CHAPTER VI

COHORT STUDIES

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The case-control study gave us the possibility of comparing healthy subjects and sick subjects in order to identify a possible link between the disease and a supposed risk factor. However, the methodological constraints are heavy, commensurate with the sophistication of the study, and the pitfalls are numerous. The list of biases is long… But these constraints are acceptable given the relative lightness of the logistics. This type of study is therefore suitable for rare diseases.

Unfortunately, one piece of important information is missing: the incidence of the disease. Compared to prevalence, incidence has an additional dimension, as it takes into account time. This is of great interest to the clinician. This notion of time makes it possible to estimate the probability of a subject encountering the event in question in the future. This is impossible with a case-control study since one starts from an artificially formed group of subjects presenting the event worthy of interest. *A new type of study is needed.*

As with the case-control study, this type of study is about risk, which provides important information to the clinician - and the patient - in terms of prediction, causation, diagnosis and prevention. This is the cohort study, with a suggestive name since among the Romans a cohort represented the tenth of a legion, i.e. 600 men, and this term lends itself particularly well to the idea of monitoring a large group of men marching towards an (uncertain) future.

The case-control study was most often retrospective, the cohort study is most often prospective. Both are observational, analytical, non-experimental studies.

Finally, the cohort study is the only one that allows us to understand survival and prognostic factors.

Cohort studies represent the most rigorous form of non-experimental epidemiological studies. They alone make it possible to evaluate the incidence of a disease, to establish a causal relationship between risk factor and disease with the least possible bias, to evaluate the latency time and the relative risk with precision maximum.

However, the accuracy and validity of the information provided are obtained at the cost of an often considerable investment in time and resources, and cohort studies, like all epidemiological studies, are subject to specific flaws that can taint their validity. Incidence indicates the percentage of new cases diagnosed over a period of time in a given population:

$$\text{Incidence} = \frac{\text{New cases diagnosed during a period p}}{\text{Total population considered}}$$

I - STRUCTURE AND IMPLEMENTATION OF THE STUDY

As opposed to case-control studies, the selection of subjects is no longer based on the presence or absence of the disease, but on the presence or absence of the supposed risk factor. The subjects are then followed over time, and the occurrence of incident events recorded (Fig. 1 and 2).

Fig. 1 - Cohort study structure:

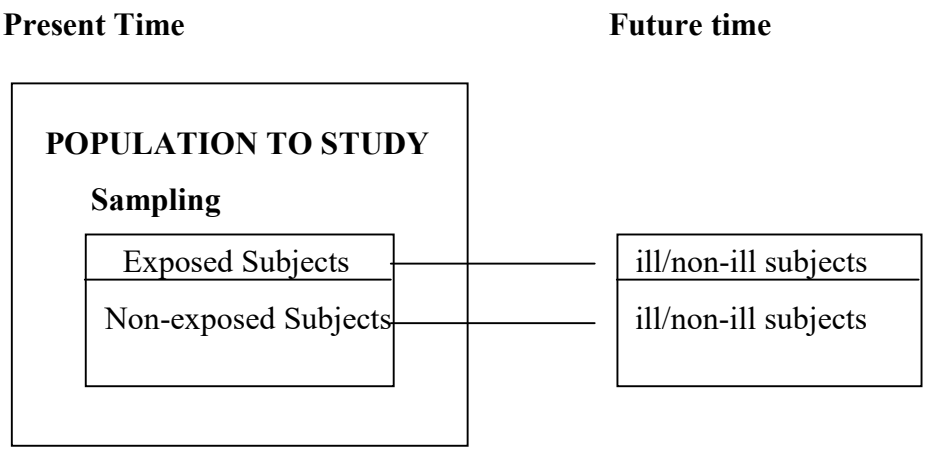
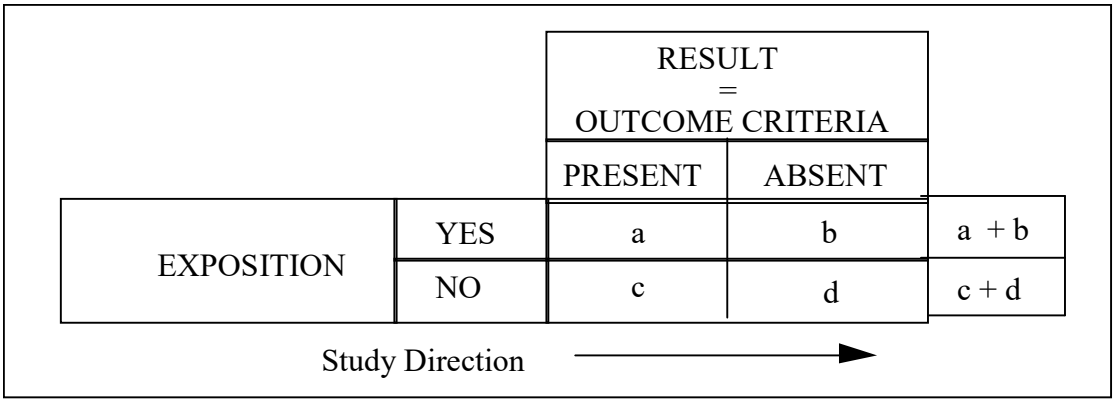
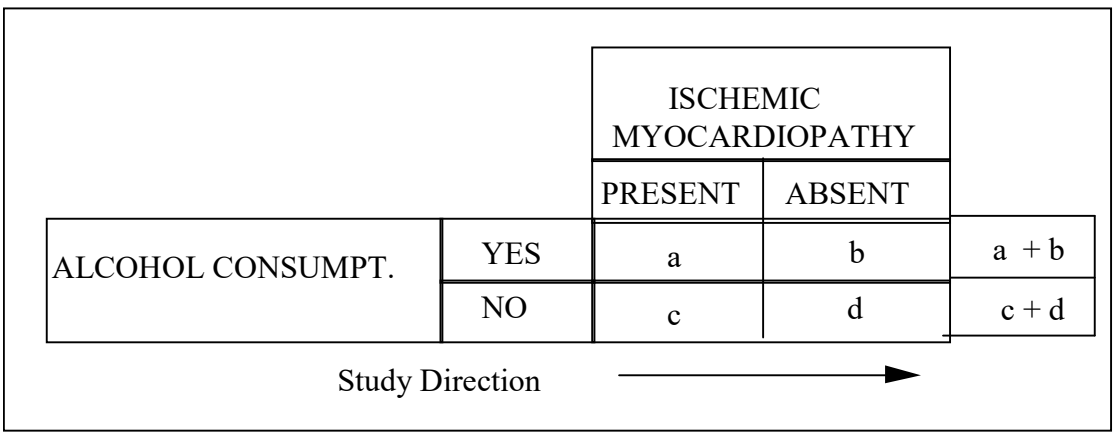


Fig. 2 - Representation of a cohort study by the 2 x 2 table: at the start of the study: (a + b) exposed compared to (c + d) unexposed





The steps are as follows:
- Precise definition of the question asked and of the population to be studied (general population, population living in a certain geographical sector, employees of a company, particular profession, specific social category, etc.).
- If the entire population cannot be studied, selection of a representative sample within this population. This sample, to be representative, must be drawn by lot, and each individual must have an equal chance of being selected. It is therefore necessary, as for prevalence studies, to have a precise list of the population studied.
- Precise definition and measurement of the assumed risk factor.
- Precise definition of incident events to be collected. Definition of the means of diagnosis implemented.
- Definition of the period of time during which the included subjects are followed. This duration depends on the risk factor, the latency period and the disease studied, and may in certain cases be very long (for example, cardiovascular diseases). The Framingham cohort has been followed for more than 40 years.
- Statement of the measures taken to avoid the loss of subject follow-up over time, the main pitfall of prospective studies.

