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1 Principles of Epidemiology

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Abstract

Epidemiology is one of the pillars of public health and contributes to guide decisions regarding policies and evidence-based medical practice. Commonly defined as the study of the distribution and determinants of health-related states and events (not just diseases) in specified populations, epidemiology, through the application of its principles, not only allows to study the use and effects of drugs in the population (effectiveness), but in the context of economic evaluations it's an important reference for the determination and planning of the allocation of necessary resources and for the definition of priority research objectives.
In this Chapter, the fundamental principles of epidemiology are discussed: from the classification of study designs (observational and experimental), their different applications and development methods, to epidemiological measures for the analysis and interpretation of data derived from the studies (measures of occurrence and association). The Chapter ends with a section dedicated to regression analysis: a statistical tool used to evaluate how certain factors can influence the outcome of interest.

1.1 Introduction

Epidemiology can be defined as the study of the distribution and determinants of events that are health-related in a specific population [Last, 2001].

Usually the epidemiologist works for:

- Identifying and controlling causes of a given event, such as death, disability, morbidity;
- Studying which behaviors and factors improve health status;
- Supporting clinical medicine in studying some diseases;
- Estimating the burden of disease in the populations with the aim of suggesting better organizational actions to decision makers;
- Assessing efficacy and efficiency of healthcare services.

1.2 Study Design

Epidemiological studies can be classified as observational (non-experimental) or experimental (Table 1.1).

The observational, or non-experimental, studies involve research that does not intervene in the relationship between exposure and outcome. The observational studies are further divided in descriptive and analytical studies. Descriptive studies can describe:

- Time: data on the basis of different time of analysis;
- Place: data stratified on the place of collection or where the event occurred;
- Person: cases according to characteristics of individuals.

Experimental studies are carried out with the aim of finding possible associations/relationships between the outcome of interest (the dependent variable) and possible risk factors for that outcome (exposure/independent variables). The key difference between observational and experimental studies is that only in this last one the casual assumption can be testified through the experiment.

Analytical and experimental studies are described in the following paragraphs.

Non-Experimental Studies

In general, non-experimental studies are purely observational and the results are descriptive. For example, researchers may investigate on the average age, sex, most common diagnoses, and other characteristics of a group of pediatric patients. In such studies, the research question focuses for example on prevalence rates, rather than causality. They may propose some associations, but cannot effectively prove them. Most non-experimental designs are retrospective. Because of retrospective nature, manipulation or randomiza-

Observational studies		Experimental studies
Descriptive studies	Analytical studies	
• Time • Place • Person	• Ecological studies • Cross-sectional studies • Case-control studies • Cohort studies	• Clinical trials (randomized/non-randomized) • Community trials • Field trials

Table 1.1. Types of study.

tion is not possible. Therefore, often the outcome has occurred before study initiation. Example of non-experimental studies are cross-sectional studies and cohort studies.

Cross-Sectional Studies

Cross-sectional studies are also called "prevalence studies" since they measure the prevalence of diseases or other attributes at a certain moment. In this study design, data concerning effect and exposition are detected at the same moment. Therefore, they provide a description of the frequency and the characteristics of a disease within a population. Cross-sectional studies are very useful in the preliminary steps of new investigations: They are easy, cheap, quick, and valid to highlight association with steady expositions in time, such as ethnic group, gender, or prolonged smoking habit.

As first step for the implementation of a cross-sectional study, a time frame (a point in time or a time period) must be identified. Afterwards, a sample of individuals from the target population must be detected: These represent the study group for the statistical analysis. Finally, researchers investigate the presence or absence of exposure and disease for each subject. As doing so, four different categories are identified:

- Persons who have been exposed and have the disease;
- Persons who have been exposed but do not have the disease;
- Persons who have not been exposed and have the disease;
- Persons who have not been exposed and do not have the disease.

Table 1.2 shows the four possible patterns of disease and exposure.

At this stage, it is possible to evaluate the patterns of exposure and disease. For example, to calculate the exposure rate, the ratio between exposed ill individuals and all the ill individuals [a / (a + c)] could be compared with the ratio between healthy exposed individuals and all the healthy individuals [b / (b + d)]. Therefore, it is possible to evaluate any significant association between the exposure and the outcome by using the Chi-squared test.

Subsequently, the strength of association between the exposure to a generic risk factor and the considered disease can be explored by calculating

Exposure status	Disease	No Disease	Total
Exposed	a	b	a + b
Unexposed	c	d	d + d
Total	a + c	b + d	a + b + c + d

Table 1.2. Patterns of exposure and disease among the population.

the Odds Ratio (OR) (see the dedicated paragraph in the section 1.5 for the full explanation of the odds ratio).

However, it is important to highlight that, in addition to detect specific information about the prevalence of a disease in a specific time, cross-sectional studies might be useful in settings where changes in exposure do not occur over time. Therefore, cross-sectional studies are usually performed before planning new interventions in order to establish health needs and priorities within a population. Also, repeated cross-sectional surveys in a defined population can provide information on the trend of a health problem and, for this reason, cross-sectional studies are sometimes called "trend studies".

Hence, cross-sectional studies are widely used since they have many advantages:

- They are quick and cheap;
- They can be used to investigate multiple exposures and diseases;
- There is no need to follow-up;
- Results can be inferred to the general population;
- They can provide valuable information about etiologic hypothesis and can help to highlight health needs of a population.

On the other hand, many are the disadvantages of cross-sectional studies due to a limitation: They do not consider events over time. With a specific focus on pharmacoeconomics, cross-sectional studies might be useful to assess the prevalence of the disease within the population, but they cannot be used for example for the approval of new drugs. At the same time, they are not useful to investigate rare exposures, rare diseases, or diseases with short duration (for the latter case-control studies are suitable) and have limited value to assess causal or temporal association between exposure factors and outcomes.

Cohort Studies

The cohort study is an investigation during which the researcher simply observes what is happening in the population and records the new cases of the studied disease. It is aimed at determining the incidence rate of a disease in an entire population or in a subpopulation (exposed-unexposed) of individuals or patients, and to assess whether it is higher in the exposed or unexposed group to a certain factor. A research protocol defines the recruitment criteria of the study population.

The type of cohort that the researcher wants to study determines the eligibility criteria of patients in the study, which may be related to demographic information, comorbidity, previous conditions, or diagnostic tests.

Depending on the time, these studies can be defined as "prospective" (the most used) if data are collected from today to the future or "retrospective" (historical cohort) if the study starts in the past (the researcher knows the amount and duration of exposure in the past and the period of follow up) and ends in the present (when the researcher performs the study).

