Modelling and Interpretation of Vaccine Cross-over Clinical Trials Data

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Abstract

In a cross-over study, subjects are randomized to a sequence of treatments over time with repeated measurements being taken after each treatment. For vaccines, such studies may be done to identify the potential for 'boosting' immune response, identifying opportune time of re-vaccination, and/or for the identification of potential alternative dosing regimens (when multiple vaccines of differing mechanism are available) amongst other reasons. Carry-over in such designs is obviously not only assumed but is also desired (unlike most drug cross-over trials where it is regarded as a nuisance). We consider the application of cross-over and carry-over modelling to vaccine cross-over designs in the context of a Balaam's design (data are disguised to actual vaccine and endpoint) based on the approaches of Jones and Kenward (2003, 2ed.). We will show that the cross-over and carry-over methods are readily applicable to vaccine studies and aid/simplify the analysis and interpretation of data arising from such studies.

Key Words: Cross-over, Carry-over, Vaccines, Balaam.

1. Introduction

In a cross-over study, subjects are randomized to a sequence of treatments over time with repeated measurements being taken after each treatment. Cross-over designs have been utilised in biopharmaceutical clinical trials for some time [10], [2] - e.g., for bioequivalence studies and for thorough QTc studies. The benefits of within-subject estimation of treatment effects are well known but may be under-utilised in some settings due to the presence of carry-over effects. That is, the effect of a prior treatment remains with the subject while evaluating the effect of a subsequent treatment.

These carry-over effects are typically regarded as nuisances, and cross-over designs have been developed to ensure treatment effects are not confounded with carry-over effects [2] amongst other parameters. However, the potential for presence of carry-over effects complicates design, and in some instances has led to recommendations to use simpler (parallel group) designs in biopharmaceutical development (see for example, [1], [5]) when a washout period [8] cannot be used to confirm the assumption that carry-over effects are negligible relative to treatment effects.

A Balaam's design [2] is one example of a design developed to ensure carry-over effects are not confounded with treatment and period effects. In such a design subjects are randomized to one of 4 sequences of treatment administration. We will denote these sequences as AA, AB, BA, and BB (see Table 1) where A denotes one treatment and B the other. The administration of each treatment is separated by a wash-out period appropriate to the products under study. While within-subject estimates of treatment effect are only possible in the AB and BA sequences, the application of the AA and BB sequences serve to allow for the estimation of carry-over effects in combination with treatment and period effects.

The value of Balaam's design rests on the assumption that there is no carryover-by-treatment interaction, i.e. that carryover from the first administration of A and from B in

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the AA and BB sequences is the same as in the change-over sequences [2].

For vaccines, such cross-over studies may be done to identify the potential for 'boosting' immune response, identifying opportune time of re-vaccination, and/or for the identification of potential alternative dosing regimens (when multiple vaccines of differing mechanism and/or coverage are available) amongst other reasons. Carry-over for vaccines (and in such designs) is obviously not only assumed but is also desired.

An in-depth discussion of the mechanisms of vaccine carry-over effects are beyond the scope of this report. We recommend the reader consult [3] and [6] for an introduction to these topics. For our purposes in this statistical assessment, it should be recognised that carry-over from a vaccine can result from multiple mechanisms - e.g., antibody may remain in the body for an extended period of time and/or elements within the body's immune system may be primed to recognise and mount an immune response to antigens even if limited or no circulating antibody remains present, or both.

Mean natural-logarithm ($\ln$) transformed antibody titre data from such a Balaam's design are plotted for 6 antibody types in Figure 1. Pre-vaccination and post-vaccination blood samples were obtained in this design in both study periods. Such immunogenicity data are typically obtained in vaccine clinical trials. These data are useful in determining the level of titre needed for protection from disease. A full discussion of the topic of correlate of protection is also beyond the scope of this report, and the reader is referred to [6] for more information. For our purposes in this report, the $\ln-$titre should be regarded as a continuous endpoint which acts as a surrogate endpoint for efficacy of vaccines.

