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THE INDICATION AND UTILITY OF THE AMBIDIRECTIONAL DESIGN IN THE STUDY OF CHRONIC CHAGAS' DISEASE

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Summary — The transition rates between the « infected non diseased » and « infected diseased » stages are not yet precisely known in Chagas' disease, neither are the determinants of that transition completely identified or quantified. Methodological difficulties of the three major classical epidemiological designs (cross-section, follow-up and case-control designs) are in part responsable for the present lack of insight in the epidemiology of chronic Chagas' disease. There exists now a hybrid design, which combines the main advantages of the prospective follow-up of the case-control design without their major deficiencies. It is shown how this design can be adapted for and applied to the study of chronic chagasic cardiomyopathy.

KEYWORDS : Chronic Chagas' Disease; Epidemiologic Methods; Ambidirectional Study Design.

I. Subject matter issues in the study of chronic Chagas' disease

Since the discovery of *T. cruzi* and the corresponding disease by Carlos Chagas in 1909, thousands of studies have been carried out on Chagas' disease, many of them of an epidemiological nature. Recently a remarkable recopilation of 5,152 papers and communications on Chagas' disease, was published by Prata & Pires de Sant'Anna (1982), 13 per cent of those articles belong to the domain of epidemiology.

That recopilation concerns only work published in the Brazilian literature till 1979. Therefore the number of 1,500-2,000 epidemiological studies on Chagas' disease carried out till now, is a reasonable guess. Although so many epidemiological studies on Chagas' disease have been done, still many questions regarding the future of an infected person and its determining factors remain unanswered. The risk of an indeterminate case to develop chronic chagasic cardiomyopathy or a digestive form of the disease, has to be determined through conditional cumulative incidence parameters. One way to get such data is through cohortstudies in which the contribution of each member of the cohort to the denominator (= person-time of observation) is exactly known. Such studies are rare, and the yearly incidence rates of chronic chagasic cardiomyopathy are rather estimates : they are about 1-2 per cent following Prata (1984).

The evolution of a chronic chagasic cardiomyopathy case is also not precisely known (Prata, 1975), although excellent cardiological evidence exists, but the major problem is one of representativeness of those clinical patients. Few data regarding the case fatality rates of Chagas' cardiomyopathy cases exist (Prata, 1975) : life table techniques have been used

in this determination (Kloetzel & Pinto Dias, 1968) but the years of healthy life lost (Ghana health assessment project team, 1981), due to Chagas' disease have not been calculated yet.

A whole list of determining factors of incidence and case fatality can be drawn from the literature. Those factors, be they risk factors (= variables related to the occurrence of the disease) or prognostic factors (= factors related to the outcome of the disease) (Kleinbaum *et al.*, 1982), are of an uttermost importance for a better understanding of the natural history of the disease and for the planning of control activities outside the framework of vector control.

The following factors are mentioned in the literature : Race : (Azevedo *et al.*, 1979; Numesmaia & Azevedo, 1973); Ecological region : there is for example some circumstancial evidence that the chagasic etiological fraction of chronic cardiomyopathies is less important and the cardiac morbidity less severe in the state of Rio Grande do Sul, Brazil than in Central Brazil (Brant *et al.*, 1957); the former assertion is being questioned by the hospital and field observations of Baruffa *et al.* (1975, 1976, 1983); Altitude (De Muynck, 1983); Degree of Parasitemia (Prata, 1975; Pifano, 1977; Castro C.N. de, 1978); Reinfection (Dias, 1963; Macedo, 1976); Sex : this variable has been studied in most surveys : there is good evidence that the incidence of chronic cardiomyopathy is affected by the gender of the patients, but not the incidence of infection (Laraña *et al.*, 1956; Fornichon, 1975; Tonn *et al.*, 1978); Strain of *T. cruzi* (Lambrecht, 1965; Miles, 1981, 1983; Tibayrenc & Desjeux, 1983); Age : the prevalence rates of chronic chagasic cardiomyopathy increase by age. This observation has been made in all endemic regions (in Brazil) e.g. Prata, 1975; Maguire *et al.*, 1982; in Bolivia e.g. Romero *et al.*, 1977; in Chile e.g. Arribado *et al.*, 1979; in Venezuela e.g. Puigbo *et al.*, 1969; etc...). In older age groups a decrease of the prevalence rates has been observed in most areas, but in Bolivia a continuing increase seems to exist (Romero *et al.*, 1977; Lafuente, 1984); Duration of infection (Moleiro & Mendoza, 1980). Many others factors have been mentioned like Profession, Climate, Mechanism of infection (Romaña, 1961) and also Genetic Characteristics of the host, Diet and Nutritional status, Stress, Physical exercise (Garnham, 1980), Migration and Life conditions in Urban centres (Medrado-Faria *et al.*, 1984).