Usually, the cohort is recruited from a source population, without necessarily having the studied disease or is formed by sick patients that are followed until the occurrence of a specific outcome (such as death in cancer patients). Based on exposure, individuals are assigned to subgroups, which generally are: exposed or unexposed to a certain factor (Table 1.3).

Individuals observed until the end of the study may not develop the disease or other outcomes under the study: this event is called "censoring". Censoring may also be due to individuals leaving the cohort for known or unknown reasons or subjects dying during the follow-up period (if death is not the outcome of interest and occurs before the outcome of interest has occurred). Even if an individual observation is censored, the individual contributes with his or her observation time to the cohort.

Individuals who develop the disease or condition under study during the follow-up period are referred to as "uncensored".

In these time-to-event cohort studies, it is possible to calculate the incidence rate (new cases of the disease, divided by the sum of the follow-up period for each individual in the cohort) and cumulative incidence (new cases of the disease in the follow-up period, divided by the total number of individuals at risk of developing the disease at the starting of the follow-up). Moreover, in a prospective cohort study another analysis can also be applied: the "Relative Risk" (RR) or "Risk Ratio", which is the ratio of the incidence rate in the exposed group and the incidence rate in the group of unexposed. A complete explanation of the relative risk, that needs also to consider the confidence interval, is provided in paragraph 1.5.

Generally, the possible presence of confounding variables can be controlled through the multivariate analysis, which allows to estimate the relative risk eliminating the effects of confounding factors.

Exposure status	Disease	No Disease	Total
Exposed	a	b	a + b
Unexposed	c	d	c + d
Total	a + c	b + d	a + b + c + d

Table 1.3. 2×2 contingency table.

In addition to the time-to-event cohort studies, there are also cohort studies in which a precise outcome is not expected, but specific parameters are evaluated, such as the monitoring of blood glucose in a population of diabetic patients over time.

Cohort studies have the disadvantage of being very expensive and requiring very large study populations and/or very long follow-up time in the case of rare diseases. On the other hand, they are considered the most robust studies after the RCTs and superior in quality compared to case-control and cross-sectional studies because they provide more reliable results and allow to study several outcomes and several exposure-disease associations. They also allow to assess the attributable risk, that is the proportion of illness among the exposed that can be attributed to exposure to the risk factor and can be avoided if the exposure was eliminated.

Experimental Studies

Experimental epidemiology differs from observational epidemiology because the aim is not just observing but creating a modification of health outcomes through experimental interventions [La Torre, 2010].

In these studies, the researchers make an intervention (e.g., administration of a drug, a surgical treatment, a diagnostic procedure) in accordance with methodological criteria (randomization, blinding) to test hypotheses or assumptions. The experimenters measure the safety and the efficacy of the health intervention (on specific health outcomes in a population of patients or individuals).

In order to perform the intervention, the study must receive the ethical approval; the verification of the existence of these assumptions is performed by ethics committees composed of experts who assess if the protocol of the experimental study fulfils the ethical requirements.

There are generally three types of experimental studies categorized as follows:

- Clinical trials, that evaluate therapeutic interventions on one or some groups of patients;
- Field trial, which is a field experimentation of primary prevention interventions on healthy subjects;
- Community intervention trial, that consists of preventive interventions on entire communities (as water fluoridation).

Randomized Controlled Trials (RCTs) represent the "gold standard" in the evaluation of effectiveness of a health intervention which can be preventive,

therapeutic, or rehabilitative. Clinical trials foresee an active intervention and measure their effects in the follow up period, thus are prospective studies.

Randomized Studies

The Structure of an Experimental Study

An experimental study must be designed and structured according to a rigorous and correct methodology.

Consolidated Standards of Reporting Trials—CONSORT Statement "is an evidence-based, minimum set of recommendations for reporting randomized

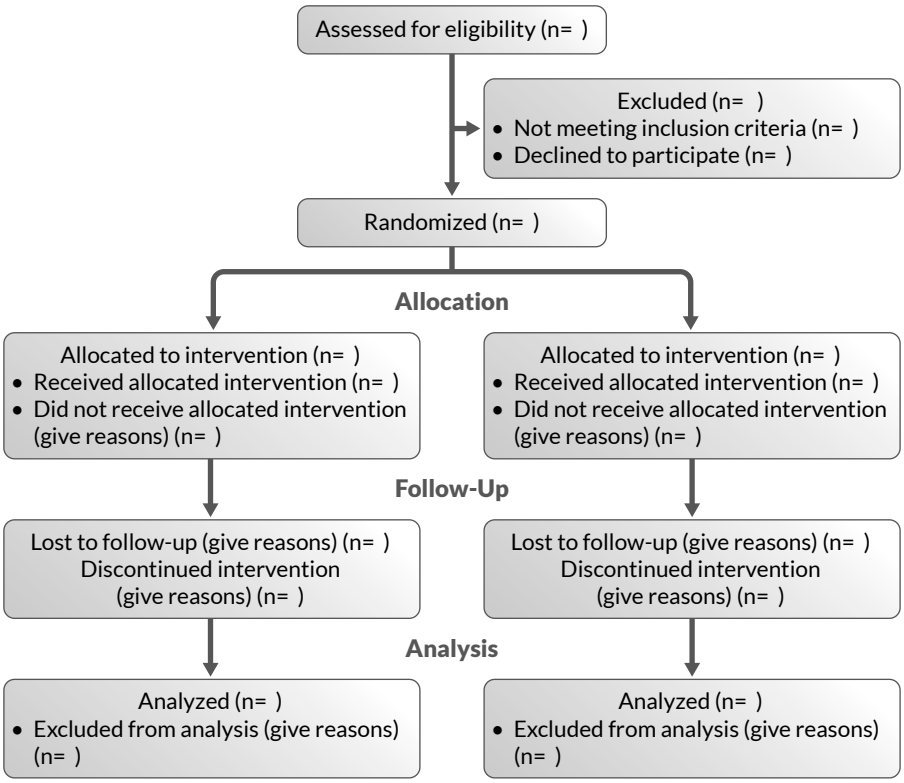

Figure 1.1. CONSORT 2010 Flow Diagram [CONSORT group. CONSORT 2010 Flow Diagram, 2010].

trials. It offers a standard way for authors to prepare reports of trial findings, facilitating their complete and transparent reporting, and aiding their critical appraisal and interpretation".

The methodological phases of a controlled clinical experimentation are the following: definition of the study population through clear inclusion and exclusion criteria, selection of participants based on inclusion criteria, request to potential participants of informed consent, randomization procedure, follow-up, and analysis of data. At the beginning of the study, it is fundamental to define the outcome of interest. Outcomes can be qualitative (as decrease of symptoms) or quantitative (e.g., laboratory parameter, mortality). Depending on the outcome, the result of a study, as well as the possibility of comparing studies, may vary.