We first consider the study design and resulting in data in more detail in Section 2. In Section 3, the traditional approach to analysis is described, and in Section 4, we describe the application of cross-over models to this data set. Our conclusions regarding the utility of cross-over modelling to vaccine cross-over data are given in Section 5.

2. Data

As described in [6], an antibody titre is 'a measurement of the amount of antibody in a serum sample, expressed as the reciprocal of the highest dilution of the sample that delivers a certain assay read-out.' In this case, the antibody titre reflects the dilution resulting in 50% killing of the antigen in question (types 1-6).

Titre results for 6 vaccine-type antibodies were assessed for the purposes of this report. As the intent of this work is to consider the modelling of such data, treatment group (i.e., actual vaccine) and the specifics of the endpoints (i.e., serotype) are disguised. Titre data are typically assumed to follow a log-normal distribution [6] and are $\ln-$transformed for analysis on the normal scale, and this practice was followed in this report.

Antibody titre data were combined across two randomized, double blind, clinical studies in the same nation with identical inclusion and exclusion criteria (healthy subjects aged 60-64 years at time of first vaccination), controls (e.g., allowance for concomitant vaccination, assays), and vaccination/sampling schedules and materials. Time between vaccinations varied by a minimum of one to a maximum of four years across the studies. The total sample size was $n = 1113$ subjects ($n = 284$ for sequence AA, $n = 404$ for sequence AB, $n = 236$ for sequence BA, $n = 189$ for sequence BB). While in this report the time between vaccinations is referred to as a 'wash-out', as a practical matter, a lengthy time period is generally used between such vaccinations to ensure an appropriate immune response is provoked by the body, and can be provoked in subsequent vaccinations. Also, as a practical matter, vaccinations in adults typically can occur at roughly one year intervals as a maximum (i.e., when undertaking an annual physical exam).

All available data for protocol adherent subjects were included in this analysis. Protocol

violators and subjects with data collected outside protocol specified windows prior to and following each vaccination were excluded from analysis prior to unblinding and analysis. One hundred forty eight subjects provided data in only 1 study period after vaccination (6 subjects provided no data in period 1, 142 in period 2).

In practice, estimates and confidence intervals from analysis on the $\ln -$transformed scale are typically exponentiated (back-transformed) to describe the data (or to test for non-inferiority, super-efficacy, etc.) We focus on the statistics here and neglect such alternative presentations without loss of generality. All results in this report are presented on the $\ln -$scale.

SAS for Windows version 9.2 running on a Thinkpad laptop were used for all analyses presented in this report. Figure 1 was produced in the Tibco Graphical workshop environment using SPLUS 8.0 running on a Thinkpad laptop.

3. Traditional Analysis and Findings

A traditional vaccine statistical analysis of such continuous repeated-measures (cross-over) data is described in [6]. In brief, comparisons are first constructed between regimens by period to compare vaccine response following each vaccination. See Table 2. The vaccines (A and B) are compared directly in the first period (A-B) following vaccination and thereafter between sequences following repeated vaccinations.

Two of the potential comparisons of interest in period 2 between sequences are given in Table 2 to conserve space (other comparisons are on file). In the period 2 assessments, carry-over is assumed to exist, but it is not estimated. The comparisons by period are not confounded with period effects (as analysis is within period); however, the analysis of period 2 data is confounded in that treatment and carry-over are both involved in the resulting estimates across sequences.

The traditional analysis becomes more complex thereafter. In general practice [6], period 2 data are compared to period 1 data, and comparisons of interest are constructed between sequences. Period effects [2] are assumed to be null (by default) in such an analysis. This is a strong assumption, and as we will see is problematic when period effects are directly estimated later in this report. See Table 3. Carry-over and treatment remain confounded when comparing sequences of vaccine administration in this assessment. Details of the effects confounded in this setting may be found in Table 3.6 of [2][Ch. 3.3].

