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COIMBRA

Bárbara Guimarães da Silva

Selection Criteria in Clinical Trials in Healthy Subjects: Vital Signs and Electrocardiogram Parameters

Dissertação de Mestrado na área científica de Biotecnologia Farmacêutica orientada pelo Professor Doutor Sérgio Paulo de Magalhães Simões e pelo Professor Doutor José Luís de Almeida e apresentada à Faculdade de Farmácia da Universidade de Coimbra.

Outubro de 2021

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Critérios de Seleção em Ensaios Clínicos em Voluntários Saudáveis: Parâmetros dos Sinais Vitais e Eletrocardiograma

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"Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world"

Louis Pasteur

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ABSTRACT

Phase I trials are the first pharmacological studies in humans, without therapeutic objectives and often include healthy subjects administered either an active investigational drug or a placebo. Risk minimization to the lowest possible level implies the implementation of safety measures, particularly in first-in-human studies with a new compound under investigation.

The health status of participants and the compliance with the eligibility criteria must be assessed at the beginning of the trial, during the screening period, to ensure and protect the well-being of all subjects. Screening assessments include, in general, medical history, physical examination, vital signs, laboratory tests and electrocardiogram. At all times, the investigator is responsible for the clinical surveillance and medical care.

Currently, no guidelines define the acceptable ranges for the main safety parameters used for the participation of a healthy subject in a phase I clinical trial. Minor deviations from the reference ranges may be accepted in the context of eligibility for a trial as they are not necessarily pathological. The safety parameters chosen for eligibility and the values defined as acceptable will always depend on the risk of the drug, considering the target organs of toxicity.

Throughout this work, an acceptability range for vital signs and electrocardiogram parameters in one phase I center will be discussed. Data collected from 16 studies will be used to analyze electrocardiogram morphological abnormalities in the same healthy cohort, providing reliable information on subjects' safety.

Keywords: Clinical Trials; Healthy Subjects; Reference Ranges; Vital Signs; Electrocardiogram.

RESUMO

Ensaios de fase I são os primeiros estudos farmacológicos em humanos, sem objetivos terapêuticos e frequentemente incluem indivíduos saudáveis administrados com um medicamento experimental ou um placebo. A minimização do risco ao nível mais baixo possível implica a implementação de medidas de segurança, particularmente em estudos pioneiros em humanos com um novo composto sob investigação.

O estado de saúde dos participantes e o cumprimento dos critérios de elegibilidade deve ser avaliado no início do estudo, durante o período de triagem, para garantir e proteger o bem-estar de todos os indivíduos. As avaliações de triagem incluem, em geral, a história médica, exame físico, sinais vitais, exames laboratoriais e eletrocardiograma. Em todas as circunstâncias, o investigador é responsável pela vigilância clínica e cuidados médicos.

Atualmente, nenhuma diretriz define os intervalos aceitáveis para os principais parâmetros de segurança que permitem a participação de um sujeito saudável em ensaios clínicos de fase I. Pequenos desvios dos intervalos de referência podem ser aceites no contexto de elegibilidade para um ensaio, por não serem necessariamente patológicos. Os parâmetros de segurança escolhidos para elegibilidade e os valores definidos como aceitáveis dependerão sempre do risco do medicamento, considerando os órgãos alvo de toxicidade.

Ao longo deste trabalho, será discutido um intervalo de aceitabilidade para sinais vitais e parâmetros do eletrocardiograma de um centro de fase I. Os dados recolhidos de 16 estudos serão utilizados para analisar anormalidades morfológicas do eletrocardiograma no mesmo coorte saudável, fornecendo informações fidedignas acerca da segurança dos participantes.

Palavras-chave: Ensaios Clínicos; Indivíduos Saudáveis; Intervalos de Referência; Sinais Vitais; Eletrocardiograma.

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LIST OF ABBREVIATIONS

AV – Atrioventricular

BMI – Body Mass Index

BP – Blood Pressure

bpm – beats per minute

CR – Clinically Relevant

CSP – Clinical Study Protocol

DBP – Diastolic Blood Pressure

EC – Exclusion Criteria

ECG – Electrocardiogram

EMA – European Medicines Agency

FDA – US Food and Drug Administration

FIH – First in Human Trials

GCP – Good Clinical Practice

HR – Heart Rate

HS –Healthy Subjects

IC – Inclusion Criteria

IMP – Investigational Medicinal Product

mmHg – millimeters of mercury

ms – milliseconds

NCR – Non-Clinically Relevant

R&D – Research and Development

RR – Respiratory rate

SBP – Systolic Blood Pressure

SD – Standard Deviation

SOP – Statistical Operating Procedure

I. INTRODUCTION

Clinical Trials are defined as "any investigation in human subjects intended to discover or verify the clinical, pharmacological and/or other pharmacodynamic effects of one or more investigational medicinal product(s) (IMP), and/or to identify any adverse reactions to one or more investigational medicinal product(s) and/or to study absorption, distribution, metabolism and excretion of one or more investigational medicinal product(s) with the objective of ascertaining its (their) safety and/or efficacy." [1]

These studies can be classified according to their development phase or their objectives. At all stages of development, dose-response information should be obtained. [2]

Occurring through four phases, sometimes overlapping, they begin with initial human pharmacology trials that provide an early evaluation of short-term safety and tolerability, as well as pharmacodynamic and pharmacokinetic information necessary to choose a suitable dosage range and administration schedule. After these, therapeutic exploratory studies are performed in small groups of patients with the target indication and later confirmatory trials that are generally longer, made on a larger scale and that include a more diverse population. [2]

In clinical research, benefits should always be prioritized and balanced over risk. In the conduction of clinical trials, it is required to strictly comply with the Clinical Study Protocol (CSP) and with the Good Clinical Practice (GCP), to ensure subject's safety and that reliable data are obtained. [3][4]

Phase I trials are the focus of this research. The subjects are usually healthy and focus on both clinically not established substances and clinically established medicines (such as bioequivalence studies for generic medicines applications). [5]

Trials with healthy subjects (HS) require risk minimization to the lowest level and that is why defining threshold values for inclusion in a trial is so important. Considering pharmacological characteristics of the study drug may be different as well as the nature of the trial, a case-by-case decision is mandatory.