CONSORT Statement is accompanied by the explanatory document that facilitates its use. CONSORT flowchart (Figure 1.1) synthetizes the phases that structure an experimental design [Schulz, 2010]. The items to include when reporting a randomized trial are presented in a specific checklist reported in Table 1.4. Additional information is available at the website www.consort-statement.org.

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomized trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	

follows >

> followed

Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomization:			
• Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomization; details of any restriction (such as blocking and block size)	
• Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
• Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	
	13b	For each group, losses and exclusions after randomization, together with reasons	

follows >

> followed

Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analyzed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All-important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

Table 1.4. CONSORT 2010 checklist of information to include when reporting a randomized trial [CONSORT group. CONSORT 2010 Checklist, 2010].

Methodological Phases

The first methodological phases of an experimental study are aimed at selecting the study population and enrolling patients in the study. The inclusion criteria depend on the specific objectives and clinical questions of interest. Eligibility criteria, i.e., inclusion and exclusion criteria, must be clear and well defined: These requirements help to make sure that patients in a trial are similar and comparable to each other in terms of age, type and stage of disease, general health, and previous treatment, although the specific objectives and clinical questions of interest may preclude external validity (application of results to populations other than the population from which participants are drawn for the study) or generalizability to the whole population. For example, exclusion criteria may be "exclude patients with prior myocardial infarction" which will limit external validity and generalizability of the study.

When all participants meet the same eligibility criteria, researchers are given greater confidence that results of the study are caused by the intervention being tested and not by other factors. If the individual satisfies the inclusion criteria, the informed consent will be asked and is mandatory. It is a document that must be prepared with extreme care and give to patients before enrollment. It contains all the elements to decide whether to join the experimentation.

The Randomization Processes

The randomization process is the random allocation of individuals to treatment or to control group. Study population should be equal and comparable except for treatment. Consequently, the case will decide whether individuals will be enrolled in the intervention group or not.

The following types of randomizations can be applied:

- Simple randomization (causal number table);
- Balanced blocks randomization: Blocks numbers referred to sequence of randomization are assigned to patients. For example, in case of two treatments, A and B, possible sequences for a block of 6 patients are ABABAB, AABBAB, BBAABA, etc.;
- Stratified randomization, in which the participants to the trial are divided in subgroups based on clinical characteristics that are thought to have an influence on the outcome under study. After that, the strata are randomized.

The Blinding

Randomization reduces the influence of confounding factors or biases during the phase of allocation. However, it does not control confounders during the intervention's phases. Instead, blinding allows to control for confounders post-randomization. Among the confounders, there are:

- The selection bias: The assignment of patients to groups of intervention may be affected if the researcher knows the physical and non-physical conditions of these patients. Blinding allows the concealment of group allocation from one to more individuals: patients, practitioners (surgeons, physicians, nurses, etc.), data collectors, and statisticians. Consequently, the blinding allows eliminating the selection bias;
- The performance bias, i.e., affecting the intervention (care, attention...) assigned to subjects in different arms. This is also referred to as "co-intervention" which, in unblinded studies, can influence the outcome measures between groups;
- The detection bias, limited by blinding, as the outcome assessors are kept unaware about allocation status of individuals. Therefore, the investigators will be not influenced in measuring outcome variables by the assignment to different groups [Renjith, 2017; Karanicolas, 2010].

There are three kinds of blinding:

- Single blinding, e.g., patients with non-familiar dyslipidemia patients can be randomized to receive oil with high- or low-content of polyphenols: The physicians who give the oil are aware about the kind of treatment, while patients do not know what are receiving. In this case, the blinding allows that both patient with severe or mild dyslipidemia have the same probability to receive both treatment;
- Double blinding, in which neither the doctor nor the patient knows whether the patient has been assigned to the treatment or the control group. An example may be a study about treatment of essential hypertension with beta-blockers versus thiazide diuretic, where only the researcher is aware of the kind of treatment, while neither the patient nor the doctor knows it;
- Triple blinding, which, in addition to the double blinding, also who interprets the results or who makes the statistical analysis is not informed about allocation of patients.

Analysis of Data and Interpretation of Results

After the schedule follow-up of intervention and control group, the last phase is the analysis of data. Through the analysis of results, it is possible to

assess if a certain health outcome is causally related to the intervention; this is the key difference with observational studies, which analyze associations between exposition and outcomes.

Collection of data may be occurring periodically throughout the follow-up period. For dichotomous outcome variables, percentage of observed events can be calculated in the randomized treatment group under study (Experimental Event Rate—EER) and for the control group (Control Event Rate—CER), the OR (with a 95% confidence interval), the Absolute Risk Reduction (ARR), the Relative Risk Reduction (RRR) and the number of patients that must be treated in order to prevent an event (NNT = 1 / ARR).

The statistical analysis is conducted using univariate techniques. The chi-square test is used to analyze difference between groups for qualitative variables. Contingency table with two rows and two columns is constructed and then chi-square test or Fisher's exact test are used. The condition of applicability of the test before, proceeding with the calculations, is that none of the four of contingency cells have an expected frequency lower than 1. A maximum of 20% of cells is accepted with an expected frequency lower than 5. If the condition is not satisfied, the Fisher's exact test is used instead of chi-square test.

Considering the measure of differences between groups of quantitative variables, parametric (Student's t, ANOVA) or non-parametric tests (Mann-Whitney, Kruskal-Wallis) are used.

Another kind of analysis is the "time-to-event" (e.g., Kaplan-Meier survival analysis), which is a key method used to compare survival probabilities, death rates, and rates of remission or cure.

The survival analysis is used when the endpoint measures the time from randomization to a well-defined event that can be positive (end of hospitalization, diminish symptoms) or negative (death, stage of disease). The analysis compares the survival curves in the different groups and determines if the treatment had an effect in decreasing or prolonging the incidence of an event.

Non-Randomized Studies

In non-randomized control trials (quasi-experimental studies), the participants are not assigned by randomization to different treatment groups, but may for example choose group, or they may be assigned by the researchers in a nonrandom fashion.

In these studies, it is important that the researchers be able to identify and control potential confounders using multi-variable analysis to minimize the

risk of bias. The principal disadvantage of nonrandomized designs, in fact, is the potential for bias, often unpredictable, deriving from confounding. Such designs can never ensure that unmeasured variables do not account for the apparent treatment effect. For this reason, the results of nonrandomized trials must be evaluated in a setting.

Despite their limitations, nonrandomized studies are sometimes the only ethical design to conduct an interventional or experimental investigation. If the treatment is potentially harmful, it is generally unethical for an investigator to assign people randomly to this treatment. For example, in a study on the effects of unhealthy diet, it is not possible to assign subjects to one group. Researchers can only compare populations with unhealthy diet with those with healthy diet. Furthermore, nonrandomized studies are usually less expensive, because they do not require the extensive planning and control of randomized studies. Therefore, nonrandomized studies are particularly attractive in the early stages of a research.