It is worth to observe that the factor smoking has not merited much attention. Recently it was shown by Hartz *et al.* (1984), that smoking is associated with cardiac wall-motion abnormalities and transmural myocardial infarction and that these associations are independant from the coronary stenosis-smoking association. Should there exist a smoking-*T. cruzi* infection interaction in the etiology and/or in the progress of chagasic cardiomyopathy ?

II. **Design issues of the classical epidemiological studies**

In recent epistemological epidemiological literature, particular attention has been paid to the essence of causality (e.g. Rothman, 1976). A distinction has been made between sufficient, necessary and contributary causes (Kleinbaum *et al.*, 1982); a sufficient cause has an effect on the development of chagasic cardiomyopathy, independent of the effects of other

causes; a necessary cause (like *T. cruzi* infection) is indispensable for the occurrence of the disease, and a contributary cause is not indispensable for the occurrence of the problem.

Chronic chagasic cardiomyopathy probably has many sufficient and contributary causes. In order to study the role of each of them, information on all those potentional causes is needed.

Studies of the determining factors are of a complex nature and ask for a well thought epidemiological design. In essence such a design has to be of an analytic nature. But very few analytic epidemiological studies have been carried out in the field of Chagas' cardiomyopathy.

One of the basic requirements of etiologic inference is that the action of a potential riskfactor (= the exposure) took place before the occurrence of the disease. The investigator has to be sure about the temporal sequence between exposure and outcome. A good analytic design should be free of temporal ambiguity, which can be avoided by a prospective follow up of the people exposed (= those in presence of the potential risk factors) before the disease occurs.

Prospective follow-up studies in Chagas' disease epidemiology

In the study of Chagas' cardiomyopathy, the people who are exposed (let us take as an example of exposure the infection by *T. cruzi)* and diseasefree (= without cardiomyopathy) at the beginning of the study, have to be followed up prospectively in order to quantify the occurrence of new cardiomyopathy cases. In order to quantify the role of the exposure, an unexposed identical group has to be followed too (= uninfected people from the same area).

Anyone who has carried out field studies knows how difficult it is to follow-up a population : people migrate and lose their motivation to cooperate. Given that the occurrence of cardiomyopathy is a rare event, either a big population has to be followed over a certain time, or a smaller population over a much longer timespan. In both situations the operational costs are enormous and the organization of such a cohort study is a real challenge; there is also the problem of selective loss to follow-up, and the data become less representative of the original studypopulation as the survey ages.

In fact very few follow-up studies have been carried out till today. The most important are : in Brazil : Bambui, Mambai, Castro Alves, São Felipe, Virgen da Lapa; in Venezuela : Belen and Eneal.

Recently a meeting was held in Salvador, Bahia, Brazil, to coordinate the longitudinal studies on Chagas' disease in existence or in planning. As a result of that meeting it may be expected that these longitudinal studies will be carried out with standardized designs and -criteria. Besides riskfactor determinations, longitudinal studies may have descriptive objectives too; then the major outcomes of the study are incidence rates (= N° of new cases / population-time of observation).

