Observational studies nested within randomized clinical trials (OS-RCTs) are now common. In this issue of the Journal, Pacanowski et al. report the effects of the −786T>C and Glu298>Asp NOS3 gene polymorphisms on the risk of cardiovascular events (CVEs) and blood pressure control (BPC) in patients with coronary artery disease and hypertension from the INVEST trial. Their interesting study provides an opportunity to discuss issues of bias and power relevant to the design and interpretation of OS-RCT.

In large RCT, the confounding effect of prognostic factors is prevented by the random assignment of treatments. Correspondingly, in large OS-RCTs treatment is an unlikely confounder as it should not be associated with the exposure of interest. However, random assignment does not balance the distribution of prognostic factors in groups defined by exposures other than the treatment. Therefore, confounding is likely and should be controlled in OS-RCT.

Although randomization prevents its occurrence in large RCT, selection bias can occur in OS-RCT if only a fraction of all RCT participants is included in the OS-RCT. Pacanowski et al. likely avoided selection bias in their analysis of new CVE by including all cases and a conditional random sample of noncases as controls. In contrast, they risked selection bias by choosing cases and controls of BPC exclusively from those included in the analysis of CVE. If the studied polymorphisms were associated with new CVE, they would also be associated with the selection of BPC cases and controls. Therefore, selection bias would have occurred unless the sampling fractions for CVE cases and controls corresponded to their distribution in the RCT cohort or were included as weights in the analysis. Selection bias in OS-RCT could be avoided by using all cases or a random sample of all cases of each outcome as separate index groups and using separate samples of noncases of each outcome (traditional case–control study) or a random sample of all RCT participants (case–cohort study) as control group.

In RCTs treatment masking makes patient management and errors in the ascertainment of the exposure independent of treatment assignment. Unfortunately, other risk factors could still influence patient management and, in consequence, become confounding factors in OS-RCT. These factors should be identified and controlled in the analysis. Also, prognostic factors may be associated with errors in outcome ascertainment, resulting in differential misclassification bias. These two biases are unlikely in Pacanowski et al.’s study, as gene polymorphisms were ascertained after completion of the trial.

Issues of study power should be carefully considered when designing an OS-RCT. In general, the statistical power of an OS-RCT of the effect of a genetic polymorphism on the main outcome will be lower than that of the original RCT, because the OS-RCT will have smaller sample size, the effect of the genetic polymorphism will likely be weaker than that of the treatment, and it is unlikely that half the participants will have the harmful genetic variant. Although treatment-by-risk factor interactions are tested in most OS-RCT, regardless of biological plausibility, these tests usually have low statistical power.

Disclosure: The author declared no conflict of interest.