Systematic and Narrative Reviews

The Evidence-Based Medicine (EBM) is the process of systematically reviewing, appraising, and using clinical research findings to aid the delivery of optimum clinical care to patients [Sackett, 1997; Sackett, 1996; Rosenberg, 1995; Evidence-Based Medicine Working Group, 1992].

In the world of EBM, a systematic review can be seen as a review of a clearly formulated question that uses systematic and explicit methods to identify, select, and critically appraise relevant research and to collect and analyze data from the studies that are included in the review [Lunet, 2010]. In other words, this study design uses all the existing research and is sometimes called "secondary research" (research on research) [Glass, 1976].

The systematic review requires focused clinical questions, and usually the PICO is a useful tool for formulating such question. "P" stands for Patient, Population or Problem: The characteristics of the patients, the population, or the diseases under study are specified. "I" concerns the Intervention (or exposure) and describes what to do with the patient in terms of treatment, diagnosis, or observation. "C" refers to the Comparison, i.e., the comparator to the "I" (a different intervention or a placebo). Finally, "O" concerns the Outcome, in terms of morbidity, mortality, and other effects.

Sometimes, within a systematic review, it is possible to perform a meta-analysis, i.e., a statistical analysis of the results from individual studies, which generally aims to produce a summary estimate of the effect under study.

As a matter of fact, a meta-analysis can be carried out in any set of individual studies considered combinable, even if assembled without a comprehensive search of all potentially relevant articles, with the aim of answering a specific question.

It is important to underline that a meta-analysis does not change the poor quality of the data presented in the single studies, thus the statistical combination of biased or confounded estimates can give invalid estimates with spurious precision.

The meta-analysis can be seen as a two-stage process. In the first stage, getting the estimates from each study is needed. In this stage, data analysis is made by adding up the numbers from the studies as if they were all from a single large study. In the second stage, a combination of the study-specific results into a summary measure is performed, using a weighted average of the results from each study.

There is a tool that aims to help authors improve the reporting of systematic reviews and meta-analyses. This is a checklist called "PRISMA Statement" (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). It is an evidence-based minimum set of items for reporting in systematic reviews and meta-analyses [PRISMA Statement].

Furthermore, there is another "secondary research": narrative review. It differs from systematic reviews in several ways. While a systematic review is based on a protocol, thus it can be replicated, a narrative review generally tends to be descriptive, does not involve a systematic search of the literature, and thereby often focuses on a subset of studies of a topic chosen based on availability or author selection. Narrative reviews are more prone to selection bias and often do not even meet central criteria to help minimize biases. However, these are typical review articles that can be found in most journals.

1.3 Bias in Epidemiology

Bias is a systematic error that can occur in design, conduct, or analysis of a study. Bias can result in incorrect estimates of the true effect of an exposure on the outcome of interest. The systematic deviation from the true value (bias) can result in either underestimation or overestimation of the effects of an intervention.

Biases may be categorized according with two groups: selection biases and information biases. They are further described below, together with their bias subtypes.

Selection Bias

Ideally, with randomization, all participants in a study have the same opportunity to be allocated or assigned to each of the study groups. Selection bias is a systematic error relevant to the selection of the study population. Selection bias occurs when sample selection does not reflect the target population. The result is a non-representative sample selected specifically excluding certain groups from the research, whether intentionally or unintentionally.

Types of selection biases include: **the healthy worker effect, non-response bias, and voluntary response bias**.

Healthy Worker Effect

The healthy worker effect is a particular type of selection bias that occurs when, for example, the effects of occupational exposure to asbestos are studied and just employed persons are enrolled in the study. McMichael in 1976 first gave this definition to refer "to the consistent tendency of the actively employed to have a more favorable mortality experience than the population at large" [McMichael, 1976]. Goldsmith noticed that most industrially employed cohorts should be expected to have better life expectancy than unemployed persons [Goldsmith, 1975]. The most vigorous occupations had a relatively lower mortality rate when compared with the death rate in occupations of an easier character or among the unemployed population.

This bias results in considering just a specific subset instead of the whole, rendering the sample unrepresentative of the whole population.

Non-Response Bias

Non-response bias occurs when the characteristics of the respondents differ in meaningful ways from those of the non-respondents. For example, let's say that Sam hands his survey out to 100 people in the cafeteria at his college. But only 45 people choose to participate in the survey, leaving 55 people that did not respond. The people that choose not to respond to the survey have certain characteristics that will prevent Sam from inferring parameters about the whole population and creating a representative sample.

Voluntary Response Bias

Voluntary response bias occurs when members of a sample choose to respond or participate in the research. These individuals may have some similar characteristics, which would also make this group a biased and non-representative group for his research. For example, a subject may volunteer

a response because an interest in the research or because have a particular opinion (negative or positive) on the topic. These individuals may have some similar characteristics, which would also make this group a biased and non-representative group for his research.

Information Bias

Information bias, also called "measurement bias", is a distortion in the measure of association that arises when study variables are inaccurately measured or classified. Errors in measurement may result in "misclassifications" and the relevance of resulting bias depends on the type of misclassification. Misclassification occurs when subjects are assigned to a different category than the one they should be in. This may result in incorrect associations between the assigned categories and the outcomes of interest. Misclassification may be non-differential or differential. Non-differential misclassification occurs when the probability of individuals being misclassified is the same across all groups. Differential misclassification occurs when the probability of being misclassified differs between groups [Porta, 2014]. Therefore, differential misclassification occurs when the information errors differ between groups. The bias is different for exposed and non-exposed, or between those who have the disease and those who have not. Misclassification can be a result of incomplete medical records, recording errors, or misinterpretation of records.

There are several types of information bias: observer bias, interviewer bias, recall bias, reporting bias, and instrument bias.

Observer Bias

The observer bias may arise due to the investigator's pre-experiment knowledge of the hypothesis or of individual's exposure or health status. Such information may influence the way data are collected, measured, or interpreted by the investigator for each study group.

Interviewer Bias

Interviewer bias occurs when the questions asked by an interviewer influence the responses given by responders.

Recall (or Response) Bias

Recall (or response) bias can affect studies that have self-reporting such interview or survey, when respondent doesn't remember things correctly. After time (few days or years from the event), this could be normal. The qual-

ity of the data greatly depends on the patient's ability to recall properly past exposures. Recall bias may result in an underestimation or overestimation of the association between exposure and outcome.

Reporting Bias

Reporting biases occur when subjects selectively suppress or reveal information.

