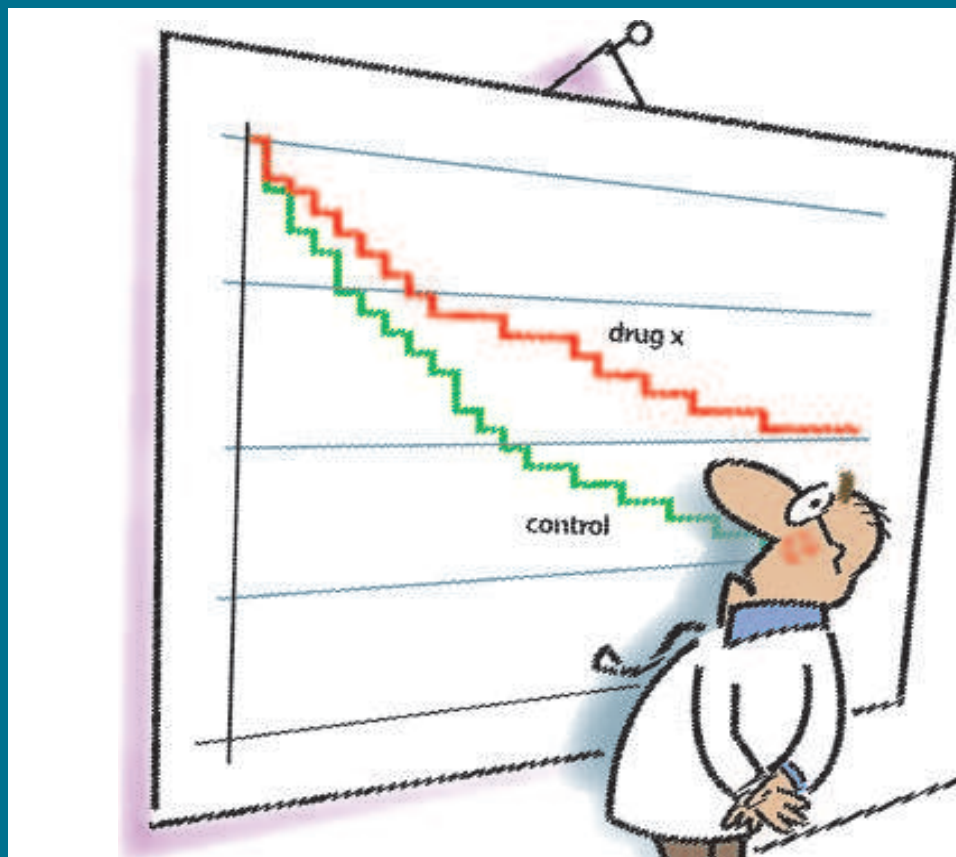


Clinical Trials

A Practical Guide to Design, Analysis, and Reporting



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Source and Control of Bias

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The aim of a randomized controlled trial is to provide unbiased evaluation of the efficacy and safety of a medicinal product or a therapeutic procedure. Unfortunately, the treatment effect estimates generated in a study are rarely free of all bias. This is due to a number of biases or errors that occur during a study from conception, through conduct, to completion of the trial, and even beyond the end of the trial when communicating trial results. These contributing factors can be classified into three categories: bias, confounding, and random error. In this chapter, we provide an overview of the ways in which bias can enter at different stages of a clinical trial and review ways of minimizing bias in clinical research.

What is bias in clinical research?

Bias is an “opinion or feeling that favors one side in an argument or one item in a group or series; predisposition; prejudice” [1]. In clinical research, bias is defined as systematic distortion of the estimated intervention effect away from the truth, caused by inadequacies in the design, conduct, or analysis of a trial [2], or in the publication of its results. In other words, in a biased trial, the results observed reflect other factors in addition to (or, in extreme cases, instead of) the effect of the tested therapeutic procedure alone.

The list of potential biases in research is long [3]. A correspondingly large proportion of the effort and skill in clinical research goes into avoiding bias. It is commonly accepted that it is impossible to completely eliminate the possibility of bias. However, it is also recognized that bias can be reduced with, among other things, careful planning and prudent study design. Although bias is typically introduced into a trial inadvertently, due consideration should be given to this problem since it can invalidate research: the mere suggestion that bias is present is often sufficient to cause the validity of a trial to be questioned. The main types of bias and ways of reducing such unwanted influences on the study outcome are listed in **Table 1** and described below.

