Editorial

Role of nested case–control studies in the difficult quest for new coronary risk factors

About half of all patients with ischaemic heart disease do not exhibit traditional risk factors.[1] Therefore further gains in the control of ischaemic heart disease will require concerted efforts, and the necessary allocation of resources, for current research to evaluate new possible risk factors and preventive interventions. These concerted efforts should proceed on several fronts simultaneously. Basic researchers provide biological mechanisms and answer the crucial question of why an agent or intervention reduces disease or death. Clinicians provide benefits to affected patients through advances in diagnosis and treatment, and formulate hypotheses from their clinical experience. Epidemiologists and statisticians formulate hypotheses from basic, clinical, and descriptive epidemiological studies and test these hypotheses in prospective studies and, where appropriate, in randomised trials.

The ultimate goal of epidemiological studies is to establish whether a cause–effect relation exists between a putative risk factor and disease. Making such a judgement involves several steps, the first being to establish whether there is in fact a valid statistical association. To conclude that an association is valid, the potential role of chance, bias, and confounding must be ruled out. If a valid statistical association is present, the question then becomes: is it one of cause and effect? To render this judgement, the totality of evidence from all sources must be considered, including the strength and consistency of the association and the plausible biological mechanisms to explain the findings.[2]

Epidemiological studies

Epidemiological studies can be either descriptive (cross sectional or case–control studies) or prospective (cohort studies or case–control studies nested within a prospective cohort).[3 4] Descriptive studies are useful primarily for the formulation of hypotheses; prospective studies may be helpful for hypothesis testing. In descriptive studies a putative risk factor is measured at or after the identification of cases who show evidence of the disease under investigation. In cross sectional studies members of a defined population are examined for the presence (cases) or absence (controls) of disease; in case–control studies cases are identified first and then controls are matched as a standard of comparison. Levels of a putative risk factor are then compared in cases and in controls: higher levels in cases suggest the possibility of an association between putative risk factor and disease. In cross sectional studies a potential bias is the erroneous recall of prior clinical events; in case–control studies a potential bias is patient selection. Furthermore, in both cross sectional and case–control studies even the convincing demonstration of an association between putative risk factor and disease does not allow us to rule out reverse causality where elevated levels of the putative risk factor can be the result of disease rather than its cause. In prospective studies a putative risk factor is measured in members of a defined population without evidence of the disease under investigation. In prospective cohort studies enrolled individuals are then followed up for a time period sufficient for a sizeable percentage of them to develop the disease. The levels of a putative risk factor at the time of enrolment are then compared in cases who will develop disease and controls who will not develop disease at follow up. In cohort studies a potential bias is represented by losses at follow up.

Nested case–control studies

In case–control studies nested within a prospective cohort the levels of the putative risk factor are compared only in cases who will develop disease at follow up and in an equal number of controls (better if the number of controls is a multiple of the number of cases) who will not develop disease at follow up; in this case risk factors do not need to be assessed in the remaining subjects which were part of the initial cohort. Nested case–control studies compared to cohort studies are less exposed to the potential bias of losses at follow up and cheaper as less measurements are needed.

In general, the presence of higher levels of a putative risk factor in cases than in controls is more convincing evidence that a cause–effect relation might exist between putative risk factor and disease when they are found in prospective studies than when they are found in descriptive studies. Indeed, in prospective studies the results are less likely to be influenced by a selection bias and are less likely to be accounted for by reverse causality. Unfortunately, even in well designed prospective studies the association between a putative risk factor and disease can be the result of chance, particularly if the number of enrolled individuals is small. Furthermore the association can be entirely due to confounding factors if a known risk factor is responsible for both increased levels of a putative risk factor and disease. In prospective studies the confounding role played by known risk factors can be unmasked using appropriate multivariate statistical models although this reduces the power of the study. It is impossible, however, to adjust the results for unknown confounding factors. When a putative risk factor is biologically plausible and the association with the disease is consistent in well designed prospective studies, the cause–effect relation can be definitely proven by randomised trials showing that interventions that reduce the levels of the putative risk factor prevent the disease.