Instrument Bias

Instrument biases occur when an inadequately calibrated measuring instrument systematically over- or under-estimates measurement. Blinding of outcome assessors and the use of standardized, calibrated instruments may reduce the risk of this bias.

Confounding

The word "confounding" is a Latin expression (*cum fundere*) that means that two variables, the exposure and the confounding factor, act together, giving a distortion (bias) in the measure of association. This refers to the case in which the measure of association between an exposure and the outcome is confused by the effect of another factor.

As defined by Rothman, "confounding" is a "distortion of the association between an exposure and an outcome that occurs when the study groups differ with respect to other factors that influence the outcome" [Rothman, 2012]. Unlike selection and information bias, which can be introduced by investigators or patients, confounding is a type of bias that can be adjusted in the analysis if the investigators possess information on potential confounding factors about study subjects.

To illustrate the features of confounding, Rothman and others use as example a study by Stark and Mantel, who studied the association between birth order and the risk of Down syndrome [Stark, 1969]. The results showed that a 5^{th} born child has roughly a 4-fold increase in risk of having Down syndrome. However, it should be considered that women giving birth to their fifth child are older than women giving birth to their first child. Therefore, the relationship between birth order and prevalence of Down syndrome is confounded by maternal age, which exaggerates the association between birth order and Down syndrome. Subsequent studies have confirmed that maternal age, irrespective of how many children a mother may have, is more directly linked to Down syndrome than birth order.

1.4 Measures of Occurrence

Describing the frequency and the distribution of diseases and other health-related events and assessing the association between possible risk factors and diseases are the main objectives of epidemiology.

Compared to other sciences centered on the study of disease, epidemiology focuses on the occurrence of diseases and aims to investigate the underlying causes of disease and other health outcomes [Rothman, 1998].

Health phenomena are described by measures of occurrence. Measures of occurrence enable us to quantify the occurrence of disease in a population and its causes, to compare the occurrence of disease between populations and to evaluate temporal trends of the occurrence of disease: These are the central points of epidemiologic research [Rothman, 1998]. The basic measures of disease occurrence are incidence and prevalence. This section will provide a brief overview of these issues.

Incidence

Incidence measures the number of new cases of disease in a specific population within the time frame established for observation and is generally calculated in cohort studies.

Incidence can be defined in two ways, through proportion and rate: cumulative incidence and incidence rate.

Cumulative incidence (or incidence proportion) is a proportion whose numerator is the number of new cases occurring in the population over a period of time, and the denominator is the total of the population at risk at the beginning of the interval. People who already have the disease or people who cannot develop the disease, such as individuals who have been fully immunized against a certain transmissible disease, are generally excluded from the denominator. Cumulative incidence is an estimate of the probability that a subject of a population gets sick (or experiences another outcome of interest) in a given period of time and it is suitable for study of closed populations.

$$\text{Cumulative incidence} = \frac{\text{Number of new cases of disease in a given time period}}{\text{Population at risk at baseline}}$$

Incidence rate describes the occurrence of new disease per unit of person-time. The numerator is the same as the cumulative incidence, while the denominator is the sum of the person-time of each individual of the population that is at risk in the considered time interval. Person-time (person-year,

person-month, etc.) means the time experienced by an individual during which that individual could experience the outcome of interest. Compared to the cumulative incidence, incidence rate allows us to consider the possibility that the population at risk varies during the time interval considered and that the same people may have experienced the event several times. The calculation of average incidence rate also takes into account censoring.

$$\text{Incidence rate} = \frac{\text{Number of new cases of disease in a given time period}}{\text{Sum of the persontime spent in population}}$$

Prevalence

Prevalence is the proportion of people in a population that expresses the studied event at a given time out of the total number of individuals in the population observed in the same period. Very often the researched event is represented by disease, thus the prevalence provides a measure of the overall burden of an illness in a population at a given time (point-prevalence or punctual-prevalence), or during a specified time interval (period-prevalence). It is generally calculated in case-control and cross-sectional studies.

$$\text{Prevalence} = \frac{\text{Number of cases with the condition in a given time}}{\text{Total number of subjects in the population}}$$

It is defined "punctual prevalence" when the frequency of illness at a specific time is measured and "period prevalence" when the measurement refers to a period of time (e.g., one week, one month, one year) and always considers the entire population unlike the incidence rate.

The prevalence is influenced by the incidence and duration of the disease: the higher the incidence or the duration, the higher the prevalence and vice versa. Some diseases, such as infectious diseases, are short-lived and have a low prevalence but a high incidence rate, while chronic diseases, such as diabetes, have a high prevalence and a low incidence. This is because infectious diseases occur more frequently but have a shorter duration compared to chronic diseases. Moreover, the prevalence depends on the severity of the disease: A serious illness causes more deaths and the prevalence decreases.

Since the prevalence also considers the already existing cases of disease and not only the new ones, it can be defined as a snapshot which describes the percentage of a population that has developed or is at risk of developing a disease and is used mainly for the planning of Health Services and the analysis of the relative costs.

1.5 Measures of Association

The measures of association are fundamental in the investigation of any cause-effect relationship. These measures are particularly useful during the study of causes of diseases or in the evaluation of health interventions effect. Association is not causation, of course, but finding significant associations between potential causal agents and outcomes can be a first step toward confirming causal links.

Associations can be investigated in cohort studies using Relative Risk (RR) and comparing the frequency of disease in the exposed group with that in the non-exposed one. The existence of an association can be measured through case-control studies comparing the frequency of exposure of cases, patients, with that in controls, non-patients; the corresponding measure is the Odds Ratio (OR).

Contingency Tables

The contingency tables are double entry tables, with the variables shown in rows and columns, and the respective combined frequencies. In statistics, contingency tables are used to represent and analyze the relationships between two or more variables.

Table 1.5 shows a contingency table about a cohort study: The left column reports the exposure status, whilst the upper row delineates the disease status of the participants.

Relative Risk

Relative risk, which sometimes is called "risk ratio", is a ratio of the probability (risk) of the event occurring in the exposed group versus unexposed group.

Referring to Table 1.3 of a cohort study, the risk of getting the disease for smokers a / (a + b) is divided by the risk for nonsmokers c / (c + d). Then,

Exposure	Disease status	
	Present	Absent
Smoking	a	b
No Smoking	c	d

Table 1.5. Example of contingency table, cohort study.

relative risk is defined as the ratio between the risk of getting the disease for exposed group and the risk of getting the disease for unexposed group:

$$RR = \frac{\left(\frac{a}{a+b}\right)}{\left(\frac{c}{c+d}\right)}$$

When there are more than two exposure variables, the RRs can be calculated taking one exposure level as the reference. The risk of getting the disease is related to each exposure category (Table 1.6).