Selection bias

Selection bias occurs if a systematic difference exists in the way in which study subjects are enrolled (accepted or rejected) into a trial or in the way that treatments are assigned to those enrolled, which in turn has an effect on the trial conclusions. For example, a trial investigator might have reason to believe that diabetic patients are less likely to respond to a new blood-pressure-lowering drug and, consequently, tend to include them in the group receiving the established (control) drug. If the investigator’s assumption proves to be correct, the results will show an exaggerated treatment difference and the trial conclusions will favor the new treatment more than they should.

Prevention of this type of bias depends, to a great extent, on how adequate the treatment allocation is. This is the main reason for the use of randomization methods in clinical trials. When randomization is employed properly, all study subjects are given the same chance of being assigned to each of the study treatments. Moreover, the treatment allocation in such a trial cannot be influenced by the investigator. Examples of good methods of treatment randomization include computer-generated codes, random number tables, and even the toss of a coin. Inadequate methods of randomization include alternate assignment and assignment

Table 1. Summary of the most common types of bias in clinical trials and methods of bias control.

Type of bias	Method of bias control
Selection bias	Randomized treatment assignment Concealment of treatment assignment
Bias in study management	Standardized study procedures Standard equipment Training and certification of research personnel
Observer ascertainment bias	Blinding or masking
Bias introduced by exclusions after randomization	Intention-to-treat analysis Worst-case scenario analysis
Publication bias	Prospective registration of clinical trials Publication of 'negative' trials

by odd/even date of birth or hospital number. However, it should be noted that, even when carried out properly, simple randomization does not guarantee the elimination of selection bias – it only reduces the possibility of this unwanted effect.

To further help minimize selection bias, stratification of randomization is used. This involves patients first being classified into subgroups, or 'strata', by one or more characteristics that may influence treatment response, such as age or severity of disease. Patients within each stratum are then randomized separately to ensure that, based on the stratification characteristics, the patients are well-balanced across the treatment groups.

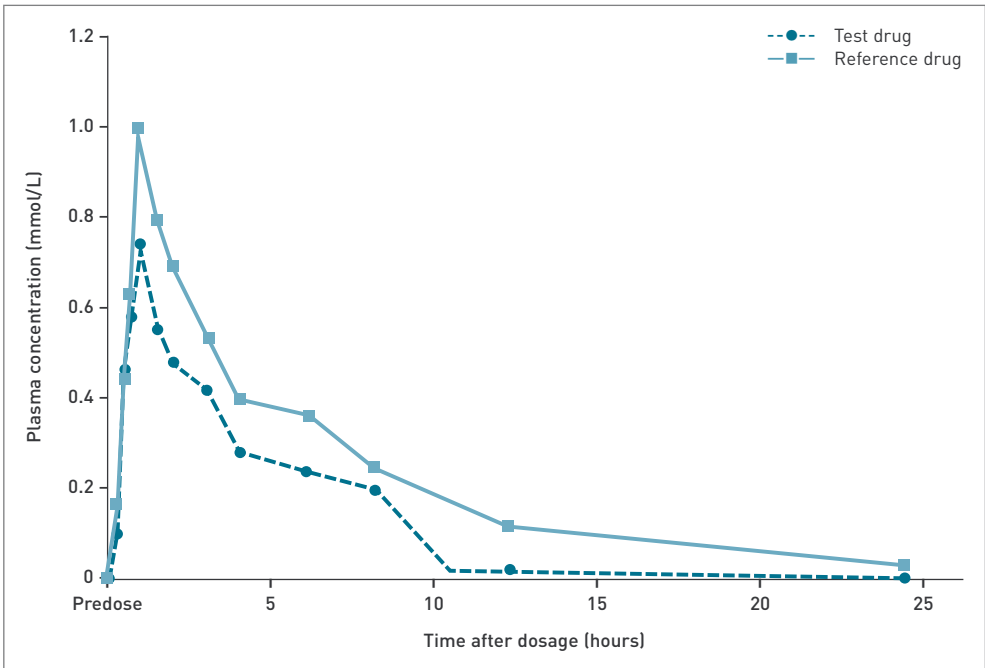
With respect to randomization, there are two processes of equal importance:

- creation of a random treatment assignment code
- concealment of that code until treatment allocation occurs

Some investigators working in clinical research appreciate the code-generating process of randomization but then disregard concealment. Without satisfactory concealment, even the best, most unpredictable randomization codes may be undermined. Chalmers et al. reported manifold overestimation of treatment effect in trials without adequate concealment of treatment allocation [4]. By using proper concealment procedures, such as keeping individual treatment codes in sealed opaque envelopes and making them accessible only to authorized personnel, those who are admitting volunteers into a study are protected from knowing the treatment allocation that will be used.

Selection bias can also be introduced if a highly selected group is enrolled into a trial (eg, in order to ease the demands of patient-recruitment or minimize inter-subject variability). The treatment effect in such a group might well be different from that

Figure 1. Mean pharmacokinetic profiles for the example bioequivalence study.

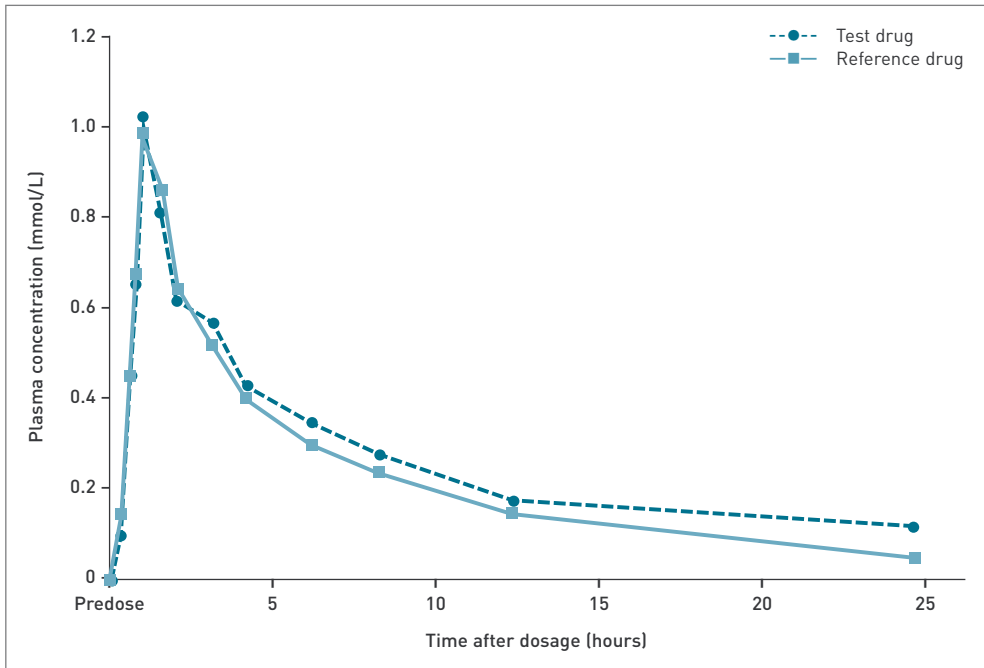


in the actual (target) population of interest. Where it is essential for the study group to closely reflect the target population, trial protocols should clearly specify and make mandatory the inclusion of a wide selection of the entire patient population.

Bias in study management

The interpretation of a randomized controlled trial relies on the assumption that any differences in outcome are the result of either chance (whose effects can be quantified) or of inherent differences between treatments. This assumption is invalid if the treatment groups are not handled equally with regard to all of the study procedures.

For instance, consider a trial conducted to compare the absorption of two dosage forms of a drug: a fast-dissolving tablet absorbed from the tongue and a regular tablet absorbed from the intestine. If, due to poorly defined study procedures or noncompliance, the subjects receiving the regular tablet are allowed to lie down following drug administration, the transit and intestinal absorption will be slowed and this might distort the overall study conclusions.

Figure 2. Mean pharmacokinetic profiles for the repeated example bioequivalence study.

