A valuable reference for all who are involved in research in the field of Dentistry

- Research project design
- Ethical considerations about research with humans
- Epidemiology
- Qualitative research
- Meta-analysis
- Clinical research methodology
- Randomized clinical trials
- Laboratory research
- Sampling of human material to conduct research studies of the oral cavity
- Basic statistical analysis for dental research
- A step-by-step guide on how to conduct a systematic review
- Bibliographic research in Dentistry: electronic information sources
- Scientific writing

LAR - Latin American Region

IADR
International Association for Dental Research

Handbook of
Scientific Methodology
A guide for the dental researcher

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SBPqO - Sociedade Brasileira de Pesquisa Odontológica

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Handbook of Scientific Methodology: a Guide for the Dental Researcher

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Preface

Sigmar de Mello Rode

President, Latin American Region, International Association for Dental Research (IADR); Head Professor, Dentistry Course, University of Taubaté (UNITAU); Adjunct Professor, School of Dentistry of São José dos Campos, São Paulo State University (UNESP).

In 1996, during a meeting at the University of Buenos Aires, Hector Lanfranchi Tiziera, José Luiz Lage Marques and Sigmar de Mello Rode realized they had a common dream, a dream of bringing together the Latin American countries to form a dental research organization prepared to discuss and find solutions for the region's problems. A seed of union and integration had been sowed. Later on, in the year 2000, during the 78th General Session & Exhibition of the International Association for Dental Research (IADR), in Washington, DC, that seed encountered fertile soil, as the idea of creating federations of participating countries gained strength.

After 7 years of meetings and negotiations, the idea matured, and the Latin American Federation (LAF) was officially founded in September 2003, during the 20th Annual Meeting of the Brazilian Society of Dental Research (SBPqO), the Brazilian Division of the IADR, in Águas de Lindóia (Brazil). The meeting was attended by delegates from Brazil, Argentina, Venezuela and Peru. At the time, the first president and vice-president of the LAF were chosen, respectively Hector Lanfranchi Tiziera (Argentina) and Sigmar de Mello Rode (Brazil). The first constitution of the federation was discussed and approved, providing for a presidency that would be held alternately by representatives of member countries, and for a board that would be established with representatives from all the participating countries, nominated by the respective Divisions/Sections of origin.

The formation of the LAF was officially approved at the IADR Council Meeting, in the 82nd General Session & Exhibition of the IADR, Honolulu

(USA), in March 2004. In November 2008, during the 7th Conference of the IADR – Venezuela Division in Maracaibo (Venezuela), the LAF Board – under the presidency of Sigmar de Mello Rode – approved the nomination of Ana Maria Acevedo (Venezuela) to be the next president starting November 2009. To comply with the changes in the IADR constitution, an amendment was also approved to change the Latin American Federation (LAF) to Latin American Region (LAR), without, however, straying from the ideals that had steered the creation of the organization.

The first meeting of the Region took place in October 2005, in the city of Mar del Plata (Argentina), the second, in September 2007, in the city of Atibaia (Brazil), and the third, in November 2009, in the city of Isla Margarita (Venezuela).

The Latin American Federation – now called Latin American Region (LAR) – is the Latin arm of the American continent in the structure of the International Association for Dental Research (IADR) – the world's most important dental research organization. It is also the realization of a dream to bring together the dental researchers working in the region's countries. Today, it has members from Argentina, Brazil, Chile, Colombia, Costa Rica, Ecuador, Panama, Paraguay, Peru, Uruguay, and Venezuela, and is a channel fostering integration and exchange of research experiences on all levels, from scientific initiation production by undergraduates to research of excellence conducted by the most experienced scholars.

The objectives of the LAR are ambitious. It aims at integrating, developing and strengthening research in Dentistry and correlating fields in all of Latin America by way of an intense scientific, academic, cultural and personal exchange, and, more importantly, underpinned by the broad international visibility provided by the IADR.

As one of its objectives, and aiming at boosting the visibility of the region's dental research, we conceived a joint venture between the LAR and the Brazilian Division of the IADR, represented by its president Katia Regina Hostílio Cervantes Dias – a dear friend and constant partner. It involved developing a Handbook of Scientific Methodology. The book would be written by Latin American authors, and would become a reference work for Latin American researchers, whether beginners or more experienced. After much work and effort, we are pleased to present the product of this endeavor in the following pages.

This task would nevertheless have been impossible without the invaluable support and incentive provided by the Johnson & Johnson Company, Consum-

er & Personal Products Worldwide, through Marcelo W. B. Araújo, Associate Director – Clinical & Professional Affairs Oral Care Research, Development & Engineering.

Our sincere thanks to all the authors who selflessly devoted time and knowledge to writing the chapters of the handbook, to the Imprensa Científica publishing house for the translation and publishing services provided, and especially to Cristiane Miranda França, who organized and closely supervised the whole project, coordinating and guiding all the parties involved.

Special thanks are also in order to Carlos de Paula Eduardo (Director of the School of Dentistry, University of São Paulo) and to Katia Regina Hostílio Cervantes Dias for their support in developing and executing the project.

With the English version of this Handbook of Scientific Methodology, we are hereby keeping one of the promises we made of leaving a legacy to Latin America. We are already working to make its contents also available in Portuguese and Spanish, on the LAR webpage.

The dream has come true, and it is up to all of us to make it thrive even further. The example of successfully integrating the dental research community in the Latin American Region is one to be followed.

Introduction

Carlos de Paula Eduardo[a]
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One of the goals of the International Association for Dental Research (IADR) is to encourage research development and promote an environment where researchers can work together. To this end, the Latin American Region of the IADR conceived a project aimed at encouraging scientific production, partnerships between research centers and the exchange of experience and information among Latin American researchers. The present book is the first fruit of this project. Most of its chapters were co-authored by researchers from different countries.

The book is a significant contribution to Brazilian research. The authors of each chapter were chosen according to criteria of excellence in the different areas of scientific methodology, applied to both laboratorial and clinical research.

The scientific community has long been awaiting a work like this, which would give due attention to research aspects of enormous importance, such as ethics, biosafety, laboratory features and funding sources, among others.

A well-substantiated presentation both of the methodology that should be applied to clinical research and observational studies, and of the ethical and legal aspects involved, places in evidence the guidelines that should be followed to give credibility to a research project, in all its developmental phases, and the importance of multicentric studies.

The emphasis given to randomized clinical trials, with its contents, protocols and use of placebos, reflects the auspicious moment we live today for

conclusively consolidating the road that leads to a growing number of clinical research studies.

The information provided in the statistics and systematic review chapters highlights two of the most important methodological tools for prospecting significant, valid and reliable evidence, and thus facilitating the mastery of these intricate subjects.

A full chapter is dedicated to the ethical considerations involved in research with human beings. These should be observed even before a study is carried out, while still in the design preparation phase. The importance of underpinning ethical evaluation with a scientific basis is also stressed, and the practice of obtaining informed consent from patients voluntarily taking part in any research is consolidated.

Several important aspects are discussed under the topic of epidemiology, such as ethical issues, sample size, eligibility criteria for participants and groups taking part in this kind of study, as well as the instruments used for collecting data and planning the study analysis of the results. The relevance of epidemiological studies, along with their principles and basic concepts, is discussed as contributing to the viability of these studies, particularly as regards the setting of sample size and sample randomization. The importance of strictly following the design of an epidemiological study, involving case-control studies and randomized controlled trials, is also stressed.

The approach used by the book to address the topic of bibliographic research in Dentistry through electronic information, in an in-depth and broad-ranging manner, points out the need for establishing a close and constant relationship between libraries and users.

There is also a discussion about the relationship between the publication of scientific studies and the proper preparation and submission of the manuscripts for these studies, reflecting the ability of authors to interpret and put on paper the results obtained in all the phases of their research projects.

In concluding this introduction, which aims merely at highlighting some of the concepts put forth by the authors of this book, we would like to stress the present importance of a work of this nature, which represents true "Basic Evidence in Dentistry" in the field of research. This kind of evidence is deemed of great value by the Brazilian funding agencies. As a result, the Brazil of today holds an outstanding position in the world research scene, both quantitatively and qualitatively.

Thanks to the individual effort of researchers and funding agencies of different countries, Dentistry in the region has progressed and gained respect and

prestige. With actions such as this one, our research will undoubtedly grow even stronger through our joint efforts to work together.

All of those who have dedicated themselves with body and soul to raising our research to a position of excellence both domestically and worldwide stand to gain.

1

Research project design

Rita S. Villena[a]

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A research design may be structurally different, depending on its objective. In general, we may define two variants, based on different concepts and objectives. One variant would be the research project and the other one, the research protocol. The project is a research proposal, which is generally submitted for obtaining approval or authorization to conduct the research in question. The project may also be used to apply for research grants. A clear example of this would be to submit a project for obtaining approval to conduct a thesis or to apply to any national and/or international agency or institution for funding or grants. In this specific case, researchers should be familiar with the potential funding sources and the approval requirements. The protocol is usually a more structured and technical document, clearly and thoroughly showing what the researcher intends to study. It also includes all the design, methodological and ethical instruments to be taken into account before conducting the study. In general, the protocol is written as a preliminary document prior to data gathering and is meant to support the researcher's work. Content should be thorough and complete. Although it is a more technical document, it contains the same sections as a research project or proposal.

Researchers who are taking their first steps in this kind of scientific work and have uncertainties or require support are advised to consult colleagues who are more experienced in developing protocols – generally members of university research committees or research associations in their countries, such as the IADR (International Association for Dental Research).

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1. Defining the research project

Before beginning a research study, it is important to thoroughly determine or define the hypothesis or problem underlying the study in clear and simple terms. A clearly defined hypothesis will make it easier to view the overall setting of the research study and to determine its practical and economic feasibility. The researcher may have an innovative idea, but if it requires costly investments and high technology to be developed, these requirements should be considered from the very beginning to determine the feasibility of conducting the study. This aspect is quite often overlooked by some beginner researchers, leading to a great loss of time in developing a project that will eventually have to be reformulated because these applicability determining factors were not taken into account from the start. Often, the lack of state-of-the-art infrastructure, the high or sophisticated technology required, the need for highly trained personnel, and the heavy investments in time and money make it difficult or unfeasible to conduct a study. However, nowadays with globalization, it is increasingly frequent to see partnerships between institutions making it possible to conduct more complex work in the region, with the support of agencies from developed countries. It is also worth noting that a more sophisticated research project, technologically speaking, is not necessarily a better one. Studies that are easy to apply and conduct may also yield very positive data and knowledge, contributing significantly to the community, city or country. This is why it is important that we, university professors and/or researchers in the region, support and encourage as many low cost, applicable and far reaching studies as possible, for the benefit of the population. The new generations of researchers must not lose their enthusiasm to produce scientific knowledge just because they do not have the resources that are available in countries offering better financial support. Therefore, it is important to have lines of research that are easily applicable in their local settings and capable of adding knowledge and contributing to respond to unanswered questions in their social reality. This would encourage new researchers to continue the search for the needed answers and solutions.

Everything mentioned in this section shows the importance of knowing how to define the object of the study previously: What to research? This requires answering other questions that are directly related with the possibility of conducting the study. The major criteria that should be considered before beginning a project are concisely shown in Table 1.

Table 1 - Criteria to consider before beginning a research project.[1,2,3]

Feasibility	• Suitable number of individuals • Infrastructure to conduct the study • Relevant technical experience • Feasible in terms of time and money (reasonable time frame to conclude the project, realistic and justifiable budget) • Manageable in terms of scope
Interesting for the researcher. Original. Applicable	• Confirms or refutes previous findings • Broadens previous findings • Delivers new outcomes • Delivers actionable and feasible outcomes
Ethics and significance	• For scientific knowledge • For healthcare clinical policy • For future lines of research • Satisfactory ethical approaches

2. Structure of a research project

The structure of a research protocol may be different in terms of presentation and descriptive thoroughness, depending on the purpose for which the document was drafted, as it can be aimed at:

- Supporting the feasibility of conducting a study before research committees and obtaining the approval from academic institutions to conduct it. The document would be mainly targeted at projects that would eventually result in undergraduate or graduate theses.
- Submitting a research project to individuals, agencies or institutions for an ethical evaluation of its applicability (ethics committees).
- Obtaining approval of or funding for the study from academic institutions, government and/or development agencies, and national and/or international foundations.
- Guiding the researcher and/or group of researchers during the process of conducting the project.[4,5,6]

Each one of the sections that make up a research project will be briefly described below. The purpose of this chapter is to be of practical use, especially for beginner researchers. The objective is to be clear, concise and as instructive as possible, since many pages and even an entire book could be written on this subject.

- Research Project Title
- Introduction/Explanation of the problem or hypothesis
- Objectives
- Background/Reference Framework

- Methodology/Material and Methods
- Work Timetable
- Required Resources and Budget
- Bibliographic References

2.1. Research project title

The project title is the first impression that the researcher's proposal will have on the reader. Therefore, it should be informative, concise and appealing, and should describe the project's content in a few words. An appropriate title should describe, as much as possible, three important aspects: type of study (for example: prevalence or cross-sectional, incidence or longitudinal, *in situ* or *in vitro*, etc.), principal variables and sample.[7,8,9]

2.2. Introduction or explanation of the problem

This section is a prelude that briefly presents the problem to the reader, by informing the most significant scientific data currently available about the research subject, the current situation and the need or rationale for study. The need for and the purpose of the study should, thus, be included at the end of this section of the text.

The following questions should be answered:

- What is the current situation?
- What has been studied up to now about this subject? (include summarized highlights)*
- What requires further research?
- For what reasons will this study be conducted or why do we intend to study this subject?
- What is the purpose or objective? (the main purpose of the study should be described at the end of this chapter, written in narrative fashion instead of following the same wording pattern of the objectives, which begin with a verb (see "Objectives"). Some research projects will not include the objectives as an additional item. This is why the purpose of the study should be clear by the time the reader finishes reading this chapter.

* This part should not exceed three or four pages in a thesis, and should not be more than one or two pages in a project. Only the strictly necessary and relevant bibliographic references should be included in the text, since an extensive review on the subject is not required.

2.3. Objectives

The purpose of a research study should describe both in general and specific terms:

1. The overall objective
2. The specific objectives

The overall objective should be directly related with the research project title. For example, if the title is: "Dental caries prevalence in 5-year-old children from the city of Lurin", the overall objective will be to assess, or investigate, the prevalence of dental caries in 5-year-old children living in the city of Lurin.

The secondary objectives will provide greater details of some of the complementary or secondary aspects to be evaluated in the research project, but they will not be included in the title. In general, the title has to be as concise as possible, as was previously discussed. According to the previous example, the objectives should not be written in narrative fashion, instead each paragraph should begin with a verb. For example: to study…, to assess…, to observe…, to determine…, to compare…, to investigate…. These are some of the verbs most frequently used to begin the wording of the objectives. In many research centers, the objectives are not presented separately as a full chapter of the research project. They are generally included at the end of the introduction, but this is a parameter that the researcher should check before presenting the project, in order to adjust to the uniform requirements of the institution where the project will be presented.[8,10]

2.4. Background / reference framework

This section will include a review of the literature to allow the reader to have an overview of previous studies (results obtained, methodologies used) currently available on the subject. This review will serve to support the proposed study and to discuss the referred studies, in light of the study results, in the discussion chapter, which is included in the final document, after the study results have been obtained.

In general, this section has a logical and historical sequence to give the reader a perspective of the events that have taken place until now, regarding the subject of the study. The authors' names, the year the study was published, and its respective bibliographic reference, allowing readers to promptly locate it if required, should be included in the text. The purpose of this is to enable the reader to follow the historical sequence of this review.

In many cases, when the research project is meant to be an initial proposal to conduct a future study, a thorough search of bibliographic references is not

required. This section may simply be a well-documented introduction of the study to be developed. Once again, it is important to take into account the uniform requirements for research protocols set by the agency or institution to which the study proposal is being submitted. The structure of a protocol is not rigid or always the same. It may vary depending on the agency/institution and type of study application (funding or grants, thesis or others).

2.5. Research methodology / material and methods

This chapter should clearly describe how the study subjects or animals were selected, as well as the material, equipment and methodology used. While describing a research project involving a sample of humans, this project chapter should preferably be entitled "Research Methodology" rather than "Materials and Methods" because the latter would lead to the inconvenience of having to include in the materials section the group of volunteers or individuals taking part in the study.

The methodology should be thoroughly described to enable the reader to understand and interpret the study results, as well as to allow other researchers to partially reproduce the methodology in future studies or replicate it to obtain similar data with the purpose of checking the authenticity, validity and reliability of the methodology, or of complementing the results with future studies following the same line of research.[3,6]

Providing references for the methods used is also necessary. The trademarks of the equipment and/or material used may also be included in the text, in parenthesis or as a footnote, followed by the ® symbol in the case of a registered trademark and, preferably, specifying the name and location of the manufacturer. If pharmaceutical drugs and/or chemical products are used, include their generic names, dose and route of administration.[10,11]

2.5.1. Ethics

When the study is conducted in humans and/or animals, the authors should state if the procedures followed comply with the ethical requirements of the pertinent (institutional or regional) committee and the 1975 Declaration of Helsinki, revised in the year 2000. In the case of projects funded by local or foreign organizations, the approval by the local ethics committee does not exempt the donor from ethical responsibility for the project and vice-versa.[1]

Study participants should be told what the objective of the study is and what their participation will involve. The decision to participate in the study should totally depend on the participant's own free will. If he or she is a minor,

his or her parents' or guardians' consent is required. Participation in the study should be authorized by a written consent letter, the template of which should be included in the annexes of the project.

The patient's name, initials or hospital code number should not be used, especially in illustrative material.[7,12]

2.5.2. Statistical analysis procedure

Statistical methods should be described in detail so that an informed reader with access to the original data may be able to check the study results.

2.6. Work timetable

This section is intended to allow researchers to plan the period of time required to conduct the study and to commit themselves to following a timetable. The project should include the sequence of the study phases and approximate duration of each phase. The time schedule should, therefore, include the time required to purchase the equipment and supplies, gather data, conduct the statistical analysis, and draft the report or full-text study in traditional format, including study results, discussion and conclusions. In some cases, a pilot study will be required and should also be mentioned in the project timetable. It is often presented graphically, making it easier for the reader to view it at a glance. Long-term project proposals (more than a year) should generally divide the study into phases on an annual basis. Although organizations will normally approve the full project, funds are generally provided annually, under the condition that study progress reports be submitted to the funding agency for examination purposes.[1]

2.7. Required resources and budget

It is very important that researchers develop this section thoroughly, after the future study is believed to be well-defined. Researchers are even advised to draft it before developing the project itself because, as mentioned in section "Defining the research project" of this chapter, their enthusiasm may lead them to attempt conducting a study that is hardly feasible in practical and/or economic terms.

The funding application or study budget should be detailed by type of expenditure, with its respective rationale.[1] The most common expenses that should be included in the project budget usually are:

- Personnel (salary, time spent on the project, etc.)
- Office/laboratory rental fees

- Equipment
- Supplies
- Patient healthcare costs
- Travel/lodging/food
- Data processing
- Transportation/postage and packing
- Secretarial expenses
- Publishing/Editing

2.8. Bibliographic references

Bibliographic references serve to offer the reader the opportunity to be aware of and able to access the original sources of the project. The objective of these references is to justify, support and/or clarify the author's ideas. This is why using basic or classic references on the subject is recommended, as well as recent and diverse references, preferably published in high impact journals. Obtaining scientific support solely or mostly from textbooks should be avoided. References should generally be numbered consecutively, in order of appearance in the text, where they should be identified with superscript Arabic numbers, in parenthesis or highlighted in some other way to allow readers to know that the quotation they have read was obtained from the indicated reference, which is described at the end of the document.[13,14] These features may vary according to the uniform requirements set by the institutions or agencies to which the research project will be submitted. Therefore, previously checking the uniform requirements for drafting or submitting a research project is important to avoid the refusal of the project because of this kind of error.[7] The Vancouver uniform requirements for bibliographic references are generally the most widely used and may be found in different websites, such as: *http://www.fisterra.com/recursos_web/mbe/vancouver.asp#electronico*.

3. Application template for research project funding or grant

The funding application template may vary depending on the institution to which it is submitted. Nevertheless, a template commonly used is presented in Figure 1. It illustrates well the step-by-step process the researcher must follow to submit a research project.

Figure 1 - Funding application template. [continued on next page]

1. Institution in charge of the project							
2. Principal researchers							
Prof/Dr/Mr./Ms.				Prof/Dr/Mr./Ms.			
Last name		Name		Last name		Name	
Position in the Institution				Position in the Institution			
Postal Address				Postal Address			
Phone #:				Phone #:			
Fax #:				Fax #:			
(Attach to this application the résumé of the principal researchers of the project. Describe in detail research activities and list of published papers, both nationally and internationally).							
3. Project title							
4. Project summary							

5. Work timetable (an illustrative and sequential timetable graph may be included)				
6. Project duration	years		months	
7. Total sum required (include taxes separately)				
8. Detailed information of requested funding (include taxes separately)				
	1st year	2nd year	3rd year	Total
a. Salaries				
b. Equipment/supplies				
c. Travel				
d. Data analysis and Secretarial expenses				
e. Transportation and others				
Annual total:				
Total investment:				
(All expenses must by described in detail and then justified)				

Figure 1 (continued) - Funding application template.

9. Ethics

a. Submit the project to the pertinent ethics committee and attach the approval letter

b. If the project includes studying humans or volunteers who are minors, a written authorization of the adult responsible for each minor should be included. If the study subjects are adults, a signed written consent from each study participant should be attached.

10. Compliance of the researchers with the code of ethics and requirements of the institution [Contract stipulations of the institution may be included, which should be signed in agreement by the principal investigator(s).]

Signature(s)	Name	Activity in the project (weekly hours)	City	Date

11. Research project proposal
(Include all the project details in accordance with the list of contents shown below)

a. Title (repeat the title presented on the first sheet)
b. Introduction
c. Objectives
d. Methodology
e. Timetable
f. Required resources and budget
g. References

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2

Ethical considerations about research with humans

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The ideas put forth in this chapter are the authors' personal views about the interactions between two important aspects of modern life – Science and Ethics. – Their aim is to draw the reader's attention to this interaction, evoking questions for further development. The very nature of Ethics and the enormous scope of modern Science are enough to quell any pretension of exhausting the subject. In addition, a very practical view of the topic is presented, based on the authors' daily experience in evaluating projects as Ethics Committee (EC) members. The text is also based mostly on the regulations imposed on scientific experimentation with human subjects in Brazil, particularly Resolution 196/96[1] of the Brazilian National Health Council, our main and closest source of ethical reference.

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Some historical facts

The importance of the ethical aspects of scientific research in humans should not be underestimated when developing a project. The concern about how a specific method might affect human beings taking part in a

research goes far back in history. Percival was already writing about it in 1802.[2] Considering the professional codes of conduct, it goes even further back.[3,4]

Why Science has to converge with Ethics

Constructing scientific knowledge requires hard work (testing, repeating, testing again, repeating again, testing yet again, repeating yet again, and writing), mental activity (setting a hypothesis, interpreting the data, and concluding) and credibility (accreditation by other scientists). These aspects form the basis of modern Science. Although not as expensive as wars, which have almost no critical supervision by society, science is a relatively expensive activity, and society has the right to oversee and control its use of resources and resulting products.

The peer-review method is the main way to control the scientific quality of research and it is used for most certifications, evaluations and authorizations in modern Science. Government institutions take part indirectly in the social control of Science, generally in the double role of granting and overseeing the scientists' work. A much smaller part of this control, although not less important, is exercised by society through non-governmental organizations, political and activist groups that try to influence the topics and the way scientists do research.

Specific commissions and supervising groups like the Institutional Review Boards (IRBs) and ECs, which include representatives of social groups, are important avenues for conducting a direct social overview of research activities. All these ways of certifying projects are valid and accepted in one aspect or another, but the supervision by an EC is the best option, since it involves scientific, social and ethical evaluation, as a whole, and also adds more credibility to the work.

Validation of scientific results

Credibility is of utmost importance in science, and scientists know their results have to convince other scientists and society in general. Projects or research papers have to be evaluated under the peer-review system of the granting institution, academic society or scientific journal before being granted funds, being presented during scientific meetings or accepted for publishing in recognized scientific journals. This system allows the necessary validation of a scientific work by other scientists, who will also perform the final testing, by repeating the experiments to confirm or refute the previous study's conclusions.

Researchers have to be extremely clear, both to themselves and to others,

about the methods used to gather and analyze data. Other scientists will judge not only the validity of the data but also the validity and accuracy of the methods used to derive the data. If someone is not forthcoming about the procedures used to yield a new result, validation of the result will be hampered. The development of new methods can be a controversial process, as scientists seek to determine if a given method can serve as a reliable source of new information.

Most of the results produced during scientific research will never leave the notebook, but the record has to be kept. In addition to the common methods of keeping formulas and data, it may be necessary to prove the authenticity of the research. Some scientific journals are starting to ask for the raw data in controversial subjects or results, and the reviewer has the assumed right to ask for it if he/she should wish to confirm the research results or the way they were analyzed. For some famous ex-researchers this may be too late, as in the cases of a very promising young physicist from Bell laboratories,[5] the discovery of HIV,[6] and the famous stem cell case from South Korea,[7] to cite just a few.

Before preparing a project for ethical evaluation

Before making the decision to do research involving humans, the authors should be aware of the basic paperwork involved. Furthermore, before preparing a project to be submitted to an EC it is fundamental to know international and local regulations. There is a list of documents, links and references at the end of this chapter which may be useful for those who want to submit a project to an EC or just to acknowledge it. A compilation of local regulations from several countries of Latin America (and other places) is available at the U.S. Department of Health and Human Services website.[8]

Although every single EC has specific requirements, most of them follow the requirements of international codes and regulations, such as the Nuremberg Code,[9] the Declaration of Helsinki,[10] and the Guidelines of the Council for International Organizations of Medical Sciences (CIOMS).[11,12] In Brazil, the necessary information is summarized by CNS Resolution 196/96 (from the Brazilian National Health Council) and its complementary resolutions.[1]

Considering international and local regulations, the minimum amount of information in a protocol to be submitted to a critical and ethical evaluation should include the scientific project, complementary ethical information, informed consent, curricular information about the researchers and a series of forms. The following sequence of information is just one of several possibilities. Other sequences, with a greater or fewer number of items, are also appropriate for local EC purposes.

Scientific project

The project should be structured in the same way as those submitted to a regular fund-granting institution. It is the main source of scientific information, and the authors should bear in mind that it will be read with utmost care, but not necessarily by a specialist in the subject under investigation. It may be helpful to describe the basic ideas sustaining the project's aim in a more didactic fashion and avoid shortcuts, specially while justifying the outline of the experiment and the methods that will be used on humans. Specialists tend to have all the details clear in their minds and it may seem unnecessary to them to explain "why" and "how." This attitude may be counterproductive and result in an evaluation report that is full of questions. In fact, as part of the philosophical principles at the very core of the ethical evaluation of scientific research projects, most ECs, if not all, have a heterogeneous composition, and the view of non-experts about the project is greatly valued. Although the following information is generally valid, the authors should always check the recommendations of their local EC for more specific details.

Identification of the research team and institutions involved in the project

The project should have a front page containing the title of the project and the name, address, location, contact telephone and other personal data of the main researcher. Occasionally, it should also include the personal data of the other professionals involved in the project. Some ECs will ask for the data on all the researchers involved. Identification and information about the institution (institutions) where the experiments will be performed should also be included. If the project has one or more sponsors, particularly companies with commercial interest in the results, complete information about them should be supplied as well.

The literature review and the rationale behind conducting the research

The body of the project should include an introduction and/or a literature review of the research subject and its supporting and antagonizing theories. This should be conducted to clarify the reader in regard to the state of the art of the relevant scientific knowledge and the gaps remaining, thus justifying the performing of the experiments. The necessary degree of detail is relative. The authors should not overdo it by quoting extensively or being too wordy. He/she should cite the more recent and/or important works, published in the more re-

spected journals, and of course restrict the literature review to the topic under study. For most protocols, a few pages of literature review are enough to reference the main ideas. If the project involves a new or non-registered drug, a new health device or a new method for diagnosis or treatment, the theoretical basis for the research must be very consistent and sometimes extensive, with 30, 40 or more pages of literature review. For new drugs and devices created in other countries – a common situation in Latin America – the authors should also clarify the registration status or study phase of the new drugs or equipment or device in the country of origin. This involves the principle of reciprocity in human research ethics, in which there is mutual justice, responsibility, benefits and risks for both countries. It is important to conclude the literature review with a summary of the research justification. It is also worth remembering that no research is free of costs, risks or some degree of discomfort; hence every single study should be well justified.

Aims

The scientific aims of the project should be clearly stated, preferably on two levels: a general aim will express the main research idea supporting the methodological choices, and the specific goals will follow and must be connected to the specific questions raised and methodologies used.

Methodology

There should be a clear description in the text of the characteristics expected or accepted for human subjects, such as number, origin, age group, gender, social level, education level, general health status and specific diseases, as well as any other characteristics that will be considered in the selection of human subjects. The inclusion and exclusion criteria are generally described along with the description of the characteristics of human subjects or just after it. Each individual method to be used during the project must be described in great detail, especially those considered standard to the field specialist. The authors must keep in mind that the readers, including the EC referees, may not be familiar with all the methods described in the project. Although the methods to be directly used on human subjects have precedence in terms of ethical evaluation, it is better to risk being didactic than laconic. All methods, even the simplest, like clinical examination, should be described and should include all planned steps, equipment involved and the time it will take to perform. If a standard procedure of any method is going to be changed, the alteration should be highlighted. The outline of the experiments should be clearly stated, including the

general characteristics of the study (longitudinal, cross-sectional, controlled, blinded, etc.), study groups, use of control or placebo groups, periods of follow-up, "wash-up" periods, and all other details. For projects involving health treatment, alternative protocols should be established in case the expected results are not observed. Clear criteria about the conditions or situations in which a method – or even the whole research – should be suspended, interrupted or terminated altogether have to be described in the text, especially if a drug or a new treatment is involved. Regardless of the nature of the material, samples or data acquired during the research, the project must clearly state the information concerning its use. If there is any possibility of stocking the material for future use, this intention should be disclosed to the EC and to the human subject, and written in the protocol and in the Informed Consent (IC). Great care is required before using any result of the research for economic-related activities or to obtain any kind of profit. Although no specific paragraph need be included, there should be no doubt as to where each method will take place, citing specifically the communities, institutions, hospitals, health centers, laboratories, clinics, schools, and other places which will be involved at one time or another. A timetable for the whole project should be available, considering that the research should start just after EC approval, and it should determine the time for the follow-up and final reports as well. The expected scientific results should be described, even though they may change as the research evolves. They should not be mistaken for the educational, economical or social results of the research, which should be described elsewhere. A proper bibliography must be included at the end of the project and all studies quoted in the text should be detailed there. The Vancouver citation system is frequently used for this purpose, but other systems may also be used.

Criteria for suspending or terminating the research

Like any other activity associated with risks, research also should have some criteria that can be applied in case the results do not come out as expected. If the methodology to be used in the project entails foreseeable risks or strong discomfort, safeguards should be established in order to protect human subjects from unnecessary harm. Major harm is commonly preceded by small problems or minor harm, which may be reversible and detectable either directly or by complementary exams. For instance, if medication known for its potential to damage the liver is to be used, the project should include the previous examination of subjects to exclude potential candidates with pre-existing liver disease, habits, history or activities that may be associated with liver problems

(viral or drug hepatitis, drug use, alcoholism, or any other sign or symptom of liver disease). Each individual should be submitted to medical and laboratory examination before being accepted as a human subject, and these examinations should be repeated from time to time during the experiment (and sometimes after it) in order to identify the first sign of alterations should it occur. In the case of positive test results, the human subject should normally be excluded from the experiment until his/her state is investigated thoroughly. If the alteration is provenly associated with the experiment, the human subject should be permanently excluded from the study and receive all the necessary care. If any harm, or its potential effects, is serious enough, it may be necessary to stop conducting the protocol until all the circumstances are clear and the safety of human subjects is definitely assured. All possibilities have to be weighed carefully to guarantee the best interest of the human subject. Suspensions and interruptions are not always related to harm, risk or pain. If the research involves treatment of a certain disease, and one of the treatments under evaluation proves to be better than the other(s), it is an ethical obligation to stop conducting the protocol and provide all subjects with that option.

Ethical comments about the project

An additional set of information and comments about the research should follow within the project or elsewhere in the protocol. It differs from the information included in the regular project because of its ethical, moral and philosophical nature. In order to describe the ethical aspects of a project properly, its authors must have some knowledge about human rights and about basic documents on human research.

The ethical comments deal mainly with the consequences the research may have on human subjects, and the conditions researchers establish to avoid or reduce the potential harm, problems or discomfort involved in participating. Since this category of information is generally not present in regular scientific projects, it is easier to describe it as a supplement to the project. The subsequent paragraphs will concentrate on the main topics that researchers should comment on in a protocol.

Process of recruiting human subjects and obtaining their informed consent (IC)

The process of recruiting human subjects should be described in detail. Starting with who is going to direct the process (whether one of the researchers or a hired professional), how and where contact will be made? Will the strategy

to recruit human subjects include contact before they undergo standard treatment in a hospital or at a local church or community group? Will the initial interview occur locally, in a clinic or elsewhere? How will the IC be presented and explained, and how will the doubts from human subjects be clarified? Projects involving people under 18 years of age should also clarify how the parents or their legal representatives will be involved. For long-term longitudinal follow-up studies, procedures to renew the informed consent should be added. What process will be used for illiterate human subjects? In short, what must be clear is how, when and who is going to interact with the potential human subjects to obtain the IC.

Participation of vulnerable groups in the research

If the participation of vulnerable individuals is planned in the project outline, the text has to include a reasoning and justification, especially if risks are involved. A person is vulnerable when a situation or fact reduces his ability to understand the project permanently or temporarily and he decides on his own to take part or not to take part in it. Underage people, mentally ill patients, people submitted to hierarchy (military, police, employees, monks, students under researcher responsibility, etc.), institutionalized individuals (prisoners, people in assisted living facilities, hospice patients, etc.), patients with serious diseases (especially if treated in the same institution to which the researcher belongs) and minorities are typical vulnerable groups. Any other situation or condition that reduces the capacity of the individual to say "no" when invited to participate in the project characterizes vulnerability. If a person is vulnerable, he or she should not participate in a research project, unless there is no one else able to participate, no other way to obtain the information, or the human subject will achieve a direct and important benefit from doing so.

Unbiased analysis of discomfort and risks

A description of any anticipated discomfort, risk and benefits should be added to this section. Whenever possible, the protocol should estimate their probability of occurring and their likely intensity. Analysis of discomfort, risk and benefits should be thought of and written about from the human subjects' point of view. The EC will always evaluate the research protocol considering the possible and likely consequences of the project methodology on the human subjects submitted to it. Furthermore, since any research performed on human subjects is potentially associated with risks or discomfort to the individual or to the community, whether they be local or systemic, immediate or

delayed, light or serious, predictable or casual, calculable or incommensurable, there will always be some level of risk weighing on humans participating in the study. Hence, a positive analysis between the foreseeable risks and the direct benefits is necessary. The researchers and the Committee have to consider if the knowledge to be generated has the potential to minimize suffering or to eliminate a disease or condition that affects humankind or part of it, especially if this part includes the human subjects participating in the study. Any attempt to hide or minimize the real discomfort and risks involved in a study, or even artificially inflate its benefits, is an attempt to fool the human subjects and should be avoided at all costs. All situations have to be analyzed by the EC individually in order to decide if the research outline is appropriate or not.

If an unforeseen risk to the well-being of the human subject is detected, at any time or in any way, his/her participation should be interrupted until all the circumstances are clarified and his/her safety is assured. In the same fashion, in clinical trials, if a proposed protocol proves clearly superior to the others, the experiment should be terminated and all human subjects should receive the best option. The research team should communicate any adverse effects promptly to the appropriate government agencies and/or the EC. It is ethically unacceptable, and may even be illegal, to require that human subjects waive their natural and legal rights under any circumstances. It is even worse if the requirement is included in the IC.

Any risks and discomfort will be directly related to the methods used on human subjects and their description should obviously be in agreement with the methodology stated in the project. Each method individually has the potential to cause harm and/or discomfort, and researchers should comment on its risks cautiously, even if the risk is small or unlikely to occur. It is not a question of considering only the "worst-case" scenario, but no possibilities should be ignored. Unfortunately, some researchers consider mainly or only the "best-case" scenario, an attitude that will certainly lead to errors. In fact, a complete list of all possible outcomes of each method is fundamental. For example, when surgery is scheduled as part of the research, all possibilities should be considered: Is there risk of bleeding during or after the procedure? Is there risk of reaction to any of the drugs used in the human subjects' preparation? Do the preevaluation exams cover all the possible problems that may increase the surgical risks? Are the clinicians really well prepared to perform the procedure? Is there enough equipment to care for the human subject and is the support team ready? Has a surgical plan been made? Should any critical conditions arise, who will take care of the human subject? Does the research team have insurance to cover

expensive procedures should they be needed?

Researchers should not assume that standard procedures (like clinical examination, complementary exams, etc.) are not associated with the research and set them aside. It is also a mistake to believe that only high-level risks, like death or physical damage to organs or tissues should be considered. Any alteration of bodily functions or well-being, even psychological or aesthetical, arising from participation in the project, must be taken into consideration, as most lawyers will confirm.

Careful discussion of risks and discomfort is not only good for the human subjects, but also for the researchers and their institution. Knowing all the possible risks and discomforts, the research team can work to avoid or reduce their impact, consequently improving their relationship with human subjects in a healthier way.

Clinicians and research teams tend to underestimate the effect that discomfort may have on people undergoing treatment or in diagnostic procedures. Discomfort is a factor frequently forgotten in the project outline, and one that may do more than just annoy some human subjects. Most projects depend on a certain degree of compliance by human subjects to follow recommendations and protocols, and suffering certainly sends the opposite message.

When considering risks and discomforts, the authors should "impersonate" the human subject, exercise empathy, taking his or her view in order to understand how a person feels when subjected to the method in question. If the authors have real experience with the proposed methods, especially if they underwent the procedures themselves, they will be more careful when describing the risks and discomforts, and more benevolent when devising ways to reduce them.

Protective measures

As a direct consequence of the previous discussion, the authors should describe which measures will be used to eliminate or reduce the risks. In fact, both items can be discussed together, listing the risks and describing in detail what measures will be taken to prevent them. If there is no predictable risk associated with the method, the authors should state this, but should never ignore the discussion about the method. It is also very important to clarify who will take care of each problem. Names, telephone numbers, home or office addresses and email addresses should be made available. If there is a life-threatening risk involved, emergency telephone numbers and those of emergency clinics should be at hand or made available in the informed consent. The longer the

planned follow-up, the more careful the planning and the safeguards against risks should be. The necessary health care should be provided to cover regular or emergency events. Research teams, sponsors and hosting institutions have the combined responsibility to provide comprehensive care to human subjects during and sometimes after the experimental period, for either foreseen or unforeseen events.

Protection of confidentiality and data handling

Even though the human subject's health is the primary concern, authors should also describe the measures to protect confidentiality. If the research produces and keeps potentially stigmatizing information, like results of lab exams, history of infectious diseases, cancer, drug use or sexual behavior, extra care should be taken to ensure data safety, during and after the research. Access to the database and clinical files should be restricted to the research team, sometimes even to a single person. Lockers, storage systems, computers, files and spreadsheets with results should not be kept in places of easy access. Most exam results have some degree of identification and should not be handled carelessly or left on desks or counters. Lab books should also be stored in safe places at the end of each working day. Methods involving pictures of the face or genital areas create a complex ethical situation. Unless such pictures are essential, they should not be taken. If they are important, the human subjects should be clearly aware of the research method used and the conditions governing picture use and storage. Research material, including pictures, is commonly used in lectures, in presentations for scientific meetings and in publications, exposing the image of the human subject to the public. This has obvious ethical consequences, although researchers and clinicians alike do not always realize the problem. It is important to give human subjects the opportunity to decide (when signing the IC) if they agree with the conditions set in regard to taking, storing and using their pictures. If possible, black strips should be placed over their eyes in order to minimize the chance of identification, although this chance will never be totally eliminated in facial pictures. In conclusion, all the data belonging to human subjects should be used for a specific purpose only, assigned by the investigation(s), and should have guaranteed confidentiality.

Expected benefits

Human subjects should be clearly informed about the direct benefits that they might reasonably expect to derive from participation in the project, and that they would not have should they chose not to participate. The benefits to

science (greater knowledge about the subject), researchers (publications, grants, and reputation), institutions or companies (prestige and profit), or even to humanity (less suffering and better quality of life) are indirect benefits and should be listed as complementary to the direct benefit. Access to a standard health treatment, complementary exams, and medication may be listed as benefits, but the authors should be very careful not to mistake what the individual is already entitled to (whether participating in the research or not) with what the individual will not have unless he or she takes part in the research. What is already a right (by Constitutional privilege, insurance or part of a job) cannot be listed as an advantage of participating in the research, since there is no benefit in giving what is already granted. The diagnostic value of some methods can also be a benefit, but this issue should be considered with caution. Authors should consider how the results will benefit the individual, if the research will be useful to a doctor treating the individual or if it will improve life quality in any way. Authors should also explain how they will deliver the results to the human subjects (through a letter or a specific form, personally, etc.), and, more importantly, how they will deal with the consequences of each possible result. Will human subjects be treated within the research protocol or will they be sent to a health service nearby? Authors must also consider the potential of a result that may lead to despair or suffering, as would the diagnosis of a disease currently without treatment.

Reimbursements and payments

Authors should comment on and produce a list of all the expenses that human subjects may have in relation to participating in the research, and also describe how they will cover or reimburse them. Reimbursements should be calculated carefully so that the human subjects are not induced to participate, especially the economically disadvantaged. Of course, a small sum to one person may be a temptation to another, and a large amount of money to recompense the individual may interfere with his/her autonomy. The same may occur if an expensive treatment, a piece of equipment or a medication not available otherwise is offered to a human subject as part of the research work. In some places, even simple medical equipment or medical attention are luxuries, and this could cloud clear thinking about the problems involved in taking part in the experiment. Reimbursement should not be confused with payment. While not universally rejected, paying human subjects for research participation is generally considered suspicious and, in some countries, even illegal. If the amount of money or any sort of advantages offered is great in the eyes of the prospective

human subject, his/her clearness of mind and consequent autonomy will be put in check, invalidating the freedom that must accompany the process. In developing countries, even small sums of money may be tempting and may push individuals who would not otherwise take part in the research to disregard their inner choices. Participation under these conditions is not only unethical but also tends to be counterproductive and untrustworthy. Research expenses should not be charged to the local public health care system, and study subjects should not take the place or the funds of regular patients.

Indemnity, compensation and reparation for harm suffered

Projects with foreseeable risks should couple those risks to actions designed to repair any harm and/or compensate for it in those cases where irreparable harm or loss occurs. Conditions and availability for indemnity, compensation or reparation for any harm should be explicitly stated in the protocol. The most obvious but certainly not the only harm is the death of a human subject during or caused by any experiments. If there is a reasonable risk of harm, and this harm cannot be repaired, the researchers or the hosting institution must have an insurance plan to address the needs of the human subjects, their families and other parties involved. This type of insurance may not be available in some countries and may have to be bought elsewhere. If the researcher does not have insurance or the financial means to provide for his/her obligation in a case of legal compensation, the institution where the research is being conducted will likely be involved in the lawsuit and will have to answer to the party claiming damages.

The enormous differences in the legal systems of different countries should be considered when deciding how to describe and determine the compensation in the protocol. In case of doubt, the opinion of an expert lawyer may be helpful. Regardless of the legal system, the right to compensation for damages associated with participation in experiments should not be denied to human subjects.

More frequently, the harm or problem caused by the experiment is not permanent and can therefore be repaired. Medical treatments, drugs, hospitalizations, surgeries, exams, and other actions can be provided in order to resolve the condition or problem and restore the health of the human subject. Repairing any problems caused by research interventions is an obligation of the research team, and may include a lawsuit, even if earlier action taken to restore the human subject to his previous state of well-being is successful.

Although not limited to the project methodology, legal compensation and

reparation are closely associated with the risks and discomforts caused by it. This is another reason why the authors must scrutinize their comments regarding the risks and discomforts associated with the proposed methodology and must establish how any harm or discomfort can be limited and is to be repaired. Professional behavior and competence in performing the methods are essential to reduce the chance of harm and to minimize the discomfort, but good rapport with the human subjects is also important to humanize the process.

Informed consent (IC)

Although information about the project and the complementary parts of the protocol are important issues, the IC is its focal point. It should be obtained in all research involving human subjects. In fact, almost all regulations and laws about research in humans consider the consent of the human subject or his/her legal representative as a fundamental condition before a research subject may enroll in the project. The IC is the source of information that will allow the individual to understand the project and elicit a clear, just and unconstrained decision. It represents the legal and moral protection safeguarding not only the human subject, but also the research team and the hosting institution, since it is the subject's open expression of agreement to participate in the experiment. The IC must be written in uncomplicated language, should be easily understood by the average human subject, and must contain a comprehensive summary of the protocol. The IC should always be prepared or at least subscribed by the chief researcher, and should be presented in a more inviting way, rather than in a formal, legal style. It must also provide the necessary contents and respect the assurances for all the parties involved. A template of the IC and of any other forms used to obtain the IC (letters, invitation folders, posters, articles for newspapers and others) should be disclosed beforehand to the EC, together with the project.

After drafting the IC, consider the possibility of asking a person not involved in the project and with the same cultural and educational background as the potential human subjects to read the IC and answer direct questions about the main points. Which methods will be used? What is expected of the human subject? What risks and discomforts are expected and how likely are they to occur? What are the human subject's rights and expected benefits while participating in the project? If any of these questions are overtly misunderstood, the text should be rethought. It is probably too complicated or lacks essential information for human subjects.

All informed consent forms should have a minimum amount of informa-

tion, as well as legal and ethical assurances, in order to be valid. A suggestion for the contents of an IC is described below. The suggested order may vary and some ECs may call for additional items. The contents of an IC can be separated in three major didactic parts: Information, assurances/guarantees and consent.

Invitation or opening section

The initial paragraph should contain the research title, full identification of the hosting institution, the names of the members of the research team, the rationale behind the research and a description of its scientific (or other) aims, and should invite the potential human subject to take part in the project.

Information

A group of paragraphs should convey the information needed to explain all the methodological aspects of the project to the individual. The information should be clear enough for anyone reading the text to have an idea of all the situations the human subject will face during or after the experiment, in a very direct way. In other words, it should allow full understanding of the project outline. If the entire research process is not known or understood, the consent will not be valid.

Methodology

The description should include all the methods that will involve human subjects directly or indirectly. Lab methodology should be included if it has the potential of affecting the human subjects. Do not undervalue simple or traditional methods, like clinical or radiographic examinations. Describe all methodology in enough details for any human subject to understand readily. Do not include details about methods that will not affect human subjects, like formulas or sequences used exclusively in the lab. A good balance between what should and should not be informed is important to achieve the goal of adequately informing human subjects without overwhelming them.

Foreseeable discomforts and risks

All predictable risks or discomforts potentially associated with participation in the research should be described in the IC. Comments made in the previous pages about risks and discomforts are also valid here, though the text should be simpler and more direct, to allow unhindered understanding. An easy way to understand what should be described is to "put yourself in the position" of the human subject and "undergo" all the proposed procedures. By being subjected

to the methods – even if only hypothetically – the researcher's empathy will be enhanced. Risk and discomfort have the highest potential for causing conflict during and after the experiments, and explaining them clearly before human subject enrollment has a strong appeasing effect on litigation. On the other hand, any unexpected pain or harm will be multiplied if the sufferer learns that this risk or discomfort was predictable and was not duly informed beforehand.