In a descriptive study the investigator describes the occurrence of cases by their classical characteristics of time, place and persons; in an analytical study the main preoccupation is causality. If a researcher tries to prove that a factor « X » (be it age, sex, race, ethnicity, reinfection by *T.*

cruzi, ...) is a riskfactor, and thus a sufficient or contributary cause of the disease, than he has to be sure that what is observed, results from the action of the factor « X » and not from the effect of some other factor « Y » that interacts simultaneously with « X » and with the disease-outcome. Such a factor is called a « confounding factor » and a major preoccupation of etiologic studies is to control for confounding, while confounding is not an issue in descriptive studies. The need to control for confounding obliges a researcher to have valid information on all potential confounding factors. This makes a prospective follow-up study with etiologic objectives operationally very difficult.

Case control studies in Chagas' disease epidemiology

A more easy way to get information on the association between exposure and occurrence of disease is through a case-control study. In a case-control design the researcher select the cases (here chronic chagasic cardiomyopathy patients) and as controls diseasefree individuals representative of the population from which the cases originated. The potential risk factors for the occurrence of the disease are explored in both cases and controls (*).

In order to get valid information on the temporal sequence between exposure and disease, only new cases of cardiomyopathy should be selected. Given that the occurrence of chagasic cardiomyopathy is generally quite silent, it is not feasible to get those cases by means of a registry, as is common practice in cancer research (Cole, 1980). Thus the whole population has to be examined regularly. This is an enormous task and only few research teams meet the operational and logistic requirements to carry out such a case-control study on incidence cases.

In fact, case-control studies on population based incident cases are rare, more common are case-control studies on hospital cases, thus on prevalent cases.

The requirement of lack of temporal ambiguity is the main reason why incident cases have to be preferred over prevalent ones, in the case-control design on chronic Chagas' disease. Prevalent cases can not provide the guarantee that the determining factors preceded the occurrence of the cardiomyopathy. It is a fact that a chronic disease has an impact on the behaviour of the affected person. Stress and migration to the city are good examples of factors which may induce chagasic cardiomyopathy (Medrado-Faria *et al.*, 1984), but may be also the consequence of the disease.

In case-control studies, recall bias are a major issue, because they may introduce serious distortions in the information on exposure factors. Recall bias is a more serious issue in the study of prevalent than of new cases.

(*) In the recent literature on Chagas' disease, the term « case control » design is also used when seropositive persons (called « the cases ») are being matched to seronegative ones (called « controls ») and the difference in the number of existing or new cases of Chagas' cardiomyopathy is being explored. In those studies the use of the name « case control » should be avoided because they are of a matched longitudinal or cross-sectional design.

Cross-sectional study design

The majority of the epidemiological studies on Chagas' disease are cross-sectional ones. The resulting measures of disease frequency are prevalence rates. Such rates are operationally easy to determine but difficult to interpret because they are composed of 3 parameters : incidence rates, migration rates and mean duration.

Suppose that in a cross-sectional study, the prevalence rates of chagasic cardiomyopathy remain identical from a certain age on; the following explanations for those stable rates could be put forward :

— the incidence rate = 0, the case fatality rate = 0, and the migration rate = 0;
— the incidence rate = the case fatality rate, thus the number of new cases is equal to the number of cases who die due to the disease; and the migration rate = 0;
— the incidence rate = migration rate and the case fatality rate = 0; there eg. is a selective outmigration of existing cases to the city, that is equal to the number of new cases;
— or any variation of those 3 parameters.

Without further information, it is difficult to figure out exactly what the dynamics of a given endemicity are, when only prevalence data are available.

For example : in many areas the chagasic cardiomyopathy prevalence rates of unselected rural populations show a decline in older age groups (e.g. in Castro Alves (Brazil), Maguire *et al.,* 1982; in Montero (Bolivia), De Muynck *et al.,* 1978). Selective mortality is the explanation commonly given, but selective outmigration could be as good an explanation. In the Bolivian Chaco such a decline in cardiac prevalence rates was not observed (Romero *et al.,* 1977) and the hypothesis of minor severity is being made, but lack of selective outmigration could also explain that prevalence picture.

For the study of the determinants of the chronic chagasic cardiomyopathy, the use of prevalent cases is also not desirable because the cases, prevalent at any point in time, are only the survivors (Cole, 1980). Thus in Chagas' disease a cross-sectional study design is not indicated when riskfactor determination is the prime concern.

