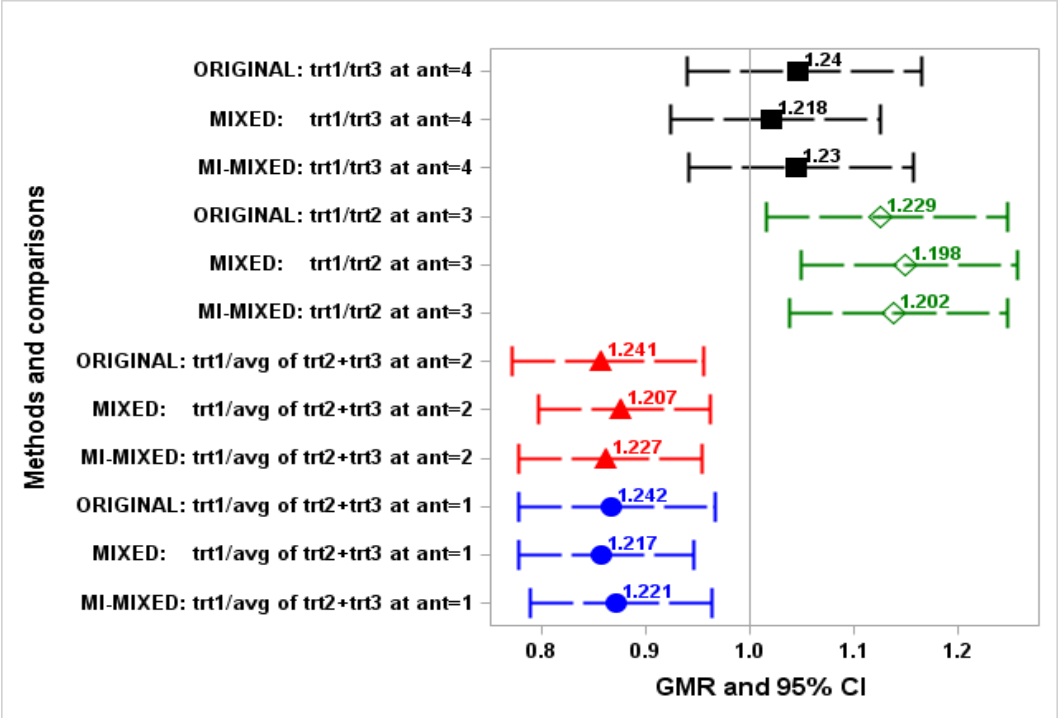

Figure 1: Results-1: GMRs (labels with CI widths)

Figure 2 shows similar results. Additionally, we make use of the study design to obtain the most information as possible. The comparisons are the same as Figure 1 for strains A1 and A2. The difference is for strains B1 and B2. Because the B1 of Trivalent-2 and the B2 of Trivalent-1 are imputed, now it is possible to pool Trivalent-1 and Trivalent-2 when comparing to Quadrivalent vaccine for the B1 and B2 strains. More information is used and this results in narrower CIs. The CI widths of the MI-MIXED method for strains B1 and B2 are 1.18 and 1.202, respectively in Figure 2 compared to CI widths of 1.202 and 1.23 for the MI-MIXED method of strains B1 and B2 in Figure 1. This approach of a 2:1:1 randomization ratio design with filling in the missing data for the B strains would potentially save money to the sponsor when determining the trial's sample size compared to a 2:2:2 randomization ratio design.

The confidence intervals not just shrink, but it is also apparent that for most of the cases, the lower confidence limit of the GMR is shifted to the right, which is what would be desired in a non-inferiority trial. More research is needed regarding this observation.

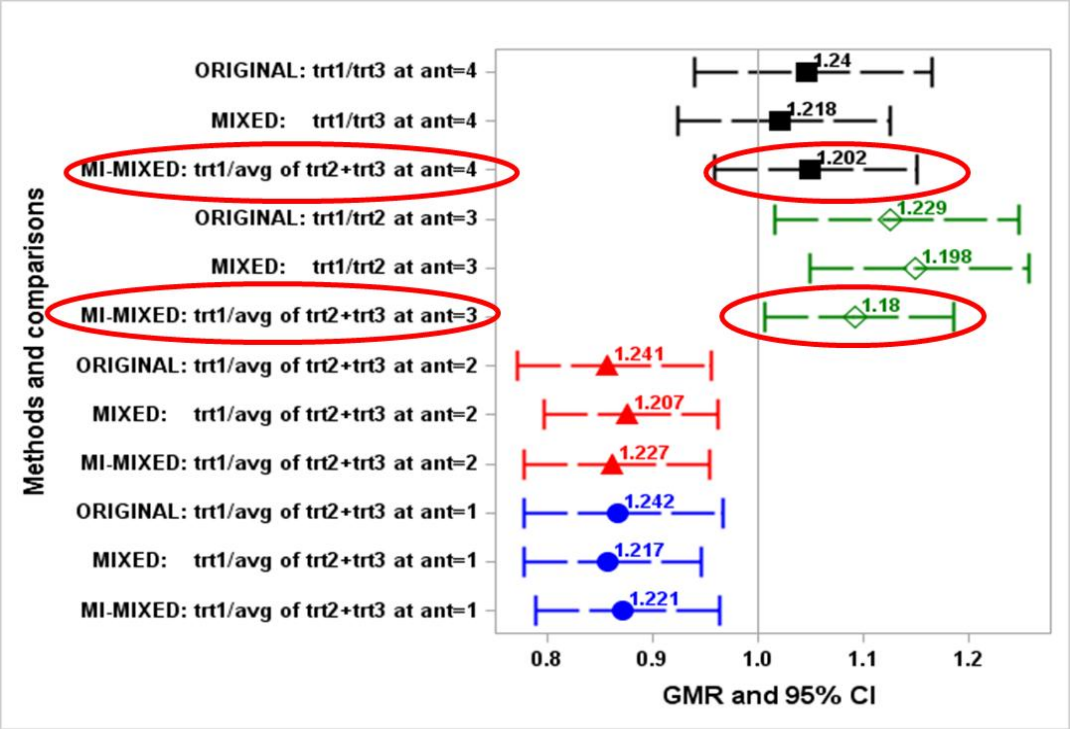

Figure 2: Results-2: GMRs (labels with CI widths)

4. Summary

In vaccine trials, very often vaccines with different number of strains are compared. It is possible to consider this as a missing data problem. The control vaccines with less number of antigens can be considered as having this data as missing compared to the investigational vaccine that has more number of antigens. Additionally, the correlations between the strains should be taken into account. In this paper, it is shown that multivariate techniques along with missing data approaches can be used to tackle this unique situation. The result is greater precision with shorter CIs. This approach can be extended to any type of vaccine, to multivalent vaccines that have occasional missing values, and to situations where there is limited amount of blood that can be tested such as vaccine trials with infants.

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