Foreseeable and direct benefits

The description of benefits in the IC should clearly mirror that of the research protocol, albeit in a more simplified and direct language. It is generally not a problem for researchers to state the benefits; the problem is usually overstating them. The most common mistake is to exaggerate real benefits or create benefits where there are none. Even for benefits that are easy to understand or are delivered directly, like medications and laboratory exams, the text should be very clear on how the benefits will be delivered, for how long, by whom and for whom. Restriction clauses should be clearly stated, never implicit. Some benefits are not necessarily good for all human subjects. The results of the genetic evaluation of hereditary conditions or the serologic evaluation of infectious diseases, for example, may even be harmful to the interests of a human subject. Appropriate explanations should be given in these cases, so that a human subject may decide in his/her best interest. It is also questionable to offer advantages as "bait" to lure individuals that will have difficulty obtaining these benefits otherwise, particularly when the circumstances involve life threatening or severely disabling diseases or conditions.

Alternative methods

Human subjects are entitled to know if there is an alternative way to obtain the information the research team wishes to produce with the project. Can the information be found in the published literature, making the current project redundant? Can the information be obtained with experiments on animals? Can a smaller number of subjects be enrolled using another method? Can a less risky or invasive method produce the same level of information? In projects where treatment is the object of the research, it is necessary to explain to human subjects what other ways there are of treating the disease or condition. Are there any other medications that can be used to treat human subjects? Is the proposed treatment likely to have any advantage over the other options? What is the rationale behind the preference of one option over the other? If it is a new treatment and the advantages or disadvantages, compared with standard

protocols, are not yet entirely clear, this should be explained unequivocally to the human subjects. In short, human subjects should not be made to believe that the proposed research protocol is the only one available, unless this is really true, and should further be informed about the available options.

Assistance and follow-up

This section should contain information about who will be responsible for providing assistance and for following the human subjects, and how these services will be provided during and, when necessary, after the experiments. Each project has a particular sequence of methods and procedures, according to its particular needs. The more complex and serious the methods and conditions or diseases involved in the project, the more sophisticated the "safety net" established by the research team must be in order to protect the human subjects involved. Simple procedures may require supervision while conducting the method, ranging from a few days of supervision to a decade or more of follow-up. Postal addresses, telephone numbers and email addresses should be made available to human subjects. It is important not to restrict contact with the research team by human subjects, although a specific person may be designated as a "support person" for the most common queries. Longitudinal projects dealing with disease treatment or complex diagnostic procedures, or any project or method with potential consequences to human subjects, should have a set of protocols to provide explanation, health care, lab examination, medication, transportation or any other way of assisting human subjects. Support may be needed after-hours if the conditions being evaluated so require. Patients with certain diseases or conditions may need medical attention at any time, at short notice. Emergency 24/7 phone numbers should be written in cards for human subjects, along with instructions for specific situations. Extremely delicate situations may require a hospital environment. This occurs in research testing for new medications or those associated with likely strong side effects. In all situations, simple or complex, instructions meant to aid human subjects should be very clear, preferably in print, and should indicate what to do, where to go, and whom to look for in the possible situations, particularly in case of emergency.

Ways to contact ECs, researchers and institutions

The IC should contain information on the proper ways to get in touch with the EC, telephone and fax numbers, postal addresses, email and website addresses. The information on where and how to contact the research team or the institutions involved in the project should include at least postal address,

telephone number and email address. Projects involving procedures that might lead to urgent problems must make after-hour phone numbers or information on emergency room facilities available. An introductory text should state what sort of information or assistance human subjects should look for in the IC or at the institutions, or request of the researchers.

Assurances and guarantees

In order to obtain ethical clearance, the research protocol and the research team should consider that all human subjects have some basic rights. These rights are the result of a historic process of learning from past mistakes – sometimes gross – of previous studies (although not necessarily conducted long ago). The suffering, mutilation, humiliation, and sometimes death of human subjects caused by scientific research studies have led society (thus, also researchers) to set some limits to protecting other individuals from the same fate. Accordingly, the basic rights of human research subjects are not granted by the research team, EC or research institution; rather, they are a result of the moral and ethical development of the entire society and are on equal standing with human rights.

Explanations about the project

All relevant information about the project must be delivered in a clear and direct manner to human subjects, before, during and after the research, even if the information may have the potential of influencing the human subject to take part or stay in the project. In fact, if the information has the potential to influence the risk of harm to human subjects or to prevent it, this must be emphasized even more in the explanations. A good example is the possibility of being selected for a control or a placebo group in a treatment protocol. In fact, information about the project's outline should not be underestimated.

One's freedom to not participate in the project

Whatever the participation in a research project may imply, it must be completely free of constraints or coercion. This is the very basis of research performed ethically. The consequence is that the individual's freedom to refuse to take part in the study, to leave it in the middle or even to ask for the removal of his/her data from the study after its completion must be very clearly guaranteed in the IC and be unconditionally respected. A suggested statement would be "You have the right not to accept taking part in this project or to withdraw your consent, at any stage of the project, without being liable to any penalty

whatsoever." Additional guarantees may apply in specific cases, e.g., when students take part in a project. In these situations, it may be useful to add a statement safeguarding against academic punishment. For patients in treatment, or awaiting it, the IC should underscore the guarantee that clinical care will not be refused to non-participants.

Confidentiality of identity and research data

This right is based on the privacy granted to most individuals in most cases and in all situations of life, including that implied in research participation. The protocol established to deal with personal data should be clear to human subjects. How will the information be stored? Will it be used in any environment outside one of research? Who will have access to the information or to the files? How will the data be reported and published? In special cases, when pictures or examinations bearing any form of identification are produced and stored during the research project, special guarantees should be established. Will these materials be displayed anywhere, published in scientific journals or presented in conferences and classes? If so, human subjects should have the option of accepting or rejecting use of their images, and also the option of accepting or rejecting participation in the project. Are there any legal issues or previously arranged conditions about data confidentiality in the project? If so, they should be explicit in the IC.

Reimbursement of expenses

As discussed earlier, participation in the research cannot entail any expenditure by human subjects. Any foreseeable expenses resulting from participation in the research should be described in the project and their reimbursement, properly planed. Unforeseen expenditures should also be reimbursed. Protocols oten fail to consider expenses involved in transportation, lab examinations or work absences. Even though these may not be primary expenses, if caused by participation, they should be compensated.

Indemnity, compensation and reparation for damage

As discussed previously, one or more protocols should be established for each risk and any probable harm or problem, explaining clearly how that problem or harm will be dealt with, who will deal with it, and where it will be dealt with. Specific addresses and telephone numbers should be made readily available to human subjects. Any foreseeable harm may not occur, and any unforeseeable harm or loss that may occur must also to be dealt with by the research

team if it occurs as a consequence of participating in the project. It should also be clear that human subjects have a natural right to seek compensation outside of the IC terms, and no clause of the IC or other research documents should suggest or imply the contrary.

IC copy

A human subject has the right to receive a copy of the IC, signed by the researcher, and the researcher should retain a copy signed by the human subject, for a legally recommended number of years.

Consent

After a potential human subject has read the IC and all the necessary related information, has clarified any pending doubts, has totally understood the research and his/her participation in it, and is completely satisfied, the IC can be signed by both parties. The IC should not be signed if any of the above conditions are not met. Blank spaces for filling out name, identity numbers, telephone numbers, and other information should be left at the end of the document.

Special cases

There are certain cases where additional care is necessary in order to guarantee ethical standards in research. Individuals that have permanent or temporary restrictions in their ability to understand the project, to make a decision or to refuse to participate are considered vulnerable individuals. These include people subject to authority (soldiers and other military personnel, institutionalized people, people involved in religious institutions, prisoners, students and employees), those with mental problems (mentally ill, mentally disturbed, under the influence of drugs, etc.), underage individuals, ethnically differentiated groups, or those under any other circumstance that may reduce their ability to refuse to participate. Illiterate subjects should mark the IC with a fingerprint or other manner of expressing their approval and agreement.

If certain scientific subjects or circumstances require that the research be done with vulnerable individuals, their participation should be clearly justified in the protocol. For some of these groups, the IC must be presented to and signed by the legal representative or guardian of the vulnerable person. This is also true for underage individuals, for the mentally ill and for native Indians. In each case, the research subjects should be involved in the process of obtaining an IC to the limit of their comprehension, and to the agreement of their legal

representative.

Indians and culturally differentiated communities should fulfill a two-level agreement process for the IC. The first level to be consulted is generally the community leader, who must agree with the research. After obtaining the agreement of the local leader, each individual should be involved in the process and personally agree with the IC. In some countries, governmental authorization is necessary before seeking culturally differentiated communities.

If any situation or condition should arise preventing the application and attainment of a proper IC, researchers should clearly explain such circumstances and request an EC's authorization to proceed with the research under a specified condition. Such circumstances are more common in projects involving long stored samples and historical files. New collections of biological samples should be properly labeled to include individual's contact information.

Some protocols require that an IC be obtained after the methods are applied, like those involving medical emergencies and educational psychological research, when knowing the procedure is part of the research may prevent the expected outcome from occurring. In these cases, an IC should always be obtained afterwards, and if the individual does not agree, his or her information should not be used in the research. These circumstances should be described and justified in the research protocol. Moreover, if the research outline requires that some information be restricted for release to human subjects in order to secure the desired results, the EC should be informed, and the requirement, dully justified.

When research is performed with methods having a high risk of hurting the human subject's reputation or countering his beliefs, like illicit drug use, illegal acts, sexual behavior or preference, or even some lab tests, researchers may set special confidentiality safeguards, e.g. non-identified ICs and questionnaires to secure complete anonymity. In these cases, an explanation should precede data collection in order to clarify the situation to the research subjects, stating that answering the questions or performing the requested actions implies agreeing to take part in the research.

Research with human parts, bones, discarded organs, aborted fetuses, and dead people is subject to additional legal regulations in most countries. Researchers should consider and follow any such regulations carefully. The consequences of not doing so may be much more direct and strict than when live subjects are involved. Authorization for research with deceased people or body parts is normally given by close relatives, if the deceased is identified, or by a legal authority, if the dead person is not identified or has no family. Occasionally,

some people may donate their bodies for research purposes post-mortem. The assistance of a lawyer may be helpful if the research team or the hosting institutions have no previous experience with these situations. Additional care should be taken not to use any material or human resources or even the premises of live patients when performing the research procedures. Similarly to the case of live people, it is fundamental to avoid unnecessary procedures and mutilations of a dead person's body. Even the dead should be given proper respect.

Declarations, forms and other paperwork

The paperwork required by each EC can vary widely, even in the same country. The research team must check with the local EC for the requirements regarding forms and declarations. Some commonly required documents are described below.

A formal request by the authors soliciting an ethical evaluation of the protocol is the first step of the process. The chief researcher should sign the request, and the text should include the title of the project and other information considered appropriate by the local EC. Acceptance of the national or international ethical regulations for research on humans may also be required, and some ECs have a specific form for this purpose.

In general, it is necessary to secure an authorization or agreement of the hosting institution before performing the research. This is important not only for opening doors, but also for legal purposes. In most countries, research teams and hosting institutions share the expenditures and legal responsibilities. The same applies to sponsors, especially in projects financed by private companies or by people with financial interest in the research results.

All arrangements providing special conditions for enrollment of human subjects, disclosure of results, patents or any restrictions should be made clear to the EC and, sometimes, to the research subjects. Publication of the results should have no restrictions, since these limitations would prevent the community from learning about the side effects or the lack of results of medication, equipment or therapeutic procedures tested during the research. In this same respect, several important medical journals now require the research project to be registered in international systems so that the data may be made available to the public independently of publication in a journal.

A detailed description of the project budget, including how much money will be made available, what its source is and how it will be spent should be provided for EC evaluation. Payment to researchers and human subjects, especially if tied to the enrollment of human subjects or to certain clinical out-

comes, should be clearly stated.

If the research team or hosting institutions are planning on setting up a bank of biologic material, this intention should be declared and the legal requirements followed. All material collected during a specific research project should be used exclusively to achieve the aims described in the project. An additional, IC should precede any new use of biological material or data collected during the research.

The résumés of the main researcher and other participants must be included in the protocol, allowing clear disclosure of the professional qualification of the researchers, particularly if the protocol includes specialized or risky clinical procedures. The EC has an obligation to evaluate this information, and has the right to question the research team if it feels that lack of research team expertise may put human subjects in danger.

The chief researchers and the hosting institution should clearly state which ethical regulations, declarations or laws will be followed. In countries with no established regulations, the declaration of Helsinki[10] and the International Ethical Guidelines for Biomedical Research Involving Human Subjects[11], and other such documents[8,9,13-16] should be followed.

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3

Epidemiology

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Has the prevalence of malocclusion been diminishing among Brazilian adolescents in recent years? Is the use of resin more effective in the treatment of caries than amalgam fillings? Is periodontal disease associated to premature birth? These and other questions either directly or indirectly affect all professionals who practice dentistry, whether researchers, clinical practitioners or public administrators. Such questions make up part of the routine work of researchers, along with the use of methodological tools needed for obtaining valid, reliable results.[1]

Epidemiology is the study of factors affecting the health and illness of populations and serves as the foundation and rationale for interventions made in the interest of public health. It is considered the most important methodology of public health research and is highly regarded in evidence-based medicine for the identification of risk factors of disease and the determination of optimal treatment approaches in clinical practice. Epidemiologic studies involve the definition of study design, data collection, statistical analysis, data interpretation and the documentation of results for submission to peer-reviewed journals.[1-4]

Epidemiology has traveled side-by-side with clinical practice since its emergence as a science. The two are complementary practices that to-

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gether focus on the health-illness process as their object of interest. They differentiate only with regard to their field of operation. While clinical practice is concerned with the health of individuals, epidemiology addresses collective health problems. As such, its work needs to cover issues related to housing and sanitation conditions, transportation and access to educational services.[3]

In the field of epidemiology, the emergence of disease is related to a series of events that can be identified and investigated. Since the occurrence of these events is not a consequence of chance, it is important for researchers to know how to identify the best route to follow in order to solve the problem at hand.

Issues connected to where a particular disease emerges are considered important clues in epidemiological investigations. The results could have repercussions for policies on local development, urban planning and transportation.[5] The 1850 study by John Snow on the ingestion of water as a cause of a cholera epidemic in London was a monumental benchmark in epidemiology. Snow identified with great precision the context in which cholera occurred and its suitability to an experimental model of investigation. The researcher demonstrated a spatial association between cholera deaths and the provision of water from different pumps of the public water supply, thereby identifying the origin of the epidemic, even without discovering its etiological agent.[1,6]

In planning and analyzing an epidemiological study, it is fundamental for the researcher to consider the distribution of a disease as well as its temporal and spatial determinants in the population, along with the influence of the social dimension on the chance of illness and death. To this end, new methodologies and analysis techniques emerge constantly and are incorporated in epidemiology. There is a considerable diversity of researchers currently working in the field of epidemiology, including healthcare professionals from different specialties and researchers from other fields, such as demographists, statisticians, geographers, lawyers and historians. Most researchers work in universities, research institutes and public healthcare services.[1,7]

The aim of this chapter is not to offer an exhaustive description of the entire subject of epidemiology. Instead, it is merely to present the reader with concepts, study designs and methodological aspects involved in epidemiological research, as well as to discuss important aspects involving the effect or impact of exposure on the population studied.

Fundaments of Epidemiology

Epidemiology is defined as a science that studies the health-illness process of a population, analyzing distribution and determinant factors of disease and

events associated to human health. It indicates specific actions regarding the prevention, control or eradication of disease, and producing valid indicators for the planning, administration and evaluation of routine actions in health promotion policies.[3] Epidemiological studies seek to identify characteristics that differentiate the occurrence of a particular disease among the groups investigated.

With the firm commitment of contributing toward the formulation and monitoring of public policies (including actions aimed at reducing social inequality), epidemiology in Brazil has undergone considerable growth and has made technical-scientific advances in the last 20 years.[2,7] This becomes evident when comparing the few hundred people who appeared for the 1st Brazilian Conference on Epidemiology held in Campinas in 1990 versus the 6,500 participants in the 7th Brazilian Conference on Epidemiology, which was held in conjunction with the 18th International Conference on Epidemiology in the city of Porto Alegre in 2008. Approximately 5,800 scientific papers were received. Regarding the field of dentistry, the event in question merits special attention, since it was the first national epidemiology event to include the oral health line of research, demonstrating the strong presence of epidemiological studies in this field.

In order to understand the innumerous phenomena involved in the health-illness process, epidemiologists have formed interdisciplinary partnerships that go beyond traditional partnerships with statisticians and clinicians. Epidemiological studies have increasingly relied on the participation of sociologists, anthropologists, economists, geographers, philosophers, bio-engineers, toxicologists, microbiologists, mathematicians, etc.[2,8]

Basic terms and concepts

Epidemiological studies begin with a systematic gathering of information on a defined population and the quantification of the data collected. The data set has a specific nomenclature.[1,9,10]

- **Datum:** Any characteristic that can be observed or measured in some way. Data are considered the raw material of statistics (observable data).
- **Variable:** The factor or condition one wishes to observe in order to draw some type of conclusion. Variables are classified in the following manner:
  a. **Qualitative (or Categorical):** Characteristic of a population that cannot be measured. Classification:
     - **Nominal:** Symbols or numbers are used to represent the data, demonstrating the group (or category) to which they pertain (male/female; living/dead; smoker/non-smoker; present/absent).

- **Ordinal:** Ordered categories in conventional degrees. The data correspond to values that denote the order of first, second, third and so on. Ex.: bad/fair/good/very good /excellent.

b. **Quantitative (Numerical):** Characteristic of a population that can be measured. Classification:
   - **Discrete:** a variable that can only take on whole values in a set of values. A discrete variable is generated by a counting process (number of medical appointments per year; number of children per household; number of students in a classroom).
   - **Continuous:** a variable that can take on a value within an interval of values. A continuous variable is generated by a measurement process [ex.: blood pressure (mm Hg); height (cm); Body Mass Index; temperature (Celsius)].

Validity of epidemiological studies

In order to carry out a valid (or reliable) epidemiological study, it is important for the researcher to be cautious in order to avoid arriving at erroneous conclusions. To this end, it is necessary to avoid methodological mistakes during the planning, execution and analysis of the results. A study is considered valid when it reproduces the truth of the facts investigated.[11] However, any investigation is subject to failure due to two types of errors: systematic error (or bias) and random error (natural in any sample process).

Internal validity is related to the use of information produced by the study to make inferences regarding the target population from which the sample was taken. External validity refers to the generalization of the results in relation to a population outside the study universe.[4]

In order to carry out a valid (representative) study, one of the main issues for researchers regards the sample size to be employed (animals, teeth, individuals), regardless of the type of study to be carried out (laboratorial, clinical or epidemiological).

Sample dimensioning

In seeking to determine a representative sample for an epidemiological study, the researcher must use a random sample and perform a sample calculation to define the number of participants. Furthermore, care must be taken in order not to commit any systematic error.[10,11]

Population (N): A set of individuals who are eligible for the study and from whom possible participants are sampled (also called sample universe, real

population or census). Based on the number of observations, the population is classified as (1) Finite: limited number of observations that can be counted; and (2) Infinite: unlimited number of observations that renders counts impossible.

For ethical reasons, limitations on human or financial resources and time constraints, it is normally not possible to analyze the entire sample universe. Thus, researchers investigate only a small portion of the population.

Sample (n): A set of individuals originating from the sample universe (not necessarily in a representative manner). The sample corresponds to a part of the population on which one intends to make inferences based on the results of the study (also called *target population*). The sample is always finite.

Main sampling methods

Epidemiology makes use of different sampling techniques. The ideal option depends on factors in the study: size and type of population, questions to be investigated, time and resources available (personnel and material), etc. The results obtained in a sample of participants only represent the rest of the population to which it belongs if the sample represents all the characteristics of the population (as if it were a photograph). To this end, the study design needs to follow an adequate methodology, making the variable investigated in the study a reflection of its behavior in the population. This means that the numeric values encountered should be similar to the corresponding population parameters.

The sampling method determines the ability to generalize the results in relation to the population studied and also the type of statistical analysis to be employed. It is important to know the characteristics of each method: (1) Non-randomized sample and (2) Randomized sample

Non-randomized sample

The selection process does not take into account the probabilities of each element to be included in the sample.

Quotas: The sample is recruited from a location of convenience for the researcher. When a possible participant shows up at the location and fulfills the eligibility criteria, he/she is asked to participate.

Accidental: The sample is recruited from a location of convenience for the researcher. When a possible participant shows up at the location, he/she is asked to participate (there are no inclusion criteria for participation in the study).

"Snowball": Individuals from a particular group are recruited. These same individuals are then asked to identify other people from this group, who are also asked to participate in the study and so on, until reaching the required

sample size.

Judgment: Only those individuals the researcher judges most adequate for the study are selected.

Randomized sample

One of the resources researchers use to obtain a representative sample for the study. The choice of the characteristics of the sample should be made through some type of random drawing.

Simple random sampling: All individuals have an equal, independent likelihood of being selected to participate in the study.

Systematic sampling: Intervals are chosen: Every n^{th} person is chosen for participation in the study.

Stratified sampling: The population is divided into strata by a variable of interest, and individuals are randomly chosen from within these strata to participate in the study.

Cluster sampling: There are two or more stages in the sampling process. Firstly, groups of units are randomly chosen. Within these groups, either all the individuals are selected or only some are selected at random.

1^{st} stage: random drawing of primary units (ex: schools)

2^{nd} stage: drawing of secondary units (ex: students)

In both stages, either simple or systematic sampling is used. This implies an operational facility in performing the drawing.

1^{st} - random drawing of number of units from the 1^{st} stage (ex: schools)

2^{nd} - drawing of units from the 2^{nd} stage (ex: students – only from the selected schools)

The sampling process by conglomerates influences the accuracy of the estimates, causing a loss of the internal homogeneity of the conglomerates. In order to correct this flaw, the researcher should include a large number of participants. This correction can be made using the cluster effect – multiplying the final sample size by a value between 1.2 and 2.

Biases

These can be defined as any distortion of the results of a study due to systematic errors. Bias is any partiality in the collection, analysis, interpretation, publication or reviewing of the data, the potential effect of which is to lead to conclusions that are different from reality. The most common types are selection bias, measurement/observer bias and confounding bias (or variable).[12]

Selection bias

A change in the estimate of the effect measure due to the way the participants were selected to make up the sample. The following selection bias situations may occur in a study:[1,10,13]

- Error in identifying the population or group studied.
- Losses or non-responses of participants.
- Insufficient sample size.
- Non-random selection of the sample in a population-based investigation.
- Losses during the follow-up of the participants.

Observer bias

Occurs in the following situations:

- Diagnostic error
  Ex: Diagnosis of dental caries
- Lack of validity of the data collection instruments

In order to avoid or minimize measurement bias, it is essential that an intra-examiner calibration step be carried out as well as an inter-examiner calibration step in studies that employ more than one examiner in the data collection process.

Confounding bias

The presence of one or more variables that are related to both the disease investigated as well as the exposure of interest. Confounding bias is present when a third variable is interposed between the exposure factor and the outcome of the study (disease investigated). In order for a variable to be characterized as a confounding bias, it must be considered a risk factor for the disease that is being studied. Ex: periodontal disease vs. cardiovascular disease (tobacco smoking: confounding variable).

Information bias

Alteration of the effect estimated due to errors in the classification or measurement of one or more variables.

Recall bias

This is more common in studies in which data are obtained retrospectively after the development of the disease. It is believed that those individuals identified with the disease investigated (cases) have a greater likelihood of recalling past exposure than those free of the disease (controls).

Causality in epidemiological studies

Epidemiological studies should identify the causes and determinants that influence the occurrence of disease and harm to health, incorporating them into the study designs. However, it is not easy to determine whether the cause should be considered necessary, sufficient or a protective factor for certain illnesses.[14]

Epidemiology employs certain theories in order to explain the origin and interaction of causal factors in the occurrence of illnesses, regardless of their being present on a biological or individual level or within a given social context. Life course theory, in particular, stands out for analyzing exposure (causal factor) that occurs at a specific moment in the course of a life and, over time, triggers a delayed effect in the etiology of a disease.[15]

The increasing presence of statistics in epidemiological studies has made researchers more attentive to risk factors of disease. From the simplest to the most complex, statistical models have become increasingly more prominent in the daily practice of epidemiologists. The contribution of multiple factors toward the emergence of disease was first stressed in 1965, when biostatistics began to be considered an essential tool in epidemiology.[16]

In order for key issues to be raised in epidemiological analysis, it is important for analytical tools to be created that are suitable to the various stages of observation (teeth, individuals) and that prioritize modeling patterns (individual, households, neighborhoods).[8,15] These investigations must incorporate a statistical model that either separately or simultaneously investigates the effects of unit characteristics on a group and individual level, and outcomes on an individual level. This model is called *multilevel analysis* and has been increasingly employed in epidemiological studies.[5,8]

Epidemiological study designs

Observational designs

Cross-sectional study design

A cross-sectional study is a photograph in time – a survey. It is carried out either at a single point in time or over a short period of time, with no structural distinction between predictors and outcomes. Participants are selected from a well-defined population.[9,13,17] A clinical examination performed during the National Children's Vaccination Day is a cross-sectional study in which the child population can be assessed on a particular day.[18] As the participants are only contacted once, such studies are relatively inexpensive. However, this feature limits their usefulness, since they are suitable for measuring descriptive information on prevalence, but not incidence.[18,19,20] Cross-sectional studies also offer

the advantage of avoiding problems regarding time and dropout rates found in a follow-up design.

Case-control study design

In a case-control study, the prevalence of risk factors in a sample of individuals identified as having the disease or other outcome of interest (cases) is compared with that of a separate sample that does not have this characteristic (controls). Allocating participants into groups according to their disease status is the basis of a case-control study. The choice of controls for any study requires careful consideration. In particular, controls should be free of the disease at the time they are serving as controls. The major principle is that the controls should represent the population at risk of the disease. More specifically, they should be individuals who, if they had experienced the disease, would have been included as cases in the study.[9,13,21] The design is simple: cases are those people with the condition and controls are those without the condition. It is a relatively inexpensive and efficient way to study rare diseases.

One problem with case-control studies is their susceptibility to sampling bias.[12] There are four approaches to reducing sampling bias: (a) sampling controls and cases in the same manner; (b) matching cases and controls; (c) carrying out a population-based study, and (d) using several control groups.[13,17]

The other major problem regarding case-control studies is their retrospective design, which makes them susceptible to measurement bias, affecting cases and controls differently. Such bias can be reduced by measuring the predictor prior to the outcome and by blinding the subjects and observers.[13,17,22,23]

Another drawback to case-control studies is recall bias, which occurs if cases and controls recall past events differently. Since the cases are actively chosen, a case-control study ensures that enough people will be found when a disease is rare. Carefully designed case-control studies can provide useful results, and such studies should be evaluated with careful consideration of the methodology employed.

Cohort study design

In a cohort or follow-up study, a healthy group of people is identified and followed-up over time in order to observe who develops the outcome of interest and who does not. The important point to remember regarding cohort studies is the time factor – at the beginning of the study, neither the people nor the researchers know who is going to develop the condition. This effectively avoids recall bias, although other types of bias may still hinder such studies.

Cohort studies involve two primary purposes: descriptive, which is typically to describe the occurrence of certain outcomes over time; and analytic, which analyzes associations between predictors and outcomes. In a prospective cohort study, the investigator defines the sample and measures the predictive variables before determining a follow-up period for the observation of the outcome. In other words, the investigator takes measurements at baseline that may predict the subsequent outcomes, and then follows up the subjects with periodic measurements of the outcomes of interest.[23] The prospective cohort design is a powerful strategy for assessing incidence and the natural course of a condition, and is helpful in investigating potential causes of the condition. It also allows the investigator to measure variables more completely and accurately than is possible retrospectively. The design of the retrospective cohort study differs from that of a prospective study in that the assembling of the cohort, the baseline measurements and the follow up have all occurred in the past. This type of study is only possible if adequate data regarding risk factors and outcomes are available on a cohort of subjects that has been assembled for other purposes.[13,17]

Experimental study

Randomized controlled trial

Finally, there is the randomized controlled trial or clinical trial, which is often called the gold standard of epidemiological studies. In clinical trials, the investigator administers an intervention and observes its effect on the outcome. The major advantage of a trial over an observational study is the ability to demonstrate causality. This trial is a human experiment in which people are randomly assigned to receive one treatment or another. Randomization, which eliminates bias due to baseline-confounding variables, should be tamperproof. Paired randomization, when feasible, is an excellent design. In small trials, stratified blocked randomization can reduce the chance of misdistributions of key predictors. It is preferable that the individual (and the healthcare professional as well) be "blind" to which treatment is being received, but this is not always possible. Blinding the intervention is as important as randomization and serves to control co-interventions and biased outcome determinations or conclusions. In clinical trials, the aim is to replicate a "real-life" situation so that the results are as close as possible to what would occur if the treatment were carried out in real life. Clinical trials tend to be extremely expensive, time consuming and address narrow clinical issues. Therefore, such trials are best reserved for relatively mature research issues, for which observational studies

and other lines of evidence suggest that an intervention may be effective and safe, but that stronger evidence is required before it can be recommended.[13,17]

Final considerations

Throughout the entire construction process of a study involving human subjects – from the design of the study protocol to data collection and the publication of the results – researchers must be aware of the ethical issues involved. The conducting of an epidemiological study enables a researcher to identify causal factors in the occurrence of disease or harm to health, but in order for these factors not to be identified and interpreted erroneously, it is important for the results to be analyzed in a precise, coherent fashion. Furthermore, it is essential for all methodological aspects of the study to be followed strictly. Regardless of the subject being investigated, the researcher must choose the best methodology executable within the actual context of the work. Every epidemiological study has limitations, regardless of whether the design is observational or experimental. When planning to carry out an epidemiological study, researchers should be aware of the following points: (1) the ethical aspects involved; (2) sample size; (3) eligibility criteria of the participants and study groups; (4) instruments used for data collection; and (5) analysis plan and reporting of results.

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4

Qualitative research

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The term "qualitative research" refers not only to certain research strategies and techniques, but also to the general theoretical-methodological perspective adopted by the researcher, that is, to the researcher's "arguments, views and logic in his or her way of thinking and doing things".[1]

In public oral health, as well as in other fields of interest related to public health in general, issues and situations requiring research come up on a daily basis. However, because of their very nature, these research subjects merit different approaches from those based on the positivist method, which in general looks for "the facts and causes of phenomena, regardless of the subjective states of the individuals experiencing these phenomena".[1]

Two examples of this kind of problem serve to illustrate the previous statement. The first example comprises the cases of very low patient compliance to good hygiene habits and in which we want to identify the reasons for this behavior. The second one includes the cases of communities with high prevalence of early childhood caries, the causes of which we also want to identify to recommend actions to help control the problem. In the first example, patient compliance to good daily hygiene habits is known to be related to multiple economic, political, social, and cultural factors, raising the need to use broader approaches to study the problem. Therefore, a qualitative study may possibly provide us with more information than one based on a quantitative approach. The second example is somewhat similar to the first one, as the issue of caries in early childhood is not believed to be an exclusively early infection problem by those who study this subject. Other determining factors have

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been highlighted as well, indicating that research on high caries prevalence in a community should include approaches that allow us to understand the views of the social actors involved, in this case the parents or care providers of the children with caries, on the causes of the phenomenon.

The intention in the following pages is not to cover the entire subject of qualitative research, as this is not possible. The available space merely allows us to comment briefly on the more general features of the qualitative approach in research work and, especially, to share with our readers the idea that this approach will always be an alternative for studying many of the issues related to public oral health.

Finally, it is necessary to clearly state that we do not view the qualitative and quantitative approaches as mutually excluding strategies. On the contrary, we believe that the tensions around the use of one or the other approach are due more to the researchers' lack of knowledge than to the apparent incompatibility between these approaches.

What is qualitative research?

As mentioned before, qualitative research is an approach rather than a specific set of techniques. The purpose of qualitative research is to "understand the phenomena from the viewpoint of those who experience them. The reality that matters is the one that people perceive as significant".[2]

In other words, this means that qualitative research focuses on the search for the "sense" or "meaning" that the phenomenon under study has for the people who experience it. Therefore, there is no superior interest in looking for the "truth" or in defining laws, based on the study findings, that may apply to entire populations to foresee their acts, customs, habits or behaviors.

This interest in searching for the meaning of phenomena accounts for some of the features of qualitative research. Firstly, the great majority of the time, qualitative research is conducted in the natural environment where the people or populations under study live in. "The researcher who applies a qualitative approach is trying to understand people within their own reference framework".[3] Secondly, the people and essential traits of the phenomenon under study cannot be reduced to variables that fragment reality, thus preventing it from being perceived as a whole. Thirdly, the qualitative approach assigns the same value to all the social actors involved, "all their views on the phenomenon surveyed are important".[2]

According to Galeano[1] (2004), qualitative research "is directed towards understanding reality as the result of a historical construction process based on

the logic of the various social actors, looking 'from within' and retrieving the uniqueness and distinguishing features of social processes."

Why should one choose a qualitative design?

Because health researchers often encounter phenomena or issues that are closely related to the experiences of people (who are not always "patients") and communities where economic, social, political, and cultural processes directly associated with the production of health and disease are gestated.

Therefore, it is our duty to understand these phenomena, by valuing what the subjects themselves think and do on a daily basis, both individually and collectively. In many occasions, this cannot be done from the perspective of the method used by the natural sciences, as their fragmentary conception of reality does not allow researchers to assess the phenomenon holistically.

Therefore, we choose to use a qualitative design because of the nature of the question, "because of the need to know and research more thoroughly the specificities, differences and contrasts [...] requiring changes in the research techniques and approaches, which are restrained by the logic of large numbers".[1]

What are the most popular modalities of qualitative research?

Qualitative research studies have been categorized into different modalities by various authors.[4-7] The modalities mentioned below do not cover all the possibilities, but were primarily selected based on the criterion of being the most widely used in the field of health research. These modalities include Phenomenological studies, Ethnographic studies, Grounded Theory studies, and qualitative Case Studies.

As was previously mentioned, these four qualitative research modalities share a common attitude in face of the nature of the knowledge and reality (a reality which is influenced by social relations) they study, and in face of the nature of the interactions between researchers and the knowledge they generate (knowledge is a shared creation based on the interaction between the researcher and the human subject).[8]

However, from the perspective of the methodological design, each modality has its specificities. Creswell[3] (1998) proposes that, while the phenomenological studies focus on understanding a concept or phenomenon *(for example, the dental care experience for teenagers)*, the grounded theory focuses on the development of a fundamental theory *(for example, how adult patients deal with the problem of edentulism)*. On the other hand, the case study focuses on

thoroughly understanding a case *(for example, the customer service barriers in a specific healthcare institution)*, while ethnography focuses on understanding human behavior culturally *(for example, the role of teachers in healthcare within the school environment)*.[3]

We recommend readers to revise *in extenso* all four modalities to be able to decide which one offers the most appropriate design for their research question and objectives. Some of the texts recommended for each case include:

- **Phenomenological Studies:**
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- **Grounded Theory:**
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- **Ethnography:**
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- **Case Study:**
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What are the most common problems encountered when deciding to conduct a qualitative study?

Overall, qualitative studies require adequately trained researchers, otherwise techniques may be applied incorrectly, as is frequently the case, potentially leading to erroneous conclusions. Inversely, a qualitative methodology is sometimes applied when a quantitative methodology would be more appropriate.

In qualitative studies, fieldwork and data analysis are very time-consuming. Therefore, in some cases, these studies may not be very practical to conduct, depending on how urgently results are required. In addition, researchers lack-

ing expertise tend to analyze qualitative study results as in a quantitative study. This leads to a not very thorough and comprehensive analysis of the study findings.

The researcher's standpoint may be the most difficult issue to handle while conducting a qualitative study. How researchers deal with their subjectivity, without introducing biases, may be a definitive factor for the validity and reliability of the results.

These and many other problems that may be encountered by researchers who choose to use a qualitative design may only be avoided if there is enough knowledge on the philosophical, theoretical and methodological differences between quantitative and qualitative studies. This is why we insist that researchers should receive previous training on these types of approaches, especially if we consider that this kind of training is generally not part of the education of healthcare professionals. These approaches have historically been studied as part of the natural science methods.

Despite all this, public oral health researchers should not feel discouraged. On the contrary, these difficulties are a challenge to enrich our object of study and achieve a better understanding of issues. This will contribute to provide policymakers, planners and populations with qualified information to help them make better decisions about policies, plans and programs developed to deal with these problems.

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5

Meta-analysis

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The speed at which new scientific knowledge is generated in today's world, both generally in all fields and particularly in dentistry, has forced researchers, clinicians, and policy and program planners to resort to methodologies that may enable them to summarize and quickly, but safely, assess available knowledge, to support decisions made to address specific problems.

These methodologies have been known for various decades as "literature reviews". Initially these reviews were conducted in a narrative rather than systematic way, i.e., they were limited to reviewing the evidence concerning a subject of interest in order to describe methodological features and report outcomes. The scientific quality of the papers was inferred based on this procedure. Subsequently, the methodology used to review the literature became more rigorous. Issues, such as selection of the papers to be included in the review and analysis of results, are now addressed more consistently, following strict criteria that leave no room for the investigator's subjectivity.

The overall objective of a systematic review is, thus, to assess the current degree of evidence there is to respond to a research question about a specific subject. When a systematic review focuses on estimating quantitatively the parameters used to assess the available evidence, we are then talking about a meta-analysis.

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What is a meta-analysis?

A meta-analysis is a type of study that gives priority to the systematic and structured statistical analysis of the pooled results from different controlled clinical trials on a specific question or is-

sue. It is expected that by consolidating the outcomes of multiple studies, it will be possible to obtain overall measurement parameters with higher statistical power than in any individual study reviewed.

This is why, in evidence-based dentistry, the meta-analysis methodology is considered to provide researchers and clinicians with a highly reliable tool for healthcare decision-making.

What are the steps involved in conducting a meta-analysis?

When researchers or clinicians decide to conduct a meta-analysis, they should take the following steps to ensure the best quality for their work:

1. Defining the question carefully in a concise, precise and clear manner.
2. Defining accurately the measure or measures that will be used (for example, relative risk, disparity ratio) to assess the effect of interest for the study.
3. Conducting a thorough and reproducible search of the original studies on the subject.

   The search for scientific articles reporting results of interest should be conducted in all the sources possible: electronic databases, the "grey" literature (not published in indexed journals), and even consulting researchers with renowned expertise in the study area in question. Finally, it is advisable to select articles regardless of the language they are written in. Often, only articles in English are selected and this may lead to a selection bias. The researcher should not forget that the quality of the final product depends largely on this step.
4. Selecting the studies that will be included in the meta-analysis: Deciding which articles found in the previous step will definitely be included in the meta-analysis requires the definition of criteria that may be used to thoroughly analyze the features of each study. Some of these criteria include study design, patient inclusion/exclusion criteria applied by the researchers, sample size, and the procedures used to select case and control subjects. Comparability between studies in terms of interventions and outcomes should also be taken seriously into account, as this is key to the success of the meta-analysis.
5. Gathering the significant and relevant information from each study: Two topics are essentially analyzed in this step, i.e., the methodological quality of the study (including the statistical analysis methods used) and the study results, with an emphasis on presenting the variables used to measure the effect of interest.

6. Analyzing the heterogeneity of the studies: This analysis is conducted by applying various statistical tests. If heterogeneity between studies is high, researchers may decide to suspend the meta-analysis.
7. Selecting the statistical procedures to combine the results of the studies included in the meta-analysis: What procedures will be selected to pool results will depend on the type of variables used to represent the effect under study.
8. Conducting a sensitivity analysis: This type of analysis is used to assess the methodological quality of the studies included in the meta-analysis. There is no agreement on which is the best moment to apply the sensitivity analysis. Some authors consider it should be conducted during the study selection phase (step four); others feel that it should be applied during the results combination and analysis phase to establish whether results were affected by the methodological quality.

What problems may I face while conducting a meta-analysis?

Based on the previous phases we may easily deduce that the most frequent problems faced by a researcher who has decided to conduct a meta-analysis are:

a. Including studies that are very different from one another, without clearly identifying the origin of their heterogeneity.
b. The publication bias, which occurs when the investigator tends to prefer a certain kind of publication to search for articles.
c. The selection bias, which occurs when the investigator does not set clear inclusion and exclusion criteria for the studies to be reviewed.
d. Defining measures to asses the effect of interest incorrectly.
e. Using statistical analysis techniques which are irrelevant for the type of available data.

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6

Clinical research methodology

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Clinical experience is unlikely to be passed on to others, except when knowledge is obtained by applying the scientific method consistently. In recent years, knowledge has increased exponentially day after day, giving rise to the pressing need to review the international literature. Evidence-based dentistry now allows us to access increasing scientific evidence, critically assess its validity and utility, and incorporate it into our clinical practice. Based on controlled clinical trials, evidence-based dentistry uses meta-analyses to select, summarize and quantify studies and results related to specific subjects. By combining various studies, a meta-analysis may increase their statistical power and lead to a single result, which is particularly important to plan future research.

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While conducting clinical research, the following aspects should be taken into account:

1. There are Ethical and Legal considerations that should be respected.
2. Proper planning based on a clear description of a logical and consistent Problem is essential, prior to begin-

ning the trial.

3. It is important to have reference parameters or controls, carrying out experiments with control groups.
4. The use of Statistics is required, as biological phenomena suffer inter- and intraindividual variations. Statistical science, which is able to account for such variability using probabilistic considerations, allows us to estimate the number of patients required to conduct the study and establish the possibilities of generalizing the study findings.

Planning a research project should begin with a logical question, while the objective of the project should be to answer it. One should bear in mind that it has to be about an original topic or an aspect of a disease that has not been studied yet. It is important to be sure about this before engaging in the research effort, as a clinical study requires diligent work. The bibliographic search should be thorough and answer the following questions: What is the current state of knowledge on this subject or topic? Why is this work supposed to be important? What is its potential contribution to knowledge? How the problem and working hypotheses are defined will depend on the researcher's conceptual richness and creativeness. Planning and organization will contribute to developing a thorough and accurate working protocol that will make the task easier. Writing the work plan is the first step required to submit the project for funding (scholarships or grants). It is also a very good habit that helps researchers organize themselves clearly, logically and efficiently.

1. Types of studies

Designing a study is a complex task. The first thing to decide is whether the researcher will play a passive role, as an observer of a phenomenon or event, to conduct a so called observational study, or an active role, applying an intervention to analyze the behavior of a variable, in what is known as a clinical trial or intervention study. For clinical research to be complete, it should actually include both types of studies.

If the objective is to thoroughly understand a problem, a single experimental study will not be enough, as results will be obtained in a limited setting. Although obtaining reliable results may be more difficult and time-consuming in an observational study, all relevant factors and interactions are present and will contribute to the study. There are multiple classifications, the most important of which are shown in Table 1.

Table 1 - Types of studies.

Criterion	Classification
According to the objective	Descriptive
	Analytical
According to handling of study variables	Observational
	Experimental
According to the population follow-up	Cross-sectional studies
	Longitudinal studies
According to the direction of the analysis Analytical Studies	Cause-Effect: Cohort studies
	Effect-Cause: Case-control studies
According to study onset	Prospective
	Retrospective

1.1. Observational studies

Observational studies are conducted to describe a phenomenon in its natural setting, in its own reality, without the independent variables being manipulated. There are many types of observational studies:

- According to the time of observation, they may be divided into cross-sectional or longitudinal studies.
- According to their objective, they may be descriptive or analytical studies. Descriptive studies make it possible to describe the frequency of a disease or feature in a group or population, as well as its distribution by sex, age, location, time, etc. They make it possible to generate new etiologic hypotheses and identify associations that may subsequently be confirmed by analytical studies. These studies, in turn, are designed to identify risk factors for a disease, estimate their effects and suggest possible intervention strategies, which will be applied in experimental studies.

  Next, we will describe the most frequent types of clinical research studies.

1.1.1. Cross-sectional, prevalence studies

These studies describe the state of one or more variables at a single point in time, and estimate the frequency of a risk factor or disease in a population. They may provide information on associations or correlations, but do not seek to establish the cause-effect relationship. Correlation studies do not examine variables separately, instead they focus on the correlation between two or more variables. It is an instant, static view of a situation, like a snapshot. One of their

main tasks in epidemiologic research is to measure or quantify the frequency of a disease. These studies are essential to health planning.

1.1.2. Longitudinal or cohort studies

They study subject groups (cohorts) over a period of time, looking at how one or more variables evolve or change in time or how they are related with one another. These studies may either have a descriptive or an analytical objective. Descriptive studies are useful to describe the incidence of certain effects or consequences in time, while the analytical studies make it possible to analyze associations between predictors and effects.

These designs may be of two kinds, depending on their directionality:

- **Prospective studies:** The researcher defines the sample and the measures of predictor variables before the effects occur. This is a very good strategy to define the incidence and study the probable causes of a disease or phenomenon. These studies are very important, especially, for example, while doing research on nutrition, as it is much more feasible to record relevant factors this way than by inquiring people about past alimentation habits. This type of study is not suitable for infrequent diseases or cases. Prospective studies have the drawback of being costly, especially if they are long-term studies.
- **Retrospective studies:** The researcher defines the sample and collects the data on predictor variables after the effects occur. This type of study is only possible when the patient cohort is selected for other purposes. In this way the influence and bias that may occur when the authors of the study investigate specific effects are avoided. Retrospective studies are less costly and time-consuming, but must adjust exclusively to variables already recorded in the past, even if these variables are not always ideal or very representative. Both prospective and retrospective cohort studies may have a case-control design. The study will be more powerful statistically if the sample subjects are chosen randomly.

The studies described above are useful to assess the prevalence and incidence of a disease. Data are collected by using censuses, records and surveys. Frequency is usually expressed in three different ways: proportion, rate and ratio or index. Prevalence refers to a specific point in time, while incidence measures the number of new cases in a risk population within a given period of time. These features are summarized in Table 2.

Table 2 - Difference between prevalence and incidence.

Prevalence	Incidence
Probability of being ill at the time of measurement	Risk of getting ill or becoming a case in a given period of time
Static concept	Dynamic concept

1.1.3. Case-control studies

A case-control study is an analytical type of investigation, in which individuals are divided into two groups: those who have the specific disease feature under study, called cases, and those who do not have it, called controls. These two groups are used to assess the relationship between the disease and the one or more variables under study (characteristics, states, events or exposure to factors). The study may investigate both present and past situations or factors. If it addresses past situations or factors, the study will have a retrospective longitudinal design. Case series studies are descriptive and make it possible to confirm associations with risk factors more clearly and quickly, by estimating odds ratios. However, these studies do not provide prevalence or incidence data. The effects are the starting point to infer probable causes and study associations.

Advantages:

- They provide abundant information with few subjects and are especially recommended for infrequent and/or long latency lesions.
- They are easier to conduct in a relatively shorter period of time and at low cost.
- Because of its retrospective design, a large number of predictor variables may be examined, which is useful to generate hypotheses about causes and new disease symptoms or features.

Disadvantages:

- Temporal associations cannot be established with certainty.
- Likelihood of bias is high.
- They are not very useful when exposure to the factor is very low.

Special care must be taken with biases, which are frequent. They especially occur because cases and controls are sampled separately and predictor variables are measured retrospectively. Ideal sampling is to select both controls and cases from the same risk population (see sampling strategies).

1.1.4. Other types of studies

Nested case-control studies: Both in prospective and retrospective cohort studies, the "randomized nested" case-control design is excellent for predictor variables that are very costly to measure. These variables can thus be evaluated at the end of the study, in a limited number of cases. Initially, a representative sample of the study population is selected. Then, initial measures are performed or corresponding samples are collected, and the follow up period begins. The researcher describes the features of the variable and identifies all the subjects who develop the disease, referred to as cases. Then, all those who do not develop the symptom, referred to as controls, are separated from the sample. Finally, the researcher performs the planned measures or tests on a randomized subsample of the case group and on another of the control group. The sample must be kept in perfect conditions during the study years.

A simple randomized cohort sample may provide controls for several case-control studies.

Multiple cohort study: It is used to study a cohort of a group exposed to a risk factor compared with another cohort of a group with no exposure to the risk factor or predictor variable in question. This is different from studying a group with a disease or problem (cases) *vis-à-vis* another group free from such disease (controls).

The validity and quality of these studies depend essentially on:

- Sample selection.
- Sample size and population representativeness.
- Types of variables studied.
- Precision of measures, using Standard criteria and a blind study design.
- Elimination of potential confounding factors.
- Minimizing the loss of cohort patients.