The same might happen if blood samples for pharmacokinetic analysis are not handled properly. For example, consider an anonymized study where two formulations of a drug are compared to see if they are bioequivalent. Both formulations are chemically unstable and break down very rapidly, hence certain chemicals need to be added to the blood samples as stabilizers. The pharmacokinetic analysis showed that the test formulation was significantly less bioavailable than the reference drug (see **Figure 1**). However, the study debrief showed that the test-drug blood samples had a lower concentration of stabilizer; this caused the test drug to break down more quickly than the reference drug and show falsely lower concentrations when assayed. When the study was repeated using the same drugs with adequately stabilized blood samples, the mean concentrations of the two drugs were very similar (**Figure 2**).

In order to avoid this type of bias, trials should be conducted according to standardized written protocols with clearly defined study procedures. Study personnel must be well trained and certified for particular tasks to ensure that the various measurements and assessments are performed identically on every subject and on each occasion. Study participants should be closely supervised wherever possible to ensure their compliance and to check that study restrictions are observed.

Observer (ascertainment) bias

When knowledge of the treatment assignment (by participants already recruited into a trial, investigators, or persons who analyze and report trial results) leads to systematic distortion of the trial conclusions, this is referred to as *observer* or *ascertainment* bias.

The patients' knowledge that they are receiving a 'new' treatment may substantially affect the way they feel and their subjective assessment. If the investigator is aware of which treatment the patient is receiving, this can affect the way he/she collects the information during the trial (eg, he/she asks certain questions). By the same token, the knowledge of which treatment the patient received can influence the way the assessor analyzes the study results (eg, when evaluating efficacy by selecting particular time points that favor one of the treatments over the other). In the case of laboratory-related outcomes, the knowledge of treatment assignment can have an impact on how the test is run or interpreted. Although the impact of this is most severe with subjectively graded results, such as pathology slides and photographs, it can also be a problem with more objective tests (eg, laboratory assays might be run subtly differently by the technician).

Blinding

The best way of avoiding observer bias is to conduct trials in a *blind* fashion. This means that some or all of those involved in the study (study participants, investigators, assessors, etc.) are unaware of the treatment assignment. It is important to recognise the difference between allocation concealment and blinding. Allocation concealment helps to minimize selection bias by shielding the randomization code *before and until the treatments are administered to subjects or patients*, whereas blinding helps avoid observer bias by protecting the randomization code *after the treatments have been administered* (see **Chapter 8**).

Blinding or *masking*, as it is often called, is immensely important for maintaining the integrity and validity of research results. Nonblinded studies typically favor new treatments over established ones. One meta-analysis has shown that nonblinded studies overestimate treatment effects by 17% [5].

Blinding can be performed by making study participants unaware of which treatment they are receiving (*single blind*) or by making both study participants and the investigator unaware of the treatment assignment (*double blind*). There is another level of study blinding called *triple blind* or *total blind*, which essentially means that all those involved in a study, including those responsible for data analysis, reporting, and study monitoring, have no knowledge of which treatment is being given to whom. It appears that total blinding is not as common in clinical

trials as it should be. The benefit of blinding those evaluating and interpreting trial data (alongside the participants and investigators) is obvious. Similarly, usually there are no practical considerations as to why we should not blind such personnel, and we can do this by treatment coding.

To achieve blinding in a drug trial it should be impossible to distinguish between the trial medications, and, to achieve this, placebos are used. Placebos are inert substances that are identical in physical appearance to the active treatment and, if taken by mouth (eg, as tablets), they should have the smell and taste of the active treatment. In the case of an intravenous infusion, the placebo is normally the vehicle used for the active medication (the medium in which the active drug would be dissolved). One use of placebos in trials is as a direct comparison with a new medication (placebo-controlled trials), typically when there is no established active treatment that is effective and can be used as a comparator.

Achieving blinding

Effective blinding is not always easy to achieve. Patients and investigators may be clued into whether patients are taking active medication or placebo by different means. This can happen through accidental or deliberate unmasking. It can also occur as a result of practical problems in treatment administration, eg, if a patient bites the tablet and finds that it tastes different. Another source of unmasking is the fact that side-effects of the active treatment, quite understandably, are often different from those of the placebo.