The risk of getting the disease for heavy smokers (more than 7 packs of cigarettes per week) is a / (a + b), while the risk for lighter smokers (less than 7 packs of cigarettes per week) is c / (c + d). Finally, the risk for nonsmokers is e / (e + f).

Considering nonsmoking category as reference, it is possible to calculate two separate RRs.

For lighter smokers:

$$RR = \frac{\left(\frac{c}{c+d}\right)}{\left(\frac{e}{e+f}\right)}$$

While for heavy smokers:

$$RR = \frac{\left(\frac{a}{a+b}\right)}{\left(\frac{e}{e+f}\right)}$$

Exposure	Disease status	
	Present	Absent
Smoking more than 7 packs of cigarettes per week	a	b
Smoking less than 7 packs of cigarettes per week	c	d
No Smoking	e	f

Table 1.6. Contingency table with multiple exposure groups.

When the measure of association corresponds to 1, there is no association between illness and exposure. A value less than 1 indicates a negative association, thus the exposure may be considered a potential protective factor for the development of disease. A ratio superior to 1 indicates the existence of a positive association; consequently, the exposure variable is a risk factor for getting the disease. The more the values deviate from 1 in both directions, the stronger the association. After calculating the relative risk, it is important to establish the 95% Confidence Interval (CI) of the RR. The limit or confidence interval is the interval of values in which the true value of RR lies. Even though, "confidence" or "security" is a relative concept, there is always a margin of error. Conventionally, the margin of error has been set at 5%.

When the RR contains the value 1, with a 95% confidence the RR is not statistically significant. The other two situations correspond to a 95% CI lower than 1 for significant protective factors and over 1 for significant risk factors.

Risk Difference

The Risk Difference (RD) is the absolute difference in the event rate of exposed and unexposed people. Given the value indicated in Table 1.3, RD is calculated as follows:

$$RD = \left(\frac{a}{a+b}\right) - \left(\frac{c}{c+d}\right)$$

It is the difference between incidence in exposed people and incidence in unexposed people. If RD is equal to zero, there is no difference between exposed and unexposed rates. Conversely, if RD is below zero, the exposure has the effect of reducing the risk of the outcome. If it is above zero, the exposure increases the risk of the outcome.

Moreover, the difference between risks can be absolute or relative:

- Absolute = risk in exposed persons – risk in unexposed persons;
- Relative = (risk in exposed persons – risk in unexposed persons) / risk in exposed persons.

The relative risk difference is also called "Risk Attributable to the Exposed" (RAE) or "etiologic fraction". It comes of the ratio between absolute risk difference, exposed people and unexposed people, and risk in exposed people. RAE indicates the proportion of unfavorable events that could be avoided in the group of exposed by removing the exposure to the risk factor among them.

Odds Ratio

The strength of the association between the exposure to a generic risk factor and the considered disease can be explored by calculating the odds ratio. This is obtained by comparing the ratio between the observed frequencies of individuals that have been exposed to the risk factor and have the disease (a) and the observed frequencies of individuals that have not been exposed to the risk factor but have the disease (c) with the ratio between the observed frequencies of individuals that have been exposed to the risk factor but do not have the disease (b) and the observed frequencies of individuals that have not been exposed to the risk factor and do not have the disease (d) (Table 1.7).

$$OR = \frac{\left(\frac{a}{c}\right)}{\left(\frac{b}{d}\right)} = \left(\frac{a}{c}\right)\left(\frac{d}{b}\right) = \frac{ad}{bc}$$

After this calculation, values between 0 and infinite are obtained. Their interpretation is quite immediate: OR = 1 indicates the absence of association between the exposure and the disease; values higher than 1 indicate a positive association between exposure and disease (likely, exposure is causal to the onset of the disease); values between 0 and 1 highlight negative association between exposure and disease (likely exposure prevents from the onset of the disease). Table 1.8 synthesizes the interpretation of OR.

Exposure	Disease status	
	Cases	Controls
Smoking	a	b
No smoking	c	d

Table 1.7. Contingency table in case-control or cross-sectional study.

OR = 1	No association between exposure and disease
OR > 1	Positive association between exposure and disease
OR < 1	Negative association between exposure and disease

Table 1.8. Interpretation of odds ratio.

Hazard Ratio

Hazard ratio is a measure of association widely used in prospective studies. It can be calculated comparing the ratio of (risk of outcome in exposed group) / (risk of outcome among unexposed group), occurring at a given interval of time. The hazard ratio is called "risk", but it is actually a hazard rate averaged over a certain time.

As for the other measures of association, a hazard ratio of 1 indicates the absence of association, a value above 1 suggests an increased risk, and a hazard ratio below 1 shows a decreased risk.

1.6 Regressions: How to Interpret Results

Within some epidemiological research studies, it may be of interest to evaluate the causal role of some factors in influencing the dependent variable. In this context, regression models are used to simultaneously control the causality and the possible presence of confounding factors. Therefore, the objective of this paragraph is to demonstrate the possibility of studying the effects of qualitative and quantitative variables on a single response variable.

The term "regression" comes from the studies carried out by the biologist Galton in 1886 [Bulmer, 1998]. He examined the heights of the children according to the heights of the parents in England and noted a functional relationship between the two variables: the higher the parents, the higher the children and vice versa. However, parents who were placed at the extremes (very low or very high) did not equally match extreme children. Galton observed that the height of these children moved towards the average and he concluded that this constituted a regression towards mediocrity and the functional relationship was therefore called "regression model". Nowadays, by "regression model" it is meant a statistical analysis that establishes a functional relationship between variables. A regression model allows to derive a simple mathematical model that describes the relationship between the variables and synthesizes them into a curve. In particular, the outcome variable, known as the "dependent variable" or the "response variable", is indicated by "y": It is numerical and continuous and represents the variable of interest that need to be related to one or more independent predictors. Conversely, these predictors, also known as independent variables, are indicated by "x_n".

The general linear model offers a flexible statistical framework that can be used to test almost any hypothesis about a dependent variable y that is measured numerically and can be described by a linear combination of x independent variables.

What varies, and consequently defines different types of statistical analysis, is the number of the independent variables and their nature, that might be numeric (e.g., age, blood pressure, cholesterol level, height, and weight) or qualitative. Among the latter, different subsets can be highlighted: Dichotomous categorical variables can assume just two different characters (e.g., male and female), or group categories that do not show any specific order (like hair colors) or that can be ordered (such as size classification from small to medium to large).

The line obtained by the regression model represents the straight line that minimizes the sum of the squares of the deviations of the individual values from the mean.

After performing the statistical analysis, the regression model will return the value of R^2, which is called "multiple determination index". This factor can be considered as a measure of the closeness of the observed points from the regression plan: the closer to 1, the smaller the dispersion of the points around the regression plane and the better the model.