1.2. Experimental Studies or Clinical Trials

1.2.1 Design

In clinical trials or intervention studies, the researcher creates an experimental situation, intentionally manipulates a so called independent variable, and observes the effect of this intervention. The term "experiment" refers to an intervention performed by the researchers, which consists in introducing or changing one or more factors, called independent variables, in a controlled manner and assessing the subsequent effect(s): the dependent variable(s) within a controled situation set by the investigator. The independent variable may be a drug therapy, surgery, dietary program or any other treatment administered

to the so called Experimental Group, which, in turn, should be compared with another group acting as control. This control group, which is essential for the study, may receive no intervention at all, a substance with no effect, called Placebo, or even a currently available reference drug.

To obtain reliable results, it is important to reduce:

- The influence of extraneous variables.
- The variation caused by error.

The ability to demonstrate causality is the major advantage of an intervention study. However, it is essential that the study has a randomized sample and that the observer is unaware of the intervention assignment (single- or double-blind design) to eliminate the principal variables that may influence the study results. These studies are generally costly, very time-consuming, they answer few questions and, sometimes, expose participants to certain risks. This is why they should be used as little as possible, even though evidence-based medicine and the progress of medical science are largely based on clinical trials. These types of studies are reserved for when observational studies and other lines of evidence suggest the need to use an intervention. (Table 3)

1.2.2. Clinical Pharmacology Trials

Drug and medication research or Clinical Pharmacology trials are a very important chapter in clinical experimentation. These studies must be conducted in compliance with the laws of the countries where they take place and should be submitted for approval to the corresponding authorities (in Brazil, to the National Sanitary Surveillance Agency – ANVISA; in Argentina, to the National Drug, Food and Medicine Technology Administration – ANMAT; and in the United States, to the Food and Drug Administration – FDA). Domestic legislation is intended to ensure that the scientific, ethical and legal aspects of

Table 3 - Objectives of different types of clinical studies.

Observational Descriptive	Observational Analytical	Experimental
Estimates the frequency of a disease or feature in a population	Checks etiologic hypotheses	Tests etiologic hypotheses
Identifies individuals with a specific feature	Generates new etiologic or causality hypotheses	Studies the efficacy of new treatments
Generates etiologic hypotheses	Suggests hypotheses on etiopathogenesis	Studies the efficacy of new interventions
Assesses the impact of population interventions	Generates preventive hypotheses or conducts	Establishes drug effectiveness

Clinical Pharmacology trials comply with the norms set by Clinical Pharmacology, in countries with high sanitary surveillance, and with the World Health Organization recommendations.

To conduct Phase I, II and III Clinical Pharmacology trials, as well as Phase IV (controlled studies, pharmacoepidemiologic and/or pharmacosurveillance studies) and Bioavailability and/or Bioequivalence studies, researchers should submit their projects for approval to the regulating agency, after meeting the requirements detailed below, which do not, however, consider studies conducted in humans without pharmacoclinical and/or therapeutic purposes.

Pre-Clinical Study - Pre-clinical pharmacology; Phase 0

In drug development, this phase corresponds to all *in vitro* and/or experimental animal studies designed to obtain the information required to decide whether there is sufficient rationale for more comprehensive studies in humans without exposing them to unjustified risks. Although many pre-clinical studies should be conducted prior to the clinical studies, those requiring prolonged periods of time or being of special nature continue through out the first phases of the clinical studies. These trials include:

- Pharmacodynamic Studies:
  - Therapeutic and other effects
  - Dose-effect, time-effect curves
  - Effects on systems
  - Pharmacodynamic interactions
- Pharmacokinetic Studies (Absorption, distribution, biotransformation):
  - With single - repeated dose
  - Distribution in normal and pregnant animals
  - Biotransformation
  - Excretion
  - Interaction
- Pre-Clinical Toxicology studies in at least two non-rodent species and with a minimum of three dose levels, the highest of which being sub-toxic.
- Mutagenic activity.

Clinical study

This is a systematic study, following entirely the guidelines of the scientific method, conducted in voluntary humans, either healthy or ill, with the use of drugs and/or medication. The objective of a clinical study is to discover or assess the effects and adverse reactions caused by the study drug and/or to inves-

tigate absorption, distribution, metabolism (biotransformation) and excretion of the active principles to establish drug efficacy and safety.

Clinical studies are classified as Phase I, II, III, and IV, as summarized below:

Phase I

A phase I study is the first time a new active principle or formulation is tested in human subjects – generally volunteers. These studies are intended to preliminarily assess the safety, pharmacokinetic profile and, whenever possible, pharmacodynamic profile of the study drug. Except for well grounded exceptions, they are conducted in small groups of healthy volunteers. The objective is to establish if there is an effective dose with minimal side effects. There is no control group and the drug is administered at different doses and in different periods of time.

Phase II - Pilot Therapeutic Study

The objectives of a Pilot Therapeutic Study are to demonstrate the pharmaceutical activity of the active principle and establish its safety in patients with a specific disease or pathological condition, in the short term. Studies are conducted in a limited (small) number of individuals and are often followed by a comparative study. During this phase, the optimal dose ranges and administration schedules are defined. If possible, the dose-response relationships will also be established to obtain solid background data on drug effectiveness, to be used in designing extended therapeutic studies (Phase III).

Phase III - Extended Therapeutic Study

These randomized clinical trials are conducted in large and diversified groups of patients and their objective is to establish:

- The short- and long-term Risk-Benefit Balance of active principle formulation(s).
- The overall (general) relative therapeutic value.

The type and profile of most frequent adverse reactions are explored in this phase, as well as the special features of a drug and/or medication (e.g., clinically significant interactions, main effect modifiers, such as age, etc.).

This type of study should preferably have a randomized, double-blind design with a placebo or control group. There are other acceptable designs, such as the long-term safety design.

In general these studies are conducted considering the normal conditions in

which the study drug or medication will be used.

Phase IV - Post-Marketing Studies

These studies are conducted after the drug and/or medication has begun to be marketed.

They focus on the approved features of the drug and/or medication. In general, these are Post-Marketing Surveillance Studies designed to establish the therapeutical value of a drug, detect new adverse reactions and/or confirm the frequency of already known ones, and define treatment strategies. In Phase IV studies, the same ethical and scientific guidelines should be followed as for previous phase studies. After a drug and/or medication begins to be marketed, clinical studies designed to assess new indications, new administration methods or new combinations (associations), among other things, will be considered studies for a new drug and/or medication.

The specific Pharmaco-Epidemiology, Pharmaco-Surveillance and Bioequivalence studies are also conducted during this phase.

Monitoring clinical trials

Intervention follow up is essential because the trial should be suspended at the slightest personal risk to prevent study subjects from developing complications during the trial. A committee made up of trained researchers with documented experience is generally in charge of study follow up. Monitors must be familiar with the study product(s), protocol, informed consent and other types of written information, sponsors, and regulatory standards. Once the scientific question has been answered, it is unethical to continue the trial. In addition, study discontinuation will entail money savings. Similarly, if the study question is considered impossible to answer, proceeding with the study is also unethical.

The items to be monitored include:

- The study objective, study design, subject recruitment, compliance, and randomization, whether single- or double-blind; and during follow up: symptoms, adverse effects and potential confounding factors.
- The need to change the protocol may arise during follow up. These changes may be excluding a study group, performing additional measurements to enhance safety, discontinuing treatment in high-risk patients, and increasing trial duration and/or sample size.
- How often monitoring should be performed must be defined on a case-by-case basis. Frequency should be enough to check study progress. When there are significant results to look at, a statistical test analysis is an ap-

propriate tool to check work progress. Monitoring should be documented in writing.

1.2.3. Applying the intervention

There should be at least an experimental group (undergoing the intervention) and a group receiving placebo or standard comparison treatment. Sometimes, more than one treatment is included, implying a longer and more complex, but surely very interesting, trial. We will next describe some types of design often used by researchers.

Crossover clinical study

This is an experimental design used to evaluate two or more treatments to be administered to all study participants consecutively. Treatment sequence order is randomly defined. A washout period should be allowed to eliminate previous treatment effects. The duration of this period will vary according to study population and treatment.

Multicenter clinical study

This type of clinical study follows a single protocol but takes place in multiple research centers. Therefore, it is conducted by more than one principal researcher, but using the same study procedures. Material and method calibration prior to the onset of the trial is essential.

The intervention may have different objectives such as testing new drugs or establishing the minimum effective or highest tolerable dose for drugs already approved (following FDA [Food and Drug Administration] guidelines).

Overall, projects with single-treatment interventions are easy to plan and implement. However, comparing combined therapies, as occurs in HIV+ patients, is complex, and conclusions are not very clear. In some cases, the doses of test drugs vary depending on the patient (age, weight, etc.), making a blind design difficult to use.

The researcher must analyze the likelihood of patient compliance with the intervention; for example, a single daily dose is better accepted than b.i.d. or t.i.d. dosing. It is important to see how much the daily routine of people is impacted by the intervention. If patients are required to change their habits, lower compliance should be expected.

Control groups should undergo all the treatment phases, but receiving placebo with all the physical features of the true medication.

1.2.4. Using placebo

While administering medication, a series of experimental stimuli of physical and psychological nature are produced, regardless of the pharmacological action of the drug in question. For example, the first effect of intravenous medication is the pain produced when the patient is placed in a certain position and injected with a solvent. In the case of topical medication, rubbing the skin surface and applying the drug vehicle *per se* are actions that produce effects on the patient. Besides, the psychological effects of feeling treated and cared for may generate hope, trust and tranquility, or fear, distrust and concern, considering that many patients do not want to be cured (self-aggression). All this is referred to as the placebo effect and comprises not only direct psychic changes, but also the resulting biochemical and somatic physiological changes. By extension, the effect produced by other non-drug interventions is also referred to as a placebo effect.

A pure placebo contains inert substances only. An active placebo is a substance that has a pharmacological effect, but is not related to the one desired for the disease under study. In studies involving major diseases, such as those on prevention of myocardial infarction, the placebo may be replaced by a standard medication. These studies are known as "equivalence trials" and, ideally, the new treatment should offer advantages, such as lower cost, less frequent dosing or increased safety. The study conclusion may be that the new drug is superior to traditional therapy or not. Generally larger samples are required. While planning an experiment, the placebo effect should be taken into account, as it may influence results, and the following factors should be considered and analyzed:

1. The dentist: the more emotionally involved he or she is, the greater the influence of the placebo effect;
2. the patient: the more sensitive and susceptible he or she is to the influence of others, the higher the likelihood of a placebo effect;
3. the patient's disease: the greater the psychological component of the disease, the greater the likelihood of a placebo effect;
4. the experimental situation: the higher the expectations of patients and healthcare professionals, the more the placebo effect will influence results.

Depending on the situation, the placebo effect may multiply or antagonize the pharmacological effect. The need for placebo is evident when the control group does not receive treatment, as the response differences may be attributed to the placebo effect.

Ethical use of placebo: according to the declaration of Helsinki (1964), administering placebo would be unethical if patients failed to receive therapy unequivocally beneficial to them. However, it would be acceptable to use it

if there was no established treatment for the disease in question or if current therapy had too many undesirable effects and a new therapy was proposed, whose efficacy had to be tested. In other words, the actual therapeutic action of a treatment is honestly doubted and is "blindly" compared with "nothing".[1]

In the year 2000, the World Medical Assembly revised the Helsinki Convention and introduced a change: a drug may only be compared with another drug or medication shown to be more effective for the disease in question. Therefore, the use of placebo is virtually not allowed because there is always a conventional treatment for every disease. The FDA has strongly criticized this position because it considers that in many diseases significant improvement is achieved with placebo. In 2002, however, trials with placebo were declared to be ethically acceptable in certain cases, even when a tested therapy is available and if the following conditions are met:

- For methodological, scientific and pressing reasons, placebo use is required to establish the efficacy and safety of a preventive, diagnostic or therapeutic method.
- A preventive, diagnostic or therapeutic method will be assessed for a minor disease, and placebo does not entail additional risk, severe adverse effects or irreversible harm to the patients receiving it.

While planning the study, it is necessary to state that, after the trial has been concluded, participants will have access to the preventive, diagnostic and therapeutic procedures shown to be more beneficial in the study. In summary, all the other provisions in the Declaration of Helsinki must be met, especially an appropriate scientific and ethical review.

1.2.5. Follow up and protocol compliance

Maximizing follow up and protocol compliance to achieve better results:

- In the participant selection phase: two visits may be scheduled before randomizing participants and, thus, those who apparently will not comply with follow up procedures may be excluded.
- During treatment: drug administration and dose frequency should be taken into account.
- Visits should be scheduled close to one another in time to maintain contact with participants, but not so close as to make it tiring. They should not be scheduled for an inconvenient time or day (at night or during the weekend). Participants may be reminded of their appointment by phone or e-mail.
- The importance of follow up should be stressed.
- There should be enough professionals, and a good relationship should be

established with study subjects; expenses should be reimbursed.

- Measurements should be painless and interesting. Whenever possible, the protocol should not be discontinued because of adverse reactions or side-effects.
- Ideally, social worker services should be used to recover patients lost to follow up. However, in osteoporosis studies, 60% of randomized patients are lost.[2] Patients who abandon the protocol because of adverse effects or personal problems should also be considered. Sometimes, patients may be contacted by phone or e-mail to collect data or enhance compliance.
- A method to measure protocol compliance should be defined: by pill count, personal reports, weighing administered liquids or creams, measuring saliva or blood metabolite levels, etc.

The protocol should specify the number of follow up visits; efforts should be made to have enough professionals to avoid waiting lines; appointments should be confirmed the previous day; transportation and other expenses should be reimbursed.

1.2.6. Internal validity

In a pure experiment, the internal control or validity of the experimental situation is an essential requirement and refers to the reliability of the results. One should ensure that the change in the dependent variable is exclusively due to the variation or manipulation of the independent variable, rather than to other factors or causes. Internal control means knowing what is happening to the independent and dependent variables and controlling the influence of extraneous variables in the experiment in order to arrive to valid conclusions. In other words, it means "purifying" the relationship between the variables examined, ruling out those variables that "contaminate" the experiment.

Some sources of internal invalidity include:

1. **History:** events occurring during the experiment. For example, if an observer is awarded a prize or gets a salary raise to conduct the research, he or she will be especially motivated. The opposite will be true if the research project provides no academic, moral or economic benefit to the researcher or his/her work group.
2. **Maturation:** this refers to the participants' internal processes that affect the progress of the experiment, such as labor or family problems, tiredness, disease, hunger, and others.
3. **Testing:** an initial test may determine the application of a second test because of sensitization or prejudice. Especially while applying questionnaires,

it may be difficult to measure the pure effects of the independent variable or experimental stimulus, without including the sensitization effect.

4. **Instability:** little or no reliability of measures, variations in the individuals selected for the experiment. All the factors that may affect the sample when it is collected and during the preservation period, from the time the sample is extracted until it is processed or measured, should be taken into account.
5. **Instrumentation:** this refers to changes in the measuring instruments. For example, the effects caused by changing a reagent, a device or the experimental conditions can be easily pinpointed. Similarly, while applying a questionnaire or measuring learning abilities with different tests, this error or instability in the measurements taken at different times may occur, depending on the technique and reagents used, the temperature conditions, the seasons of the year, humidity levels, etc. What factors are relevant will depend on the study variable, but all of them should be thoroughly examined before the trial begins.
6. **Statistical regression:** this refers to the effect provoked by a trend, by which the subjects selected on the basis of an extreme score tend to shift back to a mean value in subsequent measurements of the variable they were selected for.[3]
7. **Selection:** when subjects in one group are not matched with subjects in the other groups.
8. **Experimental mortality:** it refers to the differential loss of subjects between groups. This may be due to pain, tiredness, the experiment itself, the type of subjects in the groups, or to factors not related to the experiment.
9. **Interaction between selection and maturation:** it may depend, for example, on the time of the day chosen to conduct the experiment in different Latin American countries – if the experiment is conducted at noon, some may be hungry and others may not, depending on the regional habits.
10. **Other interactions:** selection may interact with mortality, history with maturation, maturation with instability, etc. The experimenter and participating subjects may also be a source of internal invalidity, essentially because of the interaction between subjects and the experimenter. For example, certain attitudes, expectations and prejudice may influence behavior during the study, leading to lack of cooperation, hostility and criticism. In all the groups, there may be individuals with a positive attitude, but also individuals with a negative attitude who may ruin the experiment. Often patients receiving treatment for certain conditions are treated very diligently and kindly by healthcare professionals. This is called “co-intervention”.

For convenience, subjects should remain unaware of the experimental hypotheses and conditions. Their attention should be distracted away from the true purpose of the experiment, even though it will be explained to them after the study has been concluded. For example, while analyzing the action of a drug, it will be convenient to use a placebo group to adjust for the expectation effect of receiving medication.

How is internal control and validity achieved? By using randomization and a single- or double-blind design. Whenever possible, the researcher should plan the interventions in such a way that no one in the experiment (patients, staff or anybody related to them) is aware of the study assignments. This requirement is harder to meet than randomization. One way of verifying if the experiment was double-blind is to ask subjects to guess, at the end of the study, what their treatment was. If most of them guess right, this means that the blind design was only partially achieved and there may be potential biases.

1.2.7. External validity

External validity refers to the extent to which the experiment results may be extended to non-experimental settings and to other subjects and populations.

Sources of external invalidity include:

- **Reactive or test interaction effect:** when the pre-trial or pilot trial determines the subjects' sensitivity to the experimental variable or the quality of their reaction to it. For example: in surveys or while taking a pulp vitality test, the first experience affects the subsequent responses.
- **Interaction effect between selection errors and experimental treatment:** this effect may occur while recruiting volunteers, especially because of motivation.
- **Reactive effect of the experimental treatments:** when the experimental setting is atypical or very different in terms of how the treatment is usually administered.[4] The experimenter should try to make the subjects forget they are participating in an experiment. They should not feel they are being observed, as unusual reactions will occur when a patient feels controlled or cared for.
- **Interference of multiple treatments.** Especially in crossover experimental designs, the "washout period" should be allowed for. This is the time required for the effect of the previous treatment to pass.
- **Failure to replicate treatments:** this may be due to a very complex experimental situation. To avoid this inconvenient, the sample should include groups as similar as possible to the general population, and the overall set-

Table 4 - Bradford Hill Criteria.

Criteria	Explanation of Criteria
1. Strength of association	A strong association is more likely to have a causal component than a modest association
2. Consistency	A relationship is observed consistently
3. Specificity	A factor influences a particular outcome of population
4. Temporality	A factor must precede the outcome it is assumed to affect
5. Biological gradient	The outcome increases monotonically with increasing dose of exposure or according to a function predicted by substantive theory
6. Plausibility	The observed association can be plausibly explained by substantive matter (e.g. Biological) explanation
7. Coherence	A causal conclusion should not fundamentally contradict present substantive knowledge
8. Experiment	Causation is more likely if evidence is based on randomized experiments
9. Analogy	For analogous exposures and outcomes, an effect has already been shown

ting must be as similar as possible to the reality considered for generalization purposes (field or laboratory studies).

The Bradford Hill causality criteria, which may be generalized, are shown in Table 4.

2. Sampling

Participant selection is an essential aspect of the study and the protocol should specify in detail the subject sample to be included. The sample has to be representative of the population it intends to study and large enough to control randomization errors as well as errors of inference due to systematic errors. In addition, the sample must have an acceptable cost in terms of time and money.

Inclusion and exclusion criteria must be defined in great detail, as well as how subjects will be recruited. For example, inclusion criteria regarding age group and sex should be defined clearly. In general, it is desirable to work with individuals in "good general state of health". This is why one should specify what kind of patients will be included, i.e., hypertensive or not, treated or untreated, diabetic, immunosupressed, cancer patients, transplanted patients, children, pregnant women, etc. Local factors must also be defined, i.e., if patients use dentures, suffer from a given disease such as dry mouth, gingivitis or periodontal disease, undergo hygiene control, if they are smokers or non-smokers, etc. For example, including smokers and alcoholics, or alcohol users,

increases the likelihood of extending results to the general population, but patient follow up may be difficult. Depending on the study objective, the type of variables associated with the study variable must be defined precisely.

The population meeting the inclusion criteria is often large. On the other hand, a representative sample is essential. The main sampling methods or techniques include:

- **Convenience sampling:** these samples include unhealthy patients in a hospital, clinic or ward. This type of sample is inexpensive and easy to recruit. However, one must be aware that convenience samples are influenced by selection factors that make patients go to those healthcare centers. They are an excellent option to address diagnostic, treatment and prognostic issues, and have the additional advantage of eliminating biases, such as the volunteer bias, and being very useful for certain studies. Consecutive sampling, a version of convenience sampling, eliminates the influence of seasons or other changes related to geography, weather or climate.
- **Paired or matched sampling:** it consists in selecting controls that match the cases on given aspects to achieve a high degree of initial equivalence. These aspects are most frequently sex and age (± 5 years), socioeconomic level, cultural level, etc. This technique is less precise than randomized allocation.
- **Population sampling:** another way of recruiting subjects is selecting them from the community or population. These samples are costly and difficult to recruit but very important for public health. The sample may be diversified and extended by collecting data through e-mail (electronically available populations) and telephone calls, acknowledging the bias this entails.
- **Probabilistic sampling:** it is used when scientific support is required in descriptive research to generalize findings to the overall population. Probabilistic sampling uses randomization and ensures that all the members of the population are equally likely to be selected. This provides a strict basis to estimate the occurrence of a phenomenon in the population. There are several modalities:
  - Simple random sampling utilizes the random number table, which most often is computer-generated.
  - Stratified random sampling divides the population into subgroups based on given features, such as sex, race and age.
  - Sampling by groups or clusters randomly selects subjects from various hospitals, healthcare centers or cities, as in multicenter studies.
  - Systematic sampling is simple random sampling using pre-selected groups.

- Factorial Designs or group randomization are used while dealing with two or more factors in a single cohort of participants.

3. Results analysis

3.1. Planning considerations

While planning a clinical investigation, it is very important to include a detailed description of how data will be collected: through surveys, measurements, testing, etc. When planning to use archives and databases, the authorization to access them should be attached. A detailed list of what variables will be collected and why must be developed and provided. It should include type of data, source, when data will be collected, and why it is useful to have it. Furthermore, expected outcomes, explanatory variables, baseline data, confounding factors, and covariates, among other data, should be specified in the protocol.

The type of software that will be used to store and process data should also be specified, as well as the methods to ensure the quality and validity of results (double entry, cross validation, etc.). The statistical analysis that will be used to conclude the study strongly depends on the nature of the variable and type of data.

3.2. Variables

The features measured in the subjects under study are known as variables. The measurement concept is very important and means assigning a value to the observation. This is the only way to organize, present, process, and obtain information based on the data collected during the study. The validity and reach of the investigation will largely depend on the type of observation and measurement scale, which will in turn determine the statistical method to be used in the results analysis.

According to their nature, variables are classified into:

- **Qualitative or categorical variables,** which are measured on a nominal scale. They are the simplest type of measure and usually correspond to non-ordinal, often dichotomous, variables, such as sex, presence or absence of a disease or risk factor.
- **Ordinal variables,** which are different because they are measured on an ordinal scale to rank responses, for example, dental plaque and tartar indexes.
- **Quantitative variables,** which are measured as numerical values. They are classified into discrete variables, which are assigned whole numbers but not decimal values (number of caries, number of children), and continuous variables, which are measured on a continuous scale with decimal values, i.e.,

are not limited to whole numbers (salivary nitrite, pH or protein levels).

Variables may also be classified according to study objectives:

a. **Exposure variables** make it possible to measure the factors under study; there are at least two: the dependent or response variable, which corresponds to the obtained or expected results, and the independent variable, which corresponds to the introduced intervention, cause or factor having an effect to be determined on the dependent variable. For example, caries incidence (dependent variable) in a group of subjects who received fluoride topical application (independent variable). Both the dependent and independent variable may be nominal, ordinal or numerical, and this will determine which statistical method will be used. For example, the drug dose administered in a clinical study (independent variable) and the size of the lesion observed (dependent variable) are both numerical. In this case, since it is a pharmacological study, the independent variable may be manipulated.
b. **Selection variables** are used to determine whether an individual may participate in the study, based on pre-established criteria; they are called inclusion and exclusion criteria.
c. **Confounding variables** may occur in any investigation or study, leading to misinterpretation; although they are associated with the response variable, confounding variables are of little interest to the study and in some cases may not even be measured. However, if identified and measured, the confounding variable may be included in the statistical test as a covariate, thus eliminating the bias it causes on the dependent variable. For example, we may observe that mothers who smoke during pregnancy have children with lower weight compared with mothers who do not smoke. However, mothers who smoke during pregnancy may also have poor nutrition and maybe this is what actually affects the weight of their children.

3.3. Organization, storage, statistical analysis, and interpretation of results

3.3.1. General considerations

Firstly, sample size should be estimated, clearly explaining the considerations made to calculate it, i.e., type of distribution assumed, significant difference desired and reason for this, levels of significance and power considered acceptable, and formula used. Sample size may usually be estimated after a pilot study has been conducted.

The analysis strategy should consider not only the statistical test to be applied, but also a much broader process of which statistical testing is a part

and that comprises firstly data observation and description. This descriptive analysis includes graphs, tables and descriptive statistics of data (means, proportions, etc.) to ensure comparability between the analysis groups. Only then will the test of choice be applied and the results analysis performed. As was previously explained, assigning values (whether numerical or not) to the measured features is of vital importance to obtain information. Once the data are obtained, they have to be organized and stored in such a way that they may be processed and analyzed statistically afterwards.

3.3.2. Organizing data: tables and graphs

Tables make it possible to arrange data in a way that is easy to display. There are different types of tables for different types of collected data.

Tables and graphs for categorical data

Frequency tables offer a first description of the population under study and are particularly important when using categorical variables, as the subsequent statistical analysis of these variables will be based on this type of data.

The number of subjects showing a specific study-related feature is recorded in this type of table. In observational studies, the simplest way to analyze collected information is to summarize data in a contingency table. The simplest kind of contingency table is the 2 x 2 table with dichotomous exposure variables, i.e., a factor is either present or absent. These variables include risk factors (smoking or non-smoking), treatments (with or without fluoride), a feature (sex), exposure *per se* (medication), etc. Once the table is made, the strength of the relationship between two nominal measures may easily be measured.

There are two ways of estimating this association, depending on whether it is a case-control study or a cohort study.

For case-control studies, the number of cases and controls, both exposed and not exposed, are included and the potential associations between exposure and disease are estimated. For example, to establish the link of oral cancer with tobacco and alcohol use, a 2 x 2 frequency table is used to display how many cases and controls consume tobacco and alcohol and how many do not. The association is measured as a probability or odds ratio (OR). It is calculated as the quotient between the probability of a patient being exposed to the risk factor and the probability of a control being exposed to that same risk factor. An example can be seen in Table 5.

The probability of patient with cancer being exposed to the risk factor is:

- (52/77) / (25/77) = 52/25 = 2.08

Table 5 - Contingency table in a case-control study to determine the association between tobacco and alcohol consumption and oral cancer.

	Cases (with cancer)	Control (without cancer)	Total
Risk factor present (tobacco and alcohol use)	52	30	82
Risk factor absent (no tobacco - no alcohol use)	25	47	72
Total	77	77	154

The probability of a control being exposed to the risk factor is:

- 30/47 = 0.64

The OR or probability ratio is:

- 2.08/0.64 = 3.25

This means that a patient who smokes and drinks is three times more likely to develop oral cancer than those who do not.

The OR is a quantitative value varying from 0 (lower limit) to infinity (upper limit), with 1 being the value for the null or no relationship hypothesis. In other words, there is a positive association for values greater than 1.

For cohort studies, the association is measured by calculating the relative risk (RR) based on a 2 x 2 contingency table. The RR measures the strength of the exposure-disease association by calculating the quotient between the disease incidence in exposed subjects and that in non-exposed subjects. The RR can only be estimated in cohort studies or in clinical trials where patients are first divided into a risk factor group and a non-risk factor group and then studied during a period of time to see who develops the disease. Patients may also be assigned to a treatment group and a non-treatment group and then observed to see who develops the disease. For example, a group of children with no caries received topical applications of Fluoride once every 6 months and another group, also of children with no caries, did not. Both groups were followed and observed to check how many children had developed caries by the end of the first year. Results are shown in Table 6.

Caries incidence among children who received topical application of fluoride was:

- 428 / 5,350 = 0.08

Among those who did not receive fluoride, the incidence was 930 / 4,870 = 0.19

- RR = 0.08 / 0.19 = 0.42

This means that the relative risk of having caries was lower in children who received topical application.

Table 6 - Contingency table for a cohort study.

Treatment	Caries	No caries	Total
Fluoride	428	5,350	5,778
No fluoride	930	4,870	5,800
Total	1,358	10,220	11,578

Bar graphs are especially used to represent nominal data graphically. The absolute or percentage values assigned to the features of interest are depicted as bars in this type of graph. Pie charts are also used, in which the slices represent percentages. A bar graph with the data from Table 6 is shown in Figure 1.

Tables and graphs for numerical data

Numerical data may be tabulated in various ways. The simplest one is to include all the observations in a single table. Data may also be arranged in a frequency table. For this purpose, data are divided into classes and then the observations are counted to see how many there are in each class. The most suitable graph for frequency tables is the histogram, which provides a very simple measure of central tendency, type of distribution and data dispersion. This kind of graph represents the frequency of observations by the various classes in which the numerical data were divided.

Box plots, dot plots and dot-density plots may also be used. The box plot is especially useful to depict certain distribution locations, the mean, the median, and the first and third quartiles, thus providing a good notion of data distribution and dispersion. The dot plot depicts the mean and standard deviation or error, while the dot-density plot graphically illustrates all the observations.

All three graphs are especially used in clinical research, as most studies comprise more than one feature or treatment. In these cases, the classification criterion is the independent variable (on the abscissa or x-axis) and the observation is the dependent variable (on the ordinate or y-axis).

The utility of each one of these plots will depend on the data and information that one wishes to show. The dot-density plot in Figure 2 is very useful for bimodal distributions. The example shows the age distribution of a group of patients with candidiasis. Just by observing the data, it is possible to state that this condition occurs especially in children and elderly people. This conclusion would be impossible with a box plot, such as the one shown in Figure 3.

On the other hand, the dot and box plots in Figures 4 and 5, showing the salivary nitrate and nitrite concentrations, give a clear picture of the location and dispersion measures. Particularly, the box plot makes it possible to asses

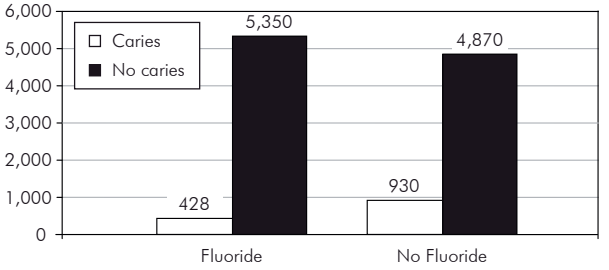

Figure 1 - Bar graph with the data from Table 6.

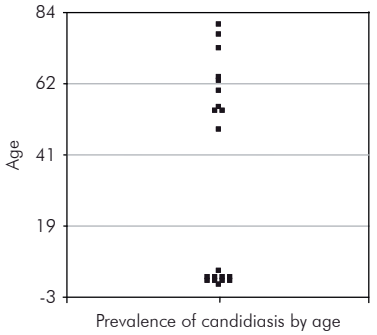

Figure 2 - Dot-density plot.

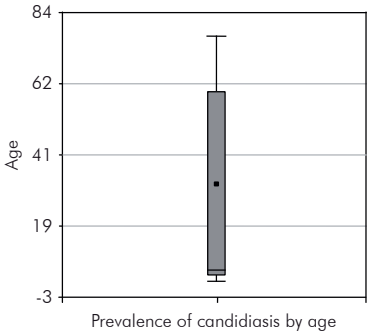

Figure 3 - Box plot.

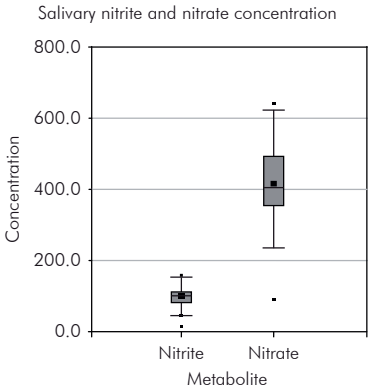

Figure 4 - Box plot.

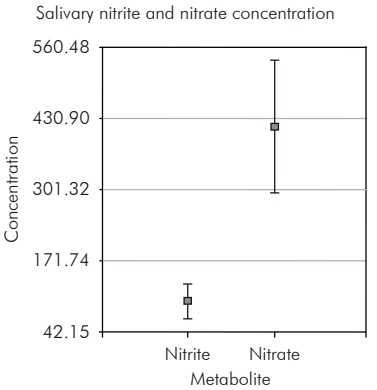

Figure 5 - Dot plot.

whether data distribution follows a normal curve by showing the differences between mean and median.

When the independent variable is also numerical, the graph of choice is the dispersion diagram.

Descriptive statistics

In addition to tables and graphs, some statistical measures are also useful to summarize data or certain data features, constituting what is referred to as descriptive statistics. These include measures of position, namely, mean, median and mode, and measures of dispersion, namely, range, percentiles, standard deviation, variance, and coefficient of variation.

Measuring the relationship between variables

For the great majority of clinical research studies, the objective is to compare at least two variables: the response or dependent variable and one or more independent variables. If both are nominal variables and the objective is to establish the association between them, this association will be estimated as previously explained (OR and RR).

Another strategy is to use hypothesis or statistical significance testing. These tests are used to demonstrate whether the relationship between the study groups is statistically significant, i.e., if the probability that the relationship is merely a coincidence is low.

While choosing the test to be used, some factors should be considered, especially the type of data, whether numerical or categorical, and the type of sample, whether independent or paired. Two samples are independent from one another when the subjects under study are divided into differing groups, for example, the subject is in a case or control, female or male group. In paired samples, the therapeutic response is measured in the same subject at two different points in time.

If a series of conditions are met, such as appropriate sample size, data with normal distribution, randomized samples, homogeneity of variance, etc, parametric methods will be used. If not, equivalent non-parametric methods, also called free distribution methods, should be used instead. Table 7 shows some of the hypothesis tests most widely used in clinical research by type of variable.

Table 7 - Summary of statistical methods for bivariate studies.

Independent variable	Dependent variable	Method
Categorical	Categorical	Chi square
Categorical (dichotomous)	Numerical	t test (paired or independent samples)
Categorical (more than 2 treatments)	Numerical	One-way ANOVA
Numerical	Numerical	Regression. Correlation

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7

Randomized clinical trials

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There are many definitions of clinical trial. For Piantadosi[1] (1997), it is simply an experiment testing a medical treatment on human subjects. In clinical trials, researchers assign participants prospectively to an intervention or comparison group in order to study the cause-and-effect relationship between an intervention and a health outcome. The term intervention is used for drugs, medical devices, surgical procedures, or behavioral modifications, among others. When study subjects are randomly allocated to intervention and comparison groups, the experiment is called a randomized controlled clinical trial (RCT).

RCTs can be long, complex and expensive studies. They require a qualified research team, composed by a principal investigator, sub-investigators, and when possible, a study coordinator. A well-structured research center is also necessary.

It is not possible to cover all aspects of RCT methodology in a single chapter. There are entire books focusing on methodological[2,3] and statistical[4] issues concerning clinical trials. This chapter has a modest objective: it attempts to acquaint under-graduate and graduate students with some of the basic concepts of RCT methodology that may be helpful in planning, conducting and reporting this type of study.

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Planning the trial

A number of diseases and conditions can affect the oral cavity. The two most prevalent oral diseases are dental caries and periodontal diseases, but other lesions, such as oral cancer, can affect the

mouth. Apart from oral diseases, there are a number of conditions that are of concern, including malocclusion and missing teeth that need replacement.

Clinical investigations in dentistry attempt to respond questions about the diagnosis, prognosis, prevention and treatment of these oral diseases and conditions. These questions are related to clinical uncertainties that the researcher wants to solve.[5] We will focus on prevention and treatment. Some examples of questions about prevention and treatment are shown in Table 1.

As discussed elsewhere in this book, well-designed RCTs are considered to be the gold-standard type of study to answer questions about treatment and prevention of diseases and conditions.[6] So, when planning a trial, keep in mind that the objective of the RCT will be to respond a well-formulated and focused question that will be translated into the trial objective.

Ethical issues and good clinical practices

When planning a clinical trial, the first important thing is to remember that this type of study is conducted in human volunteers. Thus, the rights, safety and well-being of the volunteers (study subjects) are the most important items to consider when writing a project.

Although there is a chapter in this book dedicated to ethics in research, we will briefly discuss some ethical issues that are specific to the conducting of clinical trials.

All trials conducted in human volunteers should comply with principles denominated Good Clinical Practices (GCP), which are international quality

Table 1 - Examples of questions regarding treatment and prevention of oral conditions.

Question	Disease/condition	Level
What are the retention rates of resin-based sealants?	Dental caries	Prevention
What is the best method to prevent alveolar osteitis when patients undergo dental extraction?	Alveolar osteitis	Prevention
What is the effect of bi-annual professional application of fluoride gel on caries prevention in primary teeth?	Dental caries	Prevention
Which is the most effective root-end filling material in endodontic surgery of teeth with failed conventional root canal treatment?	Endodontic treatment failure	Treatment
What is the comparative antigingivitis effectiveness of chlorhexidine and essential oil mouthrinses?	Gingivitis	Treatment
How effective is the use of low-level laser therapy in the management of temporomandibular disorder?	Temporomandibular disorder	Treatment

standards for designing, conducting, recording and reporting trials that have their origin in the Declaration of Helsinki.[7] GCP principles were published in 1996 by the International Conference on Harmonization (ICH), an international body that defines standards for human clinical trials.[8]

The principles of GCP include items related to the protection of the rights, safety and well-being of the study subjects. Any risks and inconveniences to the participants should be anticipated, and the direct benefits to the participants must justify those risks. A detailed protocol describing the trial must have received prior institutional review board (IRB) approval. Any deviations from this protocol must be communicated to this IRB.

Clinical trials involve intervention (prevention or treatment). So, investigators that participate in the trial must be qualified health care providers. Investigators should obtain freely given informed consent from all participants, and the confidentiality of the records that could identify subjects should be protected.

Investigators should ensure the accuracy, completeness, legibility, and timeliness of the data reported. All information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification. Also, procedures that assure the quality of every aspect of the trial should be implemented.

The Guidelines for Good Clinical Practices[8] are available for download at *http://www.ich.org/LOB/media/MEDIA482.pdf*. We encourage the readers of this chapter to read the full document.

Writing the trial protocol

A detailed and well-written protocol is essential for conducting an RCT. A definition of protocol can be found in the ICH's Guidelines for Good Clinical Practices: "a protocol is a document that describes the objective(s), design, methodology, statistical considerations, and organization of a trial".[8]

Protocols are important for various reasons. Protocols are legal documents that specify the responsibilities of all parties participating in a clinical trial, i.e., investigators, institutions and sponsors. Protocols ensure the quality control of trials, and allow communication to be exchanged between centers and research teams. Protocols are also required when submitting a project to the Institutional Review Board (IRB) or to a Regulatory Agency, such as the Food and Drug Administration (FDA) in the U.S., or the "Agência Nacional de Vigilância Sanitária" (ANVISA) in Brazil. They are also necessary when requesting research grants to conduct the study. But they also have an important scientific function: to help the investigator to organize the study in a logical, efficient and

objective way.

The contents of an RCT protocol generally include the following:

1. introduction
2. trial objectives
3. trial design
4. study population
5. description of the primary and secondary outcomes
6. description of the intervention
7. randomization
8. blinding or masking
9. statistical methods
10. ethical considerations

Hint: The objective of the CONSORT[9] statement, which will be described later on in this chapter, is to provide authors with a checklist for reporting a clinical trial. Nevertheless, it can also be used by less-experienced researchers, such as under-graduate and graduate students, as a checklist when writing a protocol. The CONSORT statement can be downloaded at *http://www.consort-statement.org*.

Introduction

This section should be as succinct as possible. It must provide background information about the disease/condition, the population that will receive intervention, and the intervention itself. A summary of findings from non-clinical and clinical studies that are relevant to the trial must also be included. The introduction concludes with a clear statement of the trial objectives.

Trial objectives

As in any other type of research, an RCT should answer a question, which is often related to the efficacy of an intervention. The objective of the trial also includes the nature of the study intervention, the disease/condition under investigation, and sometimes other considerations (such as the target population). Some examples of trial objectives related to treatment are shown in Table 2.

Trial design

Once the trial objectives have been carefully defined, an appropriate design must be chosen. The selection of the design depends on the trial objective.

Table 2 - Examples of randomized clinical trials objectives.

Objective	Reference
(The objective was) to evaluate the application of MTA and IRM as retrograde sealers in surgical endodontics.	Lindeboom *et al*.[10] (2005)
The objective of this study was to compare the antiplaque and antigingivitis effectiveness and the side-effect profiles of an essential oil-containing mouthrinse and a chlorhexidine-containing mouthrinse.	Charles *et al*.[11] (2004)
The objective of this study was to assess the effectiveness of low-level laser therapy (LLLT) in the management of temporomandibular joint (TMJ) pain in a random and double-blind research design.	Emshoff *et al*.[12] (2008)

The general structure of a randomized clinical trial is depicted in Figure 1. Study subjects are randomly allocated to two or more experimental groups. One group is exposed to an intervention (test group), an experimental treatment supposed to be superior to the available alternatives. The other group is exposed to a comparison intervention (control group). The control group can receive an active treatment, such as the standard therapy for the studied disease, or a placebo (no treatment). In RCTs, the experimental groups are supposed to be balanced in relation to the distribution of all predictor variables (age, gender, socioeconomic status, etc), with the exception of the intervention itself, so that differences in the outcome of the groups may be attributable to the intervention.

In RCTs, controls can be either concurrent controls, as in parallel trials, or self-controls, as in crossover trials.

In a parallel group design, each subject receives one and only one treatment (Figure 2). Comparison of the different interventions will be based on the comparison of between-subject variation. This is the most common design for confirmatory trials.[13] In RCTs testing experimental interventions with systemic effects (for instance, anti-inflammatory drugs or antibiotics), the parallel group design may be the best option.

In the crossover design (Figure 3), each subject serves as his/her own control, and the comparison of the different interventions is the comparison of the within-subject variation. Since each subject is his/her own control, prognostic factors are balanced between groups. In this study design, participants are given different treatments one after another. The sequence of assignments is randomized, and a wash-out period is required between treatments. The wash-out is a period between two treatments, necessary to allow the carry-over effects of the previous treatment to disappear.

One study design that is unique in dentistry is the split-mouth design. In this

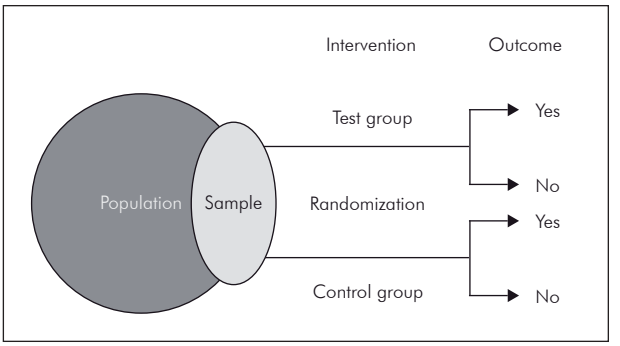

Figure 1 - Structure of a randomized clinical trial.

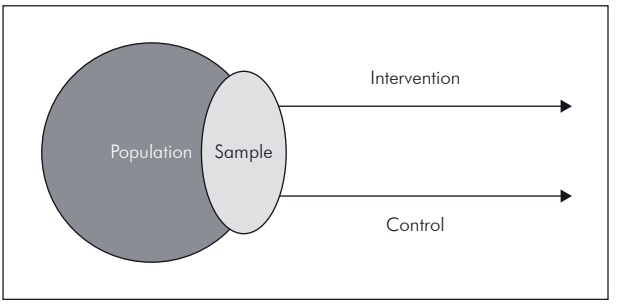

Figure 2 - Structure of a two-group parallel design randomized clinical trial.

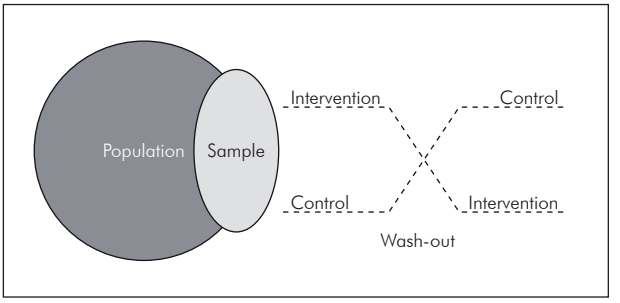

Figure 3 - Structure of a two-group crossover design randomized clinical trial.

self-controlled study, the mouth is subdivided into within-subject experimental units such as quadrants or sextants. Each participant receives all of the treatment modalities, so the number of treatment modalities should be the same as the number of within-subject experimental units. This study design should be used with caution when investigating the efficacy of drugs with systemic effects. For instance, in trials evaluating local delivery of antimicrobials in the

periodontal pocket, there is a possibility that the drug applied in a left upper molar may have a distant effect in the contra-lateral tooth.

Example: A 3-year randomized split-mouth trial was conducted to compare the caries-preventive effect of two types of sealants.[14] The authors evaluated two sealant modalities: a chemically curing glass ionomer cement (GIC) and a light-curing resin-based sealant material (RB). The permanent second molars considered to be at risk for caries were sealed randomly with either GIC or RB. The outcome measured was the caries rate of the sealed teeth and the sealant retention. The split-mouth design led to a situation where either one or two tooth pairs were observed per individual.

Study population

One of the main goals of an RCT is to provide an accurate and precise evaluation of the efficacy of an intervention for a target population with a specific disease/condition. Since in the majority of the situations it is not possible to examine all the members of the target population, statistical inference is drawn based on a representative sample of this population.

A set of eligibility criteria is used to define the candidates for inclusion in the study. Eligibility criteria consist of a set of inclusion criteria and exclusion criteria. Typical inclusion criteria are based on the studied disease/condition (diagnostic criteria, severity of the disease), demographical variables (age, sex, etc.), and comorbid conditions. Exclusion criteria are related to sources of variability (for instance: the presence of another disease or the use of medications that alter the course of the studied disease) or to conditions that jeopardize participants' safety (for instance: history of hypersensitivity to the experimental drug).

Patients that, for any reason, refuse to participate in the trial are also excluded. It is important to remember that those patients tend to be systematically different (in relation to socioeconomic status, disease severity, or other health-related problems) from the ones that agree to participate in the trial.[15]

To assess the efficacy of the experimental intervention, researchers must show that this intervention is statistically different from the comparison arm of the trial. The probability of the study of correctly detecting a meaningful difference between groups is known as the (statistical) power of the trial. For a given significance level (α), power is increased when sample size is also increased. On the other hand, the magnitude of the effect (the difference to be detected between groups) is inversely related to the sample size of the trial. Simply

put, larger samples are necessary to detect small differences between groups. Smaller samples are necessary to detect greater differences.

So, when planning a clinical trial, it is very important to pre-calculate the sample size necessary to detect the difference between the arms of the experiment. There are some strategies used for determining the appropriate sample size for a clinical trial. Although in some cases sample size calculation may be easy, in most of the cases it is recommended that the researchers consult an experienced statistician to perform this calculation.

Primary and secondary outcomes

The primary outcome is related to the primary objective, and is the variable of greatest importance in the clinical trial. It should be a reliable and validated efficacy variable, because the primary objective of most RCTs is to provide strong scientific evidence regarding efficacy. Usually, the primary outcome is the variable used in the sample size calculation.

Other outcomes of interest are defined as secondary outcomes. Secondary variables are either supportive measurements related to the primary objective or measurements of effects related to the secondary objectives. Their pre-definition in the protocol is also important, as well as an explanation of their relative importance. The number of secondary variables should be related to the limited number of questions to be answered in the trial.