In part, to attempt to account for carry-over, pre-vaccination titres may be taken into account. See Table 4. Pre-vaccination $\ln -$titres were subtracted from post-vaccination in each study period and then compared across periods between sequences.

Interpretation of Tables 2-4 may be found in Section 5.

Beyond the overly complex nature of interpretation of such multiple data analyses, these approaches highlight the need for analysis of such data accounting properly for period and carry-over effects for a quantitative understanding of the characteristics of each vaccine and repeated administration. A modelling approach based on [2] will now be described for this purpose.

4. Cross-over and Carry-over Modelling and Findings

The model chosen for application in this report for each type of $\ln -$titre y_{ijk}, separately, is derived from [2][Ch. 5.4] and is:

$$y_{ijk} = \mu_{d[i,j]} + \pi_j + \lambda_{d[i,1]} + \xi_{k(i)} + \varepsilon_{ijk}, \tag{1}$$

where $d[i,j] = R$ or T and identifies the vaccine in period (π_j, $j = 1-2$) with potential carry-over $\lambda_{d,1} = \lambda_A$ or λ_B from period 1. We assume that $\xi_{k(i)}$ and ε_{ijk} are independent random variables such that $\mathrm{E}(\xi_{k(i)}) = 0$, $Var(\xi_{k(i)}) = \sigma_B^2$, $\mathrm{E}(\varepsilon_{ijk}) = 0$ and $Var(\varepsilon_{ijk}) = \sigma_W^2$, where σ_B^2 is the between-subject variance and σ_W^2 is the within-subject variance. E denotes the expected-value (i.e., population mean) for a given parameter, and Var denotes its variance. We also assume that the $\xi_{k(i)}$ are independent among themselves and that the ε_{ijk} are independent among themselves. The usual 'centering' assumptions regarding period and carry-over effects (e.g. $\lambda_A = -\lambda_B = -\lambda$) are made for the purpose of estimation [2]. SAS code is given in [2] and is not be reproduced here.

The p-values in Table 5 denote the tests of model parameters for treatment effects $\mu_A = \mu_B$, carry-over effects $\lambda_A = \lambda_B$, and period effects $\pi_1 = \pi_2$ in columns 2 through 4, respectively. The estimates of effect correspond to $\mu_A - \mu_B$, $\lambda_A - \lambda_B$, $\pi_1 - \pi_2$ in columns 2 through 4, respectively.

Additional models may be easily explored. For example, baseline may be added as a period specific covariate following the principals described in [4][Section 4.2] with SAS code given in the appendix to said paper, and we discuss the application of such a model in Section 5.

5. Discussion and Conclusion

At best, the traditional analysis findings of Tables 2-4 should be regarded as a supplement to Figure 1 and permits additional qualitative assessments. One could conclude from the plot (and Tables 2-3) that vaccine A provides a similar or greater response relative to vaccine B for some of these endpoints (types 1-6). Administration of vaccine A prior to vaccine B results in a higher response whether one gives vaccine A or B in the second period. Finally, administration of sequence AA results in higher average $\ln -$titre relative to BB for all types. The magnitude of treatment effects relative to carry-over effects is unclear, and no potential for other confounders is however taken into direct account (unless an observer is very astute).

Inspection of pre-vaccination average $\ln -$titres in Figure 1 is sufficient to suggest that a carry-over effect is present and differentiable between vaccines (as is indeed desired).

The findings of Table 4 are very challenging to interpret in this context. Reference to Figure 1 aids somewhat in interpretation. When vaccine B is administered in period 1, average $\ln -$titres return closer to basal levels prior to vaccination in period 2. This results in a larger baseline subtracted value in period 2 if vaccine B was given first in period 1. As a generalization then, once one is vaccinated with vaccine A, $\ln -$titres are maintained higher, and there is therefore less 'boosting' with a subsequent dose of either vaccine due to greater carry-over.

