Clinical Trials A Practical Guide to Design, Analysis, and Reporting
Duolao Wang, PhD Ameet Bakhai, MBBS, MRCP

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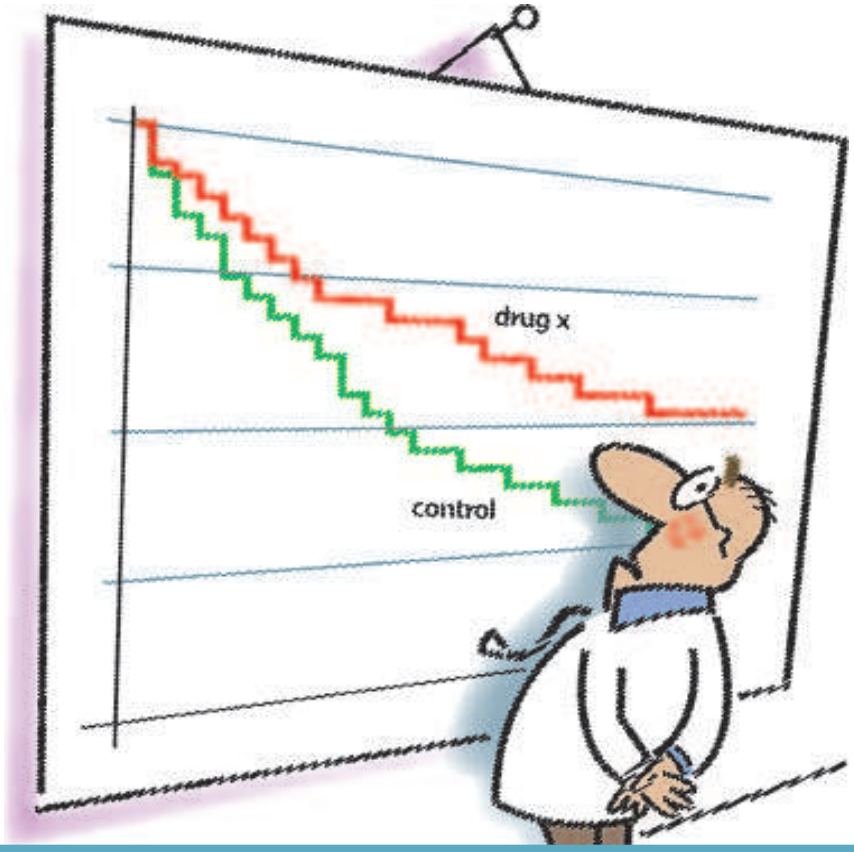

Duolao Wang, PhD
Statistician

Ameet Bakhai, MBBS, MRCP
Cardiologist

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Crossover Trials

Duolao Wang, Ulrike Lorch, and Ameet Bakhai

Crossover trials are designed so that each recruited patient receives both active and control treatments in either order for a specified duration, with a 'washout' period between treatments when no treatment is administered. In such trials, patients act as their own controls, therefore fewer patients are required to evaluate the effects of different therapies than in a trial with a parallel design. There are also limitations to the crossover design, however, and here, in this chapter, we discuss the advantages and disadvantages of crossover trials.

What is a crossover trial?

There are two commonly used types of study design in clinical research: *parallel* and *crossover*. In a parallel study design, each subject is randomized to one and only one treatment. Most large clinical studies adopt this approach. On the other hand, in a crossover trial, each subject receives more than one treatment in a specified sequence. In other words, a crossover trial is a study that compares two or more treatments or interventions in which subjects, on completion of a course of one treatment, are switched to another. This effectively means that each subject acts as his/her own control. The fundamental assumption of crossover trials is that patients usually have a chronically stable condition that will not vary between when they are taking the first and second treatments. Therefore, crossover trials are, by necessity, short-term trials.

Typically, each treatment is administered for a selected period of time and, often, there is a 'washout' or 'restabilization' period between the last administration of one treatment and the first administration of the next treatment, allowing the effect of the preceding treatment to wear off. Where possible, allocation of the treatment sequences in crossover trials is a randomized, blinded process.

Example study 1: Bioequivalence evaluation of two brands of cefuroxime 500 mg tablets (Julphar's Cefuzime® and GlaxoSmithKline's Zinnat®) in healthy human volunteers [1]

Cefuroxime axetil is a semisynthetic, broad-spectrum cephalosporin antibiotic for oral administration. A single-dose, two-treatment crossover design was carried out to evaluate the bioequivalence between two varying oral formulations of 500 mg cefuroxime axetil in 24 healthy volunteers. The two formulations used were Cefuzime as the test product and Zinnat as the reference product.