Some examples of RCT primary objectives and outcomes are shown in Table 3. We will use as examples the studies shown in Table 2.

It is preferable that the primary outcome be a definitive outcome, rather than a surrogate outcome. A surrogate outcome is one that is measured in place

Table 3 - Examples of Randomized Clinical Trials Outcomes.

Objective	Outcome	Reference
To evaluate the application of MTA and IRM as retrograde sealers in surgical endodontics.	clinical features and radiographic findings (according to Rud's classification)	Lindeboom *et al.*[10] (2005)
To compare the antiplaque and antigingivitis effectiveness and the side-effect profiles of an essential oil-containing mouthrinse and a chlorhexidine-containing mouthrinse.	Loe-Silness gingival index (GI), Quigley-Hein plaque index (PI), Volpe-Manhold calculus index (CI), Lobene extrinsic tooth stain index (SI)	Charles *et al.*[11] (2004)
To assess the effectiveness of low-level laser therapy (LLLT) in the management of temporomandibular joint (TMJ) pain in a random and double-blind research design.	TMJ pain during function	Emshoff *et al.*[12] (2008)

of the biologically definitive or clinically most meaningful outcome.[3] Generally, definitive outcomes measure clinical benefit, whereas surrogate outcomes are biological or laboratory variables that track the progress or extent of the disease. Simply put, definitive outcomes are those that are of interest to patients, and surrogate outcomes are generally of interest to clinical researchers. Investigators choose a surrogate when the definitive outcome is inaccessible due to cost, time, or difficulty of measurement.

Example: Some studies have shown an association between periodontal and cardiovascular diseases (CVD). In order to prove a causal association between the two conditions, it is important to investigate whether periodontal treatment can decrease the risk of death from adverse cardiovascular effects. However, cardiovascular events may take several years to occur, so the possible benefits of periodontal therapy can be difficult to observe in interventional studies. As a result, some investigators[16] observed the effects of periodontal treatment in surrogate outcomes, such as the level of C-reactive protein, which has been associated with CVD in medical investigations. The "chain of events" is supposed to be:

Periodontal treatment ⇒ reduces C-reactive protein levels ⇒
reduces cardiovascular events ⇒ reduces death rates

The problem in using surrogate outcomes is their validity: they may not accurately replace the definitive outcome (in this case, death from CVD). It is possible that some patients that experienced reduction of C-reactive proteins due to periodontal treatment may present, in the future, cardiovascular events.

Different scales of measurement may be used depending on the outcome being used. Scales of measurement include nominal or categorical, ordered, interval and ratio variables. These measurements should possess acceptable levels of reproducibility and accuracy. In most trials, calibration of the instrument or of the investigator responsible for the measurements is mandatory, especially in the case of subjective variables such as assessment of radiographic measurements, periodontal probing, Decayed/ Missing/ Filled Teeth (DMFT) index, etc. If one investigator is responsible for carrying out the examinations, intra-examiner calibration must be performed. If more than one investigator will carry out the examinations, intra- and inter-examiner calibration is necessary. Training and calibration of the examiners should be performed prior to the beginning of the trial. In long-term RCTs, calibration should be performed periodically during

the course of the investigation in order to guarantee reproducibility.[17]

Description of the intervention

Experimental and comparison interventions must be detailed. In dentistry, an intervention can be the use of a drug (antibiotic, anti-inflammatory drug, antiseptic, etc.), a product or device (toothbrush, sealant, restoration, dental prosthesis, dental implant, graft, etc.), a surgical procedure (tooth extraction, oral lesion removal, periodontal esthetic surgery, etc.), or an educational, motivational or behavioral intervention (oral hygiene instruction, smoking cessation program, etc.).

The protocol should describe the interventions assigned to each arm of the trial, including the control intervention. If a placebo will be used in the control arm of the trial, its characteristics and the way in which it will be disguised must also be informed. The concept of blinding will be discussed in another section of this chapter.

It is important to state the responsibilities of each member of the clinical team: who is in charge of enrolling participants, who will administer treatment, and who will assess the study outcomes.

Randomization

An adequate randomized allocation of the study subjects reduces the subjective assignment of treatment to participants. If participants are not randomized into experimental groups, these groups will probably differ in relation to measured and non-measured baseline characteristics, which will make them differ with respect to prognosis.

Important: The term "random" has a precise mathematical and epidemiological meaning. If one states that participants were randomly allocated to experimental groups, this means that each participant has a known probability of receiving each of the treatments before he/she is assigned. Treatment is determined by chance only. If participants are alternately allocated to groups A or B, or assigned by hospital number, date of birth or any other method, this cannot be called randomization, but a deterministic allocation method.

The randomization process has two stages. The first stage is the generation of a random allocation sequence. This can be achieved by tossing a coin, but a computer generated list or the use of random number tables are preferable

because these methods can be audited later.

The second stage of randomization is called allocation concealment. After the generation of the random sequence, it is very important that those responsible for recruiting subjects into the trial are unaware of the group to which a participant will be allocated, should that subject agree to be in the study. This avoids both conscious and unconscious selection of patients into the study. The sequence must be concealed from those recruiting volunteers until the individual has been recruited into the trial. It has been reported that non-randomized trials and randomized trials with inadequate allocation concealment tend to result in larger estimates of effect than randomized trials with adequately concealed allocation.[18]

Some methods used to implement allocation concealment are: use of a central telephone randomization system (by means of Interactive Voice Response Systems) and numbered containers. A simple and inexpensive method can be the use of sequentially numbered opaque and sealed envelopes.

There are different methods of randomization. Simple randomization is the most frequently used. It assigns each new treatment without regard to those already made. In a large trial (≥ 1,000 subjects), simple randomization should give a balanced number of participants allocated to each of the groups. But for smaller sample sizes the numbers allocated to each group may not be well balanced. Besides, the distribution of prognostic variables may be imbalanced too.

One approach to control the magnitude of imbalances is to use a restricted randomization method. In blocked randomization, each block contains a predetermined number of treatment assignments. For instance, each block has equal numbers of As and Bs (A = intervention and B = control, for example) and the order of treatments is randomly permuted within each block. A block of four subjects has six different possible arrangements of two As and two Bs. Similarly, treatment group is allocated to the next four patients in the order specified by the next randomly selected block. The process is then repeated. Permuted block randomization ensures balance in the number of subjects enrolled in each arm of the trial.

Another process of restricted randomization is stratified randomization. In smaller trials, groups may not be balanced in relation to important prognostic variables (such as sex, age, socioeconomic status, smoking status, severity of the disease, etc.). Such imbalances can be minimized by stratification. Each prognostic factor can define an individual stratum. Then a separate randomization process is performed within each stratum, yielding balanced prognostic factors in each treatment group.

Blinding or masking

Study subjects can modify their behavior or the way in which they relate outcomes (including adverse events) in a systematic way if they are aware of the treatment they are going to receive. For instance, they can create favorable expectation if they know they are going to receive a new experimental treatment. If they are assigned to a placebo arm, they may feel discriminated and react negatively.

Investigators can also report outcomes of the trial in a systematically biased way if they know which treatment they are evaluating. They may overestimate the effect of the intervention if they have the information that they are examining a test group subject, and they may underestimate the effect when examining a control group subject.

Finally, health care providers (dentists that are responsible for the treatment of participants) also may, consciously or unconsciously, treat the participants of each group in different ways.

When study subjects are blinded, that means they do not know which treatment they are receiving. When examiners are blinded, they do not know the treatment they are performing or evaluating, so the bias or expectations of the examiners are not likely to influence the measurements taken. When study subjects and examiners are blinded, the trial is generally defined as a double-blind trial. However, this term is ambiguous with regard to other participants, like care providers and even the data analyst. So, it is better to state who was blinded in the trial (study subjects, care providers, examiners, monitors, laboratory staff, data analyst, etc.). If there is no masking of treatments, the trial must be defined as an open trial.

In randomized placebo-controlled trials of pharmacological treatments, the placebo should be similar to the active medication in terms of appearance, taste, color and method of administration. For instance, in an RCT evaluating efficacy and safety of chlorhexidine mouthwashes, the placebo rinse must present the same appearance and be as bitter as the chlorhexidine rinse. Some investigators use quinine sulphate or quinine hydrochloride as a placebo rinse, due to its bitter taste.[19]

On the other hand, in RCTs where a surgical or other type of dental procedure constitutes the treatment, the placebo treatment should be a sham procedure.[20] A sham procedure is a procedure designed to resemble the real one and that is performed on a subject for the purpose of blinding. For instance, in many laser application trials, sham illumination is used as placebo treatment.[12,21] When performing sham procedures, it is important that the inves-

tigator that examines the subject is not the same as the one who provides the treatment.

Example: Andrade *et al*.[22] (2007) conducted an RCT that evaluated bacterial reduction after Nd:YAG laser irradiation associated with scaling and root planning for the treatment of furcation defects in chronic periodontitis patients. Investigator #1 performed all clinical measurements and collected samples for microbiological analysis. Investigator #2 was responsible for allocating randomly the experimental sites to test or control treatment, and was also responsible for the treatment itself. Investigator #1 was blinded to the treatment performed by investigator #2.

Statistical methods

Authors must present a description of the statistical methods used to estimate treatment effects, as compared to the control arm of the trial. Statistical analysis is discussed elsewhere in this book.

Ethical and regulatory aspects

After writing the trial protocol, investigators must submit it to the Institutional Review Board (IRB) or an Independent Ethics Committee (IEC). Before initiating the trial, the investigator and the institution should have written and dated approval from the IRB/IEC for the trial protocol.[8] Investigators must not start inclusion of study subjects before protocol approval.

For marketing approval of drugs, devices, cosmetics and the like, regulatory registration is mandatory. The regulatory process and requirements vary from country to country. For instance, in Brazil, Resolution 39/08 from ANVISA regulates the conduct of intervention studies in humans.[23]

Registering the trial

The debate on the transparency of clinical trials began some years ago, and one of its consequences was the publication, by the International Committee of Medical Journal Editors, of an editorial with the aim of promoting the registration of all clinical trials before they begin (i.e., before the enrollment of the first study subject).[24] This policy applies to all trials that started recruiting volunteers on or after September, 2005.

The purpose of a clinical trial registry is to ensure that everyone can find information about ongoing trials. This measure also intends to reduce publica-

tion bias. Publication bias is the tendency of clinical trials with null results (no significant differences between groups) or negative results (favoring the control arm of the study) finding it more difficult to be published than clinical trials with positive results (favoring the test arm). Negative studies have been shown to be 2.6 times less likely than positive studies to reach publication.[25] The public registry of clinical trials is a tool that helps researchers to find studies that were started, finished, but never published.

Sites where clinical trials can be registered are: *www.actr.org.au* (Australian Clinical Trials Registry), *www.clinicaltrials.gov*, and *http://isrctn.org* (International Standard Randomized Controlled Trial Number Register). In Latin America, the LATINREC (the Latin American Ongoing Clinical Trial Register) was developed by the Colombian center of the Ibero-American Cochrane Collaboration network.

Conducting the trial

After approval by the IRB and regulatory agencies, and registration of the trial protocol, investigators can start conducting the trial. The investigator should conduct the trial in compliance with the protocol, so all aspects discussed above (inclusion criteria, randomization, blinding, etc.) must be performed according to the original protocol. All deviations from it should be communicated to the IRB that approved the study.

Informed consent must be obtained from all participants of the trial or the subject's legally acceptable representative. The investigator should ensure the accuracy, completeness, legibility, and timeliness of all data reported. Data reported on case research forms should be consistent with the source documents. The investigator is also responsible for reporting all serious adverse events (SAEs) to the IRB and regulatory agencies.

RCTs financed by a sponsor (for instance, a pharmaceutical company) may be subject to monitoring, audit and inspection. Monitoring and audit are conducted by the sponsor.[8] Inspection can be performed by regulatory authorities, such as the FDA in the United States and the ANVISA in Brazil.[26] Inspection is not common in "academic" trials with no sponsor.

It is important to report all losses to follow-up and exclusions from the trial. Although a subject is not obliged to give his/her reason(s) for withdrawing from a trial, the investigator should make a reasonable effort to ascertain the reason(s), while fully respecting the subject's rights. Some individuals might have been lost to follow-up due to adverse events associated to the treatment.

Patients can fail to comply with many aspects of treatment specification.

Treatment non-adherence is a frequent problem and it has received a great deal of attention in the clinical trials literature. Intention to treat analysis is an approach to several types of protocol non-adherence. It is a strategy for the analysis of RCTs where subjects are analyzed as part of the treatment group to which they were originally assigned, even if they did not actually receive the intended treatment.[27] There is an important debate about the advantages and problems of analyses based on treatment assigned (intention to treat), compared with those restricted to participants who fulfill the protocol in terms of eligibility, intervention and assessment of outcome (as-treated analysis).

Reporting the trial

After completion of the trial, the next step is to write a report about it and publish the results. Reporting the results of a trial is one of the most important aspects of clinical research. Investigators have an obligation to the scientific community, the study participants, and the society to communicate the findings from a trial. Also, investigators should remember that assessment of the quality of an RCT is based on three sources: protocol, conduct of the study, and the clinical trial report.

As stated before in this chapter, well-designed RCTs can provide the highest level of evidence on the evaluation of prevention and treatment. However, poorly designed and reported trials have been associated with exaggeration of treatment effects.[28] Many RCTs fail to accurately report important methodological issues. Robinson *et al*.[29] (2006) conducted a systematic review of RCTs comparing powered *versus* manual toothbrushes. They observed that, of 42 included RCTs, only 15 adequately reported generation of randomization sequence and 16 performed adequate concealment of allocation. Intention-to-treat analysis was reported in only five studies.

A set of recommendations for authors reporting RCTs was published in 1996 and revised in 2001: the CONSORT statement.[30] The CONSORT (*Con*solidated *S*tandards *o*f *R*eporting *T*rials) is a checklist of fundamental methodological items that should be included when reporting an RCT, facilitating its critical appraisal and interpretation (Table 4). The main CONSORT Statement is aimed at reports of "standard" two-group parallel designs. However, there are other types of RCTs, with different designs and interventions. Thus, the CONSORT group has published some extensions of the first statement. For instance, an extension to the CONSORT Statement for cluster RCTs was developed and published in 2004, with recommendations for the report of these trials.[31] In cluster trials, interventions are randomized to groups of patients

Table 4 - Checklist of items to include when reporting a Randomized Trial – adapted from Moher *et al.*[30] (2001). [continued on next page]

Title and abstract		How participants were allocated to interventions (e.g., "random allocation," "randomized," or "randomly assigned").
Introduction	Background	Scientific background and explanation of rationale.
Methods	Participants	Eligibility criteria for participants and the settings and locations where the data were collected.
	Interventions	Precise details of the interventions intended for each group and how and when they were actually administered.
	Objectives	Specific objectives and hypotheses.
	Outcomes	Clearly defined primary and secondary outcome measures and, when applicable, any methods used to enhance the quality of measurements (e.g., multiple observations, training of assessors).
	Sample size	How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules.
	Randomization: Sequence generation	Method used to generate the random allocation sequence, including details of any restriction (e.g., blocking, stratification).
	Randomization: Allocation concealment	Method used to implement the random allocation sequence (e.g., numbered containers or central telephone), clarifying whether the sequence was concealed until intervention.
	Randomization: Implementation	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.
	Blinding (masking)	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated.
	Statistical methods	Statistical methods used to compare groups for primary outcome(s); methods for additional analyses, such as subgroup analyses and adjusted analyses.

(families, medical practices, hospitals, schools, communities, etc.) rather than to individual patients.

The CONSORT guidelines have been endorsed by the World Association of Medical Editors (WAME), the International Committee of Medical Journal Editors (ICMJE), the Council of Science Editors (CSE), and well over 200 journals worldwide.[32] Plint *et al.*[33] (2006) conducted a systematic review on the impact of using the CONSORT to improve the reporting of RCTs in journal articles. They concluded that journal adoption of the CONSORT Statement is associated with improved reporting of randomized trials. Nevertheless, they observed that poor reporting remains common.

Table 4 (continued) - Checklist of items to include when reporting a Randomized Trial – adapted from Moher *et al.*[30] (2001).

Results	Participant flow	Flow of participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.
	Recruitment	Dates defining the periods of recruitment and follow-up.
	Baseline data	Baseline demographic and clinical characteristics of each group.
	Numbers analyzed	Number of participants (denominator) in each group included in each analysis and whether the analysis was by "intention to treat." State the results in absolute numbers when feasible (e.g., 10 of 20, not 50%).
	Outcomes and estimation	For each primary and secondary outcome, a summary of results for each group and the estimated effect size and its precision (e.g., 95% confidence interval)
	Ancillary analyses	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.
	Adverse events	All important adverse events or side effects in each intervention group.
Discussion	Interpretation	Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision, and the dangers associated with multiplicity of analyses and outcomes.
	Generalizability	Generalizability (external validity) of the trial findings.
	Overall evidence	General interpretation of the results in the context of current evidence.

Most of the high-impact dental journals have endorsed the CONSORT statement. So, if an investigator intends to publish an RCT in one of these periodicals (such as Journal of Clinical Periodontology, Journal of Dental Research, British Dental Journal, Caries Research, Oral Diseases and International Journal of Paediatric dentistry, among others), he/she will have to report the trial using the CONSORT guidelines.

It is important to remember that some medical journals will only accept an RCT for publication if it was adequately registered, as discussed above. Some dental journals are encouraging authors that submit manuscripts of RCTs to register the trial in any of the free, public clinical trials registries. The clinical trial registration number and name of the trial register will then be published with the paper.

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8

Laboratory research

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Research can be defined as a process whereby experiments are used to respond to a question, an idea or a set of propositions. These experiments involve procedures and criteria that are standardized so that our personal beliefs, sensations or perceptions will not interfere in the observation and description of results.

The concept mentioned above is valid when the experiments are well designed and conducted correctly to obviate errors that will prevent correct analysis of the data obtained. Experimentation errors may be traced back to several different causes. For example, the errors may stem from the equipment in use, either because it is being used incorrectly or because it has not been well-gauged. The errors may also be due to factors that favor a slanted result, that is to say, a systemic error. Since no measurement can be entirely accurate, we have ways of estimating, and, in some cases, reducing research errors.

Among the ways we have of reducing errors in research, there are some factors inherent to the methodology used and others related to the work environment. Errors related to methodology result mostly from experiments conducted inadequately or designed improperly. Those related to the work environment will be considered here, since they may affect the result of the experiments and be directly related to the safety of the researchers.

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Laboratory characteristics

Generally speaking, there are two types of laboratories, those intended mainly for teaching – clinical laboratories – and those designed to conduct research. In dentistry, there are many different types of laboratories, including those for biological research, dental materials, biochemistry, information technology, cell culture, microscopy (electronic or light), microbiology, molecular biology, and histopathology, among others, and these laboratories perform methodologies common to all these different specialties on their premises.

All laboratories have a team that is headed by a supervisor or a responsible technician, and that is made up of technicians in different number and with different responsibilities. Depending on how the laboratory is structured, it may have researchers responsible for secondary laboratories, managers, coordinators, secretaries and other staff members. Other members of a laboratory may include researchers in training, whether basic training, like undergraduate scientific initiation students, or more advanced training, like graduate students.

Responsibilities

In order for research to proceed smoothly and to prevent both errors in experiments and work accidents, all those who use a laboratory must observe certain precautions. When using the laboratory, those in it should observe the safety regulations, whether recommended, advised or guided by good sense. The use of personal safety equipment, like gloves, glasses, mask, foot protectors (or closed footwear) and a long-sleeved, closed lab coat, are always a good precaution, as a rule, although their use is usually related to specific types of laboratories or to the type of experiment being conducted. All conditions that may pose a safety problem, or incidents that have occurred, must be reported to the lab supervisor so that the necessary measures may be taken to resolve the issue. Incorrect use of personal safety equipment or of lab equipment, or improper behavior for a lab environment should be everyone's concern. Should it occur, it must be immediately brought to the attention of the individual engaging in the improper practice. In a lab or any other work environment, it is important that everyone be responsible for everyone else, within the dictates of politeness and mutual respect. In any case, it is important that any individual, whether in training or not, be briefed about the lab and be given all necessary information on it before beginning his/her activities in it. Should the student or researcher be omissive or decide not to take any action in relation to the error or misbehavior, the supervisor should be notified.

Safety principles

In addition to appropriate use of the personal safety equipment, there are other safety-related issues that should be borne in mind. The foremost safety consideration is that one's work should be conducted seriously, without playing around, and with great attention and calm. All lab users should refrain from eating and drinking, and even smoking, inside the workplace. Use of such substances not only detracts one's attention in conducting an experiment, but may also contaminate the work environment or any food being ingested. Those who have long hair should wear a cap to keep their hair from coming into contact with the experiment, and also to prevent its movement from interfering in procedures or in one's concentration, or even from contaminating the experiment, one's skin, or one's own hair. Other good personal safety practices include keeping one's beard protected and one's nails cut, avoiding the wearing of accessories and contact lenses, and washing one's hands before and after the experiment, even though gloves may have been worn.

Even greater precautions should be taken when using chemicals in a laboratory. For example, although less common nowadays, the pipetting of reagents by mouth is not allowed; the handling of chemical substances that produce gases, fumes or aerosols should be made with an appropriate hood; after concluding an experiment, gloves should be disposed of and one's hands washed. An important consideration is that all researchers or lab users must know the chemical and safety characteristics of the product being handled. If you are using the lab and do not know the characteristics and properties of a reagent, you should seek to acquire more knowledge about it. If the necessary conditions for using a specific reagent are not available, it should not be used. Chemical reagents should be stored adequately and in the appropriate environment, especially if concentrated, and should also be disposed of suitably. Should the conditions for disposal of the material not be known, consult the in-house safety committee of your school or company. Before leaving the laboratory or after concluding the experiment, you must make sure that everything is clean and put away in its proper place, that the reagent containers are sealed and in their proper place, that air and gas outlets are closed and that all the equipment has been turned off. If you wish to learn more about chemical safety, there are Internet sites and books that you may consult. Examples of titles are given in the bibliography at the end of the chapter.

Inadequate use of lab equipment not only jeopardizes the experiment and leads to incorrect interpretation of its results, but often also leads to violation or inobservance of safety conditions. Each piece of equipment has its own tech-

nical characteristics that should be known by those using the equipment. It is not uncommon for users unfamiliar with certain equipment to use it without the adequate operating information. Before using any equipment, the responsible technician or researcher must be informed of its use so that he can provide even such basic information as what electric outlet it should be plugged into. Never change equipment settings, ignore indicator lights or force equipment parts. Inattention is another reason for improper use of equipment. Not paying due attention to what you are doing constantly leads to safety problems, when not compromising the experiment outright by mixing reagents incorrectly, following an incorrect sequence of procedures, using incorrect quantities of a substance, among other reasons.

Labs that work with radioactive products require special considerations in regard to the containment, handling and disposal of such substances, and shall not be addressed here.

Biosafety

Biosafety can be defined as a set of technical, administrative and educational measures designed to prevent, control or avoid damage to the health of man or animals and to environmental conservation, caused by the use of physical, chemical and biological agents. Biosafety levels refer to the precautions that must be taken by laboratories working with infectious agents (see Table 1).

Specific biosafety measures should be adopted by labs, based on national and international regulations governing the transportation, conservation and handling of pathogenic microorganisms. The traditional lab safety regulations require the use of good practices in regard to work, adequate containment equipment, well-designed areas, and administrative actions that minimize the risk of individual or collective contamination of lab users and that also prevent contamination of the environment. Among these measures, and in addition to the practices stated above, we can mention:

1. Ensuring that access to areas where biological, chemical or radioactive agents are being used is controlled.
2. Freezers, refrigerators and other containers in which these agents are stored must be locked or constantly checked for organization, contents and use.
3. Containers holding the types of agents mentioned above must be handled by people who are experienced or by people who are under their supervision.
4. The transportation of these agents to other areas must be carried out by experienced and skilled people, and in compliance with national (ANVISA, CTNBio) and international regulations.

Table 1 - Recommended Biosafety Levels (BL) for Infectious Agents (Oswaldo Cruz Foundation).

	Description	Practical requisites	Agents
BL-1	This is the laboratory containment level applying to laboratories of basic sciences where microorganisms belonging to risk class 1 are handled.	No requisite of specific design is needed except for good spatial and functional planning and the adoption of good laboratory practices.	The risk to individuals or to the community is non-existent or, at most, very low, meaning that the microorganisms handled have a very low likelihood of causing infection in man or animals. Example: *Bacillus subtilis*.
BL-2	This refers to a containment laboratory handling risk class 2 microorganisms.	This applies to clinical or hospital laboratories of primary diagnostic level, making it necessary to have not only the adoption of good practices, but also the use of primary physical barriers (biological safety cabin and individual protection equipment) and secondary physical barriers (lab design and organization).	The risk to individuals is moderate and the risk to the community is low. The microorganisms handled may cause infection, but a number of effective therapeutic and prophylactic measures are available and the risk of spreading is limited. Examples: Yellow fever virus and *Schistosoma mansoni*.
BL-3	A laboratory used for working with risk class 3 microorganisms or for handling large quantities and high concentrations of risk class 2 microorganisms.	This level of containment requires not only the items referred to in BL-2, but also a special lab design and construction. Strict control must be kept of the operation, inspection and maintenance of the facilities and of the equipment. Moreover, the technical personnel must receive specific training on safety procedures to manipulate these microorganisms.	The risk to individuals is high, but the risk to the community is limited. The pathogen may cause serious infections in man and animals, and may spread from individual to individual. However, there are therapeutic and prophylactic measures that can be taken. Example: Venezuelan equine encephalitis virus, *Mycobacterium tuberculosis*, *Bacillus anthracis* and HIV.
BL-4	A maximum containment laboratory for manipulating risk class 4 microorganisms, where there is the highest level of containment. Units of this type are also geographically and functionally separated from other areas.	Laboratories of this type require not only the physical and operational requirements of containment levels 1, 2 and 3, but also containment barriers (protection facilities, design and equipment) and special safety procedures.	The risk to both the individual and the community is high. The microorganisms handled represent serious risk to man and animals because they are highly pathogenic and spread easily, and also because there are no prophylactic or therapeutic measures available. Examples: Marburg virus and Ebola virus.

5. As for using genetically modified products, laboratories must follow the directives laid down by the National Technical Biosafety Committee (CTNBio) and request issuance of a Biosafety Quality Certificate (CQB).

One important principle that applies to both biological and chemical agents is to know the characteristics of the agent to be used. In the case of microbiological agents, important information includes degree of pathogenicity, power of invasion, resistance to sterilization, virulence and mutagenic capacity. All this information should be known before handling any microorganisms so that basic precautions may be taken, thus minimizing risk situations that commonly occur.

Organization and cleanliness

As a rule, in order to maintain the lab environment suitable for daily use and to avoid contamination, everyone using the lab should always observe the basic rules of cleanliness and organization. The counter and work places must always be uncluttered, clean and organized according to the characteristics of each lab. Remember that leaving the counter and environment organized and clean for the next user fosters teamwork and productivity, and also allows everyone to concentrate primarily on the experiment. Containers for disposal of materials should be made available and be correctly labeled as to their purpose. Among the containers for disposal that should be made available in a lab are those appropriate for disposing cutting and piercing instruments, so that both lab users and staff members will suffer no injuries.

A very important issue in organizing a laboratory is that all containers, flasks, tubes, etc., should be labeled with the name of the contents, the concentration, the date of preparation and the name of the responsible party, so that any lab user may identify the container and who is responsible for it. Other codes or aids for identifying or positioning are also useful and inherent to the characteristics of each lab.

Organization and positioning at a lab counter are also important. Whenever possible, work sitting down, with the counter at elbow level and one's hands below one's shoulders. It is important to keep focused and also keep a constant work pace, periodically changing one's work position and relaxing one's back and shoulders. Another aspect that helps us work better is to keep all objects at the reach of our hands, with objects least used at a distance, and to take care to keep containers with caustic or heated substances within sight and away from regularly used containers.

Obtaining funding for projects and equipment maintenance

Those who are responsible for a lab or who work in one often see themselves in need of funds to undertake their research or to maintain their equipment. A

list of possible funding sources for research is given at the end of the chapter. Bear in mind that the diversity of institutions listed implies equally diverse specific funding conditions, like project characteristics, region where the project will be developed, and end purpose, among others. Part of the success of obtaining funding for a research project entails understanding the mission of the funding agency and the types of projects it funds. A search must be made to see what programs fit the project, what the cap is on funding, how long the funding will be made available, how project analysis is carried out, what items the project must include in its description, what the minimum requirements are for submitting a project, what type of funding can be requested (for example, for national or international consumption material, for national or international permanent material, for outsourced services, for travel, for scholarships or for infrastructure funding), and what the requirements are for the period following funding cutoff. If possible, find out beforehand what forms must be filled out and what requirements are implied in the fields to be filled out.

If a researcher is submitting his initial requests, he should consider showing the project to one or more experienced researchers. Discussion of ideas, goals and experiment design, for example, may contribute substantially to project acceptance. In any case, well-accepted projects have original ideas, clear presentations, good scientific fundaments, coherence of concepts, and a well-focused and precise research plan, with a detailed description of the experiment, an execution schedule and a realistic funding proposal. The researcher should have experience with the main methodologies adopted in the work being carried out so that he will not risk having difficulties in executing the work and possibly not meeting the deadline for concluding the research. Should the agency to which the project is being submitted consider pertinent such information as an explanation for the permanent materials being requested, this information may contribute to project approval.

Planning the experiment

One of the most important stages of laboratory activity is planning the experiment. Well-planned experiments have fewer chances of going wrong and of complications or accidents occurring. When planning an experiment, we must have a clear idea of the basic question we strive to answer. With this in mind, it is important to know what the variables are and to make sure they are kept constant so that the experiment can be reliable – factors like temperature, humidity, pH, and time, among others. Most experiments require that controls be analyzed while the experiment is in process – the so-called positive and nega-

tive controls – and sometimes more than two controls may be necessary. At the same time, for interpretation to be correct, it is important that only one condition be tested, or that, when modifying other variables, all variables may be analyzed adequately without jeopardizing the final observation of the data obtained. In addition to the controls, experiments usually should be performed at least in triplicate. This condition facilitates statistical analysis later and establishes consistency in obtaining the data. If an experiment made in triplicate offers excessively disparate results, there are probably factors that have not been considered interfering in the reaction or in the experiment.

In addition to the design of the experiment itself, it is necessary to know what materials and how much of these are needed for the experiment. It is never too much to stress that the source and characteristics of these materials should be well-defined, well-known and maintained the same until the end of the experiment. Obviously, it is important to be familiar with the equipment to be used, and to know whether it is fully operative and whether it was properly gauged by the manufacturer or authorized technician.

Experiments commonly follow well-defined protocols usually validated previously by the labs where they will be performed. If you wish to change your research protocols to make improvements or to add reagents or conditions described in more recent literature, the Internet has a great many pages dedicated to disclosing such information. We have provided a list of pages at the end of this chapter, which can be consulted, although many other pages exist. It is important to bear in mind that all the experiments disclosed on these pages, or even in reference books, must be validated with control samples before they can be used with your test samples.

If there is a well-defined protocol or sequence of procedures to be followed, each stage should be checked, taking care to make sure all the reagents and materials needed are available. Another important condition is planning how long it will take to conduct an experiment, since it is not uncommon for this variable to interfere with the quality of the final data obtained. It should also be taken into consideration that most procedures take longer than expected. Also on the issue of time, if the experiment is expected to take a long time, you must make sure that any undesirable external or local conditions do not interfere with conducting it. As a last part of planning the experiment, it is necessary to have a well-defined way of observing these results; it is important to clearly define the methodology to be used in documenting the results obtained, and possibly also how and with what methods the results will be counted or measured.

It should also be mentioned that laboratory work is usually a team activity.

With this in mind, planning is also important so that a scheduled experiment of one's lab colleague will not be delayed, or unfavorable work conditions will not be created for one's teammate.

Logbook

A logbook is deservingly a topic of its own, since it is often forgotten or relegated to the sidelines by researchers or those in training. The entire experiment, from the planning, through each step of its execution, gathering of results and final interpretation should be rigorously taken note of in an appropriate notebook. Everything should be written down, like volume levels, concentrations, calculations, reagents used (including batch number, expiry date and manufacturer), changes in protocol, dilutions, sources consulted to perform the experiment, date, time, title, clear identification of each experiment, name of possible participants, unexpected delays, baths, treatments, and other data. The record should be clear, complete, in chronological order, legible, in pen (never pencil), and nothing should be omitted. Never remove a page or even part of a page. Often, a researcher finds himself having to go back through his notes to compare experiments, check conditions tested earlier, or check results obtained previously. With this in mind, the entire documentation of each experiment should be added to the notebook, including figures, tables, photographs, parallel notes and even remarks or ideas. Furthermore, logbooks usually belong to and are an important attribute of the lab, so that others who repeat the same experiment do not make the same mistakes.

Bioethics

All clinical research, or research conducted in laboratories, involving both human beings and animals and material collected from them, should be approved by an ethics research committee. Actually, Resolution 196/96 adopted by the National Health Council states, in its preamble, that it is founded on the main international documents that have given rise to declarations and directives on research involving human beings, like the Nuremberg Code, the Declaration of Human Rights and the Helsinki Declaration. In relation to human beings, it states that "all procedures of any nature involving human beings, whose acceptance is not yet acclaimed in scientific literature, shall be considered as research and, therefore, must comply with the guidelines of the present Resolution. The procedures referred to include those of an instrumental, environmental, nutritional, educational, sociological, economic, physical, psychic or biological nature, among others, whether pharmacological, clinical or surgi-

cal, and having a preventive, diagnostic or therapeutic purpose."

As for animals, their use in research should be guided by some steering principles, such as that of their importance to human beings and the justification of scientific experimentation itself using this type of model. Since Brazil does not yet have specific legislation in place in this respect, the Universal Declaration of Animal Rights – UNESCO/1978 or the regulations of the Brazilian College of Animal Experimentation (COBEA) may be used as a base for using animals in research.

These regulations and resolutions should be consulted before submitting the research project to the Ethics Research Committee (CEP), which is subject to the regulations of the National Ethics Research Commission (CONEP). To submit a project to the Ethics Research Committee (CEP), the following documents are required:

- Coversheet of registration at SISNEP (National System of Information on Research Ethics Involving Human Beings)
- Research project in Portuguese
- Detailed financial budget and researcher's stipend
- Informed consent
- Résumés of both the chief researcher and of the other researchers

When the research is conducted abroad or in cooperation with a foreign body, a document attesting to the approval by the ethics committee in the country of origin, or a justification and a list of the participating centers abroad, should be submitted to the National Ethics Research Commission (CONEP), in addition to the forwarding letter of the Ethics Research Committee of the Institution in question, and the document attesting to Ethics Research Committee (CEP) approval, with its duly substantiated report.

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Cienfuegos F. Segurança no Laboratório. Rio de Janeiro: Interciência; 2001.

Hirata MH, Mancini Filho J. Manual de biossegurança. São Paulo: Manole; 2002.

Teixeira P, Valle S. Biossegurança: uma abordagem multidisciplinar. Rio de Janeiro: Fiocruz; 1996.

List of Internet pages for additional reading

http://conselho.saude.gov.br/comissao/conep/resolucao.html

http://conselho.saude.gov.br/comissao/eticapesq.htm
http://www.bdbiosciences.com/pharmingen/protocols/
http://www.biosseguranca.com/
http://www.cellbio.com/protocols.html
http://www.cobea.org.br/index.php
http://www.ctnbio.gov.br/
http://www.fiocruz.br/biosseguranca/
http://www.molecularstation.com/
http://www.nap.edu/readingroom/books/labrats/
http://www.protocol-online.org/

Possible research funding sources

ABC – Agência Brasileira de Cooperação – *www.abc.gov.br*
BNDES – Banco Nacional de Desenvolvimento Econômico e Social – *www.bndes.gov.br*
CAPES – Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – *www.capes.gov.br*
CENPEC – Centro de Estudos e Pesquisas em Educação, Cultura e Ação Comunitária – *www.cenpec.org.br*
Comissão Fulbright – Comissão para intercâmbio entre os Estados Unidos e o Brasil – *www.fulbright.org.br*
CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico – *www.cnpq.br*
EMBRATEL – Empresa Brasileira de Telefonia - *www.embratel.net.br*
FAPs – Fundações de Amparo à Pesquisa – *www.gestaoct.org.br/orgsist/FAPs/org_membros_faps.htm*
FB – Fundação Bradesco – *www.fb.org.br*
FBB – Fundação Banco do Brasil – *www.fbb.org.br*
FINATEC – Fundação de Empreendimentos Científicos e Tecnológicos – *www.finatec.org.br*
FINEP – Financiadora de Estudos e Projetos – *www.finep.gov.br*
FIOCRUZ – Fundação Osvaldo Cruz – *www.fiocruz.br*
FRM – Fundação Roberto Marinho – *www.frm.org.br*
FUBRAS – Fundação Franco-Brasileira de Pesquisa e Desenvolvimento – *www.fubras.org*
FUNDEP – Fundação de Desenvolvimento da Pesquisa – *www.fundep.ufmg.br*
GIFE – Grupo de Institutos, Fundações e Empresas – *www.gife.org.br*
IBAMA – Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – *www.ibama.gov.br*
IPEA – Instituto de Pesquisa Econômica Aplicada – *www.ipea.gov.br*
MCT – Ministério da Ciência e Tecnologia – *www.mct.gov.br*
MEC – Ministério da Educação – *www.mec.gov.br*
MMA – Ministério do Meio Ambiente – *www.mma.gov.br*
MRE – Ministério das Relações Exteriores – *www.mre.gov.br*
OPAS – Organização Pan-Americana da Saúde – *www.opas.org.br*
PETROBRAS – Petróleo Brasileiro – *www.petrobras.com.br*
RNP – Rede Nacional de Ensino e Pesquisa – *www.rnp.br*
ROTARY – Rotary International - *www.rotary.org.br*
SBPC – Sociedade Brasileira para o Progresso da Ciência – *www.sbpcnet.org.br*
SEBRAE – Serviço Brasileiro de Apoio às Micro e Pequenas Empresas – *www.sebrae.com.br*

9

Sampling of human material to conduct research studies of the oral cavity

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1. Oral mucosa biopsy. Fundaments and techniques.

A biopsy is a surgical procedure involving the removal of tissue from a living organism for histopathological testing to diagnose the tissue under study. Given the oral cavity's good accessibility and visibility and the simplicity of the technique, the intraoral biopsy is a simple procedure that provides valuable information to the patient and group of healthcare professionals in charge of patient follow-up. The purpose of a biopsy is to determine the nature of the lesion, establish the diagnosis and prognosis, and help decide which therapy should be used. In addition, a biopsy is a document with forensic value.

Prospective research articles make it possible to adequately record clinical and histopathological aspects and their potential correlation with molecular, immunohistochemical and other kinds of studies. This is why it is important to bear in mind some general and specific considerations about the technique used to remove the test material.[1,2,3]

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2. General considerations

- Importantly, the biopsy and the rationale for its use should be specified in the research protocol, which in turn should be approved by the Ethics Committee of the institution, medical

service or research center where the study will be conducted. An informed consent signed by each patient before a witness should also be included in the project.

- It is a priority to consider that the biopsy should not only be a requirement to conduct the research, but ethically it should be beneficial for the diagnosis, treatment and follow-up of the patient.
- Depending on the specific features of the disease under study, the biopsy may be conducted directly on the lesion, when there is direct access to it, or indirectly, when open surgery is required to access the target tissue and remove a tissue sample (minor salivary glands, bone tissue lesions, etc.). According to the amount of tissue removed, a biopsy may be classified as incisional (when only a portion of the lesion is removed) or excisional, when the entire lesion is removed.
- The clinical history record is a confidential and legal document which should include a thorough and detailed description of all the personal identification data, anamnesis, personal history, family history, clinical examination, and complementary tests (radiographs, laboratory tests, biopsy, etc.).
- An essential and very important issue to assess prior to a biopsy is the decontamination of the lesion, which involves administering the therapies required to eliminate the contaminating agents (opportunistic microbial infections, chronic trauma, bacterial plaque control, etc.) causing an overlapping inflammatory process that may complicate or mask the histopathological diagnosis and course of the lesion.
- The choice of technique and instruments may vary, depending on the nature and location of the disease. However, there are constant aspects regarding the technical procedure and patient that should be considered. For example, the technique should be applied with caution and precision to preserve the cell morphology and the architecture of the original lesion, so that a proper histopathological study may be conducted. The material removed should be enough for testing purposes and representative of the disease; and sampling must not put the patient at risk.
- Clinical examination, medical imaging and other laboratory tests, such as microbiological or biochemical tests, provide the data required by – and sometimes critical for – a pathologist to make a correct histopathological diagnosis.
- The anesthetic infiltration should be applied far enough from the lesion to avoid morphological changes in the tissue to be excised. In addition, it is convenient to mark the selected representative area with a dermographic

pencil, as in some cases the anesthetic infiltration may mask the visualization of the chosen site.

- Regarding incision design, elliptical incisions facilitating posterior suturing should be preferred. In contrast, incisions perpendicular to the muscle fibers should be avoided. While taking gingival biopsies, the incisions should preserve the gingival papillae and, if possible, extend along the sulcus bottom to prevent the formation of inflammatory infiltrate, typical in this tissue.
- Importantly, caution should be taken to ensure the selection of the most representative areas of the lesion, including healthy margins. Sampling the central area of the lesion only is the wrong thing to do, as in certain lesions this area suffers necrosis and will not provide information of diagnostic value (e.g., carcinomatous ulcer). In a biopsy, sampling both the epithelial and connective tissue is essential to thoroughly study the lesion.
- The size of the biopsy specimen should be at least 0.5 cm in diameter, as smaller samples seldom provide diagnostic information.
- During the procedure, actions that may alter the sample, such as applying anesthetic infiltration to the lesion or handling the dissecting scissors in such a way that leads to excess trauma, should be avoided. The biopsy specimens must immediately be placed in a bottle with the fixation solution selected for the study and sent to the pathologist together with the previously gathered information.
- As regards the instruments to be used, the biopsy may be taken with a cold scalpel or a punch. The choice of instrument will depend on the surgeon's ability to use them.

3. Specific considerations

3.1. Biopsy of minor salivary glands

The biopsy of minor salivary glands is a simple and quick procedure, used to diagnose sarcoidosis, amyloidosis and Sjögren's syndrome. It is preferably taken from normal looking lower lip mucosa because, in that site, the minor salivary glands are more numerous and their morphology is easier to distinguish from that of other structures. This type of biopsy follows the general criteria for incisional biopsies, and complications are rare.[4,5]

3.1.1. Procedure sequence

1. The site of choice for biopsy sampling will be an area of normal lower lip mucosa. A normal appearance is important because an inflammatory process of a different origin on the mucosa may produce a cell infiltrate that

may mask or complicate the diagnosis of the disease.

2. Local anesthesia is applied to the incision area. An anesthetic combined with a vasoconstrictor is used to minimize bleeding in the surgical field and facilitate the surgeon's visualization.
3. The incision should be made to the right or to the left of the midline of the labial mucosa, approximately midway between the sulcus bottom and Klein's line (the limit between the labial mucosa and semimucosa). This incision should have a horizontal and linear design, 1.5 to 2 cm long; depthwise, it should only involve the epithelium.
4. Starting from the incision margins, the lamina propria will be divulsed with a blunt instrument to release the glands from the epithelium towards the surgical field.
5. Approximately five minor salivary glands will be carefully dissected without damaging the adjacent sensory nerves. The biopsy should sample a minimum glandular area of 12-15 mm^2 to enable the focus score to be assessed. The amount of glands required may be estimated based on the diameter of each excised gland (Table 1).
6. The glands should be placed in neutral buffered formol saline for fixation.
7. The surgical margins will be repositioned with absorbable suture (000 or 0000), avoiding any damage to the remaining salivary glands.

3.2. Biopsy for direct immunofluorescence technique

The direct immunofluorescence (DIF) test is a complementary diagnostic technique for immunologic diseases and should be used together with the clinical and anatomopathological diagnoses. In oral mucosa diseases, the DIF technique is recommended for the diagnosis of pemphigus vulgaris, pemphigoids, linear IgA disease, epidermolysis bullosa, polymorphous erythema and oral lichen planus (Table 2).

Table 1 - Determination of glandular area in relation to the diameter of the gland specimen.

Glandular diameter	Glandular area
1	~ 1 mm^2
1.5	~ 2 mm^2
2.0	~ 3 mm^2
2.5	~ 5 mm^2
3.0	~ 7 mm^2

Table 2 - Immunofluorescence findings in oral diseases.

Disease	Mucosal specimen	Result
Lichen planus	Lesional and perilesional	Linear deposit of fibrinogen, in the dermal-epidermal junction, and IgM, in cytoid bodies
Erythematous lupus	Lesional or normal	Granular deposit of IgG, IgM and C3 in the dermal-epidermal junction
Pemphigus	Perilesional	Intercellular epidermal deposit of IgG and C3
Ampullary pemphigoid	Perilesional	Linear deposit of IgG and C3 in the dermal-epidermal junction
Mucosal membrane pemphigoid	Perilesional Oral, genital and ocular mucosa	Linear deposit of IgG, IgA and C3 in the dermal-epidermal junction
Lichen planus pemphigoid	Perilesional	Linear deposit of IgG and C3 in the dermal-epidermal junction
Dermatitis herpetiformis	Perilesional or normal Distant from mucosa	Granular deposit of IgA, with or without C3 in the dermal-epidermal junction
Linear IgA dermatosis	Perilesional or normal Distant from mucosa	Linear deposit of IgA with or without C3 in the dermal-epidermal junction
Acquired ampullary epidermolysis	Lesional, new	Linear deposit of IgG and C3 in the dermal-epidermal junction
Leucocytoclastic vasculitis	Lesional	Deposit of IgG, IgM, C3, and fibrin in and around blood vessels

Adapted from: Guzmán, Fernández Blanco[6] (2007)

3.2.1. Technical considerations

- Testing should be conducted on fresh material to preserve the antigen-antibody complexes.
- The biopsy should preferably be taken from lesions within 48 hours of their development.
- Specifically in ampullary diseases, it is sometimes useful to cause a new lesion by scraping, using a bulb aspiration device or insufflating pressurized air tangentially to the mucosa.
- The biopsy site of choice will depend on the clinical manifestations of the disease. The biopsy should be perilesional both in ampullary lesions and polymorphous erythema, as well as in the erosive and ampullary variants of oral lichen planus. Biopsy sampling should be intralesional in reticular and atrophic oral lichen planus.
- How the material will be transported will depend on the time required to take the biopsy specimen to the laboratory and on room temperature. A sterile gauze soaked in saline solution may be used, placing it in a clean and

dry container, a styrofoam container with dry ice or common ice, or Michel's transport medium. The latter is recommended for samples that will be processed days or even a week after they are obtained.

- Importantly, the DIF technique complements the clinical and anatomopathological diagnosis, and healthcare professionals are advised to contact the laboratory prior to taking the biopsy and shipping the material for testing.

3.3 Mycological testing

3.3.1. Examination of mucosal secretions

The objective of this test is to diagnose superficial mycosis affecting the buccopharyngeal mucosa and the labial semimucosa. It deals almost exclusively with the diagnosis of superficial candidiasis in these sites, even though deep mycosis may also be diagnosed.

3.3.2. Sampling

Mucosal swabs: Previously soak a swab in saline solution (SS), rub it on the lesion and place it in a sterile tube with 0.5 to 1 ml of SS. A transport medium may be used, such as the Stuart's transport medium, but it is not advisable. Then the fresh material should be examined directly using KOH if necessary, as explained below, and cultured in glycosated Sabouraud Agar (GSA). This type of biopsy specimen is only useful to diagnose superficial mycosis caused by yeasts.

Scraping of mucocutaneous lesions: scrape the mucosa deeply with a blunt sterile scalpel. Then proceed to examine the fresh sample material directly, with KOH aggregate, special staining and culture, as detailed below. This sample makes it possible to diagnose primary pathogen fungi because of the low contamination level. For example: *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Cryptococcus neoformans* and *gatti*, *Pseudallescheria boydii* and *Sporothrix schenckii* and opportunistic agents (*Candida albicans* and other yeast species).