Assessments of significance in Tables 2-4 may therefore be misleading as the p-values are confounded between multiple effects (treatment, period, and carry-over.) They serve to indicate that something statistically significant is happening, but an accurate and precise analysis is needed to pick out which are the key contributing factors for a given comparison of interest.

The traditional approach is admittedly quite complex. However, more importantly, it is non-quantitative with respect to the effects of interest in such a design-space. That is, treatment, period, sequence, and carry-over effects are in part confounded in these analyses unless strong assumptions are made regarding period effects in particular. The assumption of null period effects is quite unsafe - if for no other reason than findings from other therapy areas [7].

Period effects should be expected in such vaccine trials due to the duration of wash-out, in combination with other factors, making an assumption of null unlikely. This is not surprising if considered carefully. As is well known, immunity amongst populations varies over time naturally and in response to outbreaks of disease. Moreover, when a significant part of a population is vaccinated, herd immunity may develop. Additionally, changes will likely be made to an assay over the course of years (e.g., reagents must be changed upon expiration or when amounts run-out). It would also be surprising if clinical conditions could be maintained with an exactness necessary to nullify period effects (e.g., staff changes are to be expected over the years of such vaccine trials). If nothing else, the shipping company taking blood samples to the lab may change between period 1 and 2 (with potentially different storage conditions). All such potential factors may and and of themselves contribute to a period effect. Therefore, vaccine cross-over designs should expect and protect against their occurrence by design.

The need for a model-based approach such as [2] seems justified given the complex, confounded findings of traditional analysis. It is found from Section 4 that a single model can supply all of the information required by multiple analyses of the same data set. This is true however if and only if one is willing to set up the experimental conditions to permit. If one were not permitted to administer the BB sequence for example due to ethical concerns with re-administration of the B vaccine, then a proper quantitative understanding of such data is not possible.

Turning now to the cross-over model's findings from Section 4, in this case, the simple model-based analysis was observed to be quite informative relative to the traditional approach.

Conclusions with regard the effect of vaccination with A-B are altered when accounting for period effects and carry-over. Type 1 changes from statistically significant to non-significant for comparison of treatment effects of vaccine A to B. This is probably real given the more accurate and precise nature of the within-subject comparison. This finding is not of concern as the effect is still non-inferior relative to vaccine B (data on file).

Notably, period effects were significant for all types except for 1 and 5. The magnitude relative to traditional estimates of A-B from period 1 are interesting. With the exception of type 5, the absolute effect appears to be quite a percentage of the traditional estimate of the treatment effect (see Table 2).

Carry-over is significant across all types 1-6 and supports that dosing with Vaccine A results in benefit in terms of higher maintenance of $\ln$-titres over time for these endpoints.

Baseline-adjusted model-based assessment (as described in [4]) improves the precision of the model but does not change any of the fundamental conclusions as regards treatment, period, and carry-over effects (data on file).

In summary, the purpose of this report was to interrogate whether a model-based approach such as [2] resulted in findings which represented an improvement over the traditional method(s). A 'simple' [8] approach to carry-over was adopted to assess whether this was possible and if so, whether value was added by taking such an approach. More precise methods are available [9]. While such carry-over models have been the subject of debate in the study of drugs, we are not aware of previous quantitative comparative reports in the area of vaccines using a Balaam design. Especially in a therapy area like vaccines, where wash-out may not be physiologically possible given priming of the body's immune system, we deem it important to account where possible for an effect like carry-over, even if the approach taken is not precisely consistent with the body's processes and mechanisms involved in generating immune responses.

While it would be interesting to study the potential for multi-period carry-over in this context [9] (i.e., the effect of vaccine in period 1 carrying over to period 2 and there-after

to a theoretical period 3, etc.) to better understand this phenomenon (and apply better models), in practice, this would be challenging in clinic as one of the vaccines involved cannot be given more than twice under formal expert recommendations due to observed safety side effects. Two regimen, three- or four-period cross-over designs where period and carry-over effects are orthogonal to treatment effects are well known, and further discussion and specifications may be found in [2]. However, even if it were possible to conduct such a design, it is somewhat questionable as a practical matter whether a trial that runs for many, many years in such a manner can be kept well controlled given its duration and competing risks.