The definition of exposition and disease, and therefore the recognition of exposed, unexposed, sick and non-ill subjects, poses the same type of problems as in cross-sectional studies or case-control studies.

Sensitivity, specificity, reproducibility, positive and negative predictive value of diagnostic criteria must be tested and measured. This is all the more critical as a cohort study is generally long, costly, cumbersome to conduct, and it is necessary to optimize its chances of success.

All these concepts, presented in the chapters 'Prevalence study', 'Case-control studies', 'Evaluation of a diagnostic test' apply to the first stages of the construction of a cohort study, with particular care given the heaviness of the study that we are going to undertake: these are the foundations of an 'epidemiological cathedral', which we want to be solid over time.

Subjects being classified on the basis of their exposure, it is necessary to include a large number of people in the initial phase to have, at the end of the study, a sufficient number of cases having contracted the disease during follow-up to put in evidence of an effect, if any.

Cohort studies are therefore studies involving a very large sample of the population. Following 100,000 people on a regular basis over several years represents a task of a certain magnitude…!

Example: Sir Richard Doll in Great Britain started a cohort study just after the Second World War focusing on the harmful effects of smoking. It included 100,000 doctors and followed them over the long term... that is to say, until today, which represents a follow-up of more than 50 years! He is the first to have highlighted the significant increase in the risk of lung cancer in smokers, with a relative risk of 6 compared to non-smokers. This cohort of course also allowed him to study the occurrence of other pathologies, but also, as we will see, the complications of other risk factors [1, 2].

II - POTENTIAL BIAS

Despite their apparent strength, cohort studies are also subject to the possibility of biases that can modify the results, present at each stage of the study.

A - Selection bias

Are the subjects included in the study truly representative of the population from which they come?

Example: The response rate to the various follow-up questionnaires sent to the 100,000 doctors in Richard Doll's cohort has always been close to 95%. In 1991, the author became interested in alcohol consumption in his cohort and its potential effects. The response rate immediately fell to 73% [3]. During the next questionnaire, again focusing on cancers, cardiovascular and pulmonary pathologies, the response rate rose to its usual values [4]. What happened ? Who are the 27% who did not answer a sensitive question, while answering the more socially and individually neutral questions? We can probably think that non-drinkers responded well, as well as -very-moderate drinkers. The inveterate consumers probably deemed it more prudent not to answer, while remaining faithful to the cohort otherwise, as the following questionnaires showed!

There is therefore very certainly a selection bias, and the cohort finally selected de facto by the non-responses is certainly not representative of the population that we proposed to study.

B - Misclassification bias

It exists when the subjects have not been classified in the category (exposed, not exposed, sick, not sick) which corresponds to them. It can relate to the exposure or to the illness that occurred during follow-up.

Two examples:

- In Richard Doll's study on alcohol consumption, it is likely that the -not very moderate- drinkers have... moderated their response, at least in the quantitative assessment of the alcohol ingested, and that some heavy drinkers have quite simply replied that they were drinking… absolutely nothing. This induces a bias of misclassification of exposure, which will affect the calculation of the relative risk of occurrence of pathologies complicating alcohol consumption [3].
- Anatomo-pathological diagnosis is often considered the gold standard for diagnosis. However, the analysis of the lesion can be difficult, including to differentiate malignant, dysplastic, or benign pathology. Reading the temporal artery biopsy for the diagnosis of Horton's disease (Giant Cell arteritis) is supposed to be easy. However, the kappa coefficient of reproducibility between two experienced pathologists does not exceed 86% for the recognition of a positive versus negative biopsy, and 70% for the recognition of the giant cells characteristic of the lesion and the disease, which leaves a significant percentage of potentially misclassified patients [5].

