

A comment on the reference series with regard to multiple exposure evaluations in a case-referent study

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AXELSON O, FLODIN U, HARDELL L. A comment on the reference series with regard to multiple exposure evaluations in a case-referent study. *Scand j work environ health* 8 (1982): suppl 1, 15-19. Case-referent (case-control) studies permit multiple exposures to be considered within the same series of cases and referents (controls) with regard to the etiologic contribution to a certain disease. Specific aspects of validity should be considered however, one being the utilization of an adequate reference series. Should the exposure cause, prevent, or otherwise have a certain relationship to disorders other than the one being studied, individuals with such other disorders cannot be uncritically included in the reference series if obtained from a hospital register, a register of causes of deaths, or some other source of subjects with relations to the individuals' health status. If a general population register is utilized, this problem is avoided since individuals obtained from such a register would adequately reflect the exposure frequency of the source population of the cases. These aspects are well illustrated by a consideration of the use of drugs among cases and, on one hand, referents with some sort of disorder as compared to referents (whether healthy or not) obtained from a population register on the other hand. Studies of industrial exposures seem to be somewhat less sensitive to bias of this type however, since industrial exposures are not primarily disorder-related, whereas drug exposures are.

Key terms: cancer, case-control, comparability, drugs, industrial exposures, methodology, reference entity.

Case-referent (case-control) studies have the advantage of easily permitting several exposures to be considered within the same series of cases and referents with regard to the etiologic contribution to a certain disease, eg, with the purpose of providing hypothesis-generating clues for further investigations. Concern has arisen about such multiple comparisons, however, since a number of small p-values might be obtained by chance alone, ie, a so-called "mass-significance" phenomenon might occur. The issue of reserpine causing breast cancer (1, 4, 10), as originally brought up

through multiple exposure comparisons between cases and referents, has attracted considerable criticism in this regard (11, 13, 14). However, it has also been advocated that the advance credibility of a relationship, rather than the number of comparisons, would be of primary interest for the evaluation of an observed association (5, 14).

In case-referent studies, there are several aspects of validity, one being the utilization of an adequate reference series. In the context of multiple exposure comparisons, the reference series should be suitable for all of these comparisons. In principle, one has to consider carefully what diagnoses can be accepted as reference entities, since the exposure might also cause, prevent, or have certain relations to disorders other than the one taken as the case entity of the study. If referents are

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drawn from a hospital register, the conditions under which such diagnoses were obtained should be noted, since, say, a diagnosis of hypertension at a department of internal medicine tends to have a direct relationship to antihypertensive treatment, whereas the registration of hypertension at a surgical department is a secondary notation in the context of some other disorder. Should individuals with disorders that have a direct relationship (positive or negative) to the exposure(s) under study appear in the reference series, then the exposure frequency in the source population of the cases would not be properly reflected by the referents. When the potential etiologic role of a number of various exposures is explored, it must be considered whether the selected reference entities could have primary relationships to some of these exposures but not to others. In other words some of the multiple comparisons of the exposure frequencies could be biased, whereas others would be appropriate. As further delineated later, a reference series drawn from a general

population register could be utilized to avoid this type of bias. However, healthy persons might be less cooperative than individuals suffering from a disorder, eg, in responding to questionnaires or interviews. Sick individuals may also respond in a manner more similar to the cases (3, 5). Furthermore, if the cases derive from a hospital or hospitals serving particular subgroups of the population, eg, with regard to social class, the use of hospital patients to obtain comparability between cases and referents with regard to admission criteria might be necessary.

To avoid potential inadequacies in reference entities, certain diagnoses have to be excluded, or, even better, only individuals with specific diagnoses without a known relation to the exposures at issue should be enrolled. Practical problems, like the number of available referent candidates and ignorance of the possible effects of an exposure, tend to favor the utilization of all individuals after the obviously necessary exclusions. If referents are obtained from a population register, no

Table 1. Use of antihypertensive drugs among living cases of colon cancer and referents from the population register (7).

Age (a)	Treated	Not treated
25—55		
Cases	2	8
Referents	9	80
56—65		
Cases	7	9
Referents	11	40
66—75		
Cases	11	27
Referents	16	37
76—85		
Cases	8	17
Referents	3	17
Total		
Cases	28	61
Referents	39	174
Crude rate ratio	2.0	1.0
Standardized mortality ratio	1.6	
Rate ratio (Mantel-Haenszel)		
Point estimate	1.6	
95 % confidence interval	0.9—3.0	

Table 2. Use of antihypertensive drugs among deceased cases and referents. Three cases and two referents were excluded due to a lack of information (7).

Age (a)	Treated	Not treated
25—55		
Cases	1	5
Referents	2	16
56—65		
Cases	7	11
Referents	9	17
66—75		
Cases	5	14
Referents	15	28
76—85		
Cases	8	11
Referents	11	22
Total		
Cases	21	41
Referents	37	83
Crude rate ratio	1.1	1.0
Standardized mortality ratio	1.1	
Rate ratio (Mantel-Haenszel)		
Point estimate	1.1	
95 % confidence interval	0.6—2.1	

such exclusions would be appropriate, however, not even of individuals that might turn out to suffer from a disease (other than the case entity) that might have a bearing on relations to the exposure or exposures under study. Thus, unlike when the referents are chosen from a hospital register or from a register of causes of deaths, those taken from a population register are not obtained conditionally on health status and do therefore reflect nothing but the exposure frequency of the source population of the cases. It might be added that the referents should preferably be taken within the study period rather than at the end, especially if there could have been changes in the exposure frequency over time. Moreover, there is not necessarily a need for the rare disease condition to be fulfilled for the case-referent approach to be applied (12).