This method makes it possible to view the fungal elements better because of the use of targeted sampling.

3.3.3. Steps

Mycological testing is made up of various steps, which are detailed as follows:

a) Direct examination (for mucosal swabs and scrapings of mucocutaneous lesions)

1. In fresh conditions:

- It is used for all specimens requiring mycological testing, as most fungal agents can be seen by simple microscopic observation, except for those requiring clarification with KOH because of their density. The material is placed directly between the glass slide and cover slip and is observed with an optical microscope (OM), first with a low (100 X) and then with a 400 X objective lens.
- In the case of specimens of deep mycosis, it is important to inform the predominant cellularity (leucocytes, erythrocytes, cells, macrophages, etc.).

2. By clarification with 20%-40% KOH (for mucosal swabs and scrapings of mucocutaneous lesions):

- It is used to examine thick, viscous or opaque specimens requiring softening and/or clarification with KOH prior to microscopic observation.
- The material to be observed is placed in the center of a clear glass slide. Then a drop of glycerinated water in distilled water (1:1) and a drop of KOH are added to this material, which is covered with a cover slip. The hydroxide acts directly on the specimen. Since digestion is slow, observation conditions are optimal after 24 hours.
- If an observation is required immediately, the cover slip is gently pressed against the glass slide, then it is repeatedly heated with a mild flame (without boiling), to speed up the action of the KOH, and left to rest for 20 minutes.
- The material is observed with reduced microscope light and 100 X and 400 X objective lenses. If fungal structures are not observed, repeat the observation process 48 hours to 72 hours later. The glycerinated water prevents the preparation from dehydrating.

3. Smears for direct examination with staining (scraping of mucocutaneous lesions):

- These are used to test sputum, bronchial washes, pus, etc. A drop of the material to be examined is placed in the center of a clear glass slide and spread carefully with a spatula. The smears are left to dry and, then, stained with Giemsa, Gram and Kinyoun. The specimen is observed with a 400 X and 1,000 X objective lens.

b) Seeding of material

1. To test superficial mycosis:

Mucosal swabs: They will only be processed when taken from wet lesions

that are consistent with mucocutaneous candidiasis or other mucocutaneous lesions. It is convenient to immerse the swab in 0.5 ml of sterile SS to keep it wet.

To seed the specimen, the swab should be rotated in a tube containing Sabouraud Agar with chloramphenicol (to inhibit bacterial growth) GSA-C.

2. To test deep mycosis:

Scraping of mucocutaneous lesions: The specimen should be seeded at a ratio of 0.1 ml per test tube; 2 tubes of GSA-C, 2 tubes of Sabouraud Agar with chloramphenicol and cycloheximide (cycloheximide inhibits environmental fungus growth) GSA-CC, and 2 tubes of Sabouraud Agar without antibiotic (enabling the development of *Nocardia filamentous* bacteria to occur) GSA-W.

c) Incubation

The material seeded to test superficial mycosis (mucosal swabs) is incubated at 26 ± 1ºC (mycology culture incubator) during 28 ± 2 days.

The specimens suspected of deep mycosis (scraping of mucocutaneous lesions), which are seeded in duplicate, should be incubated at two temperatures, namely, 36 ± 1ºC and 26 ± 1ºC (a set of test tubes for each temperature) during 28 ± 2 days.

If the presence of very slow growing fungi is suspected (*Histoplasma capsulatum*), incubation time may be extended up to 60 days at a temperature of 26 ± 1ºC.

3.4. Bone tissue biopsy

Particularly in the case of lesions involving or located on bone tissue, clinical examination together with the analysis of imaging tests will enable preliminary diagnoses to be established prior to the biopsy, making it easier to decide which is the most adequate way of sampling material from the lesion and to select the approach route to and most representative disease site for the biopsy.

A partial tissue sample from a bone lesion may be taken with a surgical biopsy (also called open biopsy) or with a punch biopsy (also called closed biopsy).

3.4.1. Conventional surgical biopsy

This is a surgical procedure and, as such, includes the same steps as an operation: dieresis, exeresis and synthesis. A surgical biopsy requires minimal fundamental surgical and instrumental knowledge.

3.4.2. Punch biopsy

It is ideal for sampling bone lesions from the maxillae because the proce-

dure is virtually atraumatic.[7]

The advantages of this technique include:

- Simple method
- Access to deep lesions
- Sampling of specimens from different sites and depths (central and peripheral)
- Slightly traumatic
- Reduced bleeding
- Reduced contamination
- Not risky
- Use of local anesthesia in outpatients (except for children)
- Optionally image-guided
- Reduced cost and time
- It may be repeated if required
- Does not exclude surgical biopsy

a) Thin needle punch

The thin needle punch involves the use of a conventional needle, normally varying from 18 to 23-gauge caliber, attached to a Luer-type syringe. The fine needle punch uses the negative pressure generated when the embolus is drawn out of the syringe to facilitate removal of material.[8]

This technique is used in radiolucent osteolytic lesions and cystic lesions.

b) Cutting needle punch

It removes tissue specimens in the form of cylinders, which preserve the histoarchitecture of the lesion *in situ*, facilitating the histological testing of these specimens. An 11 or 13-gauge, resistant steel, beveled and sharpened point, Jamshidi-type needle is used.[9] This kind of biopsy may be carried out combined with negative pressure. These needles are indicated for radiolucent lytic lesions or lesions with a mixed radiographic pattern.

c) Trephine punch

It involves a needle that has an active tip with a circular saw to remove biopsy specimens of highly mineralized tissue.

This technique cuts hard material in the form of a cylinder, which can be fixed and demineralized for routine histological testing, or may be immersed in methyl methacrylate without previous demineralization, to obtain histological cuts made with a special microtome. Therefore, both the organic and inorganic

component of the bone tissue may be assessed.[10]

3.5. Electron microscopic studies

The biopsy specimens should be fixed in glutaraldehyde, and the tissue cuts should be 9 nanometers in thickness. The cuts are stained with uranyl and lead, and then subjected to ultrastructural analysis. This makes it possible to visualize the basement membrane in autoimmune ampullary diseases; the protein material in deposit diseases; the Donovan bodies in leishmaniasis; and the structures characteristic of benign and malignant tumors. Table 3 shows some indications for use of electron microscopy in oral diseases.

3.6. Stains available for testing biopsy tissue specimens

Stains available for testing biopsy tissue specimens are shown on Tables 4, 5 and 6. Table 7 shows indications for use of immunohistochemistry in oral diseases.

Table 3 - Indications for use of electron microscopy in oral diseases.

Entity	Ultrastructural findings	Diagnostic problem
Ampullary epidermolysis	Cytolysis of basal lamina (simple form) Cleavage under the dense lamina (distrophic form)	
Histiocytosis X	Birbeck granules	Xanthomatous histiocytic infiltrates
Fusocellular melanoma	Premelanomas	Fusocellular carcinoma. Sarcomas
Neuroendocrine carcinoma	Dense granules, Intermediate filaments	Lymphomas. Lymphoepithelial carcinoma
Apocrine carcinoma	Duct formations, Glandular secretion	Metastasis of adenocarcinoma

Adapted from: Guzmán, Fernández Blanco[6] (2007)

Table 4 - Stains available for testing biopsy tissue specimens. Specific stains. [continued on next page]

Stain	Utility/Detection	Color
Hematoxylin-eosin	Anatomopathological diagnosis	Red/blue
Masson's trichrome	Collagen, smooth muscle fibers	Blue
Fontana-Masson	Melanin	Black

Adapted from: Guzmán, Fernández Blanco[6] (2007)

Table 4 (continued) - Stains available for testing biopsy tissue specimens. Specific stains.

Stain	Utility/Detection	Color
Silver impregnation	Treponema pallidum, Melanin, nerves, reticular fibers	Black
Methamine silver or Gomori-Grocott	Fungi, Donovan bodies, Frisch bacillus or *Klebsiella rhinoscleromatis*	Black
Verhoef-Van-Gieson	Elastic fibers	Black
Warthin-Starry or Levadite Giemsa	Treponemae, Donovan bodies, Leishmania, mastocytes, eosinophils, acid mucopolysacharides (mucin)	Black
Fite-Franco	Acid-alcohol resistant bacillus	Red
Hotchkiss-McManus or Schiff periodic acid (SPA) and diastase	Fungi, neutral mucopolysacharides, mucin, reticuline and glycogen	Red

Adapted from: Guzmán, Fernández Blanco[6] (2007)

Table 5 - Stains available for testing biopsy tissue specimens. Special stains.

Stain	Utility/Detection	Color
Alcian blue	Mucin	Blue
Toluidine blue	Mucin, mastocytes	Purple
Crystal violet	Amyloid	Purple red
Perls' potassium ferrocyanide	Hemosiderin	Blue
Congo red	Amyloid	Green (polarized light)
Scarlet red	Lipids (frozen)	Red
von Kossa	Calcium	Black

Adapted from: Guzmán, Fernández Blanco[6] (2007)

Table 6 - Stains available for testing biopsy tissue specimens. Special stains for muscle fibers and fatty tissue. [continued on next page]

Stains	Muscle fibers	Color
Gomori's, Masson's and Mallory's Trichrome	Smooth fibers	Fibers: red Collagen: green Nuclei: blueish-black
Gomori's, Masson's and Mallory's Trichrome, Mallory's phosphotungstic acid hematoxylin (PTAH)	Striated fibers	Fibers: blue with well-delimited transverse striae. Collagen: red Nuclei: blue or black
Metallic impregnation with gold, silver nitrate, osmium and manganese	Reticular fibers	Fibers: dark black
Weigert, Verhoeff and Hart	Elastic fibers	Fibers: blueish-black or black Collagen: pink or red

Adapted from: Guzmán, Fernández Blanco[6] (2007)

Table 6 (continued) - Stains available for testing biopsy tissue specimens. Special stains for muscle fibers and fatty tissue.

Stains	Muscle fibers	Color
Weigert, Verhoeff and Hart	Elastic fibers	Nuclei: blue or black colors Other tissues: yellow (counterstained with Van Gieson's stain)
Gomori's, Masson's and Mallory's Trichrome	Collagen fibers	Fibers: red, green, blue
Van Gieson	Collagen fibers	Fibers: red
Sudan II Sudan IV Scarlet R Sudan Black B Red oil	Fatty tissue	Fat: red Nuclei: blue
Sudan black	Tissue	Fat: blue Nuclei: black

Adapted from: Guzmán, Fernández Blanco[6] (2007)

Table 7 - Indications for use of immunohistochemistry in oral diseases.

Primary antibody	Expressed cell or tissue	Disease
Cytokeratin	Epithelial cells	Epidermoid carcinoma
S 100 protein	Melanocytes, Schwann and Langerhans cells	Melanoma, schwannoma, Langerhans cell histiocytosis
Vimentin Actin	Fibroblasts, endothelial cells, lymphocytes, melanocytes	Sarcomas, melanomas, lymphomas
Common leucocyte antigen	Leukocytes	Lymphomas, leukemias
Neuron-specific enolase	Neural tissue	Merkel cell tumor
HMB 45	Melanocytes	Melanoma
Factor VIII	Endothelial cells	Vascular tumors
Desmine	Skeletal, smooth muscle	Leiomyosarcomas
Actin	Striated muscle	Rabdomyosarcomas

Adapted from: Guzmán, Fernández Blanco[6] (2007)

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10

Basic statistical analysis for dental research

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First, it is important to acknowledge that statistics is not an easy topic for most people. This statement is especially true for dentists who focus most of their efforts on learning biology-related subjects instead of mathematics. As a consequence, most researchers do not like statistics, most professionals do not use it in their appraisal of the medical literature and most students are not willing to learn it. This is an unfortunate truth with known causes and consequences. Among the causes, the classic mathematical approach to teaching statistics has special bearing on this issue. Too often, great emphasis is placed on formulas and calculations performed by hand in statistics courses for health professionals. Among the consequences of having researchers, professionals and students who are not proficient in statistics are their inability to undertake a critical reading of the literature and their difficulties in designing research (from sample size calculation to proper explanation of the results).

Today, statistics is part of our daily life, and biostatistics is an essential part of Dentistry and dental research. The "romantic era" of dental research is mostly over. The time when someone would have an interesting idea, would get a couple of observations (there is a legend that says that if something is difficult to research the sample size should be around 10; if it is easy, then the sample size shoud be around 1000; any other study should

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have around 100 observations), and *voilà,* a discovery would be made is long gone. Thus, there is a very real need to find ways for people involved in research to teach and learn this subject. In this chapter I will try to focus on the concepts and uses of biostatistics. Mathematics will be kept to a minimum and will be discussed only when it is essential to understand key concepts. We will try to keep the wording casual and the explanations as intuitive as possible. Outputs generated using SPSS will be presented to exemplify the analysis, but this does not mean any particular software endorsement.

Let's start with the basics. What is statistics? In a broad sense, statistics can be defined as "the science and art of collecting, summarizing, and analyzing data that is subject to random variation."[1] Some people would argue that statistics is the art of "torturing" the data, stating that "if you 'torture' your data long enough, it will tell you whatever you want to hear."[2]

Statistical analysis is too broad a subject to be discussed in one chapter. Thus, we will use an approach of presenting one simple analysis from beginning to end, while discussing concepts, strategies and interpretation of the results. Afterwards, we will discuss some other issues that are important in dental data analysis. There are different approaches that can be used successfully to perform statistical analyses. We will focus here on four basic steps that should be adjusted according to the nature of the study:

a. Understanding the research project
b. Data checking
c. Performing the data analysis
d. Communicating results

a) Understanding the research project

The first step to performing an appropriate data analysis is having an in-depth understanding of the research project. In the best case scenario the person performing the statistical analysis should know the subject and have an "insider's" view of the research project as a whole. An alternative would be to have someone with knowledge in the research area guiding the statistician during all the steps of the analysis. This may seem obvious, but is often overlooked. Most researchers with limited knowledge of statistics feel compelled to give the statistician only the dataset and a brief explanation of the study, and then hope for the best. However, wishful thinking rarely yields good results.

We will use an example based on a widely used animal model for periodontal destruction. In this periodontitis model, silk ligatures are placed around the second upper molars of rats in order to produce biofilm accumulation. At

first, our example will have two experimental groups: the control group and the ligature group. Later on we will introduce a third experimental group. The outcome variable of our study will be the amount of alveolar bone loss that occurs after a few weeks of biofilm accumulation.

Few questions should be answered in order to gain a better understanding of a given research project:

a. What is the nature of the study? Observational or experimental?
b. How many times was the data collected? Only once or several times?
c. Are the observations independent? The study units (subjects, animals, etc) contributed to the study with more than one observation?

Let's address these three questions and try to understand their importance. First, we should focus on the differences between observational and experimental studies. Experimental studies are often concerned with comparisons. Classically, a new treatment (medication, surgical technique, etc.) is compared to a reference treatment (placebo or standard /reference treatment). On the other hand, observational studies are often concerned with parameter estimation, for instance, estimation of the frequency of an outcome (prevalence or incidence), risk assessment for a given disease or condition, etc. The first part of this chapter will deal with comparisons rather than parameter estimation.

The two other questions (b and c) are related to the independence of the observations. We will use a non-dental example to introduce this concept. Let's say that, for some reason, you have dry eyes and you want to compare two different types of eye drops. Two strategies could be used to test which eye drops are better to keep your eyes lubricated:

- You can use a different type of eye drop in each eye and see what happens.
- You can invite a friend to help and each person will use a different type of eye drop.

It is intuitive to think that if you find a difference when comparing your left and right eyes (within subject comparison), this must be attributed to the eye drops. However, if you find a difference when comparing your eyes with your friend's eyes, there is a chance that differences between subjects may explain the results. The reason seems obvious. Your left eye shares the same basic biology (genetics, anatomy, physiology, host response, etc.) with your right eye. In statistical terms, we say that your eyes are correlated, associated and dependent. In contrast, your eyes are completely independent from your friend's eyes. You (and your eyes) have no influence on the way your friend's eyes will respond to the eye drops.

Several dental data are correlated in nature (Figure 1). For instance, dif-

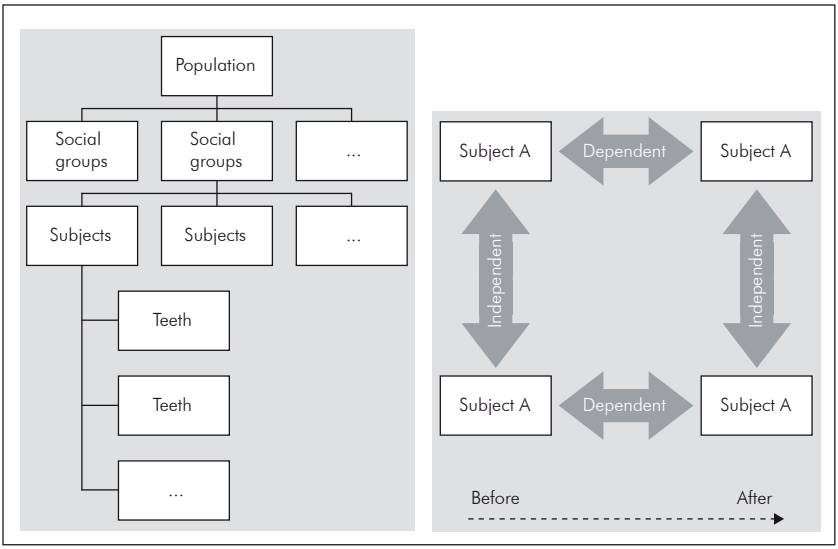

Figure 1 - Examples of correlated data in dental research.

ferent teeth within a subject's mouth will respond somewhat similarly to exposures and treatments because they share several factors, such as genetics, diet, saliva and oral hygiene. The same reasoning can be applied not only to biological factors, but also to social determinants. The same correlation can be observed in longitudinal studies, where the same subject is evaluated several times. Even though some biological variability exists over time, there is a clear relationship among several measurements performed on one person. Other study designs also having dependent observations are split-mouth and cross-over clinical studies, case-control studies, family and twin studies and multilevel studies.

Let's go back to our questions.

a. What is the nature of the study? It it experimental because the ligature is an intervention.

b. How many times was the data collected? It was collected only once: when the histological sections were evaluated.

c. Are the observations independent? They are independent because each animal contributed to the study with only one observation.

The importance of these questions resides on the suitability of each statistical method. Some methods should be used when only two groups are compared, whereas other statistical tests can be used with 3 or more groups. Some

methods can deal with dependent observations, whereas others are suitable for studies with independent observations only. We will come back to this point later.

b) Data checking

Most of the inexperienced (but sometimes also very experienced) analysts want to get fast results from the dataset. This is often a mistake. The first step to performing a good statistical analysis is that of gaining a good understanding of the data behavior. There are several ways of initiating an analysis, but a very useful one is to use distributional graphs such as scatter plots, histograms, box plots, etc.

Look at Figure 2 and think about what you see.

What probably caught your attention was the highest value observed in the ligature group. When you look at the whole dataset, this observation is clearly very different. There are few possible explanations for this finding: a typo, a measurement error or biological variability. In this case we have changed the data on purpose to show how important a scatter plot is in this stage of data checking. The correct presentation of the graph is shown in Figure 3, and there are a few conclusions you can draw from this graph:

a. Animals that received ligatures have higher alveolar bone loss than control animals.
b. There is no overlap for alveolar bone loss measurements between experimental groups (i.e., the highest and lowest values of the experimental groups do not overlap).
c. Data for the ligature group is more spread than for the control group (i.e. data variability seems to be different between groups).
d. There is one observation in the ligature group that seems to behave differently (alveolar bone loss = 726 μm).

The next possible step to be taken in analyzing this data is to check what the data distribution looks like. Most of the time, biological measurements follow the normal distribution (a.k.a. Gaussian distribution). There are several ways of checking data distribution, such as statistical tests and graphs. We will focus on graphs because they are more intuitive.

The graph most widely used to examine data distribution is the histogram with a normal curve. Alveolar bone loss measurements for the control and ligature groups are presented in Figures 4 and 5. Examining these graphs, it can be observed that the data distribution resembles that of a bell shaped curve, which is characteristic of the normal distribution. The highest alveolar bone loss value

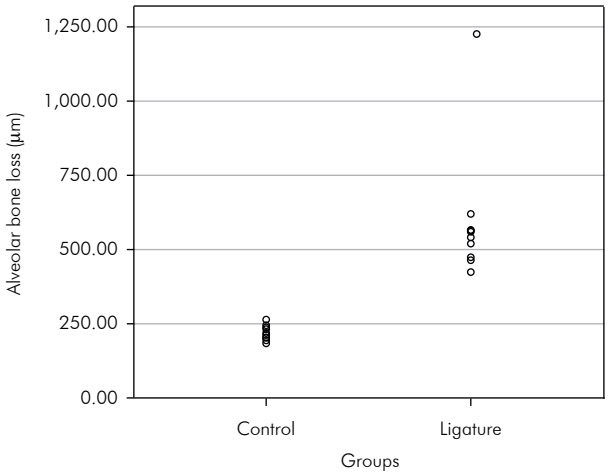

Figure 2 - Scatter plot of alveolar bone loss measurements by experimental group.

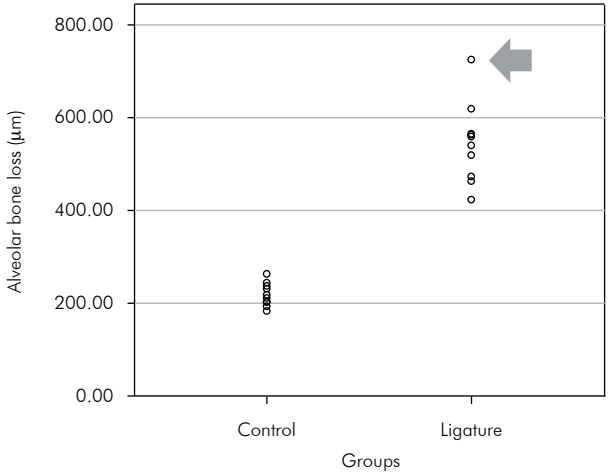

Figure 3 - Scatter plot of alveolar bone loss measurements by experimental group.

in the ligature group draws our attention because it is too far to the right in the distribution. When an observation is much smaller or larger than the rest we may call it an outlier and look for possible reasons (typo, measurement error or biological variability).

Two other graphical methods for checking normality are the P-P (probability-probability) and the Q-Q (quantile-quantile) plots (Figures 6 and 7). In both plots, the observations should cluster around the 45-degrees reference line if the data has a normal distribution. Moreover, a similar number of observations

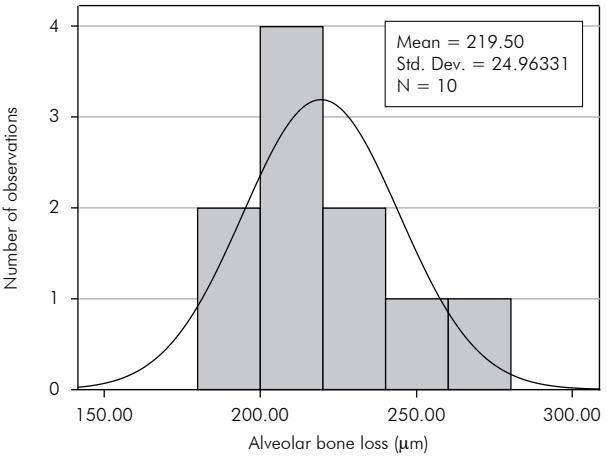

Figure 4 - Distribution of alveolar bone loss measurements in the control group.

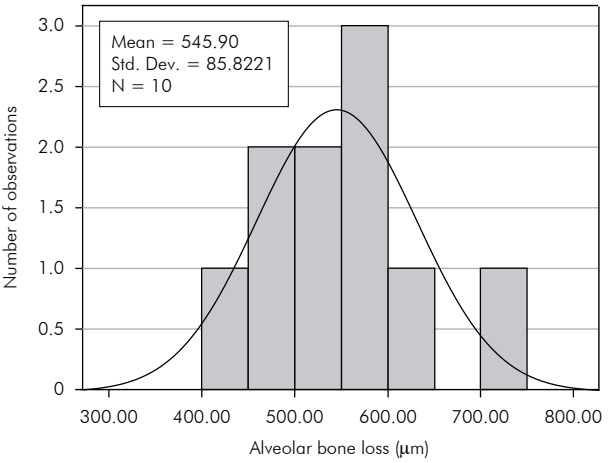

Figure 5 - Distribution of alveolar bone loss measurements in the ligature group.

should be seen above and below the reference line. The P-P plot is more sensitive to deviations in the central part of the distribution, whereas the Q-Q plot is appropriate for evaluating the tails of the distribution.

Examining Figure 6 we can see that the observations in the ligature group are distributed somewhat close to the reference line. Moreover, a similar number of observations can be seen above and below the 45-degree line. In the Q-Q plot, we see that the observation with the highest value (alveolar bone loss = 726 µm) is far away from the rest of the data. This may be another indication that we have a true outlier.

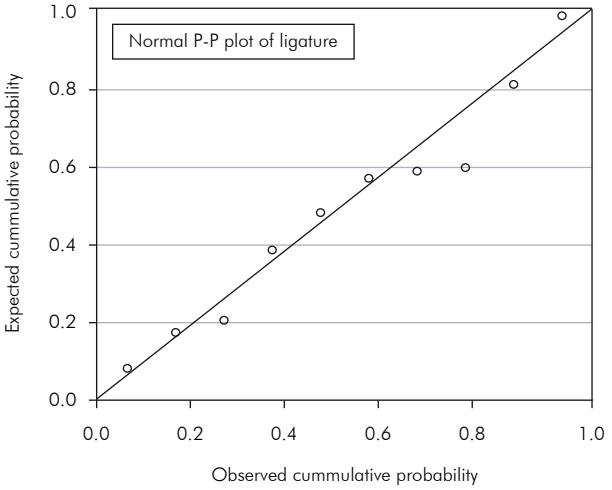

Figure 6 - P-P plot of alveolar bone loss measurements in the ligature group.

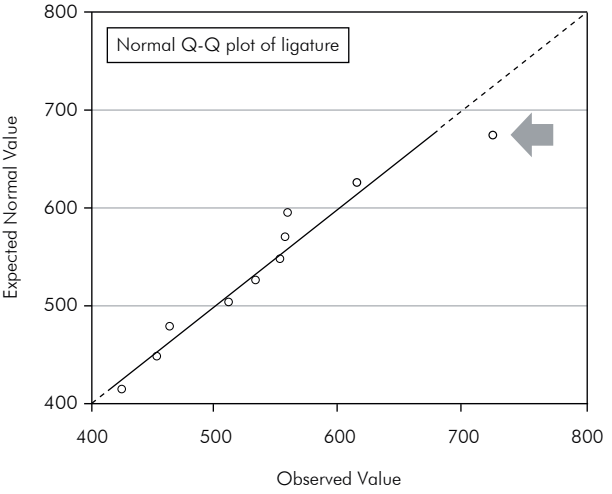

Figure 7 - Q-Q plot of alveolar bone loss measurements in the ligature group.

For the time being we will assume that the highest value observed in the Ligature group is not an outlier.

Message: make sure that your dataset is correct and then assess data distribution through visual inspection of graphs.

c) Performing the data analysis

Following our steps for performing statistical analysis, it is time to compare our experimental groups. We saw in Figure 3 that the amount of alveolar bone loss seems different between groups. However, we do not know if this difference is real or if it just occurred by chance. To test whether a numerical difference is due to a given intervention/condition/exposure or due to random variation we must perform a statistical test. This statistical test will estimate the probability (p-value) of finding this difference just by chance, and that is all a statistical test can do: provide an estimate of finding a difference just by pure luck or chance.

Message: The only information that the p-value provides is whether an observed difference occurred just by chance or not.

In order to select the correct statistical test to compare our experimental groups, we must gather the following information:

a. How many comparison groups are there? 2 comparisons versus 3 comparisons or more?
b. Is the data collected independently?
c. What is the nature of the data? Continuous or categorical?

In our example we have:

a. Two experimental groups (control and ligature)
b. The data was collected independently for each rat (as discussed before, data was collected only once and each animal contributed to the study with one observation)
c. Alveolar bone loss is a continuous variable (parametric)

If we look at Table 1 there is only one choice: independent *t*-test. Table 1 can be used in a similar fashion for all other combinations of study designs and data types.

The above mentioned strategy to select the most appropriate method for comparing groups requires one extra step. We have to consider if the data distribution is adequate for the method we have chosen. A major difference among statistical methods is whether they assume that the data will or will not behave in a certain way. Parametric statistics assumes that the data will fit a parameterized distribution such as the normal distribution discussed earlier. In contrast, nonparametric statistics does not have any assumptions about data distribution (a.k.a free distribution methods). This difference is crucial to the validity of the

Table 1 - Most frequently used comparison methods according to number of experimental groups and data type.

		Parametric	Non-parametric
Two groups	Independent (unpaired, unmatched)	Independent *t*-test	Mann-Whitney U test (a.k.a. Mann-Whitney-Wilcoxon test)
	Dependent (paired, matched)	Dependent *t*-test	Wilcoxon test (a.k.a. Wilcoxon matched pair signed rank-sum test)
3 or more groups	Independent (unpaired, unmatched)	N-way ANOVA	Kruskal-Wallis
	Dependent (paired, matched)	Repeated measures ANOVA	Friedman

results because a parametric method can yield inaccurate results when applied to data with a distribution that violates its assumptions. The good news is that if a continuous variable does not follow a normal distribution, then a nonparametric test will be more appropriate than a parametric test.

During the data checking stage, we did not find any major departure from normality in our example. Therefore, the independent *t*-test continues to be our choice of preference. If we had found any major departure from normality, we would have to have used the Mann-Whitney U test to compare the experimental groups.

At this point, you may ask: if nonparametric tests do not have assumptions about the data distribution, shouldn't they always be used? Compared to parametric tests, nonparametric tests often have less power to reach a statistically significant difference, i.e., their p-values are frequently higher. Thus, we should use nonparametric tests only when the assumptions underlying the parametric test have not been satisfied.

Before using the independent *t*-test to compare our experimental groups, there is an additional assumption that should be tested. The formulas used to calculate the independent *t*-test vary depending on whether the variances of the groups are equal or unequal. Levene's test is frequently used to compare the equality of variability in different samples. When Levene's test is significant ($p < 0.05$), an independent *t*-test that does not assume equal variance should be used. The use of Levene's test for the present data yields a p-value of 0.038 (Table 2, arrow). Thus we have to assume that the two experimental groups do not have equal variances. This means we have to look at the second line of Table 2 for the p-value (rounded rectangle). In our case, it would not make a big difference which formula was used because both calculations yielded very

Table 2 - SPSS output for the independent *t*-test used to compare the control and ligature groups.

				Alveolar bone loss (μm)	
				Equal variances assumed	Equal variances not assumed
Independent samples test	Levene's test for equality of variances	F		5.027	
		Significance		.038	
	t-test for equality of means	t		-11.548	-11.548
		df		18	10.512
		Significance (2-tailed)		.000*	.000
		Mean difference		-326.40000	-326.40000
		Std. Error Difference		28.26411	28.26411
		95% Confidence Interval of the Difference	Lower	-385.781	-388.963
			Upper	-267.019	-263.837

*p-values smaller than 0.0001 appear as 0.000 in the output.

small p-values. However, if the p-value is borderline (i.e. close to 0.05), different p-values (slightly below or above 0.05) may be obtained if the wrong formula is used.

Using an independent *t*-test to compare the control and ligature groups, we find a much lower p-value ($p < 0.0001$). This means that the probability that the difference between the experimental groups being due to chance is less than 1 in 1,000. If we set the significance level at 5% ($p < 0.05$) we can conclude that rats with ligatures have statistically significant higher alveolar bone loss than rats without ligatures. If Levene's test significance was lower than 0.05, we must look at the second line of Table 2. In our example the p-value is the same for both calculations of the independent *t*-test.

As you may have guessed, I'm playing tricks with the numbers in order to perform an analysis that favors the goals of the present chapter.

d) Communicating results

You are probably wondering why we have not calculated means and standard deviations yet. This was obviously intentional. Strictly speaking, this does not have to be done until we are ready to communicate our results. It is obvious that the use of descriptive statistics can help improve our understanding of the data, but if not used carefully, it can also divert the analyst's attention.

Table 3 - Mean alveolar bone loss (standard deviation) according to experimental group.

	Control	Ligature
Mean ± SD	219.50 ± 24.96	545.90 ± 85.82

Most of the time, it is impossible to provide the complete dataset to the reader for him to draw his own conclusions about the results of a study (even if the sample size is small). And at times, it is not possible to make sense of the data, even if it is in front of our eyes (especially large datasets of clinical and epidemiological studies). How can we understand or communicate the general trend of the data? One possibility is to use the graphical approach outlined previously. Another approach is to use numbers that can provide information about the distribution of the data. Two commonly used forms of statistics are the mean and the standard deviation. They provide information about the central tendency and variability of the data, respectively.

In our example, the mean alveolar bone loss is 219.50 μm for the control group and 545.90 μm for the ligature group (Table 3). The mean should give an idea of the point around which most of the data is centered. If we look at Figure 3, we will see that this assumption is true for the control group, but not true for the ligature group. In order to communicate how the data is spread around the mean, we can use the standard deviation. As expected, the standard deviation is much smaller in the controls. The same information can also be observed in the form of a graph (Figure 8).

It is always important to remember that the mean and the standard deviation are not actual measurements. These are statistics used merely to express data distribution concisely. Instead of providing the whole dataset, you give the reader a mathematical formula that can represent the results of a study (with various degrees of accuracy).

In conclusion, rats that received ligatures showed higher alveolar bone loss than controls, and this difference was unlikely due to chance ($p < 0.05$). In other words, ligature-induced periodontal disease significantly increases alveolar bone loss.

What if we had used a nonparametric test?

In our example above, we used an independent *t*-test because we looked at the data and decided that it did not violate any assumptions. What if we concluded after checking the data that the distribution did not follow a normal pattern, some observations were outliers, etc.? In this case we could use

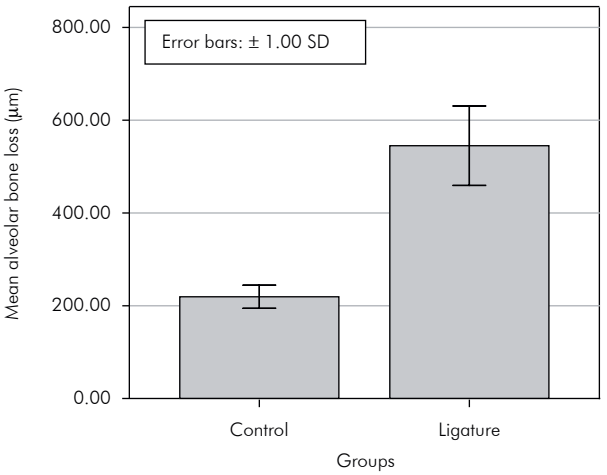

Figure 8 - Mean and standard deviation for alveolar bone loss according to experimental group.

Table 4 - SPSS output for the Mann-Whitney U test used to compare the control and the ligature groups.

Test Statistics[b]	
	Bone loss
Mann-Whitney U	.000*
Wilcoxon W	55.000
Z	-3.781
Asymptotic Significance (2-tailed)	.000
Exact Significance [2 × (1-tailed Significance)]	.000[a]

*p-values smaller than 0.0001 appear as 0.000 in the output. [a]Not corrected for ties. [b]Grouping variable: Groups.

a nonparametric test that makes no assumptions about data distribution. The appropriate alternative to the independent *t*-test is the Mann-Whitney U test (Table 1). Using this test to compare the control and ligature groups yields a very low p-value (Table 4). Here the SPSS output for the Mann-Whitney U test is not so clear. We should focus on the asymptotic significance which, in this case, is < 0.0001.

Instead of using means and standard deviations, medians and percentiles may be used. The median simply divides the data in two sets of observations with an equal number of observations. Half (50%) of the observations are above the median and the other half (50%) are below it. The 25 and 75 percentiles are often used and they divide the data in quarters. Table 5 shows the estimates for the control and ligature groups. Similar information could also

Table 5 - Median alveolar bone loss (25-75 percentiles) according to experimental group.

	Control	Ligature
Median (25% - 75%)	215.50 (200.75-239.75)	550.50 (471.50-579.50)

be shown in a box-plot (Figure 9). Box-plots have 5 percentiles: 2.5, 25, 50 (median), 75 and 97.5.

As expected, the use of a nonparametric test for our example did not change the results dramatically. However, if the parametric test assumptions are seriously violated, nonparametric tests can yield very different results.

A little bit further

Let's extend our example and include a third experimental group. The third experimental group received a medication that supposedly can prevent alveolar bone loss in animals with ligatures. Figure 10 shows the distribution of the data. The medicated group clearly has more alveolar bone loss than the control group, but the distribution of this group seems similar to that of the ligature group. No extreme values can be seen and the spread of the data seems reasonable.

The next step is to look at the distribution of the data. When we want to compare 3 or more groups, the distribution of the data should be assessed using residuals rather than the actual observations. In this context, a residual is the difference between the experimental group mean and each observation. Remember that we discussed previously that the mean is a mathematical representation of our data, and that there is always a certain degree of error involved in using it. This difference is called residual error. We will calculate the residual error for every observation in the dataset. For instance, the residual for the highest observation in the ligature group is 180.1, i.e., 726 (highest observation) – 545.90 (group mean).

Figure 11 shows the residuals of the three experimental groups. Observations close to zero represent small residuals, meaning that the observations were very close to the group mean. We can then use residuals to test the distribution of the data. Figure 12 shows the histogram of the residuals and the normal curve. It seems that no serious violation of the normal distribution exists. P-P and Q-Q plots were also used to assess the data distribution and no serious departure from normality was observed.

If we look at Table 1, ANOVA should be used to compare the 3 experimen-

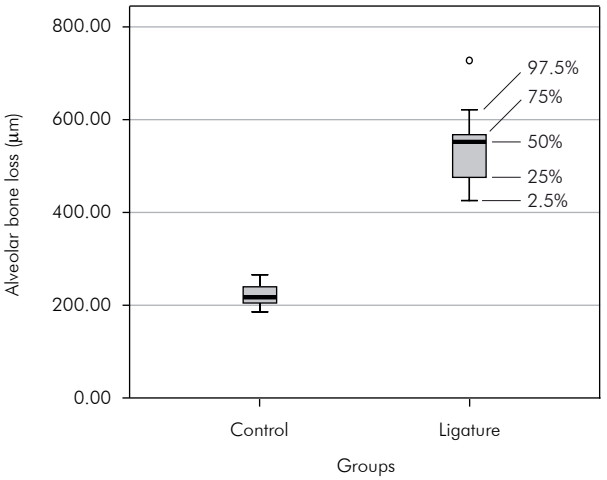

Figure 9 - Box-plot of alveolar bone loss according to experimental group.

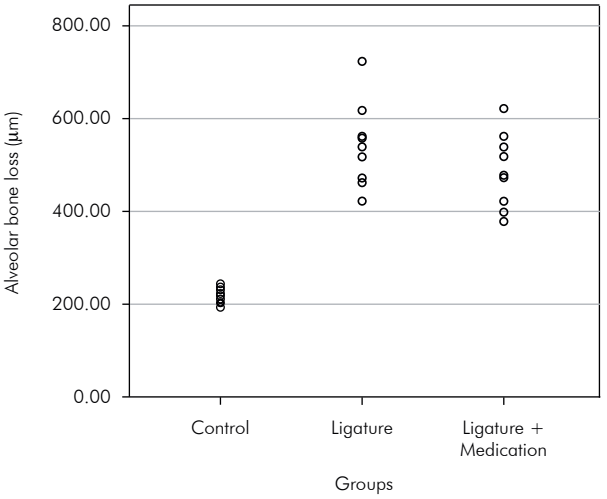

Figure 10 - Scatter plot of alveolar bone loss measurements by experimental groups.

tal groups, with independent observations and a continuous outcome. ANOVA provides an overall statistical test of the data without performing pair-wise comparisons. In Table 6 we can see that the ANOVA p-value for our example is very low ($p < 0.0001$), which means that at least two groups are statistically different.

A very tempting way of comparing our 3 groups is to perform 3 independent *t*-tests (control vs. ligature, control vs. medication, ligature vs. medica-

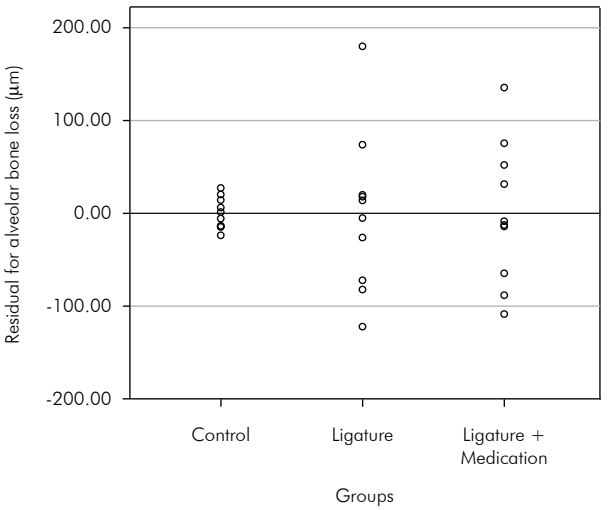

Figure 11 - Scatter plot of residuals by experimental groups.

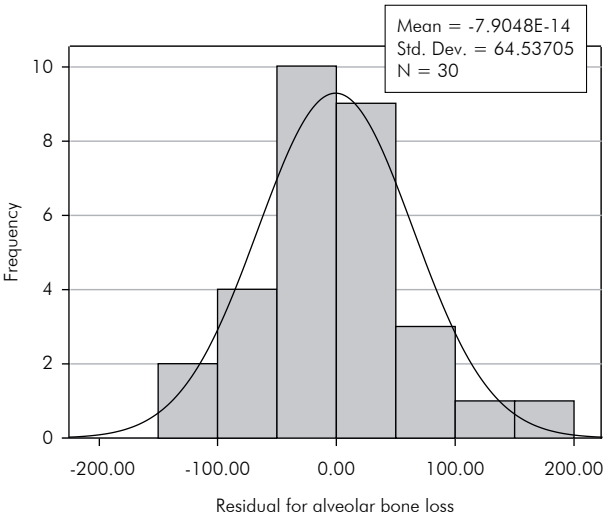

Figure 12 - Distribution of the residuals of all experimental groups.

tion). The problem with this multiple testing is that the final p-value is no longer 5%. The overall p-value for this comparison is 16%. This would be equivalent to performing an ANOVA and accepting that a p-value of 0.16 is statistically significant.

We know that at least two groups are different, but we do not know which groups they are. Before experimental groups are compared two-by-two we

Table 6 - SPSS output for the ANOVA test.

ANOVA					
Alveolar bone loss (μm)					
	Sum of squares	df	Mean square	F	Sig.
Between groups	607054.0	2	303526.977	66.194	.000*
Within groups	123805.9	27	4585.403		
Total	730859.8	29			

*p-values smaller than 0.0001 appear as 0.000 in the output.

Table 7 - SPSS output for the Levene's test of homogeneity.

Levene's test of equality of error variances[a]			
Dependent variable: alveolar bone loss (μm)			
F	df1	df2	Sig.
3.050	2	27	.064

Tests the null hypothesis that the error variance of the dependent variable is equal across groups. [a]Design: Intercept + Groups.

must test the homogeneity of variances. As before, Levene's test of homogeneity can be used for this purpose. In Table 7, we can see that the p-value for this analysis is not statistically significant ($p > 0.05$).

Several *post hoc* multiple comparison tests have been developed. Each method has advantages and limitations depending on the study design and type of analysis. In dentistry, Bonferroni, Tukey and Scheffé are often used for *post hoc* comparisons. These tests can be used to compare groups when the homogeneity test was not significant. Bonferroni is a fairly simple procedure to adjust for multiple comparisons. The adjusted p-value is calculated by dividing 0.05 by the number of comparisons. For instance, if 3 comparisons will be performed then the adjusted p-value according to this technique would be 0.017. Any comparison with a p-value below 0.017 would be significant. Similarly, the adjusted p-value can be calculated for each comparison by multiplying each p-value by the number of comparisons. For instance, a p-value of 0.015 would yield an adjusted p-value of 0.045 if 3 comparisons were performed.

As a general rule, Kleinbaum *et al.*[3] (2007) suggest that Scheffé can be used when experimental groups have different sample sizes and comparisons have not been planned in advance (for instance, exploratory analysis, subgroup analysis, etc). Tukey could be used when the experimental groups have the same sample size and the comparisons have been planned *a priori* (*i.e.* beforehand).

There are several specific multiple comparison tests for data with non-homogeneous variances, such as Dunnett's tests. However, Bonferroni and Scheffé are fairly robust tests and can often be used when the variances of the experimental groups are not homogenous.

We will present the 3 methods for the sake of being complete, but Tukey's test would be our choice. Table 8 shows all possible pair-wise comparisons using the 3 methods. The way that results are shown in this Table can be very confusing. Let's try to understand it. In the first line we have a comparison between group 1 (control) and group (2) using Tukey's method, and the p-value is presented in the column with an arrow on it. Thus, the p-value for the statistical comparison between the control and ligature groups is 0.0001, which is exactly the same p-value we saw before in our independent *t*-test (Table 2). The same p-value is observed when group 1 (control) is compared to group 3 (medication). However, when groups 2 and 3 are compared in the fourth line of Table 8, the p-value is 0.158. This confirms what we observed in our preliminary analysis; i.e., the ligature and medication groups have greater alveolar bone loss than the control group, but no significant difference exists between them.

One difference between multiple comparison tests can be seen when we look at the p-value for the ligature and medication comparison. The p-value is 0.158 for Tukey's test, 0.183 for Scheffé's test and 0.204 for Bonferroni's test. We can say that Bonferroni is more restrictive (conservative) because its p-value is higher than that of the other two methods. In other words, it is more difficult to find a significant difference using this method.

Two considerations should be made before we go forward. First, most *post hoc* tests are conservative, i.e., it is difficult to reach significance. It is therefore possible to obtain borderline statistical significance in an ANOVA test and no significance when comparing groups with *post hoc* tests. No clear solution exists when this occurs. Second, some statistical packages have the Least Significant Difference test as a *post hoc* pair-wise comparison procedure. However, this procedure is sometimes implemented with no correction for multiple comparisons yielding unadjusted p-values. This should be avoided for the reasons stated above.

Message: Test overall significance and then use a *post hoc* test to perform pair-wise comparisons.

Table 8 - SPSS output for the multiple comparison tests used to compare the experimental groups.

Multiple comparisons							
Dependent variable: alveolar bone loss (μm)							
						95% Confidence Interval	
	(I) Groups	(J) Groups	Mean difference (I-J)	Std. Error	Significance	Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	–326.40000*	30.28334	.000**	–401.4850	–251.3150
		3.00	–268.81900*	30.28334	.000	–343.9040	–193.7340
	2.00	1.00	326.40000*	30.28334	.000	251.3150	401.4850
		3.00	57.58100	30.28334	.158	–17.5040	132.6660
	3.00	1.00	268.81900*	30.28334	.000	193.7340	343.9040
		2.00	–57.58100	30.28334	.158	–132.6660	17.5040
Scheffe	1.00	2.00	–326.40000*	30.28334	.000	–404.8348	–247.9652
		3.00	–268.81900*	30.28334	.000	–347.2538	–190.3842
	2.00	1.00	326.40000*	30.28334	.000	247.9652	404.8348
		3.00	57.58100	30.28334	.183	–20.8538	136.0158
	3.00	1.00	268.81900*	30.28334	.000	190.3842	347.2538
		2.00	–57.58100	30.28334	.183	–136.0158	20.8538
Bonferroni	1.00	2.00	–326.40000*	30.28334	.000	–403.6970	–249.1030
		3.00	–268.81900*	30.28334	.000	–346.1160	–191.5220
	2.00	1.00	326.40000*	30.28334	.000	249.1030	403.6970
		3.00	57.58100	30.28334	.204	–19.7160	134.8780
	3.00	1.00	268.81900*	30.28334	.000	191.5220	346.1160
		2.00	–57.58100	30.28334	.204	–134.8780	19.7160

*The mean difference is significant at the .05 level. ** p-values smaller than 0.0001 appear as 0.000 in the output.