Each treatment was administered to subjects after an overnight fast on two treatment days separated by a 1-week washout period. After treatment administration, serial blood samples were collected for a period of 8 hours. Various pharmacokinetic parameters including $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, C_{max}, T_{max}, $T_{1/2}$ and λ were determined from plasma concentrations of both formulations. The results demonstrated that Cefuzime was bioequivalent to Zinnat since the 90% confidence intervals for the test/reference ratios of the relevant pharmacokinetic parameters were within the bioequivalence acceptance range of 80–125% (see **Chapter 13** for more about bioequivalence studies).

Table 1. A summary of the key features of two example crossover studies.

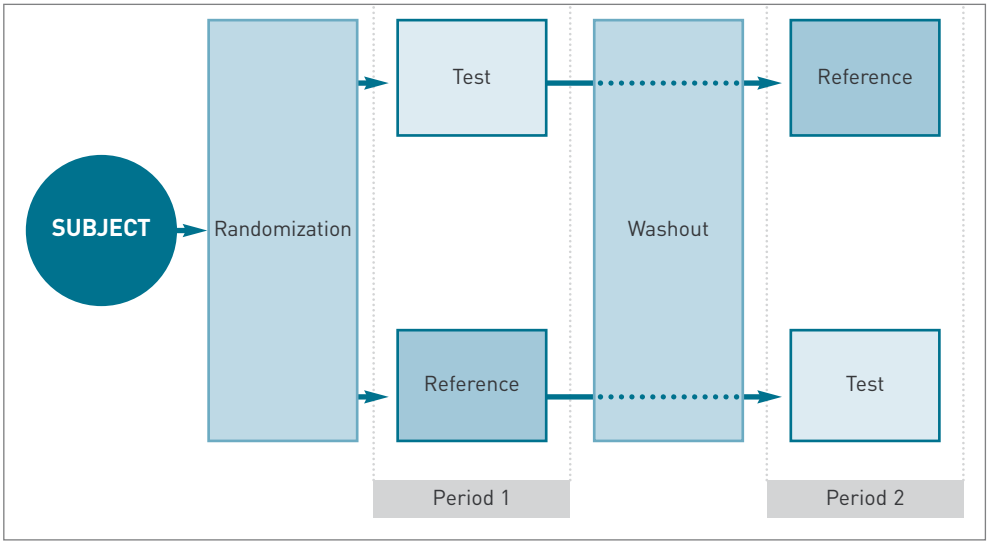
	Study 1	Study 2
Design	*2* × *2* crossover	*2* × *2* crossover
Objective	Bioequivalence evaluation	Efficacy assessment
Endpoint	Pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, and λ)	Plasma concentration of activated protein C
Treatment A	500 mg Cefuzime tablets	150 mg levonorgestrel and 30 mg ethinylestradiol
Treatment B	500 mg Zinnat tablets	150 mg desogestrel and 30 mg ethinylestradiol
Sequence 1	AB	AB
Sequence 2	BA	BA
Period 1	1 day	2 consecutive menstrual cycles
Period 2	1 day	2 consecutive menstrual cycles
Washout period	1 week	2 consecutive menstrual cycles
Sample size	24 subjects	33 subjects
Conclusion	Bioequivalence between A and B	Lower efficacy of B than A

Example study 2: Low-dose oral contraceptives and acquired resistance to activated protein C: a randomized crossover study [2]

A randomized crossover trial was carried out to assess how the use of second-generation oral contraceptives (treatment A: 150 mg levonorgestrel and 30 mg ethinylestradiol) and third-generation oral contraceptives (treatment B: 150 mg desogestrel and 30 mg ethinylestradiol) varies with respect to their resistance to the anticoagulant action of activated protein C (APC). Thirty-three healthy female volunteers between the ages of 18 and 40 years and without menstrual irregularities were assigned the two oral contraceptive preparations in random order (AB or BA).

The first oral contraceptive (A or B) was used for two consecutive menstrual cycles (Period 1) and, after a washout of a further two menstrual cycles (much longer than the half-life of each preparation), the volunteers were switched to the second preparation (B or A) for two cycles (Period 2). Blood samples were obtained between days 18 and 21 of all six menstrual cycles – one at baseline before starting either treatment, two during administration of the first preparation, one during the last cycle of the washout period, and two during administration of the second preparation. The study concluded that, compared with levonorgestrel (A), the desogestrel-containing oral contraceptive treatment (B) conferred significant additional resistance to APC. A summary of the key features of the two crossover studies is given in **Table 1**.

Figure 1. A standard two-sequence, two-period crossover design.



Classification of crossover trials

Crossover trials are classified according to the number of treatments given to a subject and according to whether a given subject receives all (*complete crossover*) or just some (*incomplete crossover*) of the study treatments.