C - Loss of follow-up

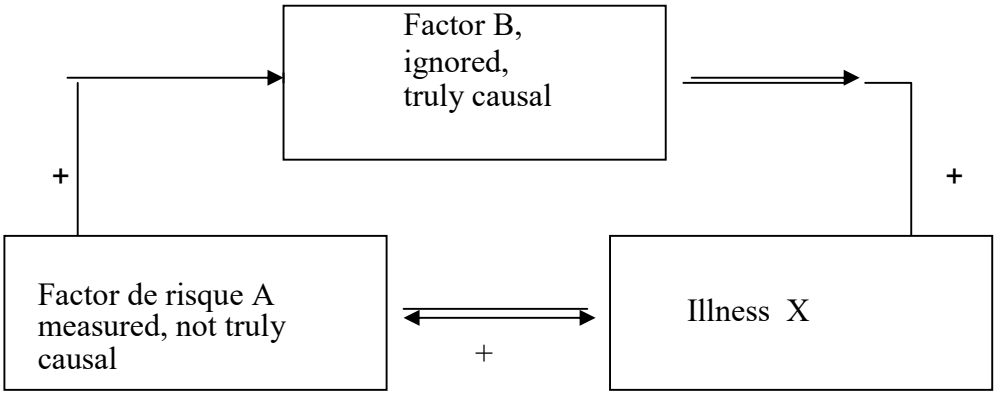
How to monitor the entire initial population over several years? People will move without leaving an address. Others will no longer answer questionnaires or show up for follow-up visits.

These lost of follow-up are not necessarily randomly distributed among the subjects initially included, but may belong more particularly to a subgroup (for example, exposed patients).

If this is the case, the conclusions of the study are distorted regardless of the precautions taken at the previous stages. If the rate of loss of follow-up exceeds 20%, the study is necessarily open to criticism. A rate of 15% probably leads to a number of discussions.

Example: 27% of subjects questioned at the start did not answer the 'Alcohol' questionnaire, and we can imagine that they are mainly among the "heavy drinkers" (very reasonable assumption). They are, in fact, 'lost to follow-up' for all that concerns the measurement of the incidence of alcohol-related pathologies, even if they will then answer questionnaires relating to different pathologies (let us not forget that these are doctors, knowing the risk factors and their consequences). How can the results of an otherwise well-conducted study be interpreted under these conditions? Was it possible to avoid the pitfalls, and what safeguards should have been foreseen before launching this part of the study? The answers are not easy to provide when one is interested in risk factors or pathologies marked by a certain social stigma.

D - Confounding factors (fig. 3)



As in case-control or cross-sectional studies, confounders are those factors associated with both the putative risk factor and the disease under study. They cause the disease, but are not apparent in the study and contribute to falsely attributing the causation of the disease to the supposed risk factor which is measured.

Example: again in Richard Doll's study, hepatic cirrhosis of course, but also hepatocarcinomas, cancers of the upper aero-digestive tract, accidents and trauma occur more easily in heavy alcohol consumers. Does this mean that alcohol as a molecule is really the causal factor of the disease? Unless it is undernutrition, often associated with alcoholism, or multiple deficiencies secondary to undernutrition, or even psycho-pathological disorders of which alcoholism is only the reflection or the consequence, or finally the putative factor genetic predisposing to alcoholism (?) which, to varying degrees, are the cause(s) of the observed complication?

If the potential confounding factors have not been predicted and measured alongside the supposed risk factor, how can their impact and their role in the genesis of the disease be assessed? How to control them?

Aren't there often confounding factors that are overlooked and therefore impossible to predict? In Figure 3, factor B is a confounding factor, not measured in the study, but actually explaining the observed positive association between factor A and disease X, as there is a chance association between A and B. The apparent association between A and X is therefore explained by a confusion bias.