Although not considered here (as a correlate of protection for this particular data type and set of vaccines is not yet available), sero-conversion and sero-protection may also be modelled in such data sets. These are binary data, and cross-over models from [2] are readily available. The type of log-normal data described in this report are regarded as surrogate marker for efficacy (termed a correlate of protection in vaccine trials). As a precise understanding of magnitude is needed for assessment for clinical use of a vaccine (or combinations of vaccines), traditional analysis (ignoring period effects and non-quantitative with respect to estimation of carry-over effects) is dangerous in terms of defining the protective effects of subsequent vaccinations. We recommend the use of the modelling approaches of [2] as an informative alternative.

As a practical matter, therefore, period effects should be expected in vaccine cross-over trials and estimated in analysis. Carry-over effects also should be accounted for in design and in modelling of vaccine cross-over data by default, in some manner, as they are desired and expected. It should be noted that the simple model applied in this report should not be regarded as adequate in all such situations. It is starting point to understand such data, in combination with the pre-vaccination titres, and should be viewed as useful for developing a quantitative understanding of the data.

In conclusion, we design vaccine cross-over trials to allow for possible period and carry-over effects and so, unless we have very good reasons not to do so, the analysis should match the design.

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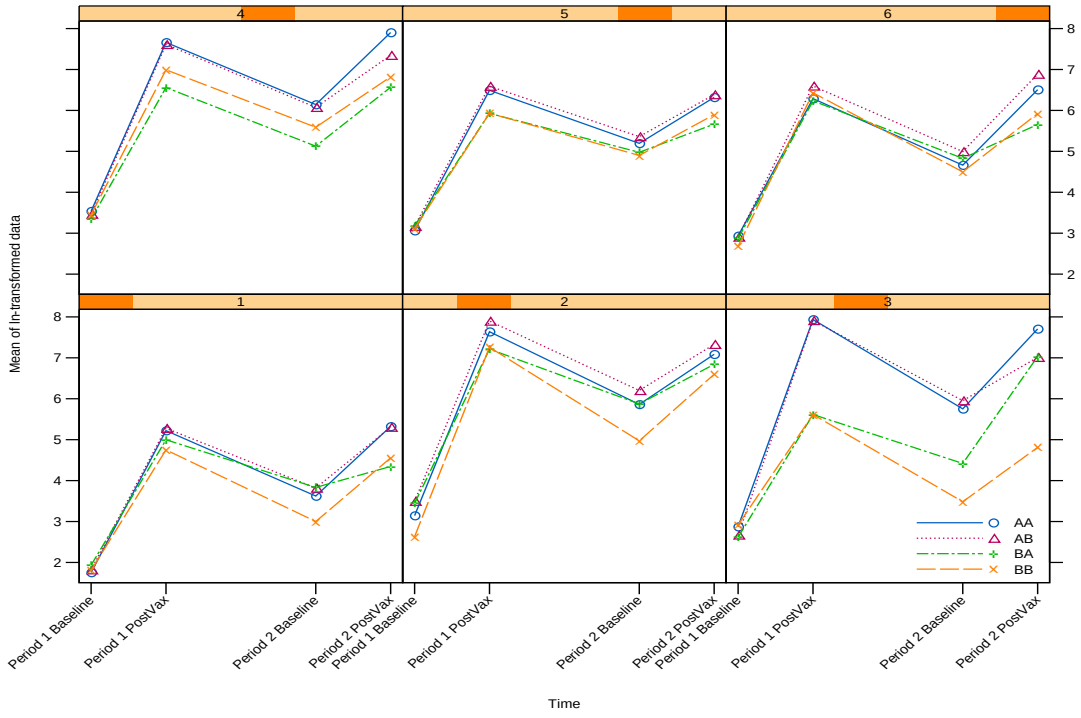