III. **Alternative design**

The ultimate goal of an epidemiological study on chronic Chagas' disease should be the control of the problem. Therefore measures of disease frequency and insight in its determinants should be the result of the study. Descriptive and case-control designs cannot provide both outcomes simultaneously. Theoretically a follow-up study could produce both outcomes, but operationally it is extremely difficult to get information on all important riskfactors.

There exists now a hybrid design, proposed by Miettinen (1982) from Harvard University, and called Ambidirectional or Nested case control design.

Although hybrid designs are attractive from coststand point, they are considered by Anderson & Mantel (1983) as a mixed blessing from the scientific standpoint. In the following chapters it will be shown how the ambidirectional design is also scientifically attractive.

The principle of the ambidirectional design is to combine the benefits of both the prospective follow-up and the case-control design while avoiding their major inconveniences. The information gathered on all followed-up persons is kept to a strict minimum and only those persons who develop the incidence of interest as well as a representative sample of the candidate population (= the non cases), are explored in detail through a case-control technique. In this paper the ambidirectional study design will be adapted to chronic chagasic cardiomyopathy (CCC). But as an extension of the general principles this design can also be used in the study of :

— the rates and the determinants of the progress of CCC cases;
— the CCC case fatality and the determinants of death due to Chagas' disease;
— the incidence of the megasyndromes and of the neurological lesions in chagasic patients and their determinants.

The characteristics of this hybrid design are summarized in a table and are compared to the 3 basic epidemiological designs (Table 1).

TABLE 1

Characteristics of the main epidemiological designs on chronic Chagasic cardiomyopathy

Characteristics	Design			
	Cross-sectional	Follow-up	Case-control	Ambi-directional
Description of occurrence of CCC	yes	yes	no	yes
Descriptive parameters	PR	IR PR*	no	PR* IR
Generation of aetiologic hypothesis regarding determinants of CCC	yes	may be	yes	yes
Test aetiological hypothesis	may be	yes	yes	yes
Measures of association**	PRR PRD	IRR IRD	OR	OR PRR, PRD IRR, IRD
Possibility to explore multicausality	yes	no	yes	yes
Possibility to explore multiplicity of effects	yes	yes	no	yes
Danger of temporal ambiguity	yes	no	yes	may be
Validity problems :				
— selective intake of exposure groups	yes	no	no	no
— selective survival	yes	no	yes	no
— selective loss to follow-up	no	yes	no	yes
— selective intake of disease groups	yes	no	yes	no
— information bias	yes	yes	yes	yes
— confounding bias	yes	yes	yes	yes
Feasibility	good	low	good	good
Cost	+	+++	+	++
Main problem	interpretation of PR	loss to follow-up information on all risk factors at to	representati-veness of controls recall bias	loss to follow-up

* : prevalence rates determined at the to survey.
** : PRR = prevalence rate ratio; PRD = prevalence rate difference; IRR = incidence rate ratio; IRD = incidence rate difference; OR = odds ratio; PR = prevalence rate; IR = incidence rate.

A. *Initial survey* (at time t_0)

At the onset of the study a single population has to be defined, without reference to the studyfactors information.

In practice this means that at the onset of the study only is needed :

— a delineation of the study population (by means of a census and a mapping of the dwellings);
— information on the variables : sex, age, ethnicity, provenance and seropositivity (only necessary if initially specific prevalence rates are wanted);
— information on the CCC status of all people.

This initial survey can be carried out by auxiliary personnel, trained in taking blood samples and ECG's and in census techniques.

Prevalence rates of seropositivity and chronic cardiomyopathy by age, sex, ethnic groups and provenance are obtained; the rate ratio and rate difference of cardiomyopathy prevalence rates between seropositive and seronegative individuals can be calculated.

As such, this initial study coincides with a simplified version of a cross-sectional study.

The question of the minimal sample size of the studypopulation at time t_0 has to be raised. The required sample size depends on the expected rate difference between the seropositive and the seronegative individuals, the α error and the desired power. In order to get an idea of an order of magnitude, the minimal sample size of the study population should be estimated at some 2,000 people, if the follow-up duration is limited to three years only (panel surveys at t_1, t_2 and t_3), the difference of annual incidence rates is 2 per cent, α error $= 0.05$ and power $= 90$ per cent.