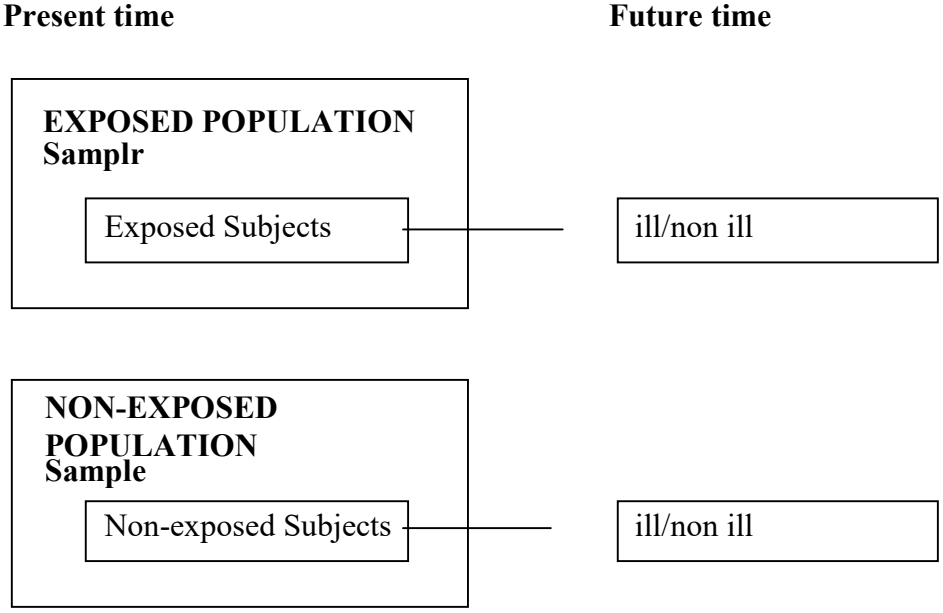
One solution is to try to control, in each study, all the foreseeable confounding factors by measuring them and taking their presence into account in the analysis of the data (stratification of the population studied according to the confounding factors, integration of these factors in a logistic or linear regression equation). Another solution is to repeat the studies in different populations, with different investigators. Finally, the concordance of the results of epidemiological studies with the results of biological studies (plausibility of the results) represents a third argument in favor of their validity.

III - STRUCTURAL VARIATIONS

A - Double cohort studies

When the risk factor is rare, even the inclusion of a very large sample of the population may not be sufficient to include the necessary number of exposed subjects. It is then necessary to use a double cohort (fig. 4) and carry out a double selection. A first sampling is carried out within the exposed population, and a second within the unexposed population. Two cohorts instead of one are then followed in parallel.

Fig.4- Double Cohort Study



The advantages of this double cohort are obvious: the number of exposed subjects is such that it guarantees a sufficient number of sick subjects if the exposure factor is truly a risk factor. This increases the chances of drawing informative conclusions.

However, there are drawbacks. The main one is that exposed and unexposed subjects no longer come from the same population, and may therefore differ by factors other than the exposure factor alone. This increases the risk of confounding factors, which, if unrecognized and uncontrolled, can lead to false conclusions.

Imagine a cohort study aimed at highlighting the carcinogenic role of asbestos, and comparing two cohorts assembled separately. The first is made up of miners working to extract asbestos, the second of employees of a large electrical construction company. Are these two cohorts similar with regard to other carcinogens, such as tobacco, the tobacco-alcohol association, the consumption of animal fats, etc.? On the other hand, aren't there among the employees of the electrical construction company a certain degree of exposure to asbestos through the handling of insulators, which risks underestimating relative risk? What are the factors of difference between the two cohorts not directly visible, likely to distort the results?

To avoid this bias and the resulting confounding factors, there remains the possibility of carrying out a single cohort study. In practice, however, how many people from the general population will it be necessary to include in order to have enough people exposed? Is this feasible and at what cost?

Double cohort studies therefore pose a problem similar to that encountered in case-control studies: that of the unforeseen similarity or differences existing between the two groups, which is at the origin of the presence of possible confounding factors.

B - Retrospective or historical cohort studies: registries.