Univariate Linear Regression Model

In order to help the reader in a simple and intuitive understanding of the concepts, we will start by presenting the simplest model, called in fact "univariate linear regression model". The following equation is used to investigate the linear relationship between a single independent variable (x) and the study variable (y) normally distributed equation of this type:

$$y = \alpha_0 + \alpha_1 x + \varepsilon$$

In this equation:

- α_0 represents the intercept, or the value that y assumes when the x is equal to zero;
- α_1 represents the coefficient of x and describes the slope of the line indicating how y varies in function of each change of x;
- ε represents the error due to the uncertainty of the statistical data.

The angular coefficient of the model explains the magnitude of the linear correlation between the considered variables. When the coefficient assumes

negative (or positive) values, there is a negative (or positive, respectively) linear relationship between the two variables. When the coefficient assumes values close to zero, it highlights that there is no linear relationship between the two variables. In case of linear correlation (Pearson's correlation coefficient ≥ 0.3), α_1 predicts change of dependent variable with one unit change of independent variable.

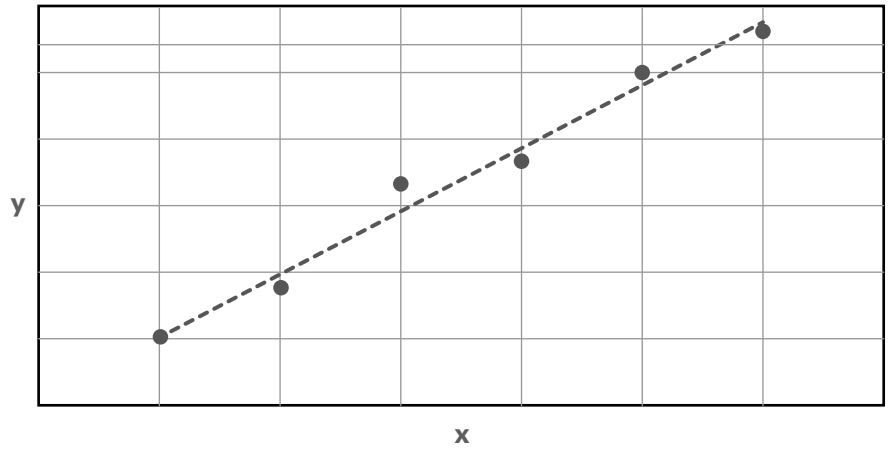

Figure 1.2. Simple linear regression model characterized by positive coefficient.

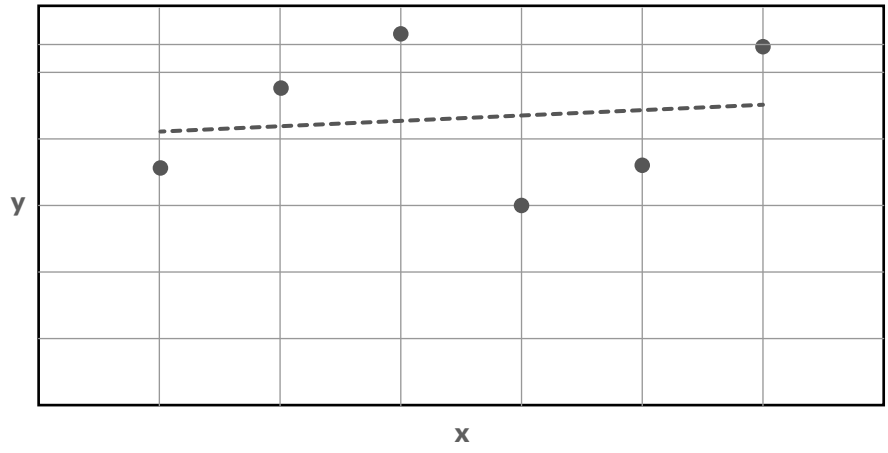

Figure 1.3. Simple linear regression model characterized by a coefficient that is close to zero.

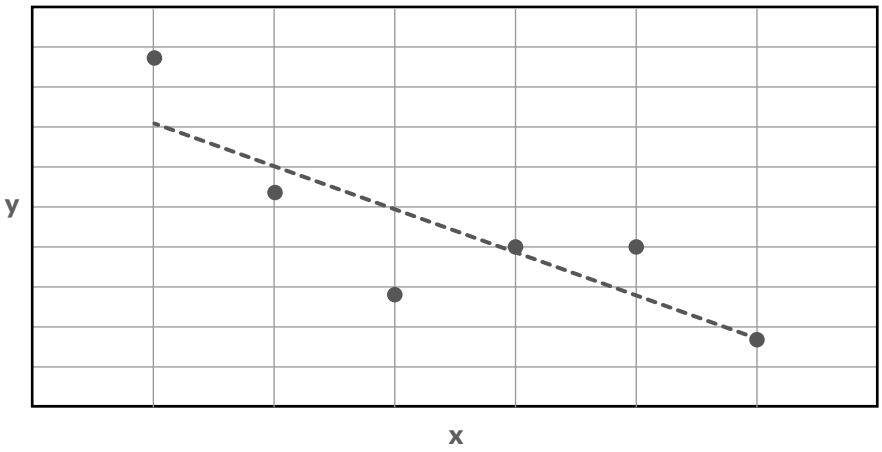

Figure 1.4. Simple linear regression model characterized by negative coefficient.

Figure 1.2, Figure 1.3, and Figure 1.4 show an example of graphic representations of simple linear regression models with positive, zero, and negative coefficients.

Multivariate Linear Regression Model

However, it is often necessary to conduct an analysis that considers several independent variables (x) at the same time to study how they affect the dependent variable (y). In this case, the statistical model used is called multivariate linear regression model. In this situation, the equation that describes the new model will look implemented with many (x) and could appear like this:

$$y = \alpha_0 + \alpha_1 x\,1 + \alpha_2 x\,2 + \ldots + \alpha_n x_n$$

In this equation:

- α_0 represents the intercept, or the value that y assumes when the x is equal to zero;
- α_1, α_2, and αn represent the coefficient of the independent variables and describe the shape of the line, predicting how y changes in answer to changes of these predictors;
- ε represents the error due to the uncertainty of the statistical data.

In the graphical representation of the multivariable linear regression model, it is necessary to imagine the points as localized within a multidimensional

space. In the case where two independent variables are taken into consideration, for example, the space will be three-dimensional and one variable describes the dependent variable (y) and the other two are representative of the two independent variables (x).