What does this all mean? Making sense of the results

A frequent mistake in data analysis occurs when researchers and readers focus on statistical significance (p-values) rather than on the interpretation of results. This has been called the "Tyranny of the p-value." In this regard, it is important to remember the meaning of p-values: the probability that a given finding was achieved by chance. Thus, after we have found a significant difference, we have to judge the results in relation to their importance and relevance. Is this statistically significant difference relevant from a biological, clinical or epidemiological stand point? In other words, are the expected benefits of the new treatment greater than those of the reference treatment?

Figure 13 illustrates a decision tree for the adoption of a new treatment. If no significant difference is found between the new treatment and the standard treatment, we should ask if the sample size was large enough to reveal a difference, if such a difference were to exist. Most clinical studies provide some information about sample size calculation and power analysis. If the study did not have a large enough sample size to detect a significant difference, then the results of the study will be inconclusive. And, the only solution is to increase the sample size of the study or to pool the results of several studies, as done in a meta-analysis. On the other hand, if the sample size was correct and no significant differences were observed, then there is no reason to adopt the new treatment.

If a significant difference was observed, then we have to judge whether the observed benefit is clinically relevant (Figure 13). It is obviously difficult to define what we believe is a relevant result. Experimental, clinical, epidemiological and scientific relevance are very subjective concepts and most of the time no

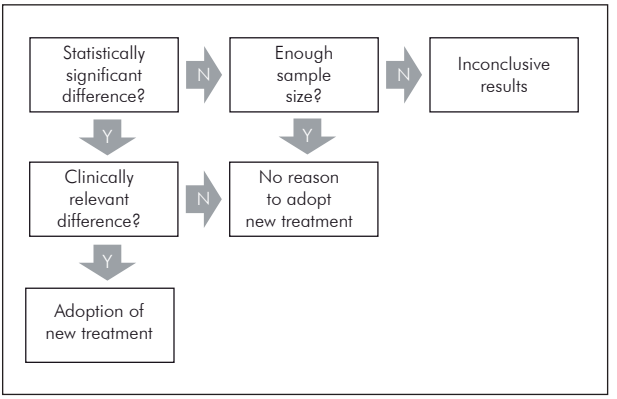

Figure 13 - Decision tree for the adoption of a new treatment.

consensus can be reached. How much periodontal regeneration, caries prevention, pain reduction, etc., is relevant? For instance, Guided Tissue Regeneration yields a mean clinical attachment gain of 1.22 mm when compared to Open Flap Debridement.[4] This may seem like a small improvement, but we should keep in mind that we are dealing with averages. Additional clinical attachment gains of 1-2 mm are clinically meaningful, but issues such as cost, safety and ease of use should also be addressed before a final judgment can be made. If we assume that the differences between a new and a standard treatment are not only statistically significant, but also clinically relevant, then we should adopt the new treatment. On the other hand, if no clinically relevant improvement can be observed, there is no reason to adopt the new treatment even though it may be "statistically better" than the standard treatment.

In our example, it is even more difficult to define the relevance of the results. What is the meaning of an additional alveolar bone loss of 300 micrometers in rats? In pre-clinical studies it is safer to avoid extrapolations of the results to humans. Whenever possible, comparisons between different procedures or exposures using the same animal model could be used to get a sense of the relevance of the results.

An intuitive way of comparing the results of different groups is to make relative comparisons. For instance, the ligature group had 2.5 times more alveolar bone loss than the controls (545.90 μm vs. 219.50 μm), and this is certainly a relevant experimental difference. On the other hand, the medicated group still had 2.2 times more alveolar bone loss than controls (488.32 μm vs. 219.50 μm). Moreover, no significant differences were found between the ligature and the medication groups. Overall, we could conclude that the ligature causes significantly more alveolar bone loss, and this significant difference is biologically meaningful. The medication did not have any positive effect on bone loss.

Message: Test for statistical significance and then assess the experimental relevance of the results.

Regression analysis

Regression analysis is a statistical technique used to assess the relationship between variables. It is often used to assess the effect of explanatory variables on an outcome variable. For instance, which factors are associated with caries, periodontal disease, fluorosis, etc., in a given population.

There are several regression methods that are more appropriately used depending on the characteristics of the data. The most widely used regression methods are linear regression for continuous outcomes and logistic regression for categorical outcomes (yes/no, health/disease, life/death) (Table 9). The most important feature of a regression model is its ability to adjust each estimate for the other variables in the model. When two or more factors are entered in a regression model, the analysis is called a multiple or a multivariable analysis.

We will use a logistic model taken from one of our studies[5] to exemplify how to interpret a multivariable analysis. The aim of the study was to assess risk indicators of tooth loss in a representative young urban population from South Brazil (Table 10). We will focus on three factors, but other variables

Table 9 - Most frequently used regression methods according to outcome type and independence of observations.

	Continuous outcome	Categorical outcome
Independent observations	Linear regression	Dichotomous, multinomial and ordered logistic regression
Dependent observations	Linear regression with standard errors adjusted for clustering of observations	Conditional logistic regression and extensions

Table 10 - Univariable and multivariable logistic regression analysis of the association of demographic, socioeconomic, and behavioral data, with the occurrence of ≥ 4 missing teeth in subjects age 14-29.

		Univariable logistic regression			Multivariable logistic regression		
Risk indicators	Group	OR	95% CI		OR	95% CI	
Age	14 – 19	1.0			1.0		
	20 – 24	3.6	0.8	16.1	3.5	0.8	16.0
	25 – 29	7.2**	3.3	15.9	6.0**	2.6	13.9
Socioeconomic level	High	1.0			1.0		
	Middle	2.1	0.9	4.7	2.3	1.0	5.4
	Low	4.6**	2.2	9.7	4.2**	1.9	9.4
Smoking	Never-smoker	1.0			1.0		
	Light	1.0	0.3	3.3	0.8	0.2	3.2
	Moderate	1.9	0.7	5.0	1.3	0.4	4.0
	Heavy	3.7**	2.0	6.9	2.2*	1.2	3.8

*p < 0.05; **p < 0.01.

could have been used. Smoking is a well known risk factor for several systemic and oral diseases, and it has been consistently associated with tooth loss and periodontal disease. If we look at the odds ratios (OR) presented in Table 10 we can see that heavy smokers were 3.7 times more likely to have tooth loss than never-smokers. This univariable estimate does not take into consideration the effect that other variables have on the relationship between tooth loss and smoking. After we include age and socioeconomic status in the multivariable model, the OR decreases and heavy smokers are 2.2 times more likely to have missing teeth than never-smokers. We may say that smoking is associated with tooth loss in young subjects after adjusting for age and socioeconomic status. The odds ratio for heavy smokers decreases from 3.7 to 2.2 because age and socioeconomic status partly explain the effect of smoking on tooth loss. Older subjects have a higher lifetime exposure to smoking than younger subjects, and subjects of low socioeconomic status smoke more than better-off individuals. In a multivariable model, each factor is adjusted for all other factors in the model. Therefore, in our case, age estimates were adjusted for socioeconomic status and smoking, and socioeconomic status estimates were adjusted for age and smoking as well.

The linear regression model could have been used in this example (Table 11). For instance the linear coefficient (also called beta coefficient) of the univariable analysis for smoking would be 1.4. This means that light smokers have 1.4 more tooth loss, on average, than never-smokers. Similarly, heavy smokers have 1.4 more tooth loss, on average, than moderate smokers. After adjusting for age and socioeconomic status, the coefficient decreases from 1.4 to 1.1 teeth lost per category of lifetime exposure to smoking.

More recently, flexible linear models have been developed to account for various types of data distribution, outcome types and clustering of observations. Among these methods, the Generalized Estimating Equations and Mixed Linear Models have been used steadily in dental research. We refer the reader to other sources for a full discussion of these methods.

Table 11 - Univariable and multivariable linear regression analysis of the association of smoking with the occurrence of tooth loss in subjects age 14-29 years.

	Univariable linear regression		Multivariable linear regression	
	β	SE	β	SE
Smoking	1.4*	0.21	1.1**	0.11

*Crude estimates. **Adjusted for age and socioeconomic status. SE: standard error.

Multilevel analysis

This is an emerging field in statistics and has become somewhat popular in the last few years. Several dental research data are multilevel (hierarchical, clustered, correlated) in nature: sites, teeth, subject, subpopulation, population, etc. Traditional statistical methods treat the units of analysis as independent observations. Thus, if data is collected at the tooth level and a standard *t*-test is used to compare two treatments, each subject will contribute to the analysis with 32 observations. If we use a sample of 10 subjects for each experimental group, instead of comparing 20 observations, we will compare 640 observations. The problem of conducting an analysis of this type, without taking into consideration the clustering of observations within subjects is that the variability of the data (standard deviation and standard error estimates) will be underestimated. This will lead to an inaccurate estimation of the p-values increasing the chances of reaching statistical significance.

There are different ways to deal with multilevel data, and we will discuss 2 of them:

a. **Data aggregation:** This is the most widely used method in dentistry and consists of using measures of central tendency (means and medians) to aggregate the lower-level data (teeth) to the higher-level unit (subjects). The classic example would be to average at the subject-level data collected at the tooth-level and use these averages to perform the statistical analysis using well-known tests such as the *t*-test and ANOVA. There is nothing wrong with this approach, but a lot of information is lost when multiple observations are reduced to only one.
b. **Multilevel modeling:** There are two ways of dealing with the dependence between observations. One is to treat the lack of independence caused by nesting within a higher level as a nuisance, something that should be accounted for, but that the researcher has no interest in studying. The second approach treats dependence as something that is of analytical interest. For instance, what is the effect of poverty on the health of subjects that are not poor but live in poor areas (context effect)? The first approach focuses on making inferences about the units at a lower level (e.g., sites, teeth, etc.), whereas the second approach allows inferences to be made also at a higher level (e.g., subjects, animals).

Reliability

Examiner reliability is a very important issue in modern research. Different measures of reliability can be used to assess agreement depending on the type

of data.

Categorical data: The simplest way of assessing reliability of categorical data is to calculate the percentage agreement among examiners. Let's assume we have two examiners that are assessing inflammation in histological sections using a 4-score scale (Table 12). The main diagonal (dark grey) represents perfect agreement between examiners; in this case, it is 71.4%. If we allow for a one-category margin of error between examiners (light grey), then the agreement is 90.7%.

This approach, however, does not take into consideration the agreement that may occur just by chance. Let's say that the examiners just guessed the inflammation scores instead of actually measuring the histological sections. They will certainly agree on some inflammation scores even though they did not measure it. The Kappa coefficient (also known as Cohen's kappa coefficient) is the statistical method most widely used to discount the agreement that could have occurred just by chance. In short, the Kappa coefficient is a measure of perfect agreement discounted for possible chance agreement. The Kappa coefficient for this data is 0.59, which can be interpreted as a moderate agreement between examiners (Table 13).

Table 12 - Distribution of scores of examiners A and B.

		Examiner A			
	Scores	No	Mild	Moderate	Severe
Examiner B	No	17	3	2	1
	Mild	3	21	2	5
	Moderate	2	1	13	11
	Severe	3	0	7	49

Table 13 - Interpretation of the kappa coefficient.

K	Interpretation
< 0	No agreement
0.0 - 0.20	Very low agreement
0.21 - 0.40	Low agreement
0.41 - 0.60	Moderate agreement
0.61 - 0.80	Full agreement
0.81 - 1.00	Almost perfect agreement

Landis, Koch[6] (1977).

The Kappa statistics can also be called the unweighted kappa because it only considers perfect agreement. However, in several situations, some degree of error is acceptable. For instance, if we allow for a one-category margin of error between examiners (light grey), then the weighted kappa is 0.67. In these cases the weighted kappa can be used in a fashion similar to the unweighted kappa. Weighted kappa can use different weights to account for minor disagreements.

The classic kappa statistics introduced by Cohen[7] (1960) was designed to assess reliability between two examiners. While Cohen kappa statistics can be used when three or more examiners are used, a generalization of this method was introduced by Fleiss[8] (1971), and provides an overall estimate of agreement.

Continuous data: The easiest way to evaluate the examiner's reliability in working with continuous data is to calculate the mean difference between the measurements made and the standard deviation of the difference. Correlation coefficients such as Pearson and Spearman are also often used to measure reliability between examiners. However, neither coefficient takes into account the magnitude of the differences between raters, which means that two examiners can be highly correlated and very different. For instance, in Figure 14, the correlation coefficient is close to 1, indicating a high correlation between observations. However, the observations are clearly different. The explanation, in this example, is that both variables increased following the same pattern, and this is exactly what a correlation coefficient is meant to evaluate.

A more appropriate way of assessing reliability is to use the intra-class correlation coefficient (ICC).[9,10] In contrast to Pearson and Spearman, the ICC takes into account the differences between raters. For instance, the ICCs for the previous examples are very low (0.09 and 0.02, respectively), indicating that, while there is a high correlation between measures (high Spearman coefficient), there is low agreement (low ICC). Be careful, because there are several types of ICCs depending on a series of assumptions about the data. Popular statistical packages often make a distinction between ICCs for consistency and absolute agreement, with the latter being preferred to assess agreement for most of the studies. Recently, the concordance correlation coefficient (CCC) was proposed to assess agreement between two variables.[11,12] This statistic has some advantages over the ICC, but the estimates are often very similar.

In Figure 15, we have a hypothetical comparison of two examiners. Most observations are close to the 45 degree line, indicating a good agreement between examiners. Few observations are far from the reference line, and those

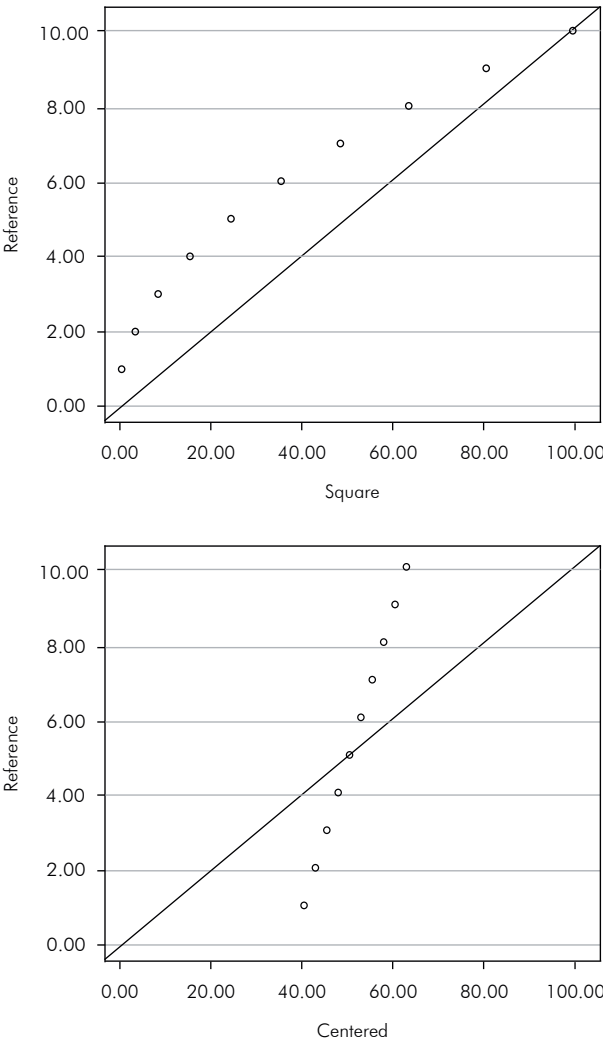

Figure 14 - Scatter plot of observations of two hypothetical examiners in relation to the line of perfect agreement.

that are far are likely to be measurement errors. Another interesting item of information that we can gather from this scatter plot is the distribution of the data below and above the reference line. It seems that examiner 2 is consistently scoring higher than examiner 2.

The average difference for this example is – 0.47 ± 1.18 indicating a small measurement error. The negative sign in the difference means that examiner 1 has lower values than examiner 2. To assess if this difference is significant we

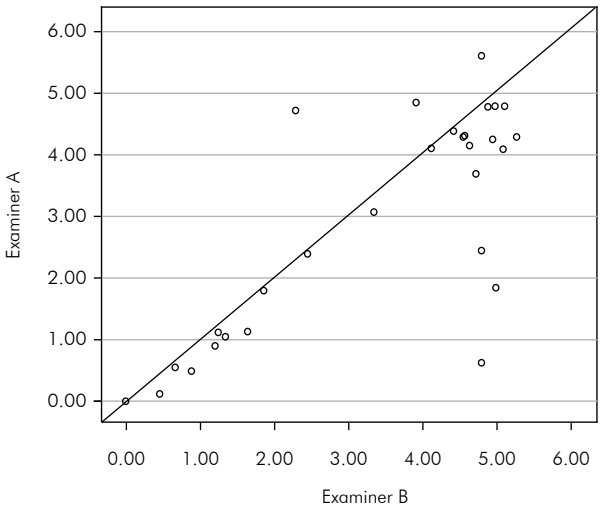

Figure 15 - Scatter plot of the observations of two examiners in relation to the line of perfect agreement.

can use a *t*-test. The p-value for this test is 0.04, indicating that this difference is not due to chance. The ICC for this dataset is 0.76 and the CCC is 0.75. The highest value for both coefficients is 1, and it indicates perfect agreement.

Today, there is a tendency to report reliability in a very concise form because journals have been limiting the number of words that an article can have. Even though it is acceptable to report few measures of reliability in an article, researchers should be aware that reliability assessment is a continuous process that should be undertaken throughout the study. In this regard, the best way to understand the reliability of the examiners is by frequency tables and graphs. As shown before, a great deal of information can be learned.

Message: Assess reliability through visual inspection of tables and graphs and report it using measures of variability and coefficients of agreement.

Last, but not least

a. Keep it simple

With the availability of new statistical methods, it is often very tempting to use an elaborate statistical analysis. However, sometimes it is better to have a simpler statistical analysis that everybody in the field understands than to have a very elaborate analysis that nobody can make sense of. In this regard the old KISS ("Keep it Simple, Stupid") principle is very important and should be kept

in mind.

b. Be careful

The use of statistical methods has increased steadily in the last few years, and part of this phenomenon is due to easier access to greater computer power and the availability of statistical packages. This is surely good news, but it can be very dangerous as well. As Hofacker[13] (1983) said, "the good news is that statistical analysis is becoming easier and cheaper" and "the bad news is that statistical analysis is becoming easier and cheaper." There is an essential difference between using a regular computer program such as Word, Powerpoint or Excel, and using a statistical program such as SPSS, STATA and SAS. Statistical analysis is based on a set of assumptions that will jeopardize the results if not fulfilled.

c. Make sense of the results

There is a certain degree of awe today toward the new advances in biostatistics. This sometimes confuses researchers and readers, removing the focus from the most basic aim of a study, which is to respond a scientific question as clearly as possible. Whenever possible, translate the results in practical terms.

Final message: Keep it simple, be careful and make sense of the results.

Last but not least, the reader should be aware that we had to simplify some concepts; consequently, there is the chance that a specific study or dataset may not follow the general rules outlined in this chapter. We hope that this chapter may encourage more people to use biostatistics in their daily professional life. With the right methodology, biostatistics can be fun and very rewarding.

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11

A step-by-step guide on how to conduct a systematic review

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A systematic review in the healthcare field is a summary of the healthcare research conducted on a given subject that uses explicit methods to perform a thorough search of existing literature and critical appraisal of individual studies to identify the valid and applicable evidence. It often, but not always, uses appropriate techniques (meta-analysis) to combine those studies considered valid, or at least uses a grading system of the levels of evidence depending on the methodology used. While many systematic reviews are based on an explicit quantitative research of the available data, there are also qualitative research reviews that, nonetheless, comply with the standards for gathering, analyzing and reporting evidence. Recent developments include realist reviews and the meta-narrative approach.[1] Unfortunately, empirical studies have shown that narrative review articles tend to be of poor quality.[2] However, clinicians have always used review articles as a source of evidence, and these studies can be useful tools if conducted properly.

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While systematic reviews are regarded as the strongest form of medical evidence, a review of 300 studies found that not all systematic reviews were equally reliable, and that their reporting could be improved by adopting a universally agreed upon set of standards and

guidelines.[3] A further study by the same group found that in a cohort of 100 quantitative systematic reviews, 4% required updating within a year of the end of the reported search period, and 11%, after 2 years. Seven percent of the systematic reviews needed updating at the time of publication. Shorter survival rates have been associated with cardiovascular topics, and heterogeneity in the original reviews.[4]

The main objective of a systematic review is to summarize the evidence on a specific clinical question.[5,6] Secondary objectives include critical evaluation of the quality of the primary studies, checking for and identifying sources of heterogeneity in results across the studies and, if necessary and possible, determining sources of heterogeneity.[5,6] Systematic reviews are also helpful in identifying new research questions.[7]

Steps in conducting a systematic review

The framework for carrying out systematic reviews will be described here in three stages: planning, reviewing and disseminating. The need for a review should be established before commissioning or commencing review work. The methodology of the review should be documented and working arrangements should be put in place to ensure that the methods can be followed. Finally, there should be a strategy for putting together a report of the review to disseminate its findings to relevant audiences and, if possible, also a strategy for updating the review.

The stages of a review and the phases within them will be described below consecutively. However, this chronology may vary during the review. It will not always be possible to complete one phase before another must be started, and sometimes it will be more efficient to work on several phases simultaneously. It is essential that good communication be maintained between those commissioning or supervising the review and those carrying it out. All the steps necessary to undertake a systematic review have been listed, but it is not possible to provide definitive advice on all of the methods. This is because the science of systematically reviewing the literature is relatively young, and many methodological issues are still being explored. The present guide is therefore meant to assist those conducting reviews in adopting a minimum standard based on the basic understanding of the subject at the time of writing.

Several steps have to be followed in order to write a systematic review. Planning the review is the first of three stages in producing a high quality systematic review, and starts with establishing the need to undertake a review. Having established a clear need for a new review, commissioning bodies may issue a

call for proposals specifying the questions to be addressed by the review. Reviewers preparing a proposal should undertake a preliminary assessment of the extent of the studies that are available, and the degree to which it can be used to answer the review questions. Convincing arguments must be included in the proposals, to the effect that the objectives of the review have been understood, that the methods to address the objectives are appropriate and feasible, and that the review team is capable of undertaking the work. In regard to securing research funds, the scientific and administrative aspects of the review should be documented in a protocol that should be discussed before commencing the review itself. Working arrangements should be put in place and adequately resourced to ensure that the methods laid down in the protocol can be followed. A diagram should be made to guide the progress of the review work (Figure 1).

When planning the review, 3 phases must be described:

1. Identifying the need for the review.
2. Preparing the proposal for the review.
3. Developing the review protocol.

Phase 1 - Identifying the need for the review

This section provides information on how to identify and appraise available reviews. This is an essential step to avoid unnecessary duplication of research and to ensure that every new review addresses the appropriate healthcare issues.[8]

Systematic reviews provide information about the effectiveness of interventions by identifying, appraising, and summarizing the results of unmanageable quantities of research.[9,10] They differ from traditional reviews and commentaries produced by content experts in that they use a replicable, scientific and transparent approach that seeks to minimize bias. Hence, rather than reflecting the views of experts, they generate balanced inferences based on a collation and analysis of the available evidence. Systematic reviews are needed to supply information for the policy- and decision-making processes applied to the organization and to delivery of health and social care. They are particularly useful when there is uncertainty regarding the potential benefits or disservices of an intervention, and when there are variations in practice. By locating and synthesizing evidence from primary studies, systematic reviews provide empirical answers to focused questions. In addition, by identifying both what we know and what we do not know, systematic reviews may also help in planning new primary research. Whenever a systematic review is being considered, efforts should be made to ensure that a good quality review in the field of interest does

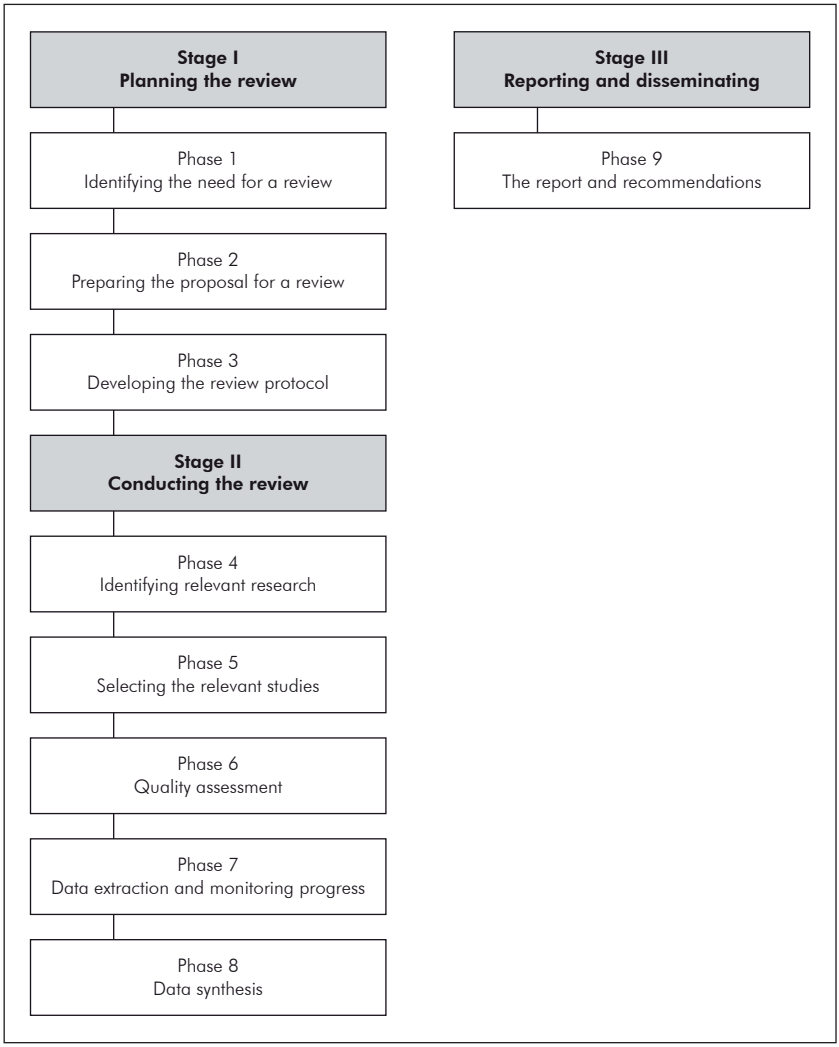

Figure 1 - Steps in conducting a systematic review. Source: Khan *et al.*[6] (2001)

not already exist. If the available reviews are outdated or of poor quality, it may then become necessary to update existing reviews or conduct a new review.[8]

The process of identifying published and ongoing reviews can involve several steps and can be most effectively undertaken jointly with experts in information retrieval, such as librarians. To ensure wide coverage, a good range of information sources should be consulted.[11]

The best single source of systematic reviews is the Cochrane Library. It contains the Cochrane Database of Systematic Reviews (CDSR),[12] the Database of Abstracts of Reviews of Effectiveness (DARE)[13] and the Health Technology Assessment (HTA) Database.[14]

Existing reviews should be assessed for quality. Until recently, reviews were generally not carried out in a rigorous manner,[15,16] and even today many reviews published in peer reviewed journals have not been conducted systematically. Regardless of source, any identified reviews should be critically appraised for quality using a checklist.[17-19] Structured abstracts included in the DARE Database[13] provide practical examples of the use of checklists to appraise and summarize reviews. The quality of a review can be defined as being confident that any bias in designing and conducting the review, as well as in analyzing its outcomes, has been minimized. Quality assessment is important because the effectiveness of interventions may be masked or exaggerated by reviews that are not conducted rigorously. The checklists for quality assessment focus on identifying flaws in reviews that might bias the results.[19]

In general, a good review should focus on well-defined questions, and the review methodology should be geared toward obtaining a valid answer. The reviewers should make a substantial effort to search for all the literature relevant to the questions posed. The criteria for selecting or rejecting studies should be appropriate so that the studies included are useful in directly addressing the question. In addition, the methodological standard of these studies should be high enough to allow the likelihood of providing a valid answer. The process of assessing study relevance and quality should be unbiased, reproducible and transparent. If these processes are not well documented, one's confidence in the results and in inferences of the review is diminished. The review should clearly display the results of all the studies included and should highlight any similarities or differences between them. It should also explore the reasons for any variations. In light of these results, and considering the populations, the interventions and the outcomes covered by the review, it should be possible to make a judgment about the applicability and value of the review findings. This critical appraisal will help identify high quality reviews. A published, up-to-date systematic review of good quality may have all the information that is needed to guide healthcare decision-making.[8]

If an initial analysis of the available literature indicates a lack of good quality reviews, then funders may feel that a systematic review should be carried out. In this case, a commissioning brief for the subject in question should be prepared. The briefing document should provide general information on the

objectives of the proposed review. It should outline the rationale for undertaking the review and the background information describing the epidemiology of the healthcare problem, as well as the patterns of use of a certain health technology and its alternatives. A quality assessment process for monitoring the progress of the review may also be stipulated.

Phase 2 - Preparing the proposal for a systematic review

This section provides an overview of how to prepare a proposal to obtain funding to conduct a systematic review. The proposal should be prepared based on the work undertaken to identify the need for a review. The briefing document provides general information on the objectives of the proposed review. The commissioners want to make sure that completion of the review will lead to a valid summary of the relevant research.

The research proposal should be based on a preliminary assessment of potentially relevant literature and should provide general information on the background of the proposed review. Reviewers should collect additional information to prepare the background of the proposal. The background should be developed by outlining the available options and arrangements for providing healthcare in the review area. It may also include information on the historical, social, economic and biological perspectives of the review problem.[20] Research questions often must be substantially refined in the proposal. Defining a question for a review is similar to formulating questions for primary research. It is a critical part of the review because other aspects of the proposal derive directly from the question.[21-23] If the proposal is successful, review questions can be clearly defined *a priori* and documented in the review protocol.[20] The Methods section of the proposal should indicate the possible inclusion as well as exclusion criteria for selecting studies. It should also include a broad strategy that can be used to search for published and unpublished research, indicating the extent of the strategy in terms of what resources will be used, how journals will be selected for hand searching and what other study identification techniques will be employed, such as citation searching.[20] The methods for study selection, quality assessment and data extraction, as well as the approaches for data synthesis should be appropriate to the objectives of the review.

The review team should have an appropriate range of expertise that can be applied to conduct the proposed review methodology, including information science, health measurement, medical statistics, health technology assessment, health economics, qualitative research, clinical epidemiology, the clinical subject area and consumer-related issues. This often means that the applicants

have to develop collaborations with other researchers and specialists, who are capable and willing to provide support in areas of expertise, and who are not available in-house. It is important that review team membership reflect a range of expertise rather than opinions.[20]

An important part of the proposal is the review budget. In order to develop a budget, help should be sought from the finance staff and the relevant members of the review team. Staff salaries are usually the most costly component of a review, particularly considering the input required from reviewers, review managers/supervisors and information officers. These staff members may be involved in the review for different lengths of time. A preliminary search carried out to estimate the size of the relevant literature should guide the costing for components related to literature searching, document acquisition and translations. Data abstraction and analysis include constructing coding forms, setting up a database using bibliographic software, preparation of summary tables and computer-technique-driven analysis. Equipment includes computer hardware and software to conduct, retrieve and store searches. In addition, computer programs may be required for data abstraction and statistical analyses.[20]

Phase 3 - Developing the review protocol

This section provides information on how a review protocol should be produced. A protocol is a written document containing background information, specification of the problem and methodology of the review. The background information and problem specification will follow directly from the work undertaken in phases 1 and 2. The details of the methodology will come from reading through the various phases described in Stage II (Figure 1).

The protocol specifies the plan which the review will follow to identify, appraise and collate evidence.[24-26] The first milestone of any review is developing and seeking approval of the protocol before proceeding with the review itself. Sometimes the protocol may be approved as part of the commissioning process. A protocol for carrying out a review is equivalent to, and as important as, a protocol for a primary research study. A systematic review is less likely to be biased if the questions are well formulated, and the methods that will be used to answer them are decided before gathering the necessary data and drawing inferences. In the absence of a protocol, it is possible that study selection and analysis will be unduly driven by the findings.[27] The protocol should state precisely the main question plus the secondary questions that will be addressed. When framing precise questions, the important factors to be considered are the population, interventions, and outcomes relevant to the objectives of the

review.[23,28,29]

Reviews provide summaries of existing data obtained from primary research of different designs on a given population, intervention and outcome. The choice of the primary research design for a particular review may have to be justified in the proposal, particularly because the validity of effect estimates is related to the study design. The preference of one study design over another should not depend on the inherent value of the design itself.[30] Instead, it should depend on the nature of the population, interventions and outcomes framed in the questions, and the core issues being addressed in the review, e.g. effectiveness, efficiency, etc. Therefore, reviewers need to explore the different ways of addressing the specific issues and choose the study designs that provide the most valid answers. A hierarchy of study designs can then be developed and a design threshold can be used as a study selection criterion. The design threshold will also depend on the findings for the literature scoping, which may reveal that only a few methodologically sound studies are available. In addition, assessment of short- and long-term outcomes may be more suited for a study conducted according to different types of designs. If the review is to include a focus on the process of implementation and/or the subjective experience of participants receiving interventions, then qualitative research may be appropriate. Assessment of efficiency will require the inclusion of economic evaluations. Hence, it might be necessary to include studies of various designs. Using the approach described above, each review question should be stated in the protocol according to the disease status of the population, the interventions being considered, the outcomes being measured, and the relevant study designs.[31]

The protocol should include a search strategy for identifying relevant research, specifying the databases and other sources that will be searched, together with the search terms. The construction of a search strategy should be based on the components of the review questions, i.e. populations, interventions, and outcomes, along with the study designs being considered. The results of the scoping search will help determine the search terms to be used. Search strategies to identify primary effectiveness studies and economic evaluations will need to be tailored to reflect the specific needs of both elements of the review (Khan *et al.*, 2001).

A study selection allowing identification of the papers found must be made in order to answer the review questions. Therefore, the selection criteria (both inclusion and exclusion criteria) should logically proceed from the questions, and they should be defined in terms of the population, the interventions, the outcomes, and the study designs of interest.[31,32,33] In order to be selected, a study

should fulfill all of the inclusion criteria and none of the exclusion criteria. It is very helpful to pilot the selection criteria on a subset of primary studies. The study selection procedure usually consists of several stages. Initially, the criteria are applied to the citations generated from searching to make a decision about whether to obtain full copies of potentially relevant references. Once copies have been obtained, the inclusion/exclusion criteria are applied and decisions are made about the inclusion of each study. Details should be given about how decisions will be made concerning the selection of individual reports, such as the number of independent assessors who will make these judgments and how disagreements between assessors will be resolved, for example by a third reviewer (Khan *et al*., 2001).

Once the studies have been selected, the next step will be data extraction and data synthesis. The synthesis strategy should take into account the presumed magnitude of the results, the size and validity of the studies, together with any factors which may explain differences between them. Finally the protocol needs to be approved by the reviewers, and they may then decide to publish the draft protocol on a dedicated website, which may allow a wide range of interested parties to provide feedback before commencing the review (Khan *et al*., 2001).

Once the review protocol has been approved, the next stage will be to conduct the systematic review, and different phases have to be considered. Although the phases within this stage are described consecutively, this sequence is not meant to follow an exact chronology. Often it will not be possible to complete a phase before others have been initiated, and sometimes it will be more efficient to work on several phases simultaneously.

Stage II includes the next 5 phases related to conducting the review.

Phase 4 - Identifying relevant research

The aim of the search is to generate a list of possible primary studies, both published and unpublished, which may be suitable for answering the questions included in the review (Goodman, 1993; Clarke, Oxman, 2000; Counsell[23], 1998). The thoroughness of the literature search is one factor that distinguishes systematic reviews from traditional reviews. It is also important to ensure that the process of identifying studies is as thorough and unbiased as possible.[35] The identification of studies depends on where and when studies are published, and if and how they are written up.

Effective searching is a skill, and it is highly desirable to involve an information expert who can design and execute sensitive search strategies. Review-

ers and librarians should work together to develop the search strategy. Initial searches conducted to identify reviews and to assess the volume of potentially relevant literature will provide input to design the search strategies. Strategies may be based on a series of trial searches, on discussions of the results of those searches performed within the review team, and on consultation with experts in the field to ensure that all possible relevant search terms are included.[36]

The search might include general databases (e.g PubMed, Cancerlitetc). These databases typically contain bibliographical details and abstracts of published material, as well as thesaurus-derived indexing terms that can be used to search for relevant articles. There are many potentially useful databases and database guides that can be consulted.[37-39] Professional Information can also help identify relevant databases. General medical databases such as MEDLINE and EMBASE can be a helpful starting point in developing a search strategy. These databases cover many of the same journals, and the extent of overlap has been estimated at approximately 34%.[40] The degree of overlap in terms of the volume of records could range from 10% to 75%, depending on the topic of review.[40-42] There is no single electronic database that is comprehensive enough, in terms of either subject or publication format coverage, to record all publications from all medical journals.[43,44] The Science Citation Index can also be used to trace citations of important papers through time, which may yield further useful references.

There is always a risk that relevant publications may be overlooked in electronic searches, due to inaccurate or incomplete indexing in the databases and weaknesses in the search strategy. Hand searching is another important way to identify very recent publications that have yet to be cited by other publications or included on electronic databases; therefore, hand searches of *Index Medicus* and *Excerpta Medica* can be undertaken.[45-47] Conference proceedings can provide information on research in progress as well as completed research. These proceedings are recorded in several databases - including the Index to Scientific and Technical Proceedings (available to the UK academic community via ISI Web of Science[48] (2000) and the Conference Papers Index[49] (2000) - in library catalogues (British Library)[50] and in large research libraries. The abstracts in conference proceedings may present limited information and there may be differences between data presented in abstracts and final reports. Attempts should be made to acquire reports of the studies from the authors before such data are included in a systematic review.[51]

After a thorough and systematic search has been conducted, a list of studies that meet the inclusion criteria should be sent to the subject experts advising

those conducting the review. They should be requested to check the list for completeness, and to provide information on any ongoing research that could be considered for inclusion in the review. It is important to contact relevant companies that may be willing to release results that have not already been published. In addition, the Internet may be a useful source of information about completed and ongoing research, particularly that which has not been formally published. However, searching the Internet can be a major undertaking. Many of the general search engines do not allow sophisticated multi-line searching and searches may produce thousands of web sites to assess. Strategies to search the Internet in a systematic manner could include using meta-search engines such as Copernic[52] and Dogpile,[53] gateways to sites with search engines such as NSABP Medical Search Engines[54] or MedNets,[55] general purpose search engines which have a medical focus such as Northern Light,[56] and gateway services to evaluated sites such as OMNI.[57]

The process of conducting systematic reviews should be replicable and transparent. Identifying relevant research should be documented in adequate detail so that readers can evaluate the thoroughness of the search for potentially relevant studies. The search should be documented as it develops, and the reasons for making changes and amendments should be noted at the time. The unfiltered search results should be saved in their entirety and retained for future potential reanalysis.[36]

Phase 5 - Selecting the relevant studies

Having completed a search for potentially relevant studies, copies of these should be retrieved and assessed for their relevance to the question included in the review. The selection process should be explicit and conducted in such a way as to minimize the risk of errors.

It is important that this selection of articles be free from biases, which occur when the decision to include or exclude certain studies is affected by preformed opinions (Goodman, 1993; Clarke, Oxman,2000).[31,32,58,59] It is essential that the decisions about the inclusion or exclusion of studies be made according to predetermined written criteria stated in the protocol. Both inclusion and exclusion criteria should proceed logically from the review question, and should also be defined in terms of the population, the interventions, the outcomes, and the study designs of interest. Only studies that meet all of the inclusion criteria and none of the exclusion criteria should be included in a review. The criteria should be piloted in such a way that they can be interpreted reliably and can classify the studies appropriately. Since the inclusion criteria ultimately deter-

mine which studies will be included in the review, it is inevitable that debate and discussion will arise as to how broad or narrow these criteria should be. The applicability of the review results may be reduced when criteria are narrowly defined. However, as the inclusion criteria for populations, interventions, outcomes and study designs are broadened, the review may contain information which is hard to compare and synthesize.[60,61] If the inclusion criteria are liberal, and if there is a large number of studies, it may be possible to investigate theories concerning the effects of differences in the study characteristics, and other effect modifiers, using mathematical modeling. The inclusion criteria specifying the type of study design stems from the desire to base reviews on the highest quality of evidence.[61] There are several areas of healthcare that have not been evaluated with methodologically sound studies. In this case, studies of methodologically lower quality may have to be included. Here it is important to note that the preference for one study design over another should depend on the nature of the questions raised in the review. Inevitably, the decisions regarding inclusion based on study design will also depend on the availability of suitable study designs in the literature.

Articles are sometimes excluded from reviews if they are written in certain languages, depending on the resources available for translation or interpretation. However, such restrictions can introduce bias and decrease precision in the meta-analysis.[62] It has also been shown that even if inclusion of studies published in all languages does not influence summary effect estimates, these studies are likely to improve effect estimate precision, an important clinical and statistical attribute of meta-analysis.[63] Therefore, whenever feasible, all suitable reports should be included regardless of language, and the influence of non-English language literature on estimation and precision of effect should be explored through a sensitivity analysis.

Study selection is a multi-stage process. Initially, the selection criteria are applied liberally to the citations generated from computer database searching. Those titles and abstracts identified as potentially relevant, resulting from searches or from inspection of bibliographies, should be provisionally included for consideration on the basis of full text articles, unless they can be deemed as definitely excludable. The reproducibility of this process should be tested in the initial stages of the review and, if reproducibility proves poor, more explicit criteria may have to be developed to improve it.

Even when explicit inclusion criteria have been specified, decisions concerning the inclusion of individual studies remain relatively subjective. It may be useful to have a mixture of subject experts and methodological experts assess-

ing inclusion. If resources and time allow, the lists of included and excluded studies may be discussed with the expert panel. In addition, these lists can be posted on a dedicated web site with a request for feedback on any missing studies, an approach used in a CRD review of water fluoridation.[64] The reliability of the decision process is augmented if all papers are independently assessed by more than one reviewer, and the decisions prove reproducible. Assessment of agreement is particularly important during the pilot phase, when evidence of poor agreement should lead to a revision of the selection criteria or to an improvement of their coding. Agreement between assessors may be formally assessed mathematically using Cohen's Kappa (a measure of chance-corrected agreement).[65-68] Many disagreements may be simple oversights, whereas others may be a matter of interpretation. These disagreements should be discussed and, where possible, resolved by consensus after referring to the protocol. If disagreement is due to lack of information, the authors may have to be contacted for clarification. Any disagreements and their resolution should be recorded. The influence of uncertainty about study selection should be investigated through a sensitivity analysis.

Phase 6 - Quality assessment

The next step is quality assessment of the included studies. This should be performed independently by two reviewers. Quality refers to internal and external validity of the studies. This is because interpretation of the findings of a study depends on design, conduct and analyses (internal validity), as well as on populations, interventions and outcome measures (external validity). These characteristics are related to the way in which the review questions are framed.[69] Assessment of study quality focuses mainly on assessing the internal validity of effectiveness studies. Other quality issues will be covered in test accuracy of qualitative research studies and health economic evaluations.

Simple assessment based on the appropriateness of the study design is often used in study selection to guarantee a minimum level of quality. The weakest study design that may be included in the review should be clearly defined in the inclusion/exclusion criteria of the protocol. This quality threshold for primary studies can be determined by generating a hierarchy of study designs and fixing a cut-off level for study selection. The hierarchy of primary study designs depends on the nature of the questions being asked, such as effectiveness, accuracy, efficiency, etc.

When assessing the effectiveness of therapy, the basic question tends to revolve around how one treatment performs in comparison with another, when

different treatments are available for the same condition. To address this issue, the preferred study design would be one that randomly assigns (concealing the assignment code) the participants having the condition of interest to alternative therapeutic interventions. This design will serve to remove selection. As a result, well-designed experimental studies tend to rank at the top of the study-design hierarchy for assessing effectiveness. Next in the hierarchy are quasi-experimental studies, where the allocation of participants is controlled, but falls short of genuine randomization and allocation concealment. However, it is not feasible to assess every therapeutic intervention on every relevant outcome using an experimental study design, particularly when randomization is unethical or impractical.[70,71] This means that when randomized trials are not possible or not available, the next best available type of evidence should be considered, as shown in the information in Table 1. It shows a commonly used hierarchy of study designs for reviews of effectiveness. It is based on the degree to which different study designs are inherently susceptible to various biases.[72-75] Reviewers often focus on randomized studies, but this emphasis may be unwarranted in some circumstances; for example, when literature scoping identifies only a few small randomized studies. In this case, it may be wise to include quasi-experimental and/or observational studies, and use study design as a basis for stratifying the analysis.

The information gained from quality assessment is crucial in determining the strength of inferences and in assigning scores to recommendations generated within a review.

Phase 7 - Data extraction and monitoring progress

Data extraction is the process by which reviewers obtain the information

Table 1 - Hierarchy of study designs for studies of effectiveness.

Study design hierarchy
1. Experimental studies (e.g., Randomized Controlled Trials with concealed allocation)
2. Quasi-experimental studies (e.g., experimental studies without randomization)
3. Controlled observational studies 3a. Cohort studies 3b. Case control studies
4. Observational studies without control groups
5. Expert opinion based on pathophysiology, bench research or consensus.

Source: Khan *et al.*[6] (2001).

they need from what is reported by primary investigators. This can be a subjective process and is prone to error. In order to minimize bias at all stages of this process, the protocol should contain a sample data extraction form that lists the data items to be extracted from each of the primary studies.[77] (Clarke, Oxman, 2000)

Data extraction, along with quality assessment, is done using data extraction forms developed after pilot testing. Reviewers usually extract information on study characteristics, methodology, population, intervention and outcomes.[7] The outcomes reported in systematic reviews vary, depending on the type of studies included. If randomized clinical trials are included, the outcomes are usually expressed as risk ratios (RR), odds ratios (OR) or differences between means for continuous outcomes. It is important that reviewers extract raw data from studies where possible. Data extraction is prone to human error and may also require subjective judgment.[77] Accuracy and consistency are extremely important in data extraction. The instruction and decision rules about coding data can be put directly on the data extraction form near the data field to avoid confusion. When multiple reviewers are participating in a project, they may need training and practice in using the form and may need to develop consensus to avoid any misunderstandings about coding. Depending on the findings of the initial piloting of the data extraction forms, additional pilot tests may be necessary.

Multiple publications on the same data should be avoided, and only the definitive results must be included in the data analysis. It may also be possible to obtain data from unpublished studies; in this case, it is important to acquire information about their quality. Furthermore, written permission should be obtained before including unpublished data in a review.[78]

Published reports usually do not provide all the information that needs to be extracted. In this case, the best option is to contact the author of the study for further information. Depending on the nature of the lacking information and on the requirements of the analysis, authors could be contacted with a specific request for completion of the standard data collection form or a request for individual patient data.[78]

Finally, communication between commissioners and reviewers constitutes an important aspect of a successful project. Therefore, several meetings should be arranged during the review work. A meeting is required before the data synthesis work can commence. Identification and assessment of the relevant studies should be completed before this meeting is held, so that the findings can be discussed. The plans and timetable for the analysis and completion of the

review can then be reviewed and finalized.[78]

Phase 8 - Data synthesis

This phase of the systematic review involves summarizing the results included in the primary studies. This can be achieved either by using a descriptive – or non-quantitative – synthesis or by using a quantitative synthesis (meta-analysis). The objective of data synthesis is to bring together the results from a primary study in a meaningful way. Most reviewers begin their analysis with a simple tabulation of the study characteristics and results. This should also be done in a systematic review, even if a meta-analysis is not performed. The process of performing a non-quantitative synthesis of the data must be explicit and rigorous.[32,79] Decisions about how the data will be grouped and tabulated should be based on the question that the review is addressing.[80]

The key elements in the descriptive approach to data synthesis may include the following characteristics:

a. Population
b. Interventions
c. Settings where the technology was applied
d. Environmental, social and cultural factors that may influence compliance
e. Nature of the outcome measures used, their relative importance and robustness
f. The validity of the evidence
g. The sample sizes, and the results of the studies included in the review.[80]

These factors should be summarized succinctly in the tables. The tables should be structured to highlight the similarities and the differences between the studies included. It should be possible to assess qualitatively, from a critical analysis of these tables, if there are differences between studies in key characteristics of the participants, interventions or outcome measures (clinical heterogeneity), in the study designs and quality (methodological heterogeneity), and in the reported effects (heterogeneity in results). Thus, it should be possible to decide whether the studies are similar enough to make it worthwhile to calculate an average estimate of effectiveness. In some cases, important factors or variables may not have been reported in the studies included. The non-quantitative synthesis should also place in evidence the problems arising from the lack of important information.[80]

Data synthesis involves computing the average effect, a process whereby the results of each study are weighted according to some measure of the importance of the study. Each study weight usually relates to its size and the result-

ing precision of the state of the effect. Statistical methods of meta-analysis are explicit numerical formulations of this process and should be used wherever possible.[80] In the absence of weighting, all studies are assigned the same weight, irrespective of their sample size. An unweighted average would be the simple average. In meta-analysis, typically, large studies (with large sample sizes and more events) are assigned more weight in computing the average.