So far, we have reviewed the design of prospective cohort studies. It is in fact possible to carry out, as paradoxical as it may seem, a retrospective cohort study.

For the structure of the cohort to be respected and for exposure to be measured before the onset of the disease, it is necessary that the exposure has been determined in the past in a precise manner (and not reconstituted in the present from memories subjects) and that all the subjects who were subjected to it (study population) are identified or identifiable.

In practice, it is often a cohort already formed during another study, for which the information initially collected included data on the risk factor that we want to examine a posteriori. It can also be a population perfectly defined at the outset, subject to a particular risk, correctly monitored and for which all incident events have been recorded. The best examples of this type are represented by the Scandinavian registers, which have systematically recorded for decades the risk factors, the incident pathologies, the results of additional examinations, and the treatments followed by the whole population of the countries considered (Norway, Sweden, Denmark). These registers make it possible to quickly obtain the answer to a question posed within historical cohorts, provided that the necessary information has been recorded initially.

Two Examples:

- The long-term consequences of gestational pathologies (pregnancy hypertension, pre-eclampsia, eclampsia) which may interfere with fetal growth and maturation are poorly understood, mainly because latency times can be extraordinarily long for certain consequences: while perinatal mortality or the prevalence of severe psychomotor disability is easy to measure, the finer and more distant consequences in terms of learning in childhood, adolescence or adult life are more difficult to grasp and require years of follow-up. On the other hand, it was possible to assess them thanks to the Danish Medical Birth Register, the Danish National Patient Register, and cognitive function measurements performed on conscripts at the time of their enlistment. The study found, in otherwise normal subjects, a slight mean decrease in IQ in young people who had been subjected to these neonatal risk factors, and a slightly larger, but significant, percentage of subjects with low IQ [6].

- Neonatal resuscitation has made great progress over the past 30 years, and children with a low Apgar score at birth have been able to be resuscitated and returned to a normal life. The Apgar score, however, reflects significant suffering during childbirth, and the long-term consequences, for the same reasons as those mentioned above, are not easy to assess. The same team looked into this question on the Danish registers, to come to the conclusion on a retrospective cohort with a 20-year follow-up, that an Apgar score of less than 7 at birth multiplied by 4 the risk of disability, long-term neurological condition, and by 1.33 the risk of a decrease in the intelligence quotient, while emphasizing that more than 90% of these children at 20 years of age were doing well and presented characteristics similar to those of the general population of the same age [7].

In the retrospective or historical cohort, the very structure of the cohort study is preserved: the exposure has indeed been determined beforehand, is well known, and is not subject to any recall biases. The incident events were then recorded, and the problem of the resulting exposure-disease time sequence does not arise. The population was well identified at the start, and it is thus possible to determine a true incidence.

Retrospective or historical cohort studies can be conducted quickly, at lower cost, and make it possible to combine the respective advantages of cohort studies (rigor, measurement of incidence, established time sequence, etc.) and the advantages of a case-control study (quick results, etc.), avoiding major pitfalls of one type or another.

Retrospective cohort studies, however, suffer from the same biases as prospective cohort studies, and often exacerbate them. The historical cohort on which they are based *was not designed for them*:

- the possible confounding factors of the new risk factor examined have not necessarily been recorded, and therefore cannot always be controlled;
- the selection biases may be different for the risk factor of the retrospective study, which does not then benefit from the "safeguards" put in place to limit the selection biases in the initial prospective study;
- the diseases of interest in the retrospective study may be different from those examined in the prospective study, and may have been recorded in the files with less rigor or accuracy. This accentuates misclassification bias. The structure put in place to ensure the best possible follow-up of patients and find the people lost to follow-up may be dependent on the diseases covered by the prospective study, and be less suited to the subject of the retrospective study designed secondarily.

The investigator of the retrospective study does not have the same quality control of the information collected as the investigator of the prospective study. He works on data already collected for another purpose, or on general data collected without a priori of their future use.