Figure 1: Mean ln −transformed Titre Data by Vaccine Sequence versus Time Matrix Plot for Antibody Types 1-6 for Periods 1-2, prior to and 1 month following vaccination

Table 1: Schematic Plan of a Balaam Design Cross-over Study

Sequence Group	Period			Number of Subjects
	1	Washout	2	
1(AA)	A	—	A	n_1
2(AB)	A	—	B	n_2
3(BA)	B	—	A	n_3
4(BB)	B	—	B	n_4
A=Regimen 1, B=Regimen 2				

Table 2: Example: Traditional Analysis of Differences in Mean ln-titre between Vaccine Groups and Following Sequences of Dosing from a Cross-over Vaccine Trial

Type	A-B Period 1 (p-value)	AA-BA Period 2 (p-value)	AA-BB Period 2 (p-value)
1	0.37(0.0006)	0.98(<0.0001)	0.77(<0.0001)
2	0.56(<0.0001)	0.25(0.0997)	0.49(0.0014)
3	2.31(<0.0001)	0.67(0.0005)	2.90(<0.0001)
4	0.88(<0.0001)	1.34(<0.0001)	1.08(<0.0001)
5	0.62(<0.0001)	0.65(<0.0001)	0.42(0.0015)
6	0.15(0.2367)	0.85(<0.0001)	0.58(0.0004)
A: New Vaccine; B: Original Vaccine			
H_0: Mean Difference between groups (or sequence) = 0			
Period 2 Comps. AA-AB, BA-BB, etc. on file.			

Table 3: Example: Traditional Analysis of Period 2-1 ln-titre Following Sequences of Dosing from a Cross-over Vaccine Trial

Type	AA-BA Period 2-1 (p-value)	AA-BB Period 2-1 (p-value)
1	0.87(<0.0001)	0.31(0.0257)
2	-0.06(0.6658)	0.20(0.1721)
3	-1.31(<0.0001)	0.62(0.0005)
4	0.29(0.0574)	0.43(0.0053)
5	0.26(0.0186)	-0.03(0.8036)
6	0.85(<0.0001)	0.66(<0.0001)
A: New Vaccine; B: Original Vaccine		
H_0: Mean Difference between sequences = 0		
Period 2-1 Comps. AA-AB, BA-BB, etc. on file.		

Table 4: Example: Traditional Analysis of Pre-Vaccination Adjusted Differences in Period 2-1 for ln-titre between Sequences of Dosing from a Cross-over Vaccine Trial

Type	AA-BA (p-value)	AA-BB (p-value)
1	0.81(0.0008)	-0.36(0.1410)
2	-0.38(0.2891)	-0.41(0.2852)
3	-2.74(<0.0001)	-1.66(<0.0001)
4	-0.68(0.0516)	-0.38(0.2872)
5	-0.16(0.5039)	-0.47(0.0490)
6	1.27(0.0001)	0.66(0.0492)
A: New Vaccine; B: Original Vaccine		
H_0: Mean Difference between Ratios across Periods between sequences = 0		
Period 2-1 Comps. AA-AB, BA-BB, etc. on file.		

Table 5: Example: Model-based Cross-over and Carry-over Analysis of ln-titre from a Cross-over Vaccine Trial

Type	Vaccine A-B (p-value)	Carry-over A-B (p-value)	Period 1-2 (p-value)
1	-0.08(0.2182)	0.69(<0.0001)	-0.12(0.0599)
2	0.24(0.0003)	0.39(0.0001)	0.34(<0.0001)
3	1.70(<0.0001)	1.19(<0.0001)	-0.45(<0.0001)
4	0.51(<0.0001)	0.72(<0.0001)	-0.27(0.0002)
5	0.17(0.0016)	0.33(<0.0001)	0.00(0.9841)
6	-0.05(0.4724)	0.83(<0.0001)	-0.30(<0.0001)
A: New Vaccine; B: Original Vaccine			