The simplest crossover design is a two-treatment, two-period crossover trial in which each subject receives either the test (A) or reference (B) treatment in the first study period and the alternative treatment in the succeeding period. These trials are often referred to as 2×2 or AB/BA trials. The order in which the treatments A and B are administered to each subject is random; typically, half the subjects receive the treatment in the sequence AB and the other half in the sequence BA. An example of a standard 2×2 crossover design is given in **Figure 1**.

Where appropriate, the crossover design can be extended to include more than two treatments per subject in consecutive periods or more than two sequences. If a trial has p sequences of treatments administered over q different dosing periods, the trial is referred to as having a $p \times q$ crossover design.

Table 2 lists some commonly used higher-order crossover designs. Each design depends on the number of treatments to be compared and the duration of the study [3].

Table 2. Examples of high-order crossover trial designs.

Design type	Order	Treatment sequence
Two-sequence dual design	*2 × 3*	ABB, BAA
Doubled design	*2 × 4*	AABB, BBAA
Balaam's design	*4 × 2*	AA, BB, AB, BA
Four-sequence design	*4 × 4*	AABB, BBAA, ABBA, BAAB
Williams' design with three treatments	*6 × 3*	ABC, ACB, BAC, BCA, CAB, CBA
3 × 3 Latin square design	*3 × 3*	ABC, BCA, CAB
4 × 4 Latin square design	*4 × 4*	ABDC, BCAD, CDBA, DACB

Advantages of crossover trials over parallel studies

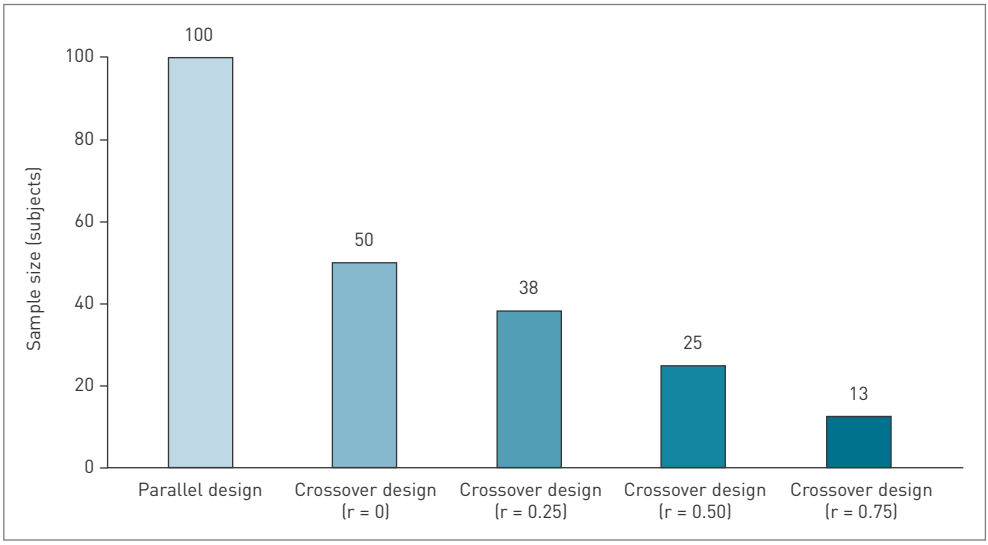
Since each subject in a crossover trial acts as his/her own control, there is an assessment of both (all) treatments in each subject. This means that treatment differences can be based on *within-subject* comparisons instead of *between-subject* comparisons. As there is usually less variability within a subject than between different subjects, there is an increase in the precision of observations. Therefore, fewer subjects are required to detect a treatment difference. If $N_{parallel}$ is the total number of subjects required for a two-way parallel trial to detect a treatment effect (δ) with 5% significance and 80% power, then the total number of subjects $N_{crossover}$ required for a *2 × 2* crossover trial to detect the same effect (δ) is approximately:

$$N_{crossover} = (1 - r)\, N_{parallel} / 2$$

where r is a correlation coefficient among the repeated measurements of the primary endpoint in a crossover trial. The above equation indicates that as the correlation increases towards 1, fewer subjects are needed for a crossover trial. **Figure 2** illustrates sample sizes for a crossover trial for some selected values of r and the sample size ($N_{parallel} = 100$) required by a parallel design trial for detecting the same clinical effect. The graph shows that:

- A crossover trial only needs half the sample size of that used in a parallel trial if there is no correlation among repeated measurements of the primary endpoint.
- If the correlation coefficient is 50%, a crossover trial only needs a quarter of the sample size of a parallel design.
- Sample size can be drastically reduced in a crossover trial if the correlation increases towards 1.

Figure 2. Sample sizes required for a crossover design to detect the same treatment effect as that seen with a parallel design with a sample size of 100, given different correlation values (r) among the repeated measurements of the primary endpoint in a crossover trial.