B. *Prospective follow-up study*

The objective is to detect all new CCC cases, therefore the whole study population (= non cases at the beginning of the study) has to be examined again at time t_1 (= one year after the initial survey). Because the development of new cases is generally silent, a registry alone is not a sufficient instrument for the detection of the incident cases.

At time t_1 the census has to be updated to know exactly the contribution in person-time of each person at risk. This is indispensable for the correct determination of the incidence rates. As a byproduct of this updating, general and specific mortality rates and also migration rates are being produced.

At time t_1 * only the people examined at t_0 should be checked, and no people who arrived in the area after t_0 should be included. This is a follow-up of a fixed cohort type.

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t_1* : study carried 1 unit of time lateron, eg. 1 year after the t_0 survey.

The original methodology of the ambidirectional design (Miettinen, 1982; Kleinbaum *et al.*, 1982) is also applicable to dynamic populations, allowing for the addition of new members during the follow-up period. But in newly arrived people prevalent CCC cases may exist, therefore a dynamic population approach is not suitable for the study of CCC. There is no need to repeat the initial serological examination at further follow-up sessions, because seropositivity does not change over time (Rassi *et al.*, 1969), and if the domiciliary infestation with triatomine vectors is being controlled, then the seronegative individuals will have a very low, if any, turnover to seropositivity, So even in the seronegative group there is no need to repeat the serological examination, if t_1 is not far away from t_0.

If at time t_1 not enough new cases are being detected, then another round of follow-up has to be planned, and a new updating of the census and an ECG examination of all people who are still CCC negative at time t_1 should be done. Such a new panelsurvey is carried out at time t_2, and if needed eventually at time 3 or even t_4, t_5.

The available data allow to determine the ratio and difference of the incidence rates of cardiomyopathy in seropositive and in seronegative individuals and also the etiologic fraction among the exposed (Miettinen, 1974). The etiologic fraction is the proportion of new CCC cases that originate among the seropositive individuals due to the fact that they are infected by *T. cruzi* (if infection by *T. cruzi* is examined as an exposure).

C. *Case-control study*

Once the incident cases are known at time t_1, then the study of their determining factors has to be carried out by means of a case-control design. For each new case of CCC, controls representative of the candidate population from which the case originated, have to be selected. A new case of CCC is defined as a person who was a non-case at t_0 and developed ECG lesions compatible with CCC in the interval between t_0 and t_1. In the choice of the suitable controls, a matching procedure may be employed. The objective of matching is to make the control group as similar as possible to the cases regarding the distribution of one or more potential confounders. The variables matched on have to be riskfactors, otherwise there is a danger of overmatching (Kupper *et al.*, 1981). In this CCC study the obvious variables to match on are : age, sex, ethnicity, serological status and neighborhood or residence. Matching has however the following negative aspects : there is a possibility of a loss of cases if no appropriate controls for each case can be found, furthermore every possibility to study the relationship between the outcome and the matching factors is lost, and there is also a loss of efficiency (relative to randomsampling) if the matching was done on factors that are not riskfactors.

Given that new CCC cases constitute rare events and that their number per unit of time is small, several controls per case are necessary. Miettinen (1969) has shown that a gain of 50 per cent in efficiency is obtained when 3 matched controls per case are used instead of one. Above 3 controls the relative gain in efficiency is mall relative to the cost. If no matching procedure is applied, then the controls should be obtained through randomsampling in a ratio of 3 controls for 1 case.

All the potential determining factors listed at the beginning of this paper should be explored.

As the number of individuals to be examined is quite small, they can be motivated to undergo indepth examinations like xenodiagnosis, 24 hours continuous ECG registration, a biochemical and immunological checkup and other appropriate examinations.

In the data analysis the factors not matched on at the design phase, have to be explored by odds ratio determination, while the control of confounding has te be done by stratification or by multivariate analysis procedures for matched data (Kleinbaum *et al.,* 1982).