The principle discussed might be illustrated through some examples. First, consider some data obtained in the context of studying various chemical exposures, particularly phenoxy acids and chlorophenols, as possibly related to specific types of malignancies (8, 9). Referents were taken from a population register, originally matching living cases, as well as from a register of causes of deaths corresponding to the dead cases. Comprehensive information on various types of exposure was then obtained through questionnaires and interviews. The referent series were primarily chosen for studies on soft-tissue sarcomas and lymphomas but were later utilized for multiple exposure comparisons with cases of colon cancer from the same district (7), both with regard to various occupational exposures and medical treatments, among which were antihypertensives. It might be added, in this context, that the antihypertensive drug hydralazine is a hydrazine derivative, and that other hydrazines are utilized to induce colon cancer experimentally.

Tables 1 and 2 display the exposure frequency of antihypertensives among the living and dead subjects of the case and reference series along with various measures of effect. Different rate ratios are obtained for the living and dead subjects, but it is obvious that many of the dead referents had suffered from cardiovascular disorders, including hypertension, ie, ref-

erence entities in the domain of cardiovascular disorders, which are apparently inadequate when exposure to antihypertensives is investigated but are likely to be appropriate in the context of considering various occupational exposures. Table 3 presents the results of the exclusion of cardiovascular diagnoses from the reference series; the rate ratios become almost identical for the living and dead subjects of the study.

A similar phenomenon was indicated in crude terms in another study on acute myeloid leukemia, background radiation, and exposure to solvents and other agents, ie, both to occupational and other factors (6). As indicated in table 4, there was a general tendency in the study towards a formal prevention with regard to the use of var-

Table 3. Use of antihypertensive drugs among deceased cases and referents as in table 2, but with 69 referents excluded as having died of ischemic heart disease, thromboembolism, or cerebrovascular disease (7).

Age (a)	Treated	Not treated
25—55		
Cases	1	5
Referents	1	9
56—65		
Cases	7	11
Referents	1	7
66—75		
Cases	5	14
Referents	5	10
76—85		
Cases	8	11
Referents	5	13
Total		
Cases	21	41
Referents	12	39
Crude rate ratio	1.7	1.0
Standardized mortality ratio	1.6	
Rate ratio (Mantel-Haenszel)		
Point estimate	1.6	
95 % confidence interval	0.7—3.7	
Combining tables 1 and 3:		
Rate ratio (Mantel-Haenszel)		
Point estimate	1.6	
95 % confidence interval	0.99—2.6	

Table 4. Status or exposure (especially risk indicators and drug exposures are displayed) among 42 cases of acute myeloid leukemia and 244 (noncancer) referents, along with estimates of the crude rate ratio. An extended table from Flodin et al (6)]^a

Status or exposure	Number of exposed individuals		Crude rate ratio
	Cases	Referents	
Male gender	23	121	1.2
Pesticides	3	6	3.1
X-ray treatment	4	20	1.2
X-ray examination	12	68	1.1
Animal contacts	16	79	1.3
Cat	14	62	1.5
Poultry	8	36	1.4
Cattle	10	43	1.5
Solvent exposure	11	13	6.3
Smoking	19	117	0.9
Rural domicile	11	59	1.1
Use of drugs			
Asthma	1	6	1.0
Cardiac insufficiency	3	44	0.3
Hypertension	5	39	0.7
Diabetes	2	25	0.4
Psychiatric disorders	2	14	0.8
Other disorders	8	56	0.8

^a It might be remarked that several exposures tend to interconfound each other. For an evaluation of the confounding, see the original report (6).

ious drugs, a phenomenon that is likely to reflect the fact that many of the (non-case) individuals had been treated with various preparations for their diseases, which directly or indirectly might have been the cause of death (the study utilized deceased cases and referents only).

To summarize, in multiple comparisons concerning exposure frequencies among cases and referents, the primary concern should not be for "mass-significance" phenomena, but rather for the validity of the reference series for the purpose of multiple comparisons. Presumably, occupational epidemiology is better off in this respect than epidemiology at large, and perhaps studies on side-effects of drugs are particularly critical. In addition, when primarily dealing with occupational health issues, many investigators might be apt to consider also the use of drugs as background information or as a sort of "dummy variable," eg, utilized in questionnaires and interviews to somewhat disguise the specific purpose of the study. However, it is essential then that discrepancies (as well as agreements) in exposure frequencies (of nonoccupational factors) be carefully evaluated and not be uncritically

used to discredit the study as a whole — nor should agreement in nonoccupational factors necessarily be taken as support for the validity of an occupational health study. In addition, there are other problems of validity in multiple comparisons, eg, interconfounding might exist between some of the exposures under consideration but not among others. Therefore, intercorrelations of various potential risk factors should also be evaluated (2, 6). However, with the proper design and analyses, the case-referent study is a very valuable tool in occupational health epidemiology and might be especially effective for the evaluation of various etiologic factors for the same disorder.

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