In the past few years case–control studies nested within a prospective cohort have played a key role in our understanding of new risk factors for ischaemic heart disease. Why has this methodologically sound and cheap epidemiological approach gained so much popularity? Probably because nested case–control studies, with a small additional effort, multiply the information which can be obtained from large cohort studies and from randomised trials. Indeed, in the last decades spare blood samples obtained at baseline were wisely collected and frozen in several prospective studies and randomised trials. These blood banks have set up the stage for nested case–control studies which could be carried out after completion of the

planned follow up. In nested case–control studies so planned it has been possible to investigate potential risk factors for ischaemic heart disease, measurable in peripheral venous blood, which were unknown[5] or were not regarded as potential risk factors[6] at the time of blood collection. Of interest, nested case–control studies can be carried out without introducing any important bias even when baseline blood samples are not available from all subjects enrolled in the initial cohort. Indeed, blood samples from selected cases and matched controls only are needed for the purpose of the study.

Homocysteine and ischaemic heart disease

The article published by Fallon and colleagues[7] in this issue of *Heart* is an excellent example of a case–control study nested within the prospective Caerphilly cohort. The results of this study do not support the hypothesis that raised blood concentrations of homocysteine are associated with an increased risk for ischaemic heart disease. The studies carried out in the past few years on the association between homocysteine and ischaemic heart disease represent a good example of how difficult it can be to prove a cause–effect relation between a putative risk factor and disease. Despite the biological plausibility—markedly increased concentrations of homocysteine have deleterious effects on the vascular wall[8 9]—and the results of several descriptive epidemiological studies consistently showing a significant association between homocysteine blood concentrations and ischaemic heart disease, prospective studies have produced remarkably conflicting results showing a strong association at one extreme and no association at all at the other.[10 11] Reverse causality, a weak average cause–effect relation, a strong cause–effect relation but limited to a small subset of patients with ischaemic heart disease, and the role played by chance, can all explain these disparate findings. Thus, the totality of available evidence indicates that only randomised trials will allow us to establish whether a cause–effect relation exists between homocysteine blood concentrations and ischaemic heart disease.

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1 Heller RF, Chinn S, Tunstall Pedoe HD, *et al*. How well can we predict coronary heart disease? Findings in the United Kingdom heart disease prevention project. *BMJ* 1984;**288**:1409–11.
2 Hennekens CH. Increasing burden of cardiovascular disease: current knowledge and future direction for research on risk factors. *Circulation* 1998;**97**:1095–102.
3 Lilienfeld DE, Stolley PD. *Foundations of epidemiology*, 3rd ed. Oxford: Oxford University Press, 1994.
4 Rothman KJ, Greenland S. *Modern epidemiology*, 2nd ed. Baltimore: Lippincot Williams and Wilkins, 1998.
5 Ridker PM, Hennekens CH, Roitman-Johnson B, *et al*. Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. *Lancet* 1998;**351**:88–92.
6 Ridker PM, Cushman M, Stampfer MJ, *et al*. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;**336**:973–9.
7 Fallon UB, Ben-Shlomo Y, Elwood P, *et al*. Homocysteine and coronary heart disease in the Caerphilly cohort: a 10 year follow up. Heart 2001;**85**: 153–8.
8 Harker LA, Ross H, Slichter SJ, *et al*. Homocysteine-induced atherosclerosis: the role of endothelial cell injury and platelet response in its genesis. *J Clin Invest* 1976;**58**:731–41.
9 Tsai JC, Perrella MA, Yoshitzumi M, *et al*. Promotion of muscular smooth muscle cell growth by homocysteine: a link to atherosclerosis. *Proc Natl Acad Sci* 1994;**91**:6369–73.
10 Christen WG, Ajani UA, Glynn RJ, *et al*. Blood levels of homocysteine and increased risks of cardiovascular disease: causal or casual? *Arch Intern Med* 2000;**160**:422–34.
11 Danesh J, Lewington S. Plasma homocysteine and coronary heart disease: systematic review of published epidemiological studies. *Journal of Cardiovascular Risk* 1998;**5**:229–32.

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December 2000;**84**:587–94 (Review)

2 **Joint British recommendations on prevention of coronary heart disease in clinical practice**
December 1998;**80**(suppl 2):S1–29

3 **Clinical anatomy of the aortic root**
R H Anderson
December 2000;**84**:670–3 (Education in Heart)

4 **Anticoagulation in valvar heart disease: new aspects and management during non-cardiac surgery**
C Gohlke-Bärwolf
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5 **Baseline predictors of tolerability to carvedilol in patients with chronic heart failure**
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7 **Heart disease in the elderly**
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November 2000;**84**:560–6 (Education in Heart)

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