If no matching was done at the designstage, the control for confounding has te be done by stratification, by multivariate statistical modelling (e.g. loglinear modelling, Reis Lopez *et al.,* 1982) or by using a confounder score (Miettinen, 1976).

IV. **Validity issues in the study of chronic chagasic cardiomyopathy**

One of the main preoccupations in observational studies is to avoid systematic errors in the effect measurements (in a CCC study : rate ratios, rate differences and odds ratios). Such a systematic error (or bias) occurs when there is a difference between what is actually being measured and the real effect measure. Bias can occur in the selection and the observation of the study objects and/or can be due to the impact of uncontrolled confounding factors. In an ambidirectional study design confounding bias will be controlled by getting the information on all potential determining factors followed by the application of appropriate statistical procedures in the analysis of data (stratified analysis, multivariate statistical modelling, stratification, matching).

Selection bias can occur in a prospective follow-up study when the follow-up of the initial cohort is not complete and when there is selective attrition of one exposure group eg more loss to follow-up of the seropositive than of the seronegative individuals. Both the ambidirectional and the prospective follow-up designs are subject to the possibility of selective loss to follow-up, therefore the observation period should be kept as small as possible and the emigrating people should be traced. Given that in an ambidirectional study design only a minimal effort is being asked from the participating people, it may be expected that the degree of refusal to participate will be lower than in a fullblown prospective follow-up study.

In the classical case-control design selection bias is *the* problem. In the ambidirectional design the controls are sampled from the same study population, thus the problem of selection bias is virtually inexistant.

Information bias occur when the measurements of the exposure and/or of the disease condition (CCC) are systematically inaccurate. Information bias due to differential anamnesis of cases and controls are more prone to occur in case-control studies on hospitalised patients than in the ambidirectional design in which the cases and the controls originate from the general population and the disease status can quite well be masked to the interviewer and even to the patient himself. A problem particular to Chagas' cardiomyopathy is the lack of observer reproducibility; there can

be misclassification in the following ways : a none case being classified as a CCC case or vice-versa and a misclassification of the serological status. The lack of reproducibility of the cardiological diagnosis has been estimated at 20 per cent (De Muynck & Romero, 1978) and is probably non-differential, therefore the obtained measures of association underestimate the real association. Consequently we may have confidence in the significant results, because it is known that in a situation of non-differential misclassification the real association is bigger than the observed one (Monson, 1981).

Anyhow it is best to avoid or to diminish this reproducibility problem by adhering to strict criteria of ECG reading and CCC compatibility, like the ones proposed by Maguire *et al.* (1982). These reproducibility problems are identical in case-control, ambidirectional and follow-up designs.

V. **Conclusion**

In the study of the occurrence of new CCC cases and of their determinants, the ambidirectional design has many advantages in comparison with the classical designs.

Simultaneously descriptive measures and etiologic information are gathered and as such this design has the basic outcomes of the cross-sectional and longitudinal studies. It has however also the same validity problems as those designs, although the loss to follow-up is probably less, relative to a fullblown cohort study.

The ambidirectional design has the same hypothesis exploring and testing potential as a case-control design, but it probably is less subject to recall and selection bias because the cases are new ones and the controls population based.

In the study of chronic Chagas' disease this ambidirectional uses the operational opportunities to full advantage and is thus quite efficient.

L'indication et l'utilité d'une étude « ambidirectionnelle » dans la recherche sur la maladie chronique de Chagas.

Résumé — Dans la maladie de Chagas les taux d'incidence de la phase cardiaque chronique ainsi que ses facteurs déterminants ne sont pas encore complètement connus quoi qu'un nombre considérable d'études épidémiologiques y ait été consacré. La raison principale de ce manque de connaissance se trouve dans les déficiences méthodologiques des « designs » épidémiologiques classiques. Il y a maintenant un plan d'étude, qui combine les avantages des études longitudinales prospectives et des études cas-témoins, tout en évitant leurs principaux inconvénient. L'article explique comment ce plan d'étude appelé « AMBIDIRECTIONAL design », peut être appliqué à l'étude de l'incidence de la forme cardiaque chronique de la maladie de Chagas et de ses facteurs déterminants.

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