C- Patient cohort studies: study of prognostic factors and survival.

Cohort studies may start from exposed or unexposed healthy subjects, and measure the incidence of consecutive pathological events, but also start from sick subjects based on the diagnosis or any other significant event in the evolution of the disease, and measure the events arising there from recovery, to improvement, to natural or iatrogenic complications, to aggravation until death in relation, or not, with the causal disease. These patient cohort studies are built on the same model as the cohort studies of healthy subjects. On the other hand, they make it possible to analyze the prognostic factors, which may be different from the risk factors.

Example: postmenopausal estrogen therapy is a risk factor for endometrial cancer. On the other hand, once the cancer has declared, it is rather a factor of good prognosis: estrogen-induced cancers evolve better than non-induced cancers.

They make it possible to take into account potentially multiple confounding factors in the evolution of patients. Thus, certain risk factors for the disease studied may also be risk factors for events occurring during the course of the disease.

Example: Horton's disease is a vasculitis affecting medium-size vessels, and central vascular accidents (CVA) as well as myocardial infarctions (MyI) on inflammatory coronary have been reported during its development. However, Horton's disease is favored by smoking, as well as non-inflammatory atheromatous stroke and MyI. A double cohort study comparing patients and controls matched by age and sex, randomly drawn from the general population was able to highlight an increased risk of stroke and MyI during the first two years in cases, however in relation with smoking and classic cardiovascular risk factors in multivariate analysis, rather than with the inflammatory disease itself. The distinction is not only theoretical, because the therapies for inflammatory damage (corticosteroids) and for the prevention of complications of atheroma are radically different [8].

Some of these patient cohort studies do not have a control group to establish a comparison of survival: they can then use statistics obtained in the general population (which in France are provided by INSEE), to compare patient survival with theoretical survival, expected in the general population. Of course, this comparison technique does not allow the control of possible confounding factors or a detailed analysis of the prognosis, since there is no personal data collected in the comparison group. However, it makes it possible to analyze whether the group of patients evolves broadly, differently or not from the population of the same age and sex (regardless of the pathologies presented by this general population).

Example: Horton's disease occurs at an average age of 75, an age at which the incidence of cardiovascular diseases, such as MyI or stroke, is particularly high. Even without a comparison group drawn at random from the general population with control for confounding factors, it was possible to know, by comparing the survival curve of the patients with that of the general population, that the survival of the patients was similar to that of the general

population, for both men and women, despite the reported cases of extremely serious complications such as aortic dissections or ruptured aneurysms [9].

Finally, specific survival analysis techniques have been developed especially for patient cohorts, in particular the so-called relative survival analysis. It has long been taught that the prognosis of Hodgkin's disease, which presents a first peak of incidence between 20 and 30 years old, and a second between 60 and 70, was more severe in elderly subjects than in young subjects: indeed, the survival curves differ radically. Simply, the survival curves of young healthy subjects also differ radically from those of older subjects... and to say that the difference, in the case of Hodgkin's disease, was linked to a particular severity of the disease in the elderly subjects came back quite simply to ignore that de facto the older subjects were… older. The mathematical tool of relative survival analysis makes it possible to take into account, in this type of comparison, age-related mortality and to differentiate it from mortality related to the added disease. It was thus able to be shown, based on data from the Côte d'Or cancer registry, that the prognosis of colon cancer in elderly subjects was the same as in younger subjects, and therefore that the disease drove similarly. Here again, the debate is not only theoretical: if the form of the elderly subject is not more serious than that of the young subject, it should certainly not be treated more aggressively... at the prize of increasing the risk of iatrogenic complications [10].