Although most of these problems can be minimized by making trial procedures more stringent and improving trial participant and personnel compliance, the challenge of distinguishable side-effect profiles appear to be the most difficult to solve. It has been suggested that use of a ‘three-arm design’ (involving a new drug, a reference drug, and a placebo) can help to overcome this problem, whereby the third ‘treatment’ may make it more difficult for the patients and study personnel to ascertain the treatment allocation [6]. For instance, even if the patients guess that they are receiving an active treatment, they may not be able to tell whether they are on the ‘old’ or new drug. Also, noninert placebos have been used to achieve the same goal (in particular in antidepressant trials) [7]. Adding a third treatment or adding the potential for toxicity to placebo (that is, using a noninert placebo) to avoid unblinding raises some ethical issues. These need to be examined carefully before such methods are applied to a particular trial. A risk–benefit assessment should be carried out, taking into account the potential benefits of properly evaluating the new treatment on the one hand and protecting patients from any unnecessary harm on the other.

Bias introduced by exclusions after randomization

It is intended that all trial participants comply with the trial protocol and complete the trial accordingly. However, in practice (and in particular during later phases of drug development), missing data can result from some of the participants dropping-out before they complete the trial. Also, data might be missing because some of the scheduled measurements were done incorrectly or, worse, not done at all. Irrespective of their origin, inappropriate handling of the missing data can lead to bias. For example, if in a treatment comparison trial the incidence of withdrawals due to adverse events is much higher with one of the treatments, excluding withdrawn subjects from the analysis would lead to an underrating of the side-effects of that drug. Analyses that exclude all subjects who were noncompliant, have missing data, or were unable to complete their assigned treatment are called *per-protocol* analyses.

There are two bias control methods that can be used to minimize the negative impact that withdrawals and exclusions can have on the interpretation of study results (eg, as seen in per-protocol analyses).

The first is known as *intention-to-treat* analysis. In this analysis, all the study participants we intended to treat are included in the analysis, regardless of whether they completed the trial according to the protocol or not. The second method is called *worst case scenario* analysis. This involves allocating the worst possible outcomes to the missing subjects or missing time points in the group that shows the most desired results, and *vice versa*. Following this, this data set is analyzed to see whether the new analysis is in agreement with the results of the initial analysis (which did not account for the missing data). As opposed to the intention-to-treat approach, which is a way of addressing the issue of noncompliant subjects (who may or may not have missing data), the worst case scenario analysis is more specifically used to deal with missing data in a trial. There are other often-suggested methods for dealing with missing data [8,9], such as '*last observation carried forward*' and '*multiple imputation*' (see **Chapter 30**).

Publication bias

It is becoming increasingly apparent that published clinical research data do not always represent the whole truth. People involved in research, including investigators, editors, and sponsors, typically prefer positive trial outcomes, which is to some degree understandable. What is not understandable is that trials generating negative or equivocal results are less likely to be published in peer-reviewed journals, and this can seriously undermine the integrity of clinical research data [10]. This tendency to favor trials with positive results is called 'publication bias'.

Although there appears to be increasing evidence that failure to publish is not a random event [11], the lack of interest in publishing ‘nonpositive trials’ might, at least partly, be a result of underpowered studies. In such a trial, negative or equivocal results effectively become indeterminate, which in turn makes them of little interest to journal editors or the scientific community.

One way of tackling publication bias is by introducing the compulsory registration of trials at initiation and ensuring that the results of all trials are published. Although it remains a long-term goal, at present such a proposal is very controversial and is the subject of intense debate. Meanwhile, the reader should bear in mind that published data alone might not always provide sufficient evidence on which to make a definitive judgment about a particular treatment.

Conclusion

The types of bias described above are the most common and, arguably, the most important in clinical research. There are, however, many other biases that can be introduced in the course of, or after, a trial, and they are comprehensively described in other publications [3,12].

Randomized controlled trials are too often assumed to produce impartial evidence by eliminating bias. The truth is that randomization, treatment concealment, blinding, standardized study procedures, and other methods mentioned in this chapter help to reduce bias, but do not eliminate it completely. We may only move closer to that goal by raising awareness among scientists, investigators, peer-reviewers, and readers about the importance of bias control in clinical research, and by applying bias-control measures wherever possible.

In this chapter, we have only addressed the issue of bias. The two other sources that could still distort the true estimates of treatment effects, random error and confounding, are discussed in **Chapters 18** and **26** of this book, respectively.

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