When there are important differences between the studies in terms of participants, interventions, outcomes and methods that potentially relate to study results, it usually makes no sense to estimate an overall average effect. However, in certain cases, subgroups of similar studies can be identified from the tabulations for which an average effect could be computed, or variables identified, which could be explored as potential explanations of statistical heterogeneity. Thus, the descriptive part of the synthesis can help plan investigations of heterogeneity.

An evaluation of the data summarized in tables can help plan the quantitative synthesis by highlighting the comparisons that could be made, the outcome that can be combined (meta-analysis) and the characteristics of the study that must be considered when investigating variations in effects (heterogeneity). Consequently, it should be determined if a quantitative synthesis is possible or appropriate. Meta-analysis is not possible when the data needed to perform a meta-analysis cannot be obtained, and it may not be appropriate when the data is sparse or when the studies are too heterogeneous to be sensibly combined. Once it is established that a meta-analysis is possible and appropriate, reviewers have to make three choices before beginning. First, which comparisons should be made? Second, which outcome measures should be used in the synthesis? Third, what measure of effect (a measure of association that quantifies the effect of intervention) should be used to describe effectiveness? These issues must be considered and stated in the protocol. The nature of the comparisons and the outcome measures should be directly related to the questions being posed in the review, and the main comparisons must already be specified.[80]

As described at the beginning of this chapter, Stage III involves the reporting and disseminating of the systematic review. The following phase analyzes how to prepare a report as an effective part of the disseminating strategy.

Phase 9 - The report and recommendations

The last step in conducting a systematic review is interpreting the results, discussing them, and writing a report for publication. A succinct report should allow readers to judge the validity and the implications of the review findings.

Preparing the manuscript of a systematic review article for publication in a peer-reviewed journal presents a unique challenge, i.e., condensing a very detailed process in order to comply with the journal's requirements. Additional disseminating strategies will be required to effectively target potential users and interested parties, so that policies and practices may be provided with the evidence contained in the review. Putting research into practice goes beyond disseminating it, because the simple fact of making the information available may not change practices. Targeted implementation strategies will usually be required to achieve this goal (Khan *et al*., 2001).

In general, the structure of a systematic review should include a concise – albeit informative – title, followed by the authors' names. A review is usually undertaken in collaboration. For this reason, the issue of determining credit and authorship should be considered seriously and early in the review process, because the criteria for authorship are often misunderstood, and this may lead to disputes.[82] Criteria for authorship include a) conception and design or analysis and interpretation of data, b) drafting the article or revising it critically for important intellectual content, and c) final approval of the version to be published.[83] All criteria must be met to qualify for authorship. Credit for conception and design of the review may be assigned at the beginning of the review. However, many other contributions, like literature searching and acquisition of studies, extraction, analysis and interpretation of data, scientific supervision, and drafting of the report and its critical revision prior to peer review, will emerge during the review. In general, acquisition of funding or collection of the data or general supervision of the review group alone is not considered sufficient contribution for authorship. A final decision about authorship may be based on scoring the contributions of each reviewer.[84] An abstract is important to attract the reader's attention, and in most journals it should not exceed 250 to 300 words.

The main text of the review should include:

i. Background information
ii. Review questions, which should be described in detail in terms of population, intervention, outcomes and research designs (phase 2)
iii. Review methods. The methods used should be described in sections covering the search process and strategies, inclusion and exclusion criteria, assessments of relevance and validity of primary studies, data extraction, data synthesis, and investigation of differences between studies
iv. Details of the excluded and included studies
v. Results of the review (see Phase 8)
vi. Discussion, which should be built on the results, help in interpreting the

data, and explore the clinical relevance of the findings[85]

vii. Conclusion

viii. Acknowledgments

ix. Conflict of interest disclosure

x. References

Concluding remarks

A design has been presented in this chapter on how to conduct a systematic review. A systematic review is considered that which provide the most reliable evidence in the existing literature on a given subject, since it summarizes the most comprehensive and up-to-date information relevant to that subject. It is aimed at fulfilling the needs of clinicians, since it allows them to critically appraise and use this reliable evidence in their clinical practice. In conclusion, the authors hope that, by reading this chapter, more clinicians will be encouraged to write systematic reviews and contribute critical evidence in their areas of expertise.

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12

Bibliographic research in Dentistry: electronic information sources

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One of the goals of libraries is to gather the human knowledge published or recorded in any kind of medium. The task of organizing information so that this knowledge can be made accessible to those that need it may seem simple at first. Nonetheless, the measures taken by the librarian to make this information available with relevance, importance and quality, are intricate and also determined by the knowledge and correct use of Library Science tools and techniques. In order for the information to reach the end user, the materials received by the library should be

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included in its collection and made available by placing it either on bookcases or on display, or else made accessible through the bibliographic records on file in the many different databases and directories that are available.

The habit of using libraries is not a strong characteristic of the Brazilian society. Moreover, organizations are known not to give priority to libraries. "[...] in a country where the reading habit is not encouraged, it is no surprise that information centers are not considered a priority in planning an organization."[1] In some cases the image of the school library used by the student during his/her childhood brings back memories which are not always pleasant. This is because libraries, in the past, unfortunately were viewed as a place where misbehaving student were grounded, or where students that, for some reason, couldn't find anywhere else to go in school, could go. This deep-rooted image of punishment still has a very negative influence on people, who also continue to view the library as somewhere apart from the real world.

If we consider that there is a "code," "symbolism," or special "communication" involving each aspect of information organization in an information center, the "mystery" involving both the library environment and the ways of retrieving information can be unraveled, as long as these factors are understood by users. Moreover, we can take advantage of knowing how this organization is done to understand how an information center works.

User training techniques, namely lectures, courses and tutorials* have been used increasingly to initiate users into the information world and, as will be seen in this chapter, also into the academic environment.[2-4]

There are so many library service opportunities offered to users, and there are users so unaware of them, that it is worthwhile gaining a better understanding of the electronic information retrieval process to enable a higher level of proficiency.

Who should I ask for information?

Whenever a user arrives at the library he should seek the reference librarian, who will aid him in his Information needs. This professional is trained to use all the available library science tools to assist the user in the process of information search and retrieval.

* Tutorials are defined as mechanisms to train users to use the virtual instructions included in databases and acting as a "Help" menu to search for tools.

Information sources

History teaches us that knowledge is a critical element in the survival of the species. Since man's early ancestors, circa 100,000 years ago, to the post-modern civilization, man uses knowledge to provide the means for his survival. Man's very survival needs have driven his development of knowledge, insofar as unresolved matters and unanswered questions impel man to create solutions. Man's needs have changed with time, and so has the reality that he has fashioned. In today's society man's needs may be different but they still depend on the knowledge he produces and consumes.

Knowledge does not exist without a source, i.e., a starting point that provides the groundwork for its construction. During the entire process of historical development of knowledge, man has depended on information sources that have changed and are still changing up to the present day. The exponential development of both information and communication technologies has been the driving force behind the increasingly faster appearance of new information sources. This requires that the quality of these sources be evaluated constantly.

An information source is defined as any means by which information is retrieved, as well as the support where this information is recorded. Thus, databases, encyclopedias, dictionaries, books, journals and magazines, theses, final term papers, dissertations, reports, multimedia (CDs, DVDs, etc.) are all examples of physical support of information sources.

In this connection, a study was conducted on the importance of using information sources in the medical field,[5] intent on presenting the new trends of medical education in Brazil, made easier mainly through the use of the Internet, where libraries "favor the exchange of information [...], thus promoting education by enabling access to and dissemination of knowledge."

This chapter intends to demonstrate how the available electronic information sources are used to conduct bibliographic research, the starting point of any academic paper.

1. The Virtual Health Library in Dentistry (BVS Dentistry)

A virtual library is an environment that organizes, processes and retrieves information in an electronic/digital support, following a subject-based criterion, removed from any real-world library connection. The difference between a virtual library and a digital library is that the latter is always linked to an institution, and its hypertext links indicate existing archives.[6]

The Virtual Health Library (BVS) was created in 1998, during the CRICS 4 (Regional Congress of Information in Health Sciences), whose main theme

was "Towards Equitable Access to Health Information." This event was held in the City of San José, Costa Rica. The Declaration of San José was drafted as the official document of the event (*http://cricS4.bvsalud.org/declesp.htm*) and it provided for the construction of a virtual health library covering the different areas of health science. The Virtual Health Library in Dentistry joins efforts with the Latin American and Caribbean Center of Information in the Health Sciences Area (BIREME), in its commitment to build the great Virtual Health Library (BVS), involving Brazil and Latin American countries, to ensure equitable and universal access to information.

The BVS in Dentistry, following the parameters set by BIREME, will be a milestone in the development of professionals in the dental field, both those involved in academic activities and those interested in continuing education as a means of personal development. Users in the field of dentistry will have access to the many information resources available both domestically and internationally, using the World Wide Web for this purpose.

The BVS in Dentistry is structured into three major blocs: Information Sources, Subjects and Highlights. Under Information Sources are the specific area-related databases providing information for bibliographic research; the purpose of the "Scientific Journals in Dentistry" is to gather in one place the several journals that provide electronic access to the full text of articles. The SCAD Copying Service is a space where the user can fill out a form to order and receive a scientific article; Health Terminology is a place where the user has access to the controlled vocabulary of the dental area for use in his/her research and/or academic production. This vocabulary is called the DeCS (Descriptors in Health Sciences), a Portuguese version of the MeSH (Medical Subject Headings), of the National Library of Medicine. Furthermore, Information Sources contains event directories, research groups and researcher directories, and also enables retrieving information available on the Web and collected in what we call the LIS (Health Information Locator).

Under Subjects, there are several pre-selected topics whose bibliographic database research is already included in the BVS in Dentistry, i.e., the results of the different bases can be retrieved by a single search.

Under Highlights, the latest area-related news can be seen, from coming events to other information of interest.

Figure 1 displays the BVS in Dentistry homepage.

2. Databases

In recent years, the existing technologies have changed the ways propound-

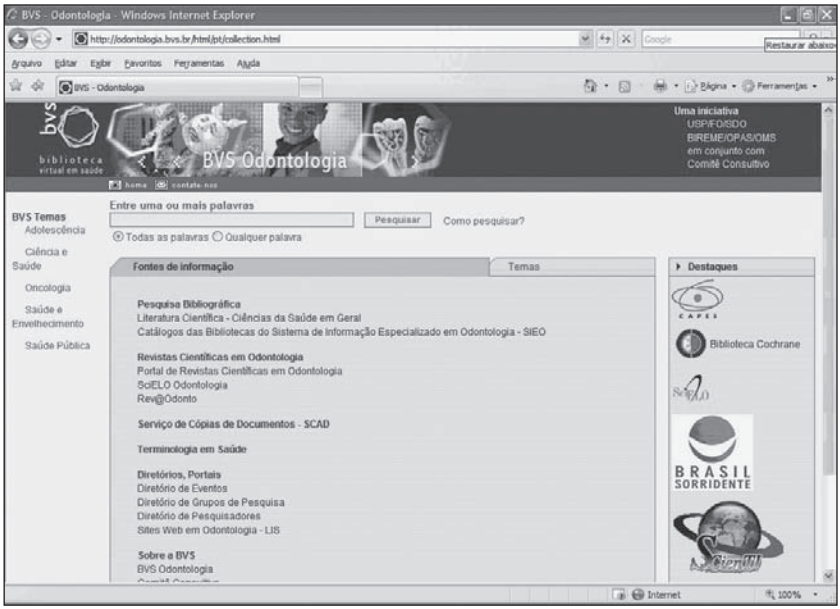

Figure 1 - BVS in Dentistry homepage.

ed for controlling bibliographic information, through which the information contained in libraries was represented by printed library directory records, bibliographic reference lists and printed indexes and abstracts. Today this information is available via remote access to electronic databases and, to a great extent, via immediate access to the full text of a given document. Considering the current ways of obtaining information by using the databases of the different areas of knowledge, the difference existing hitherto between information accessibility and physical accessibility of the document has become irrelevant.[7]

The electronic databases are understood as electronic information sources that are researchable interactively by computer.

The documentary boom, initiated as of the late 19th century, consequential to the exponentially increasing volume of documents, made it necessary to seek alternative systems to analyze and control technical and scientific production to prevent the loss of expressed and recorded knowledge or prevent this knowledge from being partially inaccessible to students, scientists and other information users.

Although the term "database" is related to the electronic format, its remote origin goes back to the bibliographic control exercised by libraries on their re-

spective collections. The directories used by libraries, in the form of file cards or listings, are examples of the first databases used to access information.

Databases are a record of the interactions and information related to the interests of the community. This information can be presented in different formats and filtered according to pre-defined search criteria. Thus, database content can be presented based on a given subject or topic, on the type of indexed documents, on the different types of users, or on the private or public nature of those who produced the information.

Databases bring together a very significant amount of material that provides the information necessary for data retrieval. Nevertheless, they cease to be effective if one is not acquainted with the ways of extracting the maximum performance offered by this information source. As a rule, databases may be consulted using simple forms (for less complex searches) or more detailed forms (for more complex searches). Conducting bibliographic research using only words or common terms may produce biased results. In the health area, the use of proper terms extracted from controlled vocabularies (MeSH – Medical Subject Headings or DeCS – Health Sciences Descriptors) is recommended to ensure greater significance and relevance of retrieval results.

In Dentistry, there are three most used databases. The Brazilian Dentistry Bibliography (BBO), the Latin American and Caribbean Literature on Health Sciences Information (LILACS), and Medline, although other databases such as EMBASE and SCOPUS also offer access to subjects in the field of dentistry.

2.1. BBO *(Brazilian Dentistry Bibliography)*

It is a database under the responsibility of the Dental Documentation Service (SDO), School of Dentistry, University of São Paulo (USP). It brings together the country's literature in the field of dentistry. It was first published in print form in 1970, with information dating back to 1966. For a few years, it was produced automatically using punch cards. As of 1982, it started to use the Microisis software, which enabled greater flexibility and storage capacity. In a joint effort undertaken with the Specialized Information System in Dentistry (SIEO), it assembled national scientific production in the field, as of 1991. The purpose of the BBO is to collect, organize and disseminate national scientific production. For this purpose, it includes the following types of materials: journal articles, specialization papers, dissertations, theses, books, book chapters, non-conventional materials and papers published in events in the form of abstracts. The BBO database is available for access and consultation on the BIREME server at the following electronic address: < *http://bases.bireme.br/cgi-*

bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&base=BBO&lang=p/ >. The journals indexed in BBO are analyzed by a Selection Committee, observing the **Selection Criteria for the BBO Database.** Today, the database has 60 indexed journals.[8]

2.2. LILACS *(Latin American and Caribbean Literature on Health Sciences Information)*

It is a cooperative database of the BIREME system, which includes the literature on Health Sciences published in the region's countries since 1982. It features articles from approximately 670 well-reputed journals from the health area, totaling over 350,000 records, as well as other documents, such as theses, theses chapters, books, book chapters, proceedings of meetings or conferences, technical-scientific reports and government publications.

Since both BBO and LILACS use the same search interface, Figures 2 through 8 demonstrate the search process in both databases.

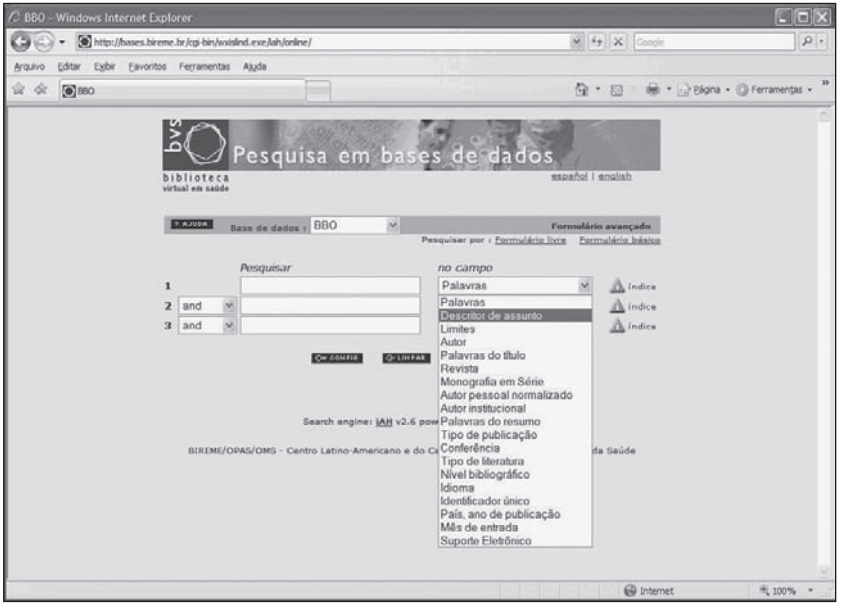

Figure 2 - Demonstration of how to use the Subject Descriptor option to start a search.

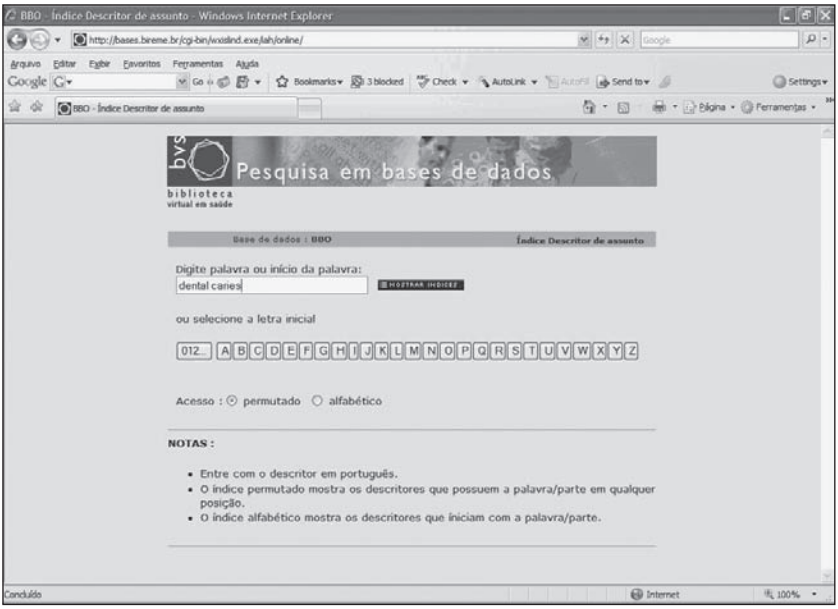

Figure 3 - Choosing the subject to be searched.

Figure 4 - Selecting the subject to be searched.

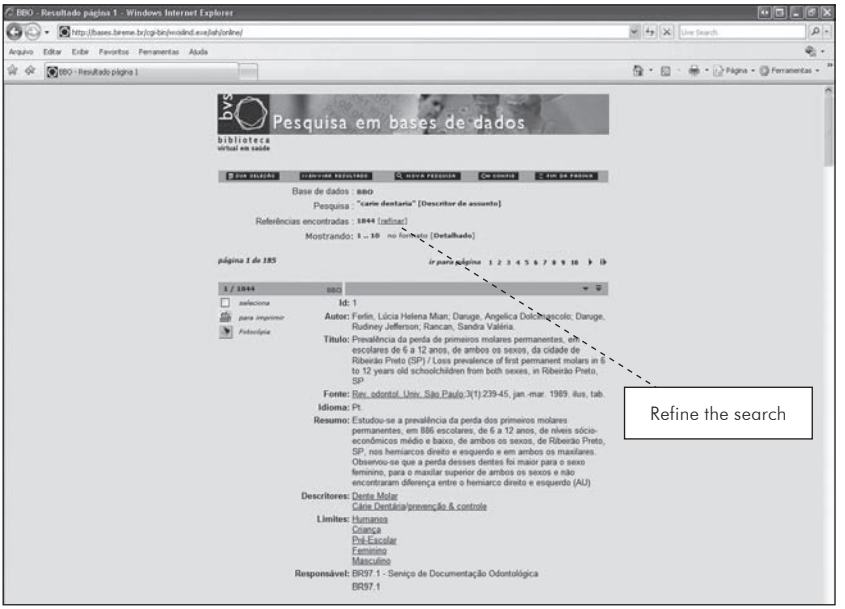

Figure 5 - Number of references located and opting to refine the search.

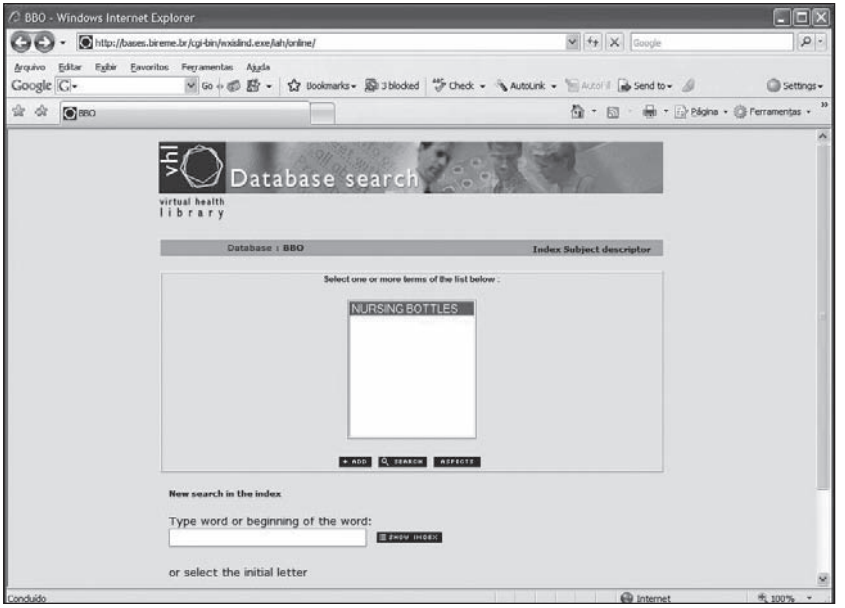

Figure 6 - Cross-checking with new words and starting a new search.

Figure 7 - Outline the search strategy and then start the search.

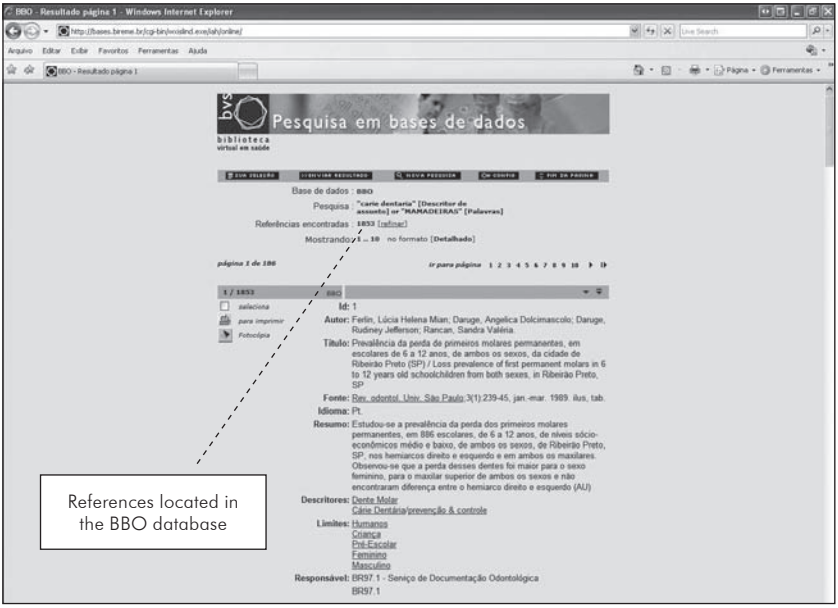

Figure 8 - Number of references located after refining the initial subject.

2.3. Medline

It is a database of the National Library of Medicine encompassing the fields of medicine, nursing, dentistry, veterinary medicine, healthcare systems and pre-clinical sciences. The Medline base covers the period of 1966 onwards and has some materials from earlier periods. It features approximately 4,800 titles of world journals in 30 languages. Access can be made directly through PubMed (*http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?DB=pubmed*).

The database allows locating subjects by Subject Descriptors, by all fields and by refining the search. The results obtained can be forwarded via e-mail, copied onto a CD or printed.

Access to PubMed is unrestricted and free of charge. Figures 9 through 11 demonstrate how to use this database.

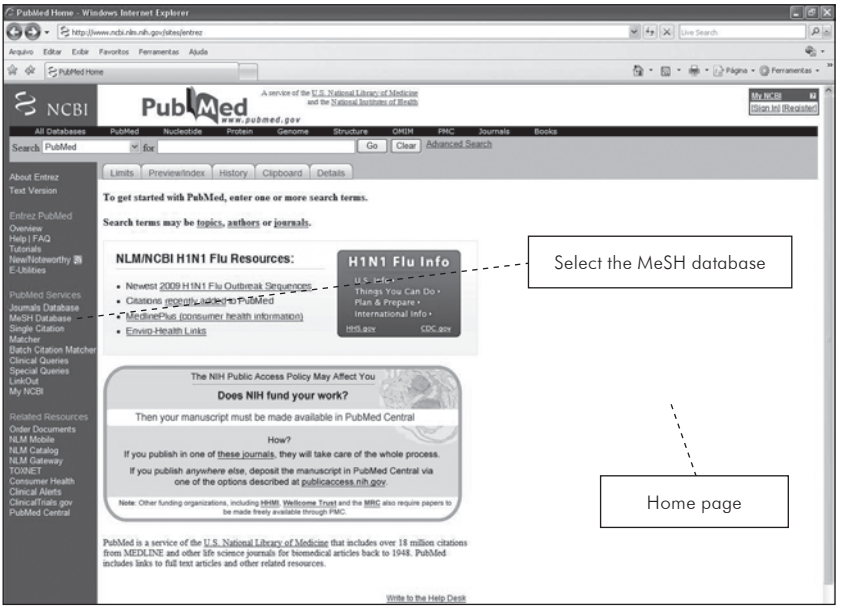

Figure 9 - The PubMed homepage.

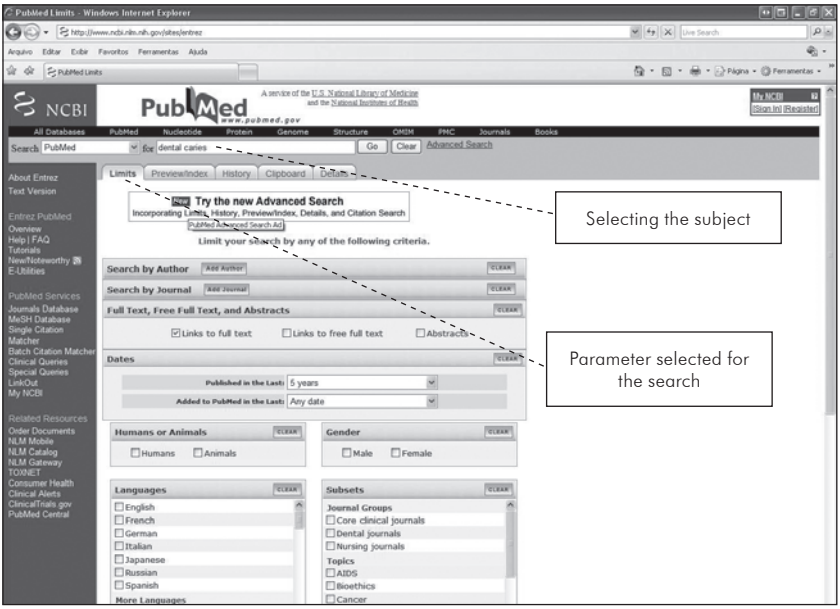

Figure 10 - Selecting the subject and parameters for refining the search.

Figure 11 - Search results after refining the search strategy.

3. Electronic journals and access to full texts

Technology has increasingly enabled changes in the ways information can be accessed. Today, an entire scientific article can be located in a few seconds, as long as its full text is made available on the Web. In the past, it took some time for a user to obtain a copy of a paper; today it can be done in a matter of minutes.

There is a trend among scientific editors to publish a greater number of issues per year of the journals under their management because of the several facilities made available by the electronic medium, thus improving their periodicity evaluations. With these advances, periodical publications can now make their full texts immediately available to the end user, right after they have been accepted, thus eliminating the procedures once required by the print publication process, obviating the sluggishness of the publishing process, and addressing the core issue of searching and retrieving information.

Initiatives such as the SciELO database (Scientific Electronic Library Online) have enabled swift and effective access to the full text of articles.

3.1. SciELO

The Scientific Electronic Library Online (SciELO) is an electronic library containing a collection of selected Brazilian Scientific Periodicals. SciELO is the result of a joint research project by FAPESP (São Paulo State Research Foundation) and BIREME (The Latin American and Caribbean Center of Information in the Health Sciences Area). In 2002, the SciELO project gained the support of the CNPq (National Council for Scientific and Technological Development). Its goal has been to develop a common methodology for preparing, storing, disseminating and evaluating scientific production generated in electronic format.

In the field of dentistry, the database has eight journal titles, four of which are Brazilian (1 - Brazilian Dental Journal, 2 - Brazilian Oral Research – continuation of the "Pesquisa Odontológica Brasileira," whose earlier title was "Revista de Odontologia da USP" –, 3 - Journal of Applied Oral Science and 4 - "Revista Dental Press de Ortodontia e Ortopedia Facial"), one title is from Venezuela ("Acta Odontológica Venezolana"), and three titles are Spanish ("Revista de Cirugia Odontoestomatognatica, Revista Espanola de Cirugia Oral y Maxilofacial e Medicina Oral, Patología Oral y Cirugía Bucal").

Figures 12 through 18 demonstrate the main steps involved in accessing the full text of the electronic journals published in SciELO.

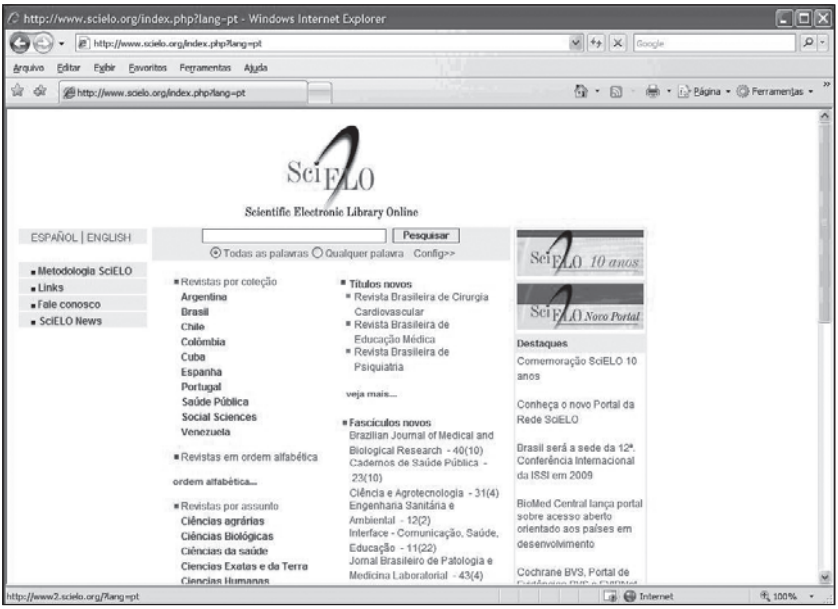

Figure 12 - SciELO's homepage.

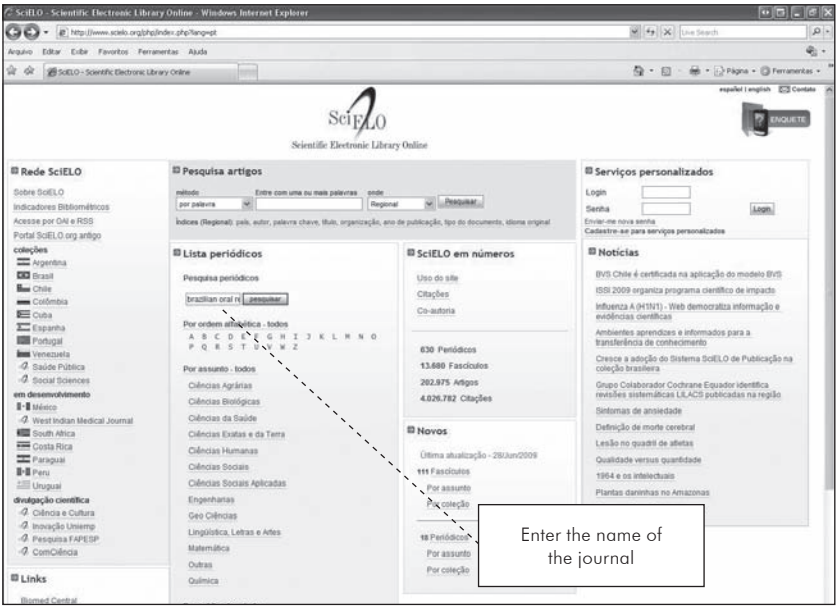

Figure 13 - Search by journal title.

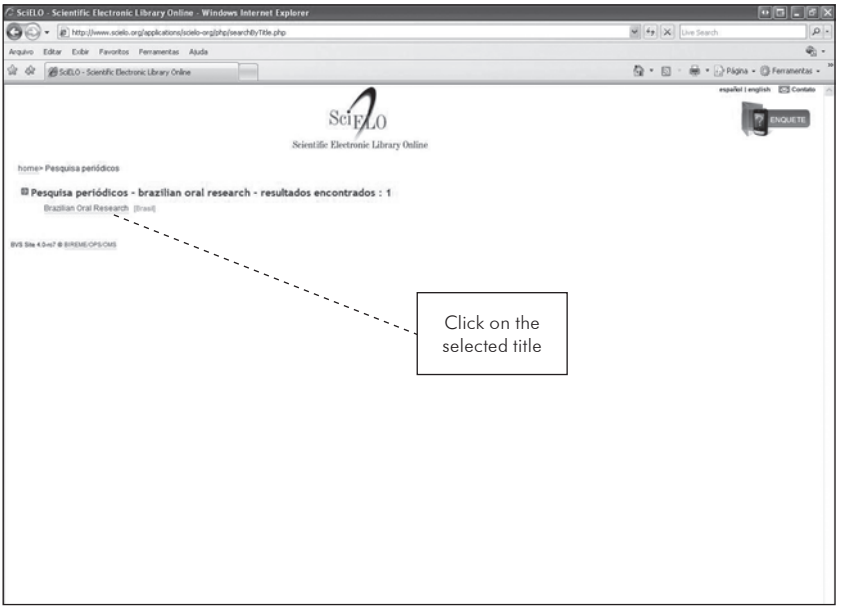

Figure 14 - Locating the desired journal.

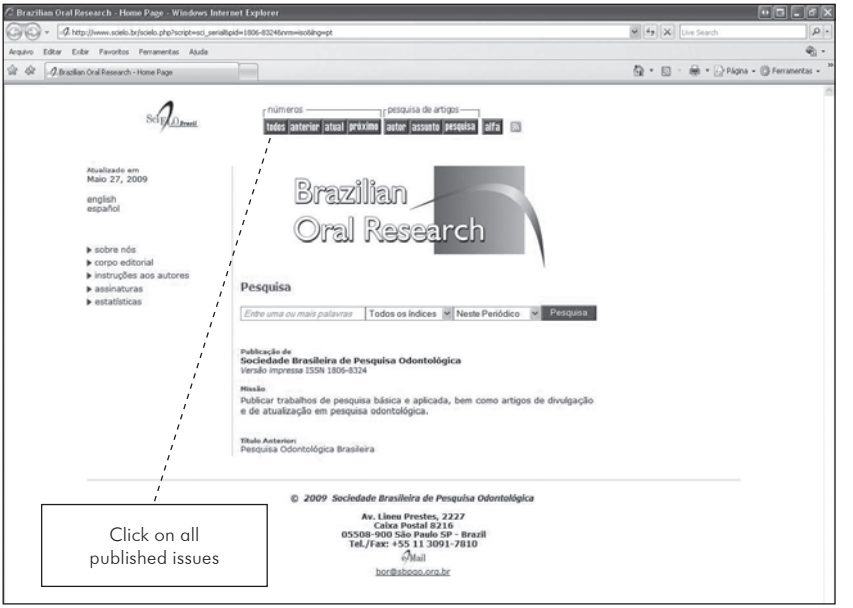

Figure 15 - Search by the issues published.

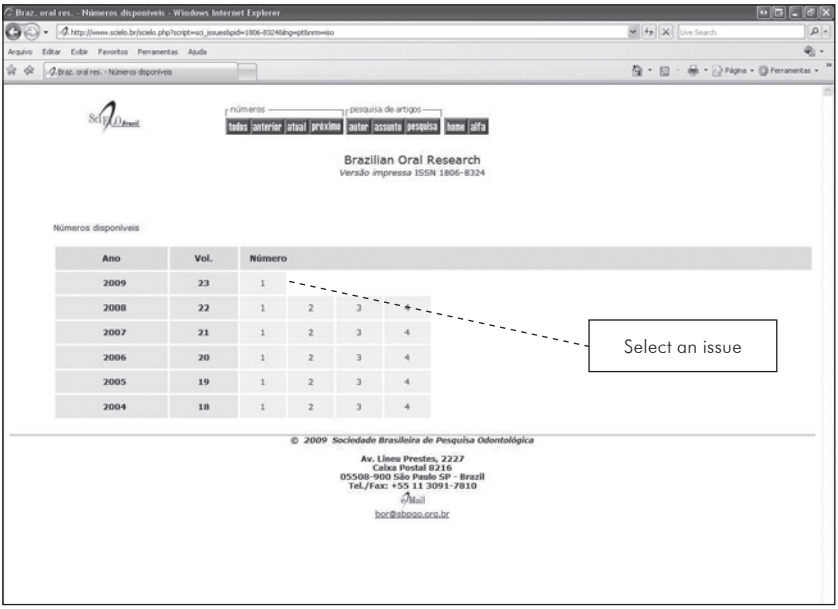

Figure 16 - Search by a specific issue of the collection.

Figure 17 - Selecting an article in "pdf" format.

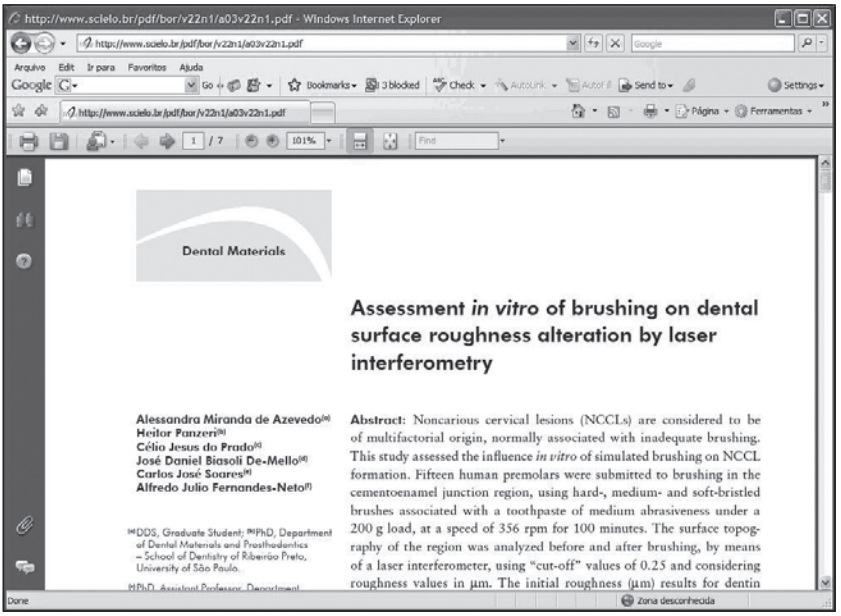

Figure 18 - Access to the full text of the article.

3.2. The CAPES Gateway

Specific policies adopted by scientific publishing houses have made it possible to gain access to the content of the articles published in their journals. Among the initiatives that have brought together the most well-reputed scientific publishers, that of the Capes Periodicals Gateway stands out especially. (*http://www.periodicos.capes.gov.br/portugues/index.jsp*). Through this portal, it is possible to locate free-access journals by choosing from a complete list of journal titles, areas of knowledge, and periodicals by publisher, as well as abstracts, patents, statistics, books and other sources, as can be read in the Gateway itself:[9]

> "Professors, researchers, students and the staff of 163 Higher Education and Research Institutions throughout the country have immediate access to the up-to-date world scientific production through this service offered by CAPES.
> The CAPES Periodicals Gateway provides access to the full texts of articles from over 11,419 international journals, both domestic and foreign, and to over 90 databases with abstracts in all areas of knowledge. It also includes a selection of important academic information sources allowing free access on the internet.
> Use of the Gateway is unrestricted and free of charge for users from the participat-

ing institutions. Access can be made from any terminal connected to the internet located in the institutions or authorized by them.
Every graduate, research and undergraduate program in the country will gain quality, productivity and competitiveness by using the Gateway, which is permanently being developed."

The procedures required for using the resources provided by the CAPES Gateway are not very different from those already seen up to this point, as shown in Figures 19 through 23.

Figure 19 - CAPES Gateway homepage.

Figure 20 - Selecting a title to be searched on the Gateway.

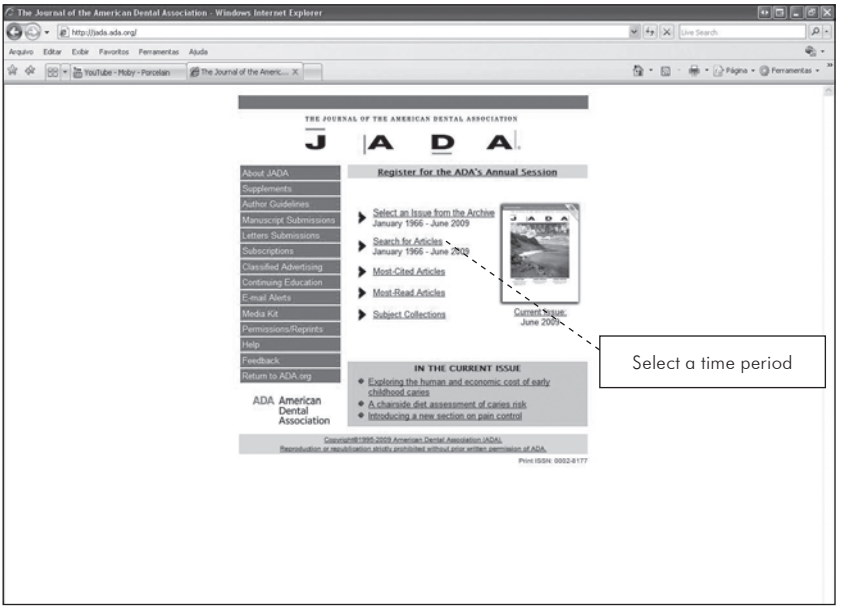

Figure 21 - Locating the title and selecting a specific time period.

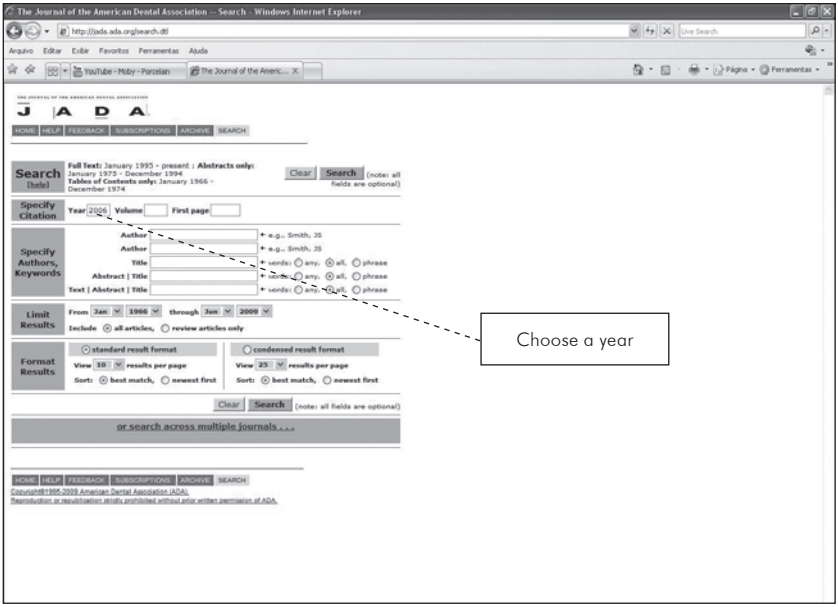

Figure 22 - Selecting the year of publication.

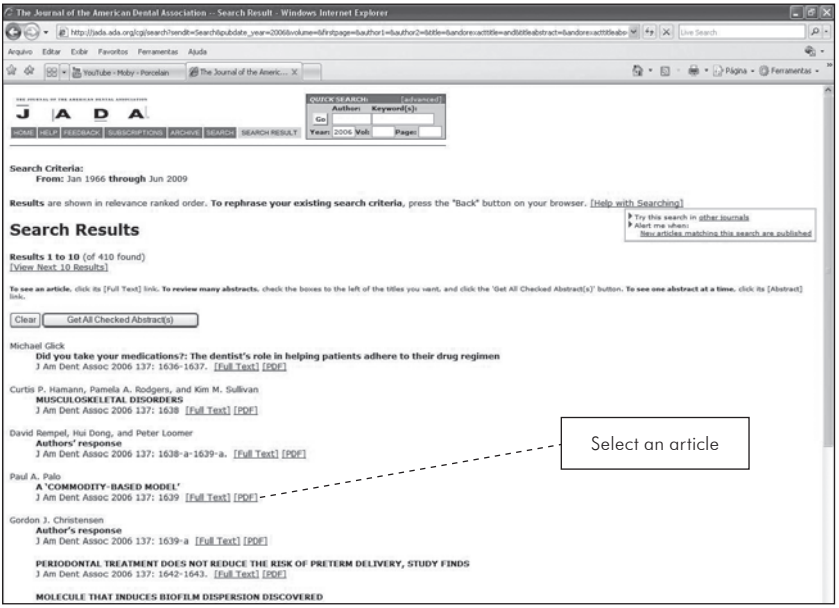

Figure 23 - Locating the article and the desired format.

It is up to the publishing houses to define the access policies to their contents. Some of them provide free access to the published texts as long as the institution buys a subscription to the print version of the title. Others provide the online version of the publication without requiring a subscription to the print version. Yet others invite institutions to subscribe to both the print and the online versions. Access to the CAPES Gateway is regulated and contingent on the IP of computers connected to State and/or Federal Higher Education Institutions. Private Higher Education Institutions may also have access to the contents of the Gateway, through a consortium called COOPERE, thus expanding the research horizon to users.

Final remarks

Library users have access to many information resources. In this chapter, a small outline has been given on how to use the electronic databases for bibliographic research.

Libraries strive to ensure that user information needs are addressed. Since technology enables changes in user conduct regarding library use, it is necessary to rethink the role played by libraries. This means that having a library full of users is no longer as important as it might have been several years ago. What is important today is to use the electronic services and products that a library has to offer. Those who believe that the library is not doing its part are mistaken, since it meets the information needs of the public using it increasingly better. A close relationship between the library and its users must be pursued in an ongoing manner, either remotely or presentially. User studies are once again being conducted to guide the dynamics of library products, and act as a reference for effecting change in its processes and for devising new forms of interaction.