D - Case-control studies included in cohort studies

It is possible, within the well-defined population of the cohort assembled beforehand, to carry out high-quality case-control studies based on cases recognized during follow-up. The controls are then selected from within the population of the cohort that has remained healthy, and the information on the risk factors studied obtained from the data recorded in the initial phase of the cohort study. This technique has several advantages:

1- The population of the underlying cohort is perfectly defined: consequently, the cases and controls can be drawn without selection bias, and be perfectly representative of the cohort: the validity of the results is considerably improved, since , in a case-control study, the results depend above all on the choice of controls (Cf. Chapter V).

2- The data used in the case-control study nested in the cohort study are those of the underlying cohort study, which were most often collected prospectively: this makes it possible to limit recall bias, or even to carry out the equivalent of a prospective case-control study.

IV - EXPRESSION OF RESULTS

Cohort studies make it possible to obtain a measure of the incidence of the disease and of the relative risk, that is to say of the count of new cases appearing during the follow-up of the cohort and a measure of the quantification of the risk in exposed people versus unexposed people. Here again, it is easier to express, in the form of a table 2x2, the results obtained at the end of the follow-up study (Table 1).

Table 1 - Relative risk computation in a cohort study; horizontal reading of the 2 x 2 table

	Ill	Non ill	
Exposed	a	b	a + b
Non exposed	c	d	c + d
	a + c	b + d	a + b + c + d

$$\text{Incidence} = \frac{(a + c)}{(a + b + c + d)} \text{ / year}$$

Let Inc-exp be the incidence in exposed subjects: $\text{Inc-exp} = \frac{a}{a + b}$ / year

and Inc-non the incidence in unexposed subjects: $\text{Inc-non} = \frac{c}{c + d}$ / year

The relative risk (RR) is the ratio of the incidence of disease in exposed subjects to that in unexposed subjects. It answers the question: how often are exposed subjects more likely than unexposed subjects to contract the disease?

$$RR = \frac{\text{Inc-exp}}{\text{Inc-non}} = \frac{a\,(c + d)}{c\,(a + b)}$$

It is also possible to calculate the number of cases, in exposed subjects, which are actually due to exposure. It is indeed reasonable to postulate that the cases occurring in the exposed population group sum up the cases which would have occurred there spontaneously, as in the non-exposed population, and the cases secondary to the exposure. The incidence "attributable" to exposure is obtained by the difference between the incidence in the exposed population and the incidence in the unexposed population.

$$\text{Inc-attr} = \text{Inc-exp} - \text{Inc-non}$$

It is possible to calculate from the number of cases actually due to exposure, the excess risk run by the exposed subjects compared to the general population (value different from the relative risk, calculated from all the cases occurring in subjects exposed).

In practice, it is necessary to take into account, in the calculation of the incidence and of the relative risk, the multiple imperfections of the cohort. There are indeed subjects lost to sight, potentially at risk of contracting the disease, and subjects who, either because they die or because they contract the disease, are no longer at risk. The rough formula given above must therefore be corrected according to the type of cohort followed and the modifications it undergoes over time.

V - ADVANTAGES AND WEAKNESSES OF COHORT STUDIES

A- Advantages

- They establish the sequence of events.
- They are not subject to most of the biases affecting case-control studies:
  - selective survival bias,
  - retrospective measurement bias of risk factors (recall bias),
  - matching bias (matching factor associated with the risk factor studied).
- They allow the calculation of incidence, relative risk and other variables assessing the risk incurred by the exposed and unexposed population.
- They are particularly suitable for the study of common diseases.
- They are the only ones able to analyze the survival of patients as a function of time, and to allow a comparison of this survival between groups of patients, or with the population

B - Weaknesses and disadvantages

- They require the inclusion of a large number of subjects in the initial phase.
- They are not suitable for the study of rare diseases, for which the number of subjects initially included would become prohibitive.
- They are long and very expensive.
- They are therefore not exploratory studies: the hypotheses tested must have acquired their scientific basis beforehand through lighter studies.
- They remain subject to the potential existence of selection bias, misclassification, or bias related to confounding factors.
- They are exposed to the possible presence of bias linked to the loss of follow-up of the subjects included.

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