All these factors make it possible to ascertain the health status of a trial subject. [5]

This way, clinical trials are a strict and highly controlled part of the drug development process that aims at making available innovative treatments to everyone, with the purpose of improved quality of life and life expectancy. [6][7]

II. CLINICAL TRIALS: OVERVIEW

2.1 Developing a New Medicine

In the developed world, the increase in life expectancy brings challenges in what concerns health, disease and quality of life so society demands a broader availability and access to medicines. Although expectation increases, new drugs and devices and the medical evolution are the results of years of research and development (R&D), which makes the development of new drugs a time-consuming process and a significant economic, professional and personal investment. [8]

Regulatory entities such as European Medicines Agency (EMA) and the Food and Drug Administration (FDA) in the United States are responsible for establishing guidance for drug testing and for ensuring their implementation. The general road to drug development and approval has been defined and regulated for decades. [9]

Based on the study of the disease, research begins with the identification of therapeutic targets and validation of the role of new molecules. The action or not of the molecule on the therapeutic target and the ability to change the course of the disease are evaluated, through a set of procedures and tests that guarantee a correct assessment of its safety and the optimization of its properties. [10]

Before testing a drug in humans, researchers must rule out the potential to cause serious harm. The two types of preclinical research are: *in vitro* (cells) and *in vivo* (animals), which intention is to obtain information, later extrapolated to the human, necessary to guarantee the safety and efficacy in the transition to the clinical phase. From a total of 10.000 molecules or compounds, only 250 will reach the pre-clinical phase and 1 or 2 to the ultimate marketing authorization stage and commercialization to patients. [11]

While preclinical research answers basic questions about drug safety, it is not a substitute for studies on the interaction between the drug and the human body. Only medicines that successfully complete the tests at the pre-clinical stage will continue and eventually access the clinical stage, which will take 7 to 12 years to complete, with a cost reported to be US$800 million for each drug. Much of this cost comes from failures, which account for 75 percent of the total research and development costs. [12][13]

The Clinical Trials act as a kind of "Do or Die" for new medicines or devices, representing the stage where most molecules "die" and simultaneously the phase of greater economic burdens and investment by the industry. [14]

2.2 Clinical Trials: Concept

According to the National Institutes of Health (NIH), a Clinical Trial is "a research study in which one or more human subjects are prospectively assigned to one or more interventions to evaluate the effects of those interventions on health-related biomedical or behavioral outcomes". [9]

Clinical trials are important to observe outcomes on human subjects under experimental conditions and help to determine which medical approaches are most effective for certain types of illnesses or specific groups of people. The term may be applied to any form of planned experiment that involves human subjects and is carefully designed to answer specific research questions and provide evidence. [9][15]

Data provided by clinical trials enables determining whether the investigational products are suitable for routine use in healthcare. These trials are very important to medical progress, to improve public health, and even for those patients who might benefit while being tested. [16]

2.3 Clinical Trial Phases

Done at hospitals and research centers, clinical trials are conducted in four phases, each with a different purpose. Researchers start with smaller groups of subjects and more focused objectives (such as safety and tolerability, human pharmacokinetics and pharmacodynamics), gradually working up to larger trials aimed at collecting additional information regarding effectiveness, risks and benefits, drug-drug interactions, among others. [9]

a) Phase I

Once preclinical studies have shown that a molecule may bring health benefit and appears safe, Phase I trials (also known as "human pharmacology" studies) begin with the

administration of a new investigational product to a small number of subjects. Studies at this stage have no therapeutic objectives and typically rely on healthy subjects to evaluate the safety and tolerability of new drugs. [9]

In practice, Phase I studies are also conducted later in the drug development process, in what regards the evaluation of food effect, drug interactions and drugs bioequivalence. As part of the design of these trials, subjects are often confined to a research unit/hospital to allow frequent procedures, such as blood collection, vital signs and electrocardiograms (ECGs), to ensure that the subjects adhere to all protocol requirements and restrictions and to maintain clinical surveillance. [17]

Phase I studies are an important part of the process of turning scientific discoveries into clinical therapies. This phase aims at establishing safety, pharmacokinetics, and dosage for subsequent testing of new drugs and they are a necessary step in building the evidence for new treatments. [18][19]

According to FDA, approximately 70% of medications move on to phase II. [20]

b) Phase II

Phase II trials, also known as "therapeutic exploratory" are conducted in a larger group of individuals than phase I trials and who are living with the condition/ disease that the new medication is meant to treat. They are designed to, firstly, evaluate the therapeutic efficacy of the drug, but may also be designed to answer questions essential to the planning of phase III trials, including determination of the appropriate dose, administration routes, and potential study endpoints. [9]

These studies are divided into two phases:

- Phase IIa are usually meant to demonstrate clinical efficacy or biological activity ("proof of concept studies").

- Phase IIb studies assess the optimal dose at which the medicine reveals biological activity while causing the fewest side effects. [21]

Phase II trials, lasting from several months to two years, are commonly randomized, in which one group of patients receives the experimental drug while the other group receives either a placebo or a drug already available on the market. Most often, they are also double-blind trials (meaning that neither the patient nor the research team knows who

receives the experimental drug, to ensure the effectiveness and impartiality of the study treatment.)

FDA estimates that about 33% of medications move on to phase III. [20]

c) Phase III

Phase III trials, being the large-scale (several hundred to several thousand subjects) controlled studies, are designed to demonstrate the efficacy of an experimental agent. [22]

In these, also referred to as "therapeutic confirmatory," "comparative efficacy," or "pivotal trial", researchers will use a larger and often more diverse target population to demonstrate efficacy, monitor side effects, compare to other standard treatments or a placebo and collect information to ensure safe use of the drug. [9]

Due to the large population sample, phase III trials are generally international, with the participation of multiple centers which highlights the importance of collecting demographic and other data, such as ethnicity and other relevant racial data, to identify possible differences in response to therapy. [23]

This is often the final stage of testing before the drug approval and the final assessment of the drug in terms of therapeutic endpoints.[23][24]

Approximately 70% to 90% of drugs that enter Phase III studies successfully complete this phase of development. [20]

d) Phase IV

Phase IV trials are long-term studies conducted after a drug has been shown to be effective and the treatment has received regulatory approval. [25]

Also known as "therapeutic use" or "post-marketing" studies, these trials imply thousands of participants and are observational studies performed on approved drugs to evaluate the long-term risks and benefits of a medicine. This phase can also determine more about the side effects, interactions with other drugs, and therapeutic efficacy when it's more widely used. [9]

At this stage, additional information on the safety, efficacy, and use of the drug or treatment is obtained. [23]

Finally, the combination of incomplete information regarding short duration and limited size of phase III (when compared to the post-marketing period), makes us realize that sometimes the balance between benefit and risk only becomes effectively clear, during phase IV, a moment of more robust clinical evidence. [25]

Table 1 depicts an overview of the drug development and approval process.

Table 1 - The drug development and approval process. [14]

Stage	No. of years	Population tested	Purpose	Estimated success rate	Capitalized cost (millions USD)
Preclinical testing and animal studies	5.5	Laboratory and animal studies	To assess biological activity and safety	5 to 20 out of 5000 to 10000 compounds	185.6
Phase 1	2	20 to 80 Healthy subjects	Determine drug kinetics, its dosage, and safety in humans	2 to 5 of above	30.5
Phase 2	2	100 to 300 Patients	Evaluate efficacy and safety (adverse events)	2 to 5 of above	41.6
Phase 3	2 to 4	1000 to 3000 Patients	Confirm efficacy and monitor for adverse events from long-term use	2 to 5 of above	119.2
FDA review	1 to 2	NA	Unbiased independent review process for approval or denial	1 compound selected	1.96
Phase 4	15	Entire user population	Additional post-marketing surveillance required by regulatory agencies or sponsor-initiated	NA	Variable

USD, United States dólar

2.4 Main Players in Clinical Trials

The development of new drugs is a complex process that involves several players, all with very different roles and interests. For clinical trials, there are a diverse set of players that work together and that will be briefly described below:

a) Subjects

Subjects are individuals who voluntarily consent to participate in clinical trials. In the case of participants with a specific disease, they may benefit from early access to new medicines. On trials conducted on healthy subjects, mainly with pharmacokinetics and pharmacodynamics objectives, participants won't get direct benefit from their participation (besides the medical check-up and care). Nevertheless, they receive monetary compensation for the time they spend on the trial. [26]

b) Research Team

Conducting a clinical trial demands a qualified team, by education, training and experience, hired by the trial center and consisting of physicians (Principal Investigator and co-investigators), nurses, laboratory technicians, and other study support staff. The research team is responsible for protecting the safety and well-being of the subjects and for ensuring the quality of the data, following the requirements of the CSP or other study-specific documents, of the GCP and other related international and/or national guidances or regulations. [26]

c) Sponsor

The sponsor is the entity that holds the experimental molecule and promotes its development. Responsible for the design, conduction, and financing of the trial, sponsors can be individuals, a pharmaceutical company, academic institution or private organizations. Whether their interest is commercial or non-commercial, they will always have to submit the protocol (and other essential documents) for approval by the Competent Authority Regulator and Ethics Committee, provide all the information about the test product and instructions to the research team and monitor the trial to make sure that all regulations are being followed. [26]

d) Ethics Committee

The Ethics Committee includes scientific and non-scientific members, with a large diversity of knowledge and experience, to ensure a comprehensive review to guarantee the protection of participants. Some of their main responsibilities are to review the study's protocol and other documents, check the adequacy of the facilities and human resources and access adverse events or any potential harm resulting from the trial to ensure the protection of the rights, safety and well-being of all participants. [26]

e) Regulatory Authorities

Testing and approving new medicines implies regulation, following guidelines and important quality assurance responsibilities. Regulatory authorities are responsible for reviewing and approving clinical trial documents and for ensuring that national and international law and guidelines are being complied with. Each country has its medicine agency. In Portugal, the authority is the National Institute of Pharmacy and Medicines (INFARMED). At a European level is EMA that regulates the process in all member states. [26]

f) Contract Research Organizations (CRO)

CROs are not a mandatory player and work in a very diversified way, depending on the functions assigned by the sponsor (all trial-related activities or only specific services such as regulatory activities, monitoring of the trial, data management, pharmacovigilance). Due to their focus, they offer a faster and qualified process as they are specialized in all the implementation and operationalization procedures. [27]

Figure 1 shows the interaction between the main players in clinical trials: The regulatory authority interacts with the sponsor and approves the trial protocol that the investigator collects. The investigator is in charge of obtaining EC approval, identifying, recruiting and following up with participants, as well as delivering study data to the sponsor. [26]

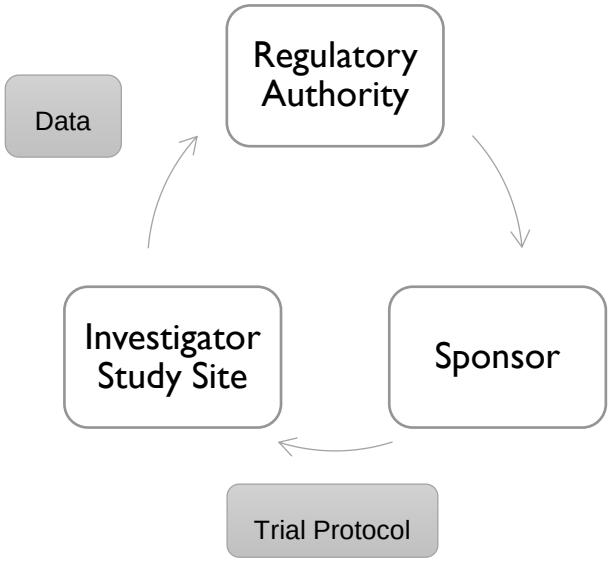

Figure 1 - Interaction between the main players in clinical trials. [26]

III. STUDY POPULATION IN A PHASE I TRIAL

3.1 What is a Healthy Subject?

Considering that this assay addresses Phase I trials, it is important to know that at this stage, a drug is being administered to healthy subjects and intends to give important information on the drug's safety. [28]

A case-by-case judgment based on a careful risk/benefit assessment should be undertaken as to whether a Phase I trial should be performed in healthy subjects or patients. Healthy subjects are easier to find than patients, are more robust, free of other medications, more likely to react uniformly, and better at completing long and complex trials. [29]

According to the Royal College of Physicians, a healthy subject is defined as "an individual who is not known to suffer any significant illness relevant to the proposed study, who should be within the ordinary range of body measurements such as weight, and whose mental state is such that he can understand and give valid consent to the study." [30]

There are several definitions of "healthy subject" in the literature. This concept applies to the beginning of the study, during screening phase and follows the eligibility criteria. Younger volunteers (aged between 18 and 45) tend to be the logical choice, since they are less likely to have co-morbidities. [5][28]

The aim of the selection process is not only to find healthy subjects, but also to discard people who have diseases or risk factors that might put them in danger or cause confusion in the interpretation of study results. Depending on the phase I goals, the selection process shall specify a list of diseases that lead to exclusion based on the potential risk to the subjects. An initial screening is always required and may become beneficial in the case of asymptomatic diseases. [31]

3.2 How Are Participants Protected?

Clinical studies are strictly regulated to ensure participants' safety. [32]

The dose administered in first in human (FIH) trials is calculated through a detailed analysis of the non-clinical research. All findings of non-clinical studies or previous human studies must be sufficient to provide information about the toxicological and physiological effects of a new drug and to show that the medicine is safe for the planned human investigation before any clinical trial is conducted. [3]

Potential participants must be given complete information about all aspects of the research, including its risks and benefits, before agreeing to participate in a clinical trial. This process is known as "Informed Consent" and involves information and discussion about the trial, finalizing in the signature of a document before enrollment in the trial. The Informed Consent Form must provide the following information:

- Details about the purpose of the trial, therapy being tested;
- Phases and procedures performed;
- Duration of the participation;
- Known or possible risks and benefits;
- Contact person in case of events or doubts;
- Financial compensation, when applicable;
- Guarantee that participation is entirely voluntary, that participants can withdraw from the study at any time, and that all study records will be kept confidential.

It is important that people decide whether to participate in a clinical trial or not, only after they have a clear understanding of the whole process and the risks that could be involved. [33]

3.3 Screening and Safety Procedures

The use of screening tests to determine if candidates are suitable subjects for inclusion in studies is an essential pre-entry activity. [34]

The full screening procedure requires at least one visit to the clinical site, where the participant's health will be properly assessed.

The investigator should select trial subjects according to the CSP and based on the following tests:

- Demographic;
- Medical history, namely previous participation in clinical trials and previous and/ or concomitant medication;
- Physical examination, including vital signs, height and weight;
- 12-lead ECG;
- Blood and urine safety tests;
- Tests for drugs of abuse (such as cannabinoids, cocaine, morphine, benzodiazepines, barbiturates, amphetamines) and alcohol;
- Pregnancy tests in women of childbearing potential.

Less frequently, according to the investigational product, other tests may be warranted, like echocardiogram, respiratory function tests, genetic tests and tests to exclude active or recent infections.

Minor out-of-range results from blood and urine safety tests parameters, as well as minor ECG variations, are normal findings and common in healthy subjects. They are not clinically relevant and do not justify the exclusion of subjects from the participation in a trial. Their clinical relevance should be evaluated by a physician based on clinical judgment or the protocol may define which values are allowed or not. [29]

3.4 Criteria for Selecting a Population

Establishing a study's population is a crucial step for the success of the entire experimental process and each study's protocol has guidelines for who can, or cannot, participate in the study. The eligibility criteria define a set of safety parameters and characteristics to attend in the evaluation of the healthy population. The inclusion (IC) and exclusion criteria (EC) are required to guarantee that high-quality CSPs are available and must be strictly met, so that the population of participants is homogeneous, as much as possible. This enhances the subject's safety and allows more valid results. [35]

I. Inclusion and Exclusion Criteria

IC are made up of a set of key features that define characteristics that the target population must mandatorily present. Typically include demographic data (age and gender), geographic, racial and general health status.

EC are conditions that, when present, make the participation unfeasible. Depending on the CSP, they usually refer to current or previous diseases or conditions or abnormalities in the exams performed that may represent a risk for the subject or impact on the data obtained.

If at least one IC is not fulfilled or at least one EC is fulfilled, the subject won't be enrolled in the study.

The choice of the appropriate eligibility criteria is an important step as they are the result of a balance between subjects and data protection and trial's exequibility.

Clinical trials should have as few EC as possible to preserve the necessary number of participants and make feasible the recruitment. [36][35]

Table 2 - Examples of eligibility criteria selection. [37]

IC	EC
Healthy male and females of non-childbearing potential, 19 to 50 years of age (inclusive).	History or presence of alcoholism or drug abuse within the past 2 years.
Body Mass Index (BMI) ≥ 18.5 and ≤ 32.0 (kg/m2) and body weight of no less than 50 kg.	Use of any prescription medication within 14 days prior to dosing.
No use of tobacco or nicotine containing products for a minimum of 6 months prior to dosing.	Participation in another clinical trial within 28 days prior to dosing.
Medically healthy with no clinically significant screening results.	Blood donation or significant blood loss within 56 days prior to dosing.
Subject understands study procedures and provides written informed consent for the tria.	Surgery within the past 90 days prior to dosing as determined by the Principal Investigator to be clinically relevant.

IV. VITAL SIGNS PARAMETERS

4.1 Vital Signs Definition

Vital signs are objective measurements of the body's basic functions. Being an area of active research and a fundamental component of the physical examination, the traditional vital signs include Blood Pressure, Heart Rate, Respiratory Rate and Body Temperature. These will be described in more detail below and are considered an important tool for diagnosis and subject protection. [38][39]

4.2 The Four Traditional Vital Signs

4.2.1 Body Temperature

Body temperature, often known as the first vital sign, is regulated in the hypothalamus and can be very helpful in providing information about the physiological state of a person. Temperature can vary depending on both internal and external factors and cannot be measured in the absence of a correlation with which part of the body the measurement is taken at, physical activities that were recently made, menstrual variation and time of the day. [40][41]

Many types of thermometers may be used to measure temperature (infrared, axillary, etc.), as well as different sites of measurement (frontal, wrist, axilla, tympan). The reference values vary according to that. Depending on the site of measurement, a fever can be considered above 38.2°C if taken orally and 37.8°C if tympanic. When the temperature is abnormal is a clinical sign. In a simple way, above 38°C is called fever, below 35°C is called hypothermia. [41][42]

The normal human body temperature can range from 36.5 to 37.5°C. [43]

4.2.2 Heart Rate

A human healthy heart supplies the body with the right amount of blood and nutrients and removes waste products. The cardiac cycle begins with deoxygenated blood going through the lungs and pumping newly oxygenated blood to the body through the aorta.

For each complete cardiac cycle, there's a heartbeat and the number of beats per minute (bpm) determines the heart rate (HR), or pulse rate (PR).

HR can be measured at several points in the body, at the wrist or at the neck where an artery is under the skin or using a stethoscope to directly listen to the heartbeat. [41]

It is important to identify whether the pulse is in a steady, regular rhythm. The normal resting HR for adults is between 60 and 100 bpm and although there is a wide range of normal, an abnormally high (tachycardia) or low HR (bradycardia) may indicate an underlying problem or be a physiological response. [40]

Acceptable ranges

The HR measurement technique should be defined in the trial protocol or in a Standard Operating Procedure (SOP) of the site. To avoid error extension, some protocols define that it should be evaluated over a 60-second cycle, when evaluated manually. [5]

A consensus document on eligibility criteria for clinical trials [5] defined a set of reference ranges of several parameters to attend when deciding on criteria. The recommendations that follow arise from this article.

I. FIH trials

As an IC, a resting HR of 50 to 90 bpm is recommended. Subjects with a heart rate of 45 to 50 bpm can be admitted if they have a normal thyroid function, no clinical symptoms of bradycardia, and no obvious signs of other diseases that cause bradycardia (as hypothyroidism). This may require additional risk-based medical evaluations of the subject. FIH trials should not include subjects with a heart rate below 45 beats per minute.

II. Trials with clinically established IMPs

50 to 90 bpm for HR at screening is appropriate. In the interval 45-50 bpm, the considerations for FIH trials also apply here. Values under 45 bpm may be an option, however, this involves confirmation of normal cardiac function through echocardiography and stress testing and, of course, the consideration of this option only is valid in the case the IMP doesn't have the potential to induce bradycardia. On a risk-based approach, the decision

to enroll subjects with a HR below the range of 50 to 90 bpm at screening may be explained in the trial protocol.

Table 3 - Heart rate: clinically acceptable ranges. [5]

First-in-human trials	- Range between 50 and 90 bpm is recommended. - Below 45 bpm is not acceptable.
Trials with clinically established IMPs (e.g. bioequivalence trials for generic medicines applications).	- Range between 50 and 90 bpm is recommended. - Below 45 bpm can be acceptable.

4.2.3 Respiratory Rate

Respiratory rate (RR) is the number of breaths a person takes per minute. In practice, the RR can be measured by counting the number of times the chest expands and contracts per minute during breathing. Abnormal changes in RR may be a sign of disorders like sleep apnea, chronic obstructive pulmonary disease, anemia, and asthma. [41][44]

Normal rates for an adult range from 12 to 20 breaths per minute. The aim of measuring RR is to determine whether the rates are higher (tachypnea) or lower (bradypnea) than normal. There is also Apnea, the absence of breathing, which refers to an interruption of airflow to the lungs for a period. [40]

In the absence of illness or response to physical exertion for example, RR should be normal or practically normal. It assumes relevance in terms of eligibility in clinical trials in the case of an IMP with the potential to cause major changes at this level.

4.2.4 Blood Pressure

Blood pressure (BP), measured in millimeters of mercury (mmHg), is an important vital sign that helps to know the condition of the patient, as an element of diagnosis (e.g., hypertension) or follow-up (clinical evolution) of several diseases. [41][46]

Two numbers are recorded when measuring BP. The first and highest is called Systolic Blood Pressure (SBP), in consequence of the heart's contraction, and refers to the pressure inside the artery when the heart contracts and pumps blood through the body. The

lower number, called Diastolic Blood Pressure (DBP), indicates how much pressure the blood is exerting against the arteries when the heart is in diastole. [45]

Appropriate enrollment ranges and measurement procedures should be specified in the protocol or in a dedicated SOP. It is a general recommendation that measurements be taken after 5 minutes of rest in a sitting position with feet flat on the floor and that the correct cuff is used in the proper position to obtain accurate and reproductible results. [5]

Blood pressure is typically written as the systolic over the diastolic pressure and BP readings of less than 120/80 mmHg are considered normal. [47][48]

4.3 Vital signs and Related Diseases Identification

A continuous variation in any vital sign can raise the suspicion of an illness. For several disorders, medical research has classified vital signs critically levels. The goal is to find trends in those changes to predict future anomalies. Table 4 shows the signs associated with the abnormal values. [49]

Table 4 - Nomenclature for vital signs abnormalities. [49]

Sign	Definition
Tachycardia	Heart rate greater than 100 bpm
Bradycardia	Hear rate lower than 50 bpm
Hypertension	Systolic BP ≥ 160, diastolic BP ≥ 90
Hypotension	Systolic BP ≤ 90, diastolic BP ≤ 60
Fever	Temperature above 37.9
Hypothermia	Temperature below 36.1

4.4 Analysis of a Population Enrolling Phase I trials

Changes in physiological parameters, or vital signs, may precede or mean health problems. As a result, a vital sign that deviates from normal thresholds must be evaluated to identify a disease or a risk. [49]

The evaluation of a parameter, in this case, a vital sign, starts with the definition of reference intervals. [50]

I. Establishment of Reference Intervals

A reference range is described as a collection of values within which 95 percent of the normal healthy population falls for the variable being measured. [51] Because 5% of healthy individuals will have values outside the intervals, the term reference interval is preferred over the term normal interval.

The first step for the establishment of reference intervals is to create a histogram, as shown in Figures 2, 3 and 4. On the *x* axis are the values of the parameter under study and on the *y* axis is the frequency with which each value was obtained. The next step is to calculate the reference limits and the final step is to compare the obtained reference interval to the ones determined in the literature. [50]

The accuracy of the dispersion measurements is dependent on the population having a normal distribution (also known as Gaussian distribution), where the distribution is symmetric around the mean. The standard deviation (SD) characterizes population dispersion. [52]

This study considered a sample of 526 healthy subjects that participated in Phase I trials conducted at a Portuguese CRO, during the year 2020, to determine the reference intervals (two SD above and below the mean of the study variable).

All subjects enrolled in this database were eligible for Phase I studies after the screening process. A total of 2083 vital signs were assessed (including systolic blood pressure, diastolic blood pressure, heart rate and body temperature), of which only 92 (4.4% of the total of tests performed) were outside the reference range and considered Non-Clinically Relevant (NCR).

a) Blood Pressure

From 01 January to 31 December 2020, 526 healthy subjects participating in Phase I trials met the eligibility criteria.

For SBP, only 11 (2.1%) subjects had abnormal NCR assessments (10 subjects had low SBP and 1 subject had high SBP). For DBP, 27 (5.1%) abnormal NCR classifications were assessed (of which 19 subjects had low DBP and 8 subjects had high DBP).

The reference range calculated for this pool of subjects (mean ± 2SD) for SBP was 86-134 mmHg and for DBP was 55-88 mmHg.

Figure 2 depicts normal distributions for BP observations (A - Systolic Blood Pressure, B - Diastolic Blood Pressure).

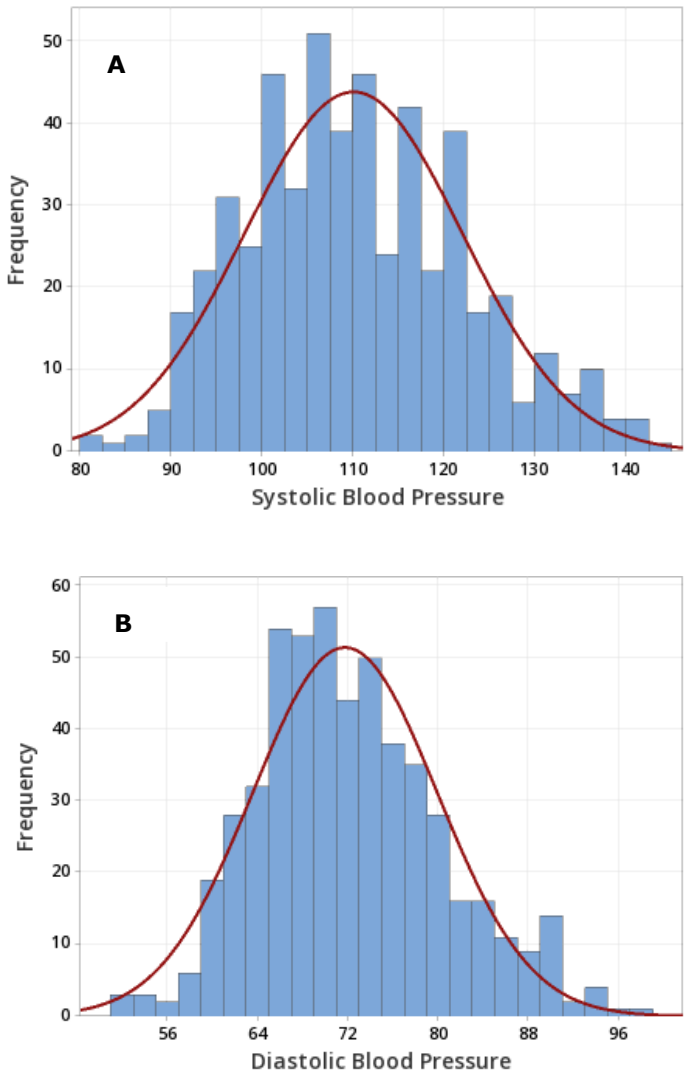

Figure 2 - Frequency distribution for Blood Pressure observations.

b) Heart Rate

The American Heart Association states the normal HR for an individual is 60-100 bpm. [53] The analysis performed on this study calculated a lower reference range for HR: 51-92 bpm.

In a total of 526 assessments for HR, there were 54 (10.3%) with abnormal NCR results (50 of them with heart rate below normal and 1 above the threshold).

However, people who practice intense physical exercise regularly often have a lower heart rate, without any clinical meaning. Because their heart muscle is in better shape and

doesn't have to work as hard to keep a steady beat, active people often have a lower resting HR. A low or moderate level of physical activity usually has a minor effect on the resting pulse. [53]

As we can see in Figure 3, this analysis also followed a normal distribution.

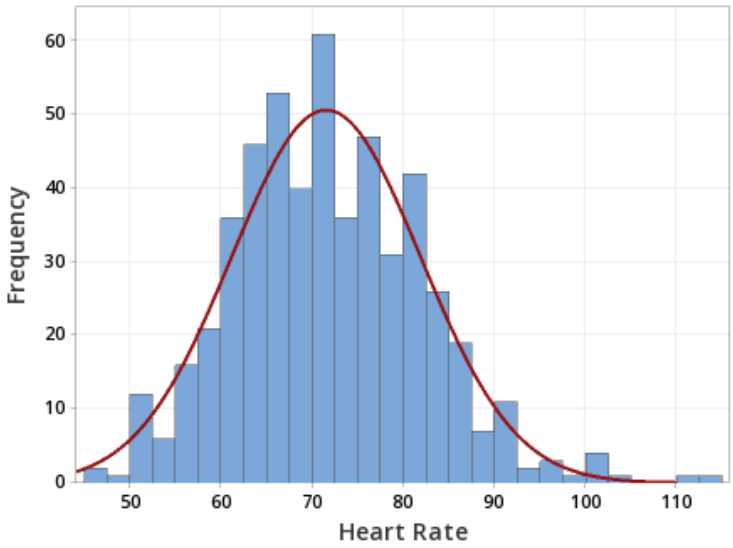

Figure 3 - Frequency distribution for Heart Rate measurements.

c) Body Temperature

Finally, this study analyzed the body temperature results in 505 healthy subjects. The reference range obtained was 36.3°C–37.5°C. There were no abnormal results assessed for body temperature in this database.

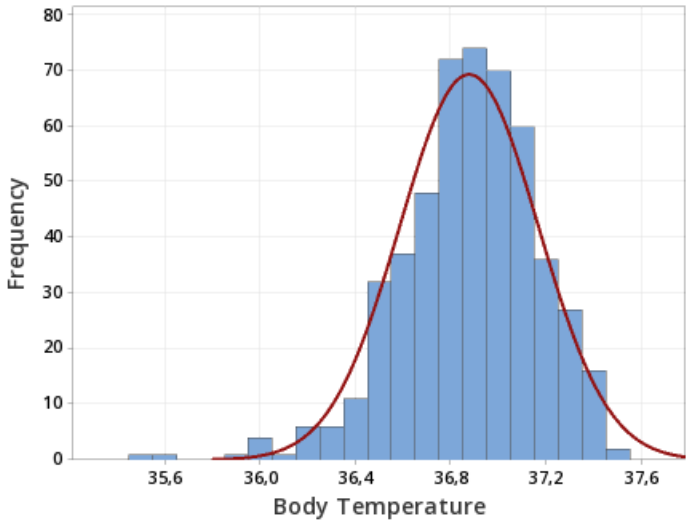

Figure 4 - Frequency distribution for Body Temperature measurement.

II. Vital Signs Classification

The classifications performed by the research physicians of the values obtained from this population are presented in Figure 4.

Heart rate has the highest number of abnormal assessments (54 subjects out of 526 - 10.3%), followed by diastolic blood pressure (with 27 abnormal classifications out of 526 - 5.1%) and systolic blood pressure presenting 11 abnormal assessments (2.1%). Body temperature values were all between the defined thresholds.

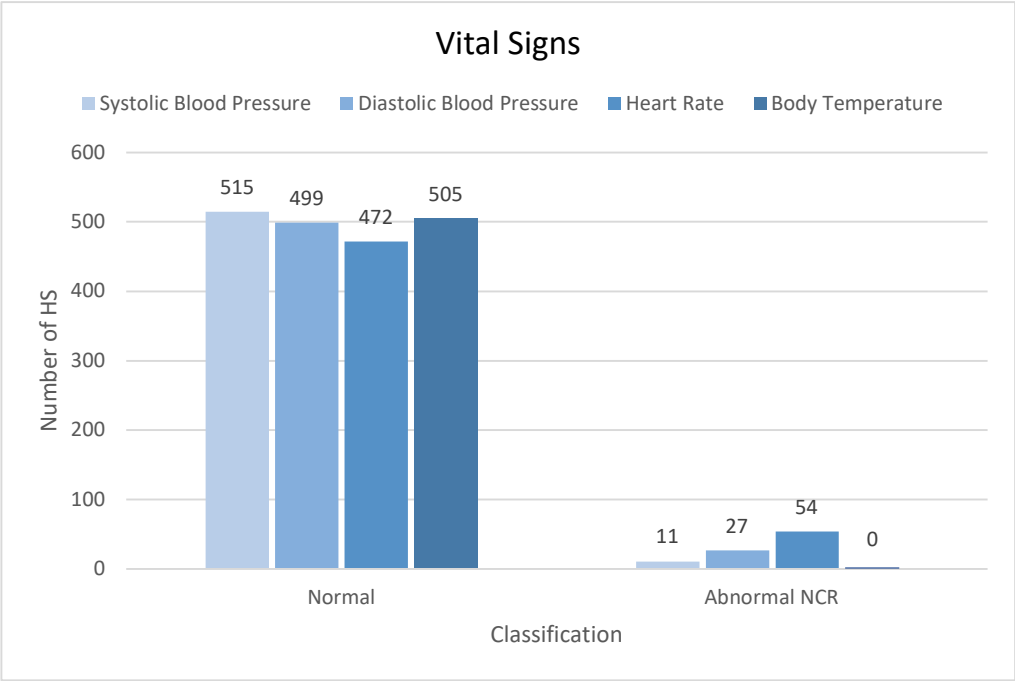

Figure 5 - Normal and Abnormal NCR classifications in this study population.

Less than 5% of the values fell outside the reference range which can be explained by the fact that normal parameters cover only 95% of the population.

All these 92 abnormal results, even considering that they are below normal, have no conceivable pathologic significance if in the absence of other abnormalities.

III. Reference Ranges Compilation

The detailed results of screening reference range from this population are presented in Table 5.

This report presents vital signs distribution and allowed the establishment of reference values, providing clinical pharmacologists data obtained under the real conditions of Phase I studies, improving the ability to screen healthy subjects and to analyze what may, or not, be Clinically Relevant (CR).

The reference values in this report are not very widespread due to the elimination of all participants with any findings that were considered not eligible during the screening process. [54]

Table 5 - Healthy subjects' population: screening reference values for vital signs.

Vital Sign	Unit	Number of subjects	Mean	Standard Deviation	Minimum	Maximum	Reference Range
Systolic BP	bpm	526	110.08	11.967	81	145	86-134
Diastolic BP	mmHg	526	71.74	8.168	52	98	55-88
Heart Rate	bpm	526	71.55	10.355	46	114	51-92
Body Temperature	°C	505	36.88	0.291	35.5	37.5	36.3-37.5

IV. Comparison to the CRO Reference Ranges defined by SOP

Table 6 illustrates the ranges obtained in this analysis and those defined by the site SOP.

Interestingly, if vital signs criteria were updated based on values derived from the normal distribution obtained under the analysis of this database, using the mean ± 2 times the standard deviation, the definition of low SBP would be revised from <90 mmHg to <86 mmHg and DBP would be revised from <60 mmHg to <55 mmHg decreasing even more the incidence of low Blood Pressure.

In case of the Heart Rate, as 50 of the 54 abnormal NCR assessments were obtained with HR below normal, if the definition of low HR would be revised from <60 mmHg to <51 mmHg, only 6 subjects would have results below the reference range, resulting in a significative decrease on the incidence of low Heart Rate.

Table 6 - Comparison to reference ranges defined by SOP.

Vital Sign	Reference Ranges defined by SOP	Reference Ranges obtained from the 2020 Database
Systolic Blood Pressure	90 ≤ PAS ≤ 140 mmHg	86 ≤ PAS ≤ 134 mmHg
Diastolic Blood Pressure	60 ≤ PAD ≤ 90 mmHg	55 ≤ PAD ≤ 88 mmHg
Heart Rate	60-100 bpm	5-92 bpm
Body Temperature	35.5-37.5°C	36.3-37.5°C

V. Comparison to Reports from Literature

The reference values established in this report are comparable to those already used by the medical community. The normal ranges used to compare with the ones obtained in this analysis are referenced in MedlinePlus Medical Encyclopedia, a service provided by the U.S. National Library of Medicine.

Table 7 - Comparison of reference ranges defined on this assay with those provided by the U.S. National Library of Medicine. [55]

Vital Sign	U.S. National Library of Medicine	Reference Ranges defined by this Assay
Heart Rate	60-100 bpm	51-92 bpm
Systolic Blood Pressure	90-120 mmHg	86-134 mmHg
Diastolic Blood Pressure	60-80 mmHg	55-88 mmHg
Temperature	36.5-37.3 C	36.3-37.5°C

As we can see in Table 7, the values obtained on this assay are similar although less rigid to those used by clinicians. That can be due to the fact that 2 standard deviations were used to define the reference range, resulting in a broader range.

It is important to keep in mind that the stringency will always depend on the IMP. If an IMP has the potential to severely affect any vital sign, that must be considered when defining the ranges for the study.

V. ELECTROCARDIOGRAM

An electrocardiogram (ECG) is a medical test commonly used to evaluate cardiac health. In the clinical trials context, it is used during screening to evaluate the health status and during the clinical trial conduct to detect potential cardiac abnormalities induced by the study drug.

ECGs provide relevant information on rhythm, conduction, ventricular repolarization, ischemia, etc. [56]

Cardiac muscle contracts in response to electrical depolarization of the myocardial cells and relax due to repolarization. This electrical activity is amplified and recorded via electrodes placed on the patient's skin and transcribed onto a graph paper to produce the electrocardiogram tracing. [57]

Electrocardiograms have a large spectrum of variability over short periods and the effect of the drug should be defined comparing to a group that received a placebo or to a baseline ECG. Variation on interval data (RR, PR, QRS and QT, which will be described forward) must be assessed to determine if there is an indicator of any cardiac effect from the drug. [56]

Interpretation of the ECG is fundamentally about understanding that normal conduction starts and propagates in a predictable pattern. Nevertheless, variants of normal are common and deviation from this pattern is not necessarily pathological. [57]

This technology is particularly attractive by being so simple, portable, cheap, and based on direct visual assessment. [58]

5.1 The Components of the ECG

There are three main components to an ECG: the P wave, which represents atrial depolarization, the QRS complex, representing ventricular depolarization, and the T wave, which represents ventricular repolarization. [59]

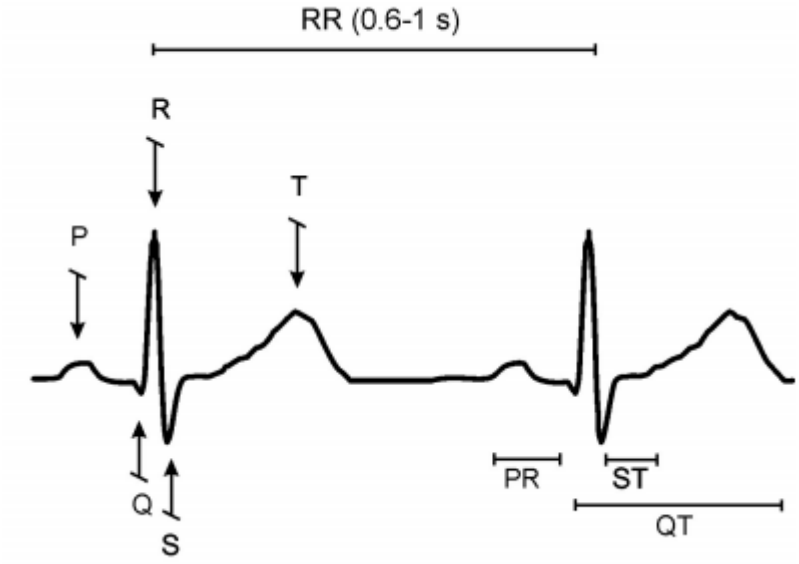

Figure 6 - Schematic representation of an ECG. [51]

To interpret an electrocardiogram, it is important to understand each individual wave and their interaction:

- QRS complex: Represents ventricular depolarization. Even though not every QRS in an ECG contains all three waves (Q, R, S), the QRS complex is always present although may or may not be considered normal. Wide complexes are due to aberrant intraventricular conduction. [52][59]
- RR interval: The interval between two consecutive R waves peak, that is the time between two QRS. It's very useful to identify an irregular rhythm. [60]
- PR interval: Also known as PQ interval (R wave is not always present), PR interval extends from the beginning of the P wave to the beginning of the QRS complex, reflecting the conduction of electrical signal from the atria to the ventricles. Alteration in the PR interval represents abnormal conduction like, for example, an atrioventricular (AV) block. [59]
- ST segment: ST segment represents the time from the end of the S wave to the beginning of the T wave and occurs when the atria are relaxed and the ventricles are depolarized. The normal ST segment is isoelectric (flat on the baseline). Variation like depression or elevation may indicate cardiac pathology, namely, myocardial ischemia. [60]