When implementing multivariate models as tools for the study of etiological research, it is necessary to respect a preliminary step that guarantees a rational approach and gives strength to the results. In fact, as first step, a stratified analysis should be performed with the aim to allow the researcher to select those variables that, based on the statistical significance obtained by the test, are likely to impact on the outcome variable. Once identified these, it will be justified to include them within the regression model.

In case of the involvement of qualitative data, the creation of dummy variables is needed. This statistical escamotage consists of creating fictitious models that attribute a dichotomous value to the presence or absence of the considered characteristics, individually or in association between them.

Although it is outside the training objectives of this text, it is worth pointing out that, as described in the introductory section of this paragraph, that regression models require specific mathematical corrections depending on the type of the independent variables taken into consideration. In the case of a dependent variable that can assume dichotomous values, for example, it will be necessary to carry out a logarithmic transformation, hence the name of "logistic regression model". Moreover, there are regression models for time-to-event data, that predict the probabilities that the considered event occurs at a given time for given values of the predictor variables.

However, we believe that it is beyond the aim of this book to provide such an in-depth knowledge, and we suggest referring to other texts for further information [Hosmer, 2013; Kleinbaum, 2010].

Questions

1. **Which epidemiological study allows to calculate the prevalence of a disease?**
   A. Cohort
   B. Cross-sectional
   C. Case-control
   D. Field trial

2. **Which of the following is not a type of randomization?**
   A. Balanced Blocks randomization
   B. Simple randomization
   C. Alternating randomization
   D. Stratified randomization

3. **Which bias occurs when a study sample is not representative of the population?**
   A. Recall bias
   B. Information bias
   C. Interviewer bias
   D. Selection bias

4. **What is meant by confounding factor in epidemiology?**
   A. A distortion of results due to excessive losses at follow-up
   B. A distortion of results due to the refusal to answer questions from the questionnaires
   C. A distortion of results due to a factor associated with both exposure and outcome
   D. A distortion of results due to instrumental errors

5. **Cumulative incidence is a ratio between:**
   A. Number of new cases of disease in a specified time period and population at risk at baseline
   B. Number of cases in a population and total population
   C. Population at risk and number of new cases in a specified time period
   D. Number of new cases in a specified time period and total population

6. **Prevalence is influenced by:**
   A. Duration of disease
   B. Incidence of disease
   C. Incidence and duration of disease
   D. None of the above

7. **Relative risk is an epidemiological measure used to evaluate:**
   A. Prevalence of disease in a population exposed to a risk factor
   B. Increasing risk of disease in population exposed compared to a population unexposed to a risk factor
   C. Incidence of disease in a population unexposed to a risk factor
   D. Incidence of disease in a population exposed to a risk factor

8. **Which of the following options indicates a quite strong linear relationship between X and Y?**
   A. $R^2 = 0.21$
   B. $R^2 = 0.75$
   C. $R^2 = 0.05$
   D. $R^2 = 0.95$

9. **The difference between multiple linear regression model and simple linear regression model depends on:**
   A. Number of dependent variables
   B. Number of regression equations
   C. Number of independent variables
   D. None of the above

Answers

1. **B**
2. **C**
3. **D**
4. **C**
5. **A**
6. **C**
7. **B**
8. **D**
9. **C**

References

- Bulmer M. Galton's law of ancestral heredity. *Heredity (Edinb)* 1998; 81(Pt 5): 579-85. https://doi.org/10.1046/j.1365-2540.1998.00418.x
- CONSORT group. CONSORT 2010 Checklist. Available at www.consort-statement.org (last accessed June, 2022)
- CONSORT group. CONSORT 2010 Flow Diagram. Available at www.consort-statement.org (last accessed June, 2022)
- Evidence-Based Medicine Working Group. Evidence-based medicine. A new approach to teaching the practice of medicine. *JAMA* 1992; 268: 2420-5. https://doi.org/10.1001/jama.1992.03490170092032
- Glass G. Primary, secondary, and meta-analysis of research. *Educational Researcher* 1976; 5: 3-8. https://doi.org/10.3102/0013189X005010003
- Goldsmith JR. Letter: What do we expect from an occupational cohort? *J Occup Med* 1975; 17: 126-31. https://doi.org/10.1097/00043764-197502000-00016
- Hosmer DV, Lemeshow S, Sturdivant RX. Applied Logistic Regression. 3rd edition. Hoboken, NJ (USA): Wiley, 2013
- Karanicolas PJ, Farrokhyar F, Bhandari M. Practical tips for surgical research: blinding: who, what, when, why, how? *Can J Surg.* 2010; 53: 345-8
- Kleinbaum DG, Klein M. Logistic regression. A Self-Learning Text (Statistics for Biology and Health). 3rd edition. New York, NY (USA): Springer, 2010
- La Torre G, Mannocci A. Experimental studies. In La Torre G (Eds): Applied Epidemiology and Biostatistics. Turin (Italy): SE*Ed*, 2010
- Last JM A dictionary of Epidemiology. 4th edition. New York (USA): Oxford University Press, 2001
- Lunet N. Meta analysis of observational studies. In La Torre G (Eds): Applied Epidemiology and Biostatistics. Turin (Italy): SE*Ed, 2010*
- McMichael AJ. Standardized mortality ratios and the "healthy worker effect": Scratching beneath the surface. *J Occup Med* 1976; 18: 165-8. https://doi.org/10.1097/00043764-197603000-00009
- Porta M. A dictionary of epidemiology. 6th edition. New York (USA) Oxford University Press, 2014
- PRISMA Statement. Available at www.prisma-statement.org (last accessed June 2022)
- Renjith V. Randomized controlled trials: What researcher's need to know? *Manipal Journal of Nursing and Health Sciences* 2017; 3(1): 45-50

- Rosenberg W, Donald A. Evidence based medicine: an approach to clinical problem-solving. *BMJ* 1995; 310: 1122-6. https://doi.org/10.1136/bmj.310.6987.1122
- Rothman KJ. Epidemiology – An Introduction. 2nd edition. New York (USA): Oxford University Press, 2012
- Rothman K, Greenland S. Modern epidemiology. Philadelphia (PA, USA): Lippincott-Raven, 1998
- Sackett DL. Evidence-based medicine. *Semin Perinatol* 1997; 21: 3-5. https://doi.org/10.1016/s0146-0005(97)80013-4
- Sackett DL, Rosenberg WM, Gray JA, et al. Evidence based medicine: what it is and what it isn't. *BMJ* 1996; 312: 71-2. https://doi.org/10.1136/bmj.312.7023.71
- Schulz KF, Altman DG, Moher D, et al. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 2010; 340: c332. https://doi.org/10.1136/bmj.c332
- Stark CR, Mantel N. Maternal-age and birth-order effects in childhood leukemia: age of child and type of leukemia. *J Natl Cancer Inst* 1969; 42: 857-66