In addition, a crossover design provides the least-biased estimates for the difference between treatments assuming that the response of subjects to treatment is consistent. Take as an example the previously mentioned Study 2: if a parallel design were used for the study, observed differences in APC between two contraceptives could be subject to unknown bias or uncontrolled effects of the menstrual cycle. By conducting this cycle-controlled randomized crossover trial, this study has effectively reduced this potential source of bias.

Main limitations of crossover trials

The main limitation of crossover trials is that they pose greater inconvenience to the subjects because multiple treatments are given and the subjects will therefore be exposed to various transitions between treatment phases. This longer period of study involvement increases the chance of subject withdrawal from the study. Censored observations due to subject withdrawal have a higher impact in a crossover design study, particularly if unequal numbers of subjects have completed different phases of the trial, meaning that even partially complete data could produce biased results.

For crossover studies, it is essential that subjects are in a comparable condition at the start of each treatment period, otherwise the validity of treatment

comparisons is compromised. Crossover design is therefore more appropriate for chronic diseases that have a stable set of symptoms (such as rheumatoid arthritis), while acute conditions (such as heart attacks) are less appropriate. Similarly, the crossover design is not suitable either for primary outcomes that are permanent or for terminal events (such as pregnancy or death).

Although crossover trials require fewer patients, this might not always be appropriate, such as for Phase III studies where a large body of evidence of patient exposure is needed to satisfy regulatory requirements regarding drug safety, tolerability, and the likelihood of unpredictable side-effects with the new treatment.

The most significant problem of crossover trials is the 'carryover' effect. The carryover effect is defined as the persistence (whether physically or in terms of effect) of treatment applied in one treatment phase of the study to subsequent treatment phases [4]. In a bioequivalence study this would arise if, for example, the pre-dose blood sample in the second period contained any measurable amount of the study drug administered in the previous period. If this is the case, it is right to conclude that the half-life of the study drug has been underestimated and, consequently, that the washout period between the two periods was not sufficiently long for there to be near-complete elimination of the drug from the subject (pharmacokinetic study) or for the subject to return to baseline values of outcome parameters (pharmacodynamic study). Psychological carryover is also possible, where some memory of the therapeutic experience under the previous treatment affects the patient's present judgment, ie, his / her perception of the next treatment. This memory may be either positive or negative [5].

Where it occurs, the consequence of carryover is that the investigators will be measuring the combined effects of two or more treatments, which in turn (if undetected) will lead to a biased evaluation. There are statistical methods that can help compensate for the lack of return to baseline for individual treatment effects in the event of a carryover [3,4,6]. However, these make further assumptions, thereby weakening the study results. The ideal scenario is to ensure that an adequate washout period is predetermined for each drug or that there is continued monitoring throughout the washout period until all subjects have returned to baseline.

Another potential problem with crossover trials is the period effect (treatment by period interaction). Even after an adequate washout interval, the effect of either treatment can be influenced simply by whether it is administered first or second. For example, in a crossover trial testing two antihypertensive drugs, both drugs might be more effective in the second period than in the first (beyond even

differences due to different patients) due to the effect of being in the trial itself. If this period effect is large, it can be minimized by allocating equal numbers of subjects to different sequences and some form of statistical adjustment might then be required [4–6].

Where are crossover trials useful?

Crossover trials are most commonly used in early drug development, especially in Phase I pharmacokinetic, bioequivalence, dose-proportionality, and dose-escalation studies (for investigating the maximum–tolerated dose), and in Phase II pharmacodynamic studies. In later phases of drug development, as well as in other clinical studies, a crossover design is suitable for trials that involve relatively stable conditions such as asthma, rheumatism, migraine, mild-to-moderate hypertension, and angina.

Treatments with a quickly reversible effect (eg, bronchodilators) are more suited for investigation under crossover design than those with a more persistent effect (eg, steroids). Furthermore, this design is more applicable to single-dose studies than to long-term repeat-administration studies.

Conclusion

The crossover design for trials is a valuable tool in clinical research when applied in the correct setting. Its main advantage is that it evaluates within-subject treatment comparisons rather than between-subject comparisons, as in studies with a parallel design. Consequently, the data variability in crossover trials is lower, which is reflected in there being more robust data with narrower confidence intervals, and which reduces the number of study subjects needed to test hypotheses in clinical settings. The main limitation of the crossover design is the possibility that a carryover effect could occur, but this can be avoided by ensuring that there is a sufficient washout interval between the different treatment periods.

Crossover trials are widely used in the earlier phases of clinical drug development (pharmacokinetic, bioequivalence, and pharmacodynamic Phase I studies) and are clinically useful in studies involving stable chronic conditions and/or drugs with short-lived effects.

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