5.2 QT Interval and Main Concerns on Clinical Trials

The QT interval is measured from the beginning of the QRS complex to the end of the T wave, expressing the whole process of ventricular depolarization until complete repolarization. [60]

This electrical activity occurs through channels that manage the rapid movement of ions (sodium, calcium, and potassium) through the cellular membrane, leading to normal myocardial depolarization. When the outflow of potassium ions of cardiac cells exceeds the inflow of sodium and calcium, repolarization occurs. If these ion channels are malfunctioning, an excess sodium inflow or a decreased potassium efflux may result. This intracellular excess of positively charged ions leads to an extended repolarization phase, resulting in a prolonged QT. A prolonged QT interval predisposes to a potentially fatal ventricular arrhythmia. It may be a consequence of ions deficiency or drugs. [61]

The QT interval has gained particular importance in clinical trials. Prolongation of the QT interval induced by drugs has been the most common cause of delays in drug development of noncardiac drugs, non-approvals, and post-marketing withdrawals by the FDA. [62]

The QT interval demonstrates daily variability, with the longest values occurring during sleep. As such, it is important to assess the impact of drugs on the QT interval at the same time of day. For even more rigorous results, other factors should be taken into account, such as food, sleep, body position, gender, and environmental factors. [63]

QT Interval Correction Formulae

The QT interval varies inversely with the heart rate. The faster the HR (or shorter the RR interval), the shorter the QT interval. That's why the measured QT intervals are adjusted for heart rate to allow correct evaluation of variations from baseline.

Various correction formulae have been proposed, the most used in clinical trials are Bazett's and Fridericia's.

(1) Fridericia's correction: $QTc = QT/RR^{0.33}$

(2) Bazett's correction: $QTc = QT/RR^{0.5}$

Since the best correction method is a point of discussion, it is of current use the application of both formulae.

In clinical practice and the medical literature, Bazett's correction is widely used. However, it is not optimal since it overcorrects at high heart rates and under corrects at heart rates below 60 bpm. In subjects with such different heart rates, Fridericia's correction is more precise than Bazett's correction. [64]

5.3 Analysis of a Population Enrolling Phase I trials

The role of ECGs as a screening tool implies the definition of normal reference ranges for key parameters (complexes, intervals, segments). Many publications provide information on the definition of normal limits for ECG parameters although they are fairly representative of a homogeneous healthy population and the data is collected from small samples of healthy subjects. [65]

I. Establishment of Reference Intervals

The reference interval of a population of 166 subjects participating in Phase I trials conducted at a Portuguese CRO, during the year of 2020, was determined by calculating two SD above and below the mean of the study variable, as described in the previous chapter for the vital signs.

All subjects enrolled in this database were accepted in Phase I studies after the screening process. A total of 508 ECG assessments were made (including Heart Rate, PR Interval, QRS Duration, QT Interval, QTcB Interval, QTcF Interval and RR Interval). Of all 166 subjects under analysis, 92 (55.4%) had results outside the normal range and considered NCR and 74 (44.6%) were normal.

a) QT, QTcB and QTcF Intervals

For QT Interval, the analysis of 20 subjects under the same conditions allowed the calculation of the following reference range, 358-455 milliseconds (ms). For QTcB, the same 20 subjects had this parameter assessed, resulting in a reference range of 354-444ms. For QTcF, a larger sample of participants was used to calculate the reference interval, with 144 subjects under analysis and resulting in a range of 358-442ms.

In Figures 7, 8 and 9 are the histograms and normal distributions for QT, QTcB and QTcF observations. This type of graph is particularly useful for visualizing how data is spread out or distributed around the mean.

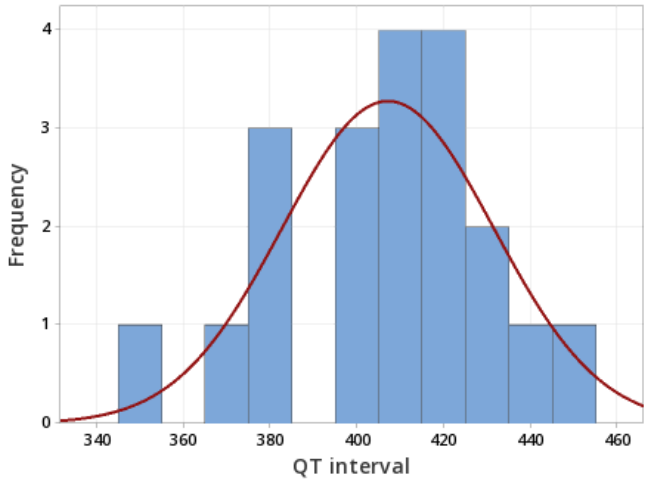

Figure 7 - Frequency distribution for QT Interval observations.

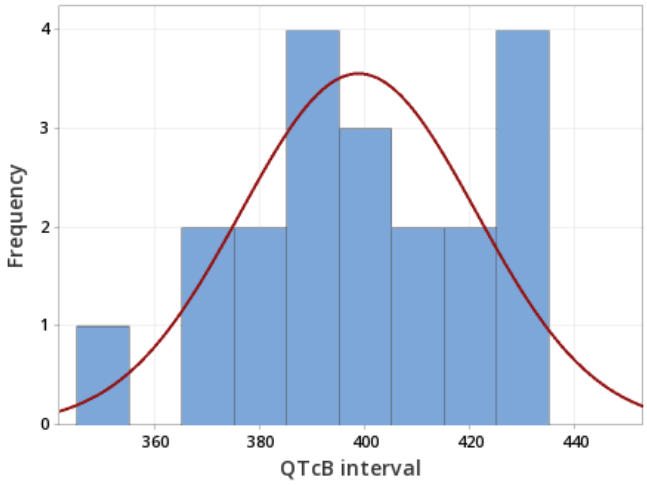

Figure 8 - Frequency distribution for QTcB Interval observations.

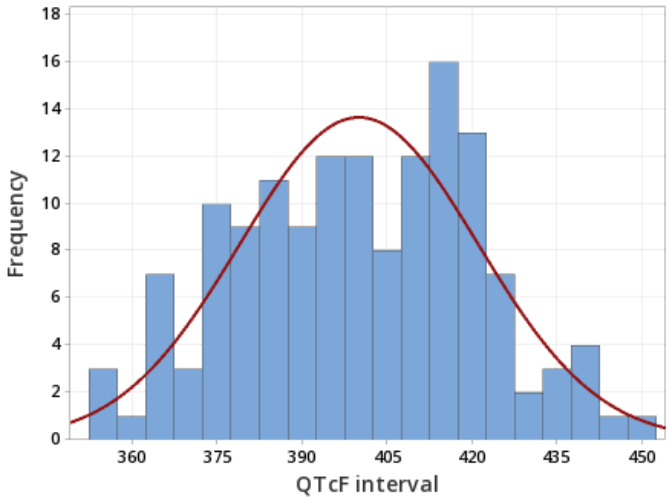