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13

Scientific writing

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Each form of communication has its own rules, laid down to enhance the understanding of those with whom one wishes to communicate. Writing is one of these forms. Writing a note in a newspaper is completely different from writing a personal letter, an email or a scientific article. This chapter will discuss the purpose and characteristics of scientific writing, focusing on the publication of articles in peer-reviewed journals. This chapter has been divided into three parts with the aim of providing a clear guideline to novice writers or to those who intend to improve their scientific writing skills. The first part presents some of the rules established by the International Committee of Medical Journal Editors (ICMJE) for writing papers to be published in medical journals. We selected mainly those items related to the statement of purpose of the ICMJE, authorship, the peer-review process, conflicts of interests, privacy and the ICMJE rules for preparing and submitting manuscripts. The entire text of the original document is available at no charge at: *http://www.icmje.org* and

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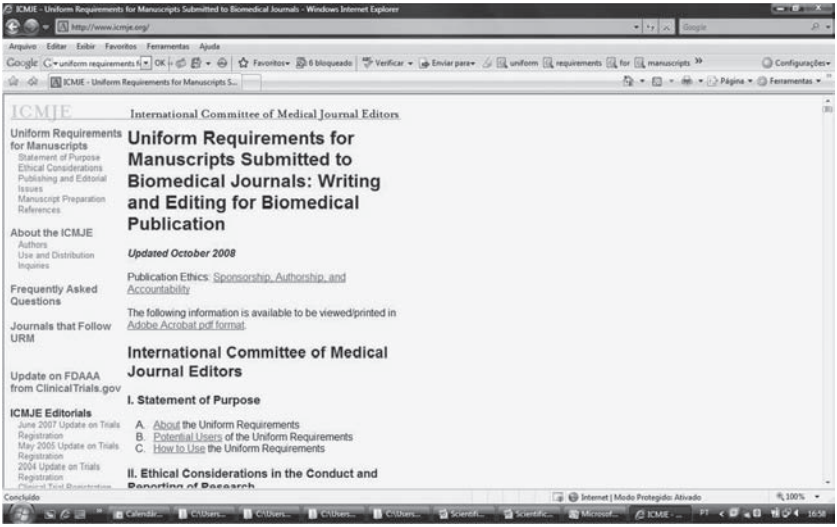

Figure 1 - Initial page of the International Committee of Medical Journal Editors (*http://www.icmje.org*).

it is mandatory that authors and editors be acquainted with it (Figure 1). The second part of the chapter discusses the role that editors play in a journal's peer-review process, their decisions and dilemmas. Lastly, the third part provides practical guidelines on the main aspects of scientific writing.

I. Uniform requirements for manuscripts submitted to biomedical journals, stipulated by the International Committee of Medical Journal Editors

Statement of purpose

A small group of editors of general medical journals met informally in Vancouver, British Columbia, in 1978, to establish guidelines for the format of manuscripts submitted to their journals. The group became known as the Vancouver Group. Its requirements for manuscripts, including formats for bibliographic references developed by the National Library of Medicine, were first published in 1979. The Vancouver Group expanded and evolved into the International Committee of Medical Journal Editors (ICMJE), which meets annually. The ICMJE has gradually broadened its concerns to include ethical principles related to publication in biomedical journals.

The ICMJE has produced multiple editions of the Uniform Requirements for Manuscripts Submitted to Biomedical Journals. Over the years, issues have

arisen that go beyond manuscript preparation, resulting in the development of a number of Separate Statements on editorial policy. The entire Uniform Requirements document was revised in 1997; sections were updated in May 1999 and May 2000. In May 2001, the ICMJE revised the sections related to potential conflict of interest. In 2003, the committee revised and reorganized the entire document, and incorporated the Separate Statements into the text. The committee prepared this revision in 2005.

The total content of the Uniform Requirements for Manuscripts Submitted to Biomedical Journals may be reproduced for educational, not-for-profit purposes without regard for copyright; the committee encourages distribution of the material.

Journals that agree to use the Uniform Requirements are encouraged to state in their instructions to authors that their requirements are in accordance with the Uniform Requirements and to cite this version. Journals that wish to be listed on the ICMJE website *(www.ICMJE.org)* as a publication that complies with the Uniform Requirements should contact the ICMJE secretariat office.

The ICMJE is a small working group of general medical journals, not an open membership organization. Occasionally, the ICMJE will invite a new member or guest when the committee feels that the new journal or organization will provide a needed perspective that is not already available within the existing committee. Member organizations open to editors and others in biomedical publication include the World Association of Medical Editors (*www.WAME.org*) and the Council of Science Editors (*www.councilofscienceeditors.org*).

Potential users of the uniform requirements

The ICMJE created the Uniform Requirements primarily to help authors and editors in their mutual task of creating and distributing accurate, clear and easily accessible reports of biomedical studies. The initial sections address the ethical principles related to the process of evaluating, improving, and publishing manuscripts in biomedical journals and the relationships between editors and authors, peer reviewers, and the media. The latter sections address the more technical aspects of preparing and submitting manuscripts. The ICMJE believes the entire document is relevant to the concerns of both authors and editors.

The Uniform Requirements can provide many other stakeholders – peer reviewers, publishers, the media, patients and their families, and general readers – with useful insights into the biomedical authoring and editing process.

How to use the uniform requirements

The Uniform Requirements state the ethical principles in the conduct and reporting of research and provide recommendations relating to specific elements of editing and writing. These recommendations are based largely on the shared experience of a moderate number of editors and authors, collected over many years, rather than on the results of methodical, planned investigation that aspires to be "evidence-based." Wherever possible, recommendations are accompanied by a rationale that justifies them; as such, the document serves an educational purpose.

Authors will find it helpful to follow the recommendations in this document whenever possible because, as described in the explanations, doing so improves the quality and clarity of reporting in manuscripts submitted to any journal, as well as the ease of editing. At the same time, every journal has editorial requirements uniquely suited to its purposes. Authors therefore need to become familiar with the specific instructions to authors published by the journal they have chosen for their manuscript – for example, the topics suitable for that journal, and the types of papers that may be submitted (for example, original articles, reviews, or case reports) – and should follow those instructions. The Mulford Library at the Medical College of Ohio maintains a useful compendium of instructions to authors.

Authorship and contributorship

Byline authors

An "author" is generally considered to be someone who has made substantive intellectual contributions to a published study, and biomedical authorship continues to have important academic, social, and financial implications.[1] In the past, readers were rarely provided with information about contributions to studies from those listed as authors and in acknowledgments.[2] Some journals now request and publish information about the contributions of each person named as having participated in a submitted study, at least for original research. Editors are strongly encouraged to develop and implement a contributorship policy, as well as a policy on identifying who is responsible for the integrity of the work as a whole.

While contributorship and guarantorship policies obviously remove much of the ambiguity surrounding contributions, it leaves unresolved the question of the quantity and quality of contribution that qualify for authorship. The International Committee of Medical Journal Editors has recommended the following criteria for authorship; these criteria are still appropriate for those jour-

nals that distinguish authors from other contributors.

- Authorship credit should be based on 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.
- When a large, multi-center group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript.[3] These individuals should fully meet the criteria for authorship/contributorship defined above, and editors will ask these individuals to complete journal-specific author and conflict of interest disclosure forms. When submitting a group author manuscript, the corresponding author should clearly indicate the preferred citation and should clearly identify all individual authors as well as the group name. Journals will generally list other members of the group in the acknowledgements. The National Library of Medicine indexes the group name and the names of individuals the group has identified as being directly responsible for the manuscript.
- Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.
- All persons designated as authors should qualify for authorship, and all those who qualify should be listed.
- Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

Some journals now also request that one or more authors, referred to as "guarantors," be identified as the persons who take responsibility for the integrity of the work as a whole, from inception to published article, and publish that information.

Increasingly, authorship of multi-center trials is attributed to a group. All members of the group who are named as authors should fully meet the above criteria for authorship/contributorship.

The group should jointly make decisions about contributors/authors before submitting the manuscript for publication. The corresponding author/guarantor should be prepared to explain the presence and order of these individuals. It is not the role of editors to make authorship/contributorship decisions or to arbitrate conflicts related to authorship.

Contributors listed in Acknowledgments

All contributors who do not meet the criteria for authorship should be list-

ed in an acknowledgments section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Editors should ask corresponding authors to declare whether or not they had assistance with study design, data collection, data analysis, or manuscript preparation. If such assistance was available, the authors should disclose the identity of the people that provided this assistance and the entity that supported it in the published article. Financial and material support should also be acknowledged.

Groups of persons who have contributed materially to the paper but whose contributions do not justify authorship may be listed under a heading such as "clinical investigators" or "participating investigators," and their function or contribution should be described – for example, "served as scientific advisors," "critically reviewed the study proposal," "collected data," or "provided and cared for study patients."

Because readers may infer their endorsement of the data and conclusions, all persons must give written permission to be acknowledged. (author's italics)

What is a peer review?

Unbiased, independent, critical assessment is an intrinsic part of all scholarly work, including the scientific process. Peer review is the critical assessment of manuscripts submitted to journals by experts who are not part of the editorial staff. Peer review can therefore be viewed as an important extension of the scientific process. Although its actual value has been little studied, and is widely debated,[4] peer review helps editors decide which manuscripts are suitable for their journals, and helps authors and editors in their efforts to improve the quality of reporting. A peer reviewed journal is one that submits most of its published research articles for outside review. The number and kind of manuscripts sent for review, the number of reviewers, the reviewing procedures, and the use made of the reviewers' opinions may vary. In the interests of transparency, each journal should publicly disclose its policies in its instructions to authors.

Conflicts of interest

Public trust in the peer review process and the credibility of published articles depend in part on how well conflict of interest is handled during writing, peer review, and editorial decision making. Conflict of interest exists when an author (or the author's institution), reviewer, or editor has financial or personal relationships that inappropriately influence (bias) his or her actions (such relationships are also known as dual commitments, competing interests, or com-

peting loyalties). These relationships vary from those with negligible potential to those with great potential to influence judgment, and not all relationships represent true conflict of interest. The potential for conflict of interest can exist whether or not an individual believes that the relationship affects his or her scientific judgment. Financial relationships (such as employment, consultancies, stock ownership, honoraria, paid expert testimony) are the most easily identifiable conflicts of interest and the most likely to undermine the credibility of the journal, the authors, and of science itself. However, conflicts can occur for other reasons, such as personal relationships, academic competition, and intellectual passion.

All participants in the peer review and publication process must disclose all relationships that could be viewed as presenting a potential conflict of interest. Disclosure of these relationships is also important in connection with editorials and review articles, because it can be more difficult to detect bias in these types of publications than in reports of original research. Editors may use information disclosed in conflict of interest and financial interest statements as a basis for editorial decisions. Editors should publish this information if they believe it is important in judging the manuscript.

Potential conflicts of interest related to individual authors' commitments

When authors submit a manuscript, whether an article or a letter, they are responsible for disclosing all financial and personal relationships that might bias their work. To prevent ambiguity, authors must state explicitly whether potential conflicts do or do not exist. Authors should do so in the manuscript on a conflict of interest notification page that follows the title page, providing additional detail, if necessary, in a cover letter that accompanies the manuscript.

Authors should identify Individuals who provide writing or other assistance and disclose the funding source for this assistance.

Investigators must disclose potential conflicts to study participants and should state in the manuscript whether they have done so.

Editors also need to decide when to publish information disclosed by authors about potential conflicts. If doubt exists, it is best to err on the side of publication.

Potential conflicts of interest related to project support

Increasingly, individual studies receive funding from commercial firms, private foundations, and government. The conditions of this funding have the po-

tential to bias and otherwise discredit the research.

Scientists have an ethical obligation to submit creditable research results for publication. Moreover, as the persons directly responsible for their work, researchers should not enter into agreements that interfere with their access to the data and their ability to analyze it independently, to prepare manuscripts, and to publish them. Authors should describe the role of the study sponsor(s), if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the report for publication. If the supporting source had no such involvement, the authors should so state. Biases potentially introduced when sponsors are directly involved in research are analogous to methodological biases of other sorts. Some journals, therefore, choose to include information about the sponsor's involvement in the methods section.

Editors may request that authors of a study funded by an agency with a proprietary or financial interest in the outcome sign a statement such as, "I had full access to all of the data in this study and I take complete responsibility for the integrity of the data and the accuracy of the data analysis." Editors should be encouraged to review copies of the protocol and/or contracts associated with project-specific studies before accepting such studies for publication. Editors may choose not to consider an article if a sponsor has asserted control over the authors' right to publish.

Privacy and confidentiality

Patients and study participants

Patients have a right to privacy that should not be infringed without informed consent. Identifying information, including patient names, initials, or hospital numbers, should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives written informed consent for publication. Informed consent for this purpose requires that a patient who is identifiable be shown the manuscript to be published. Authors should disclose to these patients whether any potential identifiable material might be available via the Internet as well as in print after publication.

Identifying details should be omitted if they are not essential. Complete anonymity is difficult to achieve, however, and informed consent should be obtained if there is any doubt. For example, masking the eye region in photographs of patients is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic pedigrees, authors

should provide assurance that alterations do not distort scientific meaning and editors should so note.

The requirement for informed consent should be included in the journal's instructions for authors. When informed consent has been obtained it should be indicated in the published article.

Overlapping publications

Duplicate submission

Most biomedical journals will not consider manuscripts that are simultaneously being considered by other journals. Among the principal considerations that have led to this policy are: 1) the potential for disagreement when two (or more) journals claim the right to publish a manuscript that has been submitted simultaneously to more than one; and 2) the possibility that two or more journals will unknowingly and unnecessarily undertake the work of peer review and editing of the same manuscript, and publish same article.

However, editors of different journals may decide to simultaneously or jointly publish an article if they believe that doing so would be in the best interest of the public's health.

Redundant publication

Redundant (or duplicate) publication is publication of a paper that overlaps substantially with one already published in print or electronic media.

Readers of primary source periodicals, whether print or electronic, deserve to be able to trust that what they are reading is original unless there is a clear statement that the article is being republished by the choice of the author and editor. The bases of this position are international copyright laws, ethical conduct, and cost-effective use of resources. Duplicate publication of original research is particularly problematic, since it can result in inadvertent double counting or inappropriate weighting of the results of a single study, which distorts the available evidence.

Most journals do not wish to receive papers on work that has already been reported in large part in a published article or is contained in another paper that has been submitted or accepted for publication elsewhere, in print or in electronic media. This policy does not preclude the journal's considering a paper that has been rejected by another journal, or a complete report that follows publication of a preliminary report, such as an abstract or poster displayed at a professional meeting, nor does it prevent journals from considering a paper that has been presented at a scientific meeting but not published in full, or that

is being considered for publication in a proceedings or similar format. Press reports of scheduled meetings will not usually be regarded as breaches of this rule, but additional data or copies of tables and illustrations should not amplify such reports. The ICMJE does not consider results posted in clinical trials registries as previous publications if the results are presented in the form of a brief structured abstract or table. The results registry should either cite the full publication or include a statement that indicates that the report has not been published in a peer reviewed journal.

When submitting a paper, the author must always make a full statement to the editor about all submissions and previous reports (including meeting presentations and posting of results in registries) that might be regarded as redundant or duplicate publication of the same or very similar work. The author must alert the editor if the manuscript includes subjects about which the authors have published a previous report or have submitted a related report to another publication. Any such report must be referred to and referenced in the new paper. Copies of such material should be included with the submitted paper to help the editor decide how to handle the matter.

If redundant or duplicate publication is attempted or occurs without such notification, authors should expect editorial action to be taken. At the least, prompt rejection of the submitted manuscript should be expected. If the editor was not aware of the violations and the article has already been published, then a notice of redundant or duplicate publication will probably be published with or without the author's explanation or approval.

Preliminary reporting to public media, governmental agencies, or manufacturers of scientific information described in a paper or a letter to the editor that has been accepted but not yet published violates the policies of many journals. Such reporting may be warranted when the paper or letter describes major therapeutic advances or public health hazards such as serious adverse effects of drugs, vaccines, other biological products, or medicinal devices, or reportable diseases. This reporting should not jeopardize publication, but should be discussed with and agreed upon by the editor in advance.

Manuscript preparation and submission

Preparing a manuscript for submission to a biomedical journal

Editors and reviewers spend many hours reading manuscripts, and therefore appreciate receiving manuscripts that are easy to read and edit. Much of the information in a journal's instructions to authors is designed to accomplish that goal in ways that meet each journal's particular editorial needs. The guidelines

that follow provide a general background and rationale for preparing manuscripts for any journal.

General principles

The text of observational and experimental articles is usually (but not necessarily) divided into sections with the headings Introduction, Methods, Results, and Discussion. This so-called "IMRAD" structure is not simply an arbitrary publication format, but rather a direct reflection of the process of scientific discovery. Long articles may need subheadings within some sections (especially the Results and Discussion sections) to clarify their content. Other types of articles, such as case reports, reviews, and editorials, are likely to need other formats.

Publication in electronic formats has created opportunities for adding details or whole sections in the electronic version only, layering information, cross-linking or extracting portions of articles, and the like. Authors need to work closely with editors in developing or using such new publication formats and should submit material for potential supplementary electronic formats for peer review.

Double spacing of all portions of the manuscript – including the title page, abstract, text, acknowledgments, references, individual tables, and legends – and generous margins make it possible for editors and reviewers to edit the text line by line, and add comments and queries, directly on the paper copy. If manuscripts are submitted electronically, the files should be double spaced, because the manuscript may need to be printed out for reviewing and editing.

During the editorial process reviewers and editors frequently need to refer to specific portions of the manuscript, which is difficult unless the pages are numbered. Authors should therefore number all of the pages of the manuscript consecutively, beginning with the title page.

Reporting guidelines for specific study designs

Research reports frequently omit important information. The general requirements listed in the next section relate to reporting essential elements for all study designs. Authors are encouraged in addition to consult the reporting guidelines relevant to their specific research design. For reports of randomized controlled trials, authors should refer to the CONSORT statement. This guideline provides a set of recommendations comprising a list of items to report and a patient flow diagram. Reporting guidelines have also been developed for a number of other study designs that some journals may ask authors to follow.

Authors should consult the information for authors of the journal they have chosen (Table 1).

Title page

The title page should carry the following information:

1. **Article title.** Concise titles are easier to read than long, convoluted ones. Titles that are too short may, however, lack important information, such as study design (which is particularly important in identifying randomized controlled trials). Authors should include all information in the title that will make electronic retrieval of the article both sensitive and specific.
2. **Authors' names and institutional affiliations.** Some journals publish each author's highest academic degree(s), while others do not.
3. **The name of the department(s) and institution(s)** to which the work should be attributed.
4. **Disclaimers**, if any.
5. **Corresponding authors.** The name, mailing address, telephone and fax numbers, and email address of the author responsible for correspondence about the manuscript (the "corresponding author"). This author may or may not be the "guarantor" for the integrity of the study as a whole, if someone is identified in this role. The corresponding author should indicate clearly whether his or her email address is to be published.
6. **The name and address of the author to whom requests for reprints should be addressed** or a statement that reprints will not be available from the authors.
7. **Source(s) of support** in the form of grants, equipment, drugs, or all of these.

Table 1 - Reporting guidelines relevant to specific research design.

Type of study	Source
CONSORT - randomized controlled trials	*http://www.consort-statement.org*
STARD - studies of diagnostic accuracy	*http://www.consort-statement.org/stardstatement.htm*
QUOROM - systematic reviews and meta-analyses	*http://www.consort-statement.org/Initiatives/MOOSE/moose.pdf*
STROBE - observational studies in epidemiology	*http://www.strobe-statement.org*
MOOSE - meta-analyses of observational studies in epidemiology	*http://www.consort-statement.org/Initiatives/MOOSE/moose.pdf*

8. **A running head.** Some journals request a short running head or foot line, usually no more than 40 characters (including letters and spaces) at the foot of the title page. Running heads are published in most journals, but are also sometimes used within the editorial office for filing and locating manuscripts.
9. **Word counts.** A word count for the text only (excluding abstract, acknowledgments, figure legends, and references) allows editors and reviewers to assess whether the information contained in the paper warrants the amount of space devoted to it, and whether the submitted manuscript fits within the journal's word limits. A separate word count for the Abstract is also useful for the same reason.
10. **The number of figures and tables.** It is difficult for the editorial staff and reviewers to tell if the figures and tables that should have accompanied a manuscript were actually included, unless the numbers of figures and tables that belong to the manuscript are noted on the title page.

Conflict of interest notification page

To prevent the information on potential conflict of interest for authors from being overlooked or misplaced, it is necessary for that information to be part of the manuscript. It should therefore also be included on a separate page or pages immediately following the title page. However, individual journals may differ in where they ask authors to provide this information, and some journals do not send information on conflicts of interest to reviewers.

Abstract and key words

An abstract (requirements for length and structured format vary by journal) should follow the title page. The abstract should provide the context or background for the study and should state the purposes of the study, basic procedures (selection of study subjects or laboratory animals, observational and analytical methods), main findings (giving specific effect sizes and their statistical significance, if possible), and principal conclusions. It should emphasize new and important aspects of the study or observations.

Because the abstract is the only substantive portion of the article indexed in many electronic databases, and the only portion many readers read, authors need to be careful that abstracts reflect the content of the article accurately. Unfortunately, the information contained in many abstracts differs from that in the text of the article.[5] The format required for structured abstracts differs from journal to journal, and some journals use more than one structure; au-

thors should make it a point of preparing their abstracts in the format specified by the journal they have chosen.

Some journals request that authors provide, and identify as such, 3 to 10 key words or short phrases that capture the main topics of the article, at the end of the abstract. These will assist indexers in cross-indexing the article and may be published with the abstract. Terms from the Medical Subject Headings (MeSH) list of Index Medicus should be used (available at *http://www.bireme.br/php/decsws.php*) (Figure 2); if suitable MeSH terms are not yet available for recently introduced terms, present terms may be used.

Introduction

Provide a context or background for the study (i.e., the nature of the problem and its significance). State the specific purpose or research objective of, or hypothesis tested by, the study or observation; the research objective is often more sharply focused when stated as a question. Both the main and secondary objectives should be clear, and any pre-specified subgroup analyses should be described. Give only strictly pertinent references, and do not include data or conclusions from the work being reported.

Methods

The Methods section should include only information that was available

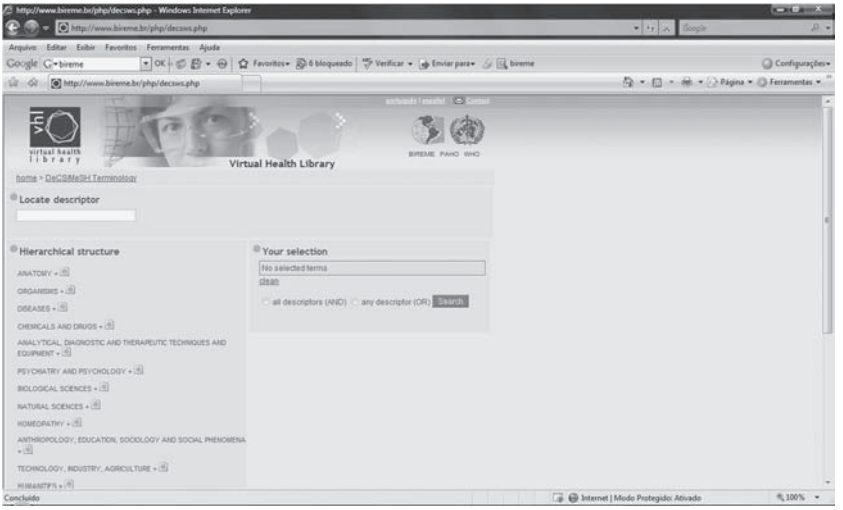

Figure 2 - Initial page of Bireme descriptors (*http://www.bireme.br/php/decsws.php*).

at the time the plan or protocol for the study was written; all information obtained during the conduct of the study belongs in the Results section.

Selection and description of participants

Describe your selection of the observational or experimental participants (patients or laboratory animals, including controls) clearly, including eligibility and exclusion criteria and a description of the source population. Because the relevance of such variables as age and sex to the object of research is not always clear, authors should explain their use when they are included in a study report; for example, authors should explain why only subjects of certain ages were included or why women were excluded. The guiding principle should be clarity about how and why a study was done in a particular way. When authors use variables such as race or ethnicity, they should define how they measured the variables and justify their relevance.

Technical information

Identify the methods, apparatus (give the manufacturer's name and address in parentheses), and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods (see below); provide references and brief descriptions for methods that have been published but are not well known; describe new or substantially modified methods, give reasons for using them, and evaluate their limitations. Identify precisely all drugs and chemicals used, including generic name(s), dose(s), and route(s) of administration.

Authors submitting review manuscripts should include a section describing the methods used for locating, selecting, extracting, and synthesizing data. These methods should also be summarized in the abstract.

Statistics

Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as the use of P values, which fail to convey important information about effect size. References for the design of the study and statistical methods should be to standard works when possible (with pages stated). Define statistical terms, abbreviations, and most symbols. Specify the computer software used.

Results

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat all the data in the tables or illustrations in the text; emphasize or summarize only important observations. Extra or supplementary materials and technical detail can be placed in an appendix where they will be accessible but will not interrupt the flow of the text; alternatively, they can be published solely in the electronic version of the journal.

When data are summarized in the Results section, give numeric results not only as derivatives (for example, percentages), but also as the absolute numbers from which the derivatives were calculated, and specify the statistical methods used to analyze them. Restrict tables and figures to those needed to explain the argument of the paper and to assess supporting data. Use graphs as an alternative to tables with many entries; do not duplicate data in graphs and tables. Avoid nontechnical uses of technical terms in statistics, such as "random" (which implies a randomizing device), "normal," "significant," "correlations," and "sample."

Where scientifically appropriate, analyses of the data by variables such as age and sex should be included.

Discussion

Emphasize the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or the Results section. For experimental studies it is useful to begin the discussion by summarizing briefly the main findings, then explore possible mechanisms or explanations for these findings, compare and contrast the results with other relevant studies, state the limitations of the study, and explore the implications of the findings for future research and for clinical practice.

Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not adequately supported by the data. In particular, authors should avoid making statements on economic benefits and costs unless their manuscript includes the appropriate economic data and analyses. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such.

References

General considerations related to references

Although references to review articles can be an efficient way of guiding

readers to a body of literature, review articles do not always reflect original work accurately. Readers should therefore be provided with direct references to original research sources whenever possible. On the other hand, extensive lists of references to original work on a topic can use excessive space on the printed page. Small numbers of references to key original papers will often serve, as well as more exhaustive lists, particularly since references can now be added to the electronic version of published papers, and since electronic literature searching allows readers to retrieve published literature efficiently.

Avoid using abstracts as references. References to papers accepted but not yet published should be designated as "in press" or "forthcoming"; authors should obtain written permission to cite such papers as well as verification that they have been accepted for publication. Information from manuscripts submitted but not accepted should be cited in the text as "unpublished observations" with written permission from the source.

Avoid citing a "personal communication" unless it provides essential information not available from a public source, in which case the name of the person and date of communication should be cited in parentheses in the text. For scientific articles, authors should obtain written permission and confirmation of accuracy from the source of a personal communication.

Some journals check the accuracy of all reference citations, but not all journals do so, and citation errors sometimes appear in the published version of articles. To minimize such errors, authors should therefore verify references against the original documents. Authors are responsible for checking that none of the references cite retracted articles except in the context of referring to the retraction. For articles published in journals indexed in MEDLINE, the ICMJE considers PubMed the authoritative source for information about retractions. Authors can identify retracted articles in MEDLINE by using the following search term, where pt in square brackets stands for publication type: Retracted publication [pt] in PubMed.

Reference style and format

The Uniform Requirements style is based largely on an ANSI standard style adapted by the National Library of Medicine (NLM) for its databases. Authors should consult National Library of Medicine's Citing Medicine for information on NLM's recommended citation formats for a variety of reference types.

References should be numbered consecutively in the order in which they are first mentioned in the text. Identify references in text, tables, and legends by Arabic numerals in parentheses. References cited only in tables or figure

legends should be numbered in accordance with the sequence established by the first identification in the text of the particular table or figure. The titles of journals should be abbreviated according to the style used in Index Medicus. Consult the list of Journals Indexed for MEDLINE, published annually as a separate publication by the National Library of Medicine. The list can also be obtained through the Library's web site. Journals vary on whether they ask authors to cite electronic references within parentheses in the text or in numbered references following the text. Authors should consult with the journal to which they plan to submit their work.

Tables

Tables capture information concisely, and display it efficiently; they also provide information at any desired level of detail and precision. Including data in tables rather than text frequently makes it possible to reduce the length of the text.

Type or print each table with double spacing on a separate sheet of paper. Number tables consecutively in the order of their first citation in the text, and supply a brief title for each. Do not use internal horizontal or vertical lines. Give each column a short or abbreviated heading. Authors should place explanatory matter in footnotes, not in the heading. Explain all nonstandard abbreviations in footnotes, and use the following symbols, in sequence:

*,†,‡,§,||,¶,
**,††,‡‡, etc.

Identify statistical measures of variations, such as standard deviation and standard error of the mean.

Be sure that each table is cited in the text.

If you use data from another published or unpublished source, obtain permission and acknowledge that source fully.

Additional tables containing backup data too extensive to publish in print may be appropriate for publication in the electronic version of the journal, deposited with an archival service, or made available to readers directly by the authors. In that event, an appropriate statement should be added to the text to inform readers that this additional information is available and where it is located. Submit such tables for consideration together with the paper so that they will be available to the peer reviewers.

Illustrations (figures)

Figures should be either professionally drawn and photographed, or submitted as photographic quality digital prints. In addition to requiring a version of the figures suitable for printing, some journals now ask authors for electronic files of figures in a format (e.g., JPEG or GIF) that will produce high quality images in the web version of the journal; authors should review the images of such files on a computer screen before submitting them, to be sure they meet their own quality standards.

For x-ray films, scans, and other diagnostic images, as well as pictures of pathology specimens or photomicrographs, send sharp, glossy, black-and-white or color photographic prints, usually 127 x 173 mm (5 x 7 inches). Although some journals redraw figures, many do not. Letters, numbers, and symbols on figures should therefore be clear and consistent throughout, and large enough to remain legible when the figure is reduced for publication. Figures should be made as self-explanatory as possible, since many will be used directly in slide presentations. Titles and detailed explanations belong in the legends, not on the illustrations themselves.

Photomicrographs should have internal scale markers. Symbols, arrows, or letters used in photomicrographs should contrast with the background.

If photographs of people are used, either the subjects must not be identifiable or their photographs must be accompanied by written permission to use them. Whenever possible, permission for publication should be obtained.

Figures should be numbered consecutively according to the order in which they have been first cited in the text. If a figure has been published, acknowledge the original source and submit written permission from the copyright holder to reproduce the figure. Permission is required irrespective of authorship or publisher except for documents in the public domain.

For illustrations in color, ascertain whether the journal requires color negatives, positive transparencies, or color prints. Accompanying drawings marked to indicate the region to be reproduced might be useful to the editor. Some journals publish illustrations in color only if the author pays the extra cost.

Authors should consult the journal about requirements for figures submitted in electronic formats.

Legends for illustrations (figures)

Type or print out legends for illustrations using double spacing, starting on a separate page, with Arabic numerals corresponding to the illustrations. When symbols, arrows, numbers, or letters are used to identify parts of the illustra-

tions, identify and explain each one clearly in the legend. Explain the internal scale and identify the method of staining in photomicrographs.

Units of measurement

Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples.

Temperatures should be in degrees Celsius. Blood pressures should be in millimeters of mercury, unless other units are specifically required by the journal.

Journals vary in the units they use for reporting hematological, clinical chemistry, and other measurements. Authors must consult the Information for Authors for the particular journal and should report laboratory information in both local and International System of Units (SI). Editors may request that the authors add alternative or non-SI units before publication, since SI units are not universally used. Drug concentrations may be reported in either SI or mass units, but the alternative should be provided in parentheses where appropriate.

Abbreviations and symbols

Use only standard abbreviations; the use of non-standard abbreviations can be extremely confusing to readers. Avoid abbreviations in the title. The spelled-out term for which an abbreviation stands should precede first mention of the abbreviation, unless it is a standard unit of measurement.

Sending the manuscript to the journal

An increasing number of journals now accept electronic submission of manuscripts, whether on disk, as attachments to electronic mail, or by downloading directly onto the journal website. Electronic submission saves time as well as postage costs, and allows the manuscript to be handled in electronic form throughout the editorial process (for example, when it is sent out for review). When submitting a manuscript electronically, authors should consult the Instructions for Authors of the journal they have chosen for their manuscript.

If a paper version of the manuscript is submitted, send the required number of copies of the manuscript and figures; they are all needed for peer review and editing, and the editorial office staff cannot be expected to make the required copies.

Manuscripts must be accompanied by a cover letter, which should include the following information:

- A full statement to the editor about all submissions and previous reports that might be regarded as redundant publication of the same or very similar

work. Any such work should be referred to specifically, and referenced in the new paper. Copies of such material should be included with the submitted paper, to help the editor decide how to handle the matter.

- A statement of financial or other relationships that might lead to a conflict of interest, if that information is not included in the manuscript itself or in an authors' form.
- A statement that the manuscript has been read and approved by all the authors, that the requirements for authorship, as stated earlier in this document, have been met, and that each author believes that the manuscript represents honest work, if that information is not provided in another form (see below).
- The name, address, and telephone number of the corresponding author, who is responsible for communicating with the other authors about revisions and final approval of the proofs, if that information is not included on the manuscript itself.

The letter should give any additional information that may be helpful to the editor, such as the type or format of article in the particular journal that the manuscript represents. If the manuscript has been submitted previously to another journal, it is helpful to include the previous editor's and reviewers' comments with the submitted manuscript, along with the authors' responses to those comments. Editors encourage authors to submit these previous communications, and doing so may expedite the review process.

Many journals now provide a presubmission checklist ensuring that all the components of the submission have been included. Some journals now also require that authors complete checklists for reports of certain study types (e.g., the CONSORT checklist for reports of randomized controlled trials). Authors should look to see if the journal uses such checklists, and send them with the manuscript if they are requested.

Letters of permission to reproduce published material, use illustrations or report information about identifiable people, or to acknowledge people for their contributions, must accompany the manuscript.

References (Part I)

1. Davidoff F. Who's the Author? Problems with Biomedical Authorship, and Some Possible Solutions. Science Editor. 2000 Jul-Aug;23(4):111-9.
2. Yank V, Rennie D. Disclosure of researcher contributions: a study of original research articles in The Lancet. Ann Intern Med. 1999 Apr 20;130(8):661-70.
3. Flanagin A, Fontanarosa PB, DeAngelis CD. Authorship for research groups. JAMA. 2002;288:3166-8.

4. Godlee F, Jefferson T. Peer Review in Health Sciences. London: BMJ Books; 1999.
5. Pitkin RM, Branagan MA, Burmeister LF. Accuracy of data in abstracts of published research articles. JAMA. 1999 Mar 24-31;281(12):1110-1.

Complementary bibliography (Part I)

Guimarães CA. Normas para manuscritos submetidos às revistas biomédicas: escrita e edição de publicação biomédica (tradução integral do texto). Rev Col Bras Cir. 2006;33(9):318-35.

World Medical Association. Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2000 Dec 20;284(23):3043-5.

Other sources of information related to biomedical journals (Part I)

Bireme *http://www.bireme.br/php/index.php*
BVS *http://bvsalud.org/php/index.php*
Cochrane Collaboration *http://www.cochrane.org/*
Committee on Publication Ethics *http://publicationethics.org/*
Council of Science Editors (CSE) *http://www.councilscienceeditors.org/*
European Association of Science Editors (EASE) *http://www.ease.org.uk/*
International Committee of Medical Journal Editors *http://www.icmje.org*
The Mulford Library, Medical College of Ohio *http://mulford.meduohio.edu/instr/*
World Association of Medical Editors (WAME) *http://www.wame.org/*

II. The role of the scientific editor and peer-reviewers

How does a scientific editor decide whether to accept or refuse a submitted manuscript? Reviewing manuscripts submitted for publication in a professional scientific journal involves the joint responsibility of authors, advisors, peer reviewers, scientific editor and editorial board. By agreeing to review a manuscript, a reviewer assumes the duty of improving – or at least maintaining – the quality and accuracy of the scientific paper that will be published in the journal. The editorial board, in turn, must consider the interests and profile of the journal's readers, given the editorial space available in the journal to address these interests.

The deadline for issuing an opinion on the submitted manuscript should be met, and it is the editor's responsibility to make sure this occurs, as well as to keep the authors posted about how this process is coming along. The authors, in turn, must have submitted the research project to an ethics in research committee and should include the committee's opinion along with their manuscript. Reviewers should make constructive comments and, in the case of a refusal, should clearly explain the weaknesses of the article substantiating the refusal. They must also treat the manuscript in a confidential manner.

The responsibilities of a reviewer can be summarized as follows:

- Provide an honest, critical assessment of the research paper, pointing out its strengths and weaknesses.
- Provide suggestions for improving the manuscript, stating clearly what should be done to raise the quality level of the paper.
- Avoid any conflict of interest, or when this can't be done, disclose it. For example, the reviewer should refuse the task of reviewing a manuscript dealing with a subject he is directly involved with or when he knows the authors.
- Avoid distortions that might influence the scientific basis of the review work. An example of this would be to favor studies with positive results, and refuse studies with negative results. Instead, the scientific merit of the manuscript should be the basis for all assessments, not its outcomes.
- Report any suspicion of fraud, plagiarism, or ethical concerns to the scientific editor, regarding the use of animals or humans in the research reported in the submitted manuscript.

Both the reviewers and scientific editor must respond to the authors in an encouraging manner. It is not pleasant to have an article refused for publication, but a carefully drafted reviewer's opinion with appropriate suggestions can be very useful.

The role of the scientific editor in this process is to analyze the submitted manuscript, check if it is within the journal's scope, appoint the appropriate expert in the specific area of the article to review it, and then analyze the reviewer's opinion. The scientific editor might also have to set up the whole peer-review process and supervise it, together with the editorial board.

The scientific editor must assess the work and academic background of the reviewers invited to form the journal's editorial board. A good starting point for a prospective reviewer would be to constructively review his own work.

Given that a scientific paper may represent months or even years of research work, reviewers' decisions are very important and should be taken carefully. With this in mind, a recommendation for refusal should be accompanied by information that could help the authors understand the basis for refusal and also be useful for improving their work. Similarly, a recommendation for acceptance should be based on scientific merit. Any suggestions for changing the manuscript should encourage and guide the author to prepare an even better manuscript. This great responsibility requires time, thought, commitment and dedication, both on the part of the scientific editor and the reviewers involved in assessing manuscripts. Only in this way will a scientific journal contribute to the development of the profession, both in research and in clinical practice.

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III. Practical guidelines

The common use of the term "to publish or perish" is generally associated with the imperatives of academic life,[1] and most institutions of higher learning have two missions: (1) to create knowledge (research), and (2) to disseminate knowledge (teaching). It is therefore expected of most academicians in most disciplines to publish, mainly in journals.[2] Thus, writing is an important part of academic life. We are always writing something: a project, a form, a report, notes, dissertations, thesis, books, manuscripts or research articles. Professional publication disseminates vital new information and research to the public and other health care professionals. It also gives the author personal satisfaction, and leads to career advancement, prestige and possibly monetary gain.[3]

The importance of publishing academic works is well known. This considered, why are only a small proportion of defended theses submitted for publication in peer-reviewed journals?

Heyman, Cronin[4] (2005) gave two main reasons. First, by the time the thesis is completed, the student often feels exhausted after addressing a topic that has consumed his or her attention for such a long period of time. It may be difficult for a student to arouse the motivation and enthusiasm needed to convert his or her work into articles for publication. Second, it may be difficult to take the large body of work contained in a thesis and condense it to write a short, succinct article that is important and interesting in its own right, rather than as part of a larger project.[1] In addition to the professionals who do not submit their work for publication, it is likely that there are many others who do submit their work but whose articles are rejected or returned with suggestions for major revisions.

Aspiring authors can improve the likelihood of having a manuscript accepted for publication by following certain guidelines. Sullivan[5] (1999) has identified the 5 "rights" of publishing: the right topic, the right journal, the right information, the right words and the right time. If an author's manuscript meets

all of these criteria, it is more likely to be published.[3]

The right topic

First-time authors sometimes think that their work may not be important to a journal because it discusses a "common" subject. Furthermore, practitioners sometimes think that because their work did not proceed "perfectly," it will not be good enough for publication. With this in mind, new authors should ask certain questions about their work before taking the time to draft a paper for publication. [3,6]

1. Will the article contribute new information?
2. What aspects of this topic have not been addressed in the literature?

A thorough review of the literature will identify information currently available about the topic and reveal how the topic has been addressed previously. There are many ways to develop a topic, but this topic must be specific to be developed thoroughly.[3]

The right journal

Depending on the nature of the topic, the manuscript may be an original research report, a review of the literature, a book review, a case study, or a "how-we-did-it" presentation.[3] The main point is that a single paper should tell one story to one audience. For example, a paper that focuses on theoretical or methodological issues designed to generate discussion may be more appropriately placed in an academic journal. A paper which aims to inform practitioners of potentially useful findings for practice might be best suited to a journal whose target audience is practitioners and clinicians.[4]

In choosing the more appropriate journal, the authors should read the mission statement of a journal, which describes its service goal and provides some information about the readership that the journal wants to attract.[3] Authors need to understand that every journal has limitations to its focus of interest: an international journal is concerned with international aspects and is not as interested in a local practice as a local journal would be; other journals publish only quantitative research; therefore, a qualitative manuscript would not be an appropriate fit.[2] Moreover, it is desirable to read some articles previously published by the journal to familiarize oneself with the style, rules and perspectives of the manuscripts. Identify two or three of the most relevant articles and read these completely for content and topic development. Use these articles as *templates* or guides to develop your own paper. Think about it: these authors are published where you want to be published.[7]

Another important consideration in deciding where to publish is the level of credibility attributed to the journal. Most readers will be familiar with the process of peer review, but another way to determine the quality of the journal is through the level of Impact Factor[8] accorded to that journal. A journal impact factor is a measure of the frequency with which the "average article" in a journal has been cited in a particular year. Some journal prestige and acceptability are based on a high impact factor.[9]

The right information

Depending on the readers of the journal, the topic can be approached from an entirely different perspective. A clear and coherent structure is an important component of any publication, independently of the audience and writing-style adopted.[1] The author should submit real and accurate information, complete citations and not falsify data or otherwise mislead the reader.[3]

A research article has five sections: (1) abstract, (2) introduction or literature review, (3) methods, (4) results, (5) discussion and conclusion (s). Although so ordered in the manuscript, these sections are often written in an alternative sequence, such as materials and methods, results, introduction, discussion, and abstract. The reason for this is that the thought process evolves through the paper and is discovered only after the results are analyzed and written out. Particularly regarding papers originating from theses, the alignment of the introduction, literature review and discussion will often require re-engineering to follow the story line. This applies mainly to the literature review of a thesis, because it will have been written with more general aims than that of an article.[7] In sum, the basic structure of the introduction is: statement of the issue, why the paper is needed and a explanation of the purpose or hypothesis.[7]

If the materials and methods section is lengthy, it should be organized under subheadings. The first subheading should refer to subjects, the second to procedures, the third to definitions and criteria, the next to data collection, and the final subheading should refer to statistical tests.[7]

The development of the results section should parallel that of the methods section. If subheadings are used in the methods section, then the same subheadings should be stated in the same order in the results section.[7]

A good discussion should include the chief results, the authors' interpretation of the results, the authors' interpretation in the context of the literature, the clinical or pathological implications of the findings, limitations of the study and a summary paragraph.[7]

Papers are often rejected because the individually solid literature review and

the data analysis sections do not tell the same story. Although obvious to the reviewers, this disharmony is not identified by the authors, who are too immersed in the detail of their project to stand back and take an overall view.[4]

Thus, we can say that a paper is well written if a reader who is not involved in the work can understand every single sentence in the paper.[6] An important part of writing, in addition to asking someone not involved in the writing process to read the paper, is the practice of putting a draft aside and letting it "cool off" so that the author can see it from a distance. This enables authors to notice any problems that can still be found in the manuscript and that may not have been evident while writing.[2]

The right words

Having clarified the story line and target audience in advance, the authors should rigorously comply with the guidelines issued by the chosen journal. Editors will expect their standard instructions to be followed. Many papers submitted for peer review are poorly presented with respect to style, grammar, punctuation and formatting. The real writing work comes after the content of the paper has first been drafted, and should take about 70% of the total time. Few authors are gifted enough to think on screen and write well at the same time.[4]

There are several ways that the author can assess and improve the quality of the writing. Many guidelines are available at bookstores or on the Internet. A good example is the *Scientific Writing Booklet*.[10] The author clearly and concisely gives guidelines for better writing; some are listed as follow:

- Interest, inform, and persuade the reader
- Write for your reader and write clearly
- Eliminate unnecessary redundancy
- Avoid digressions
- Do not over explain and avoid overstatement
- Avoid unnecessary qualifiers
- Use consistent tenses
- Use the precise word
- Simpler words are preferred over complex words, and use concrete words and examples
- Simpler sentences are preferred over more complicated sentences
- Use the active voice (except generally in methods)
- Make sure the subject and verb agree
- Use affirmative rather than negative constructions

- Avoid use of the indefinite "this"
- Use transitions
- Cite sources as well as findings
- Proofread your paper carefully; spell check does not catch everything; "there" is spelled correctly but not if you meant "their".

In general, the best writing is simple and direct. Writing that is simple and direct is most easily understood. It also tends to be the most forceful and memorable. Use no more words than necessary and never use a complicated word if a simpler one will do just as well. Many people seem to feel that writing in a complicated way makes one sound serious, scholarly and authoritative. While this type of writing may sound serious, it is no more authoritative than writing that is simple and direct. Certainly, it is more difficult to understand. Often, it sounds pompous and overbearing. If your purpose is to be understood in a way that is both forceful and memorable, adopt a style that is simple and direct.[10]

The right time

Publish promptly if you have new information or knowledge.[3] Remember: "to publish or perish".

After submitting your paper to a journal, expect the journal's editorial staff to take some time to process the submission. During this time, they are deciding if the paper is consistent with the journal's aims and Instructions to Authors, if it is of sufficient quality to merit publication and whether or not there are any special issues related to the paper such as patient consent or potential competing interests, and also whether they have been handled appropriately.[6]

If the paper passes editorial screening on the basis of these criteria, the editor will forward the paper for peer review, i.e. for review by others working in the field who will advise the editor as to its suitability for publication.[6]

In case of rejection of the paper, pick yourself (and the paper) up, dust yourself off, reformat the paper for another journal, and use the critiques of the reviewers to improve your paper. Look critically at the study for ways that the presentation can be more transparent and the purposes clearer. Remember that if you make the reviewers work too hard to understand the paper, they will not like it.[7]

If the paper is accepted pending major revision, try to accommodate all or most of the requests as best as you can.[7] Not everything that reviewers mention in their comments applies. Most often, the cry from the reviewers is "clarify, clarify, clarify". What may seem obvious to authors having worked closely on their material is not necessarily clear to anyone reading the text.[2]

A positive and conciliatory attitude in your response will likely engender a positive and conciliatory attitude in return.[7] When authors have made the necessary changes, a list of the changes made, or not made, should normally be sent to the editor, together with any necessary explanations, when the manuscript is returned after review.[2]

Bayne *et al*.[11] (2003) published an extensive tutorial for writers and reviewers involved with the preparation and evaluation of manuscripts submitted for publication in dental journals. The contents were compiled from the Instructions for Authors printed in various peer-reviewed dental journals and from feedback from 10 workshops conducted for the Editorial Review Board of the *Journal of Prosthetic Dentistry*. The tutorial presents key guidelines to ensure compliance with the principles of sound scientific writing and the expeditious review of manuscripts prepared for publication in peer-reviewed dental journals. It is very important reading for those who intend to have their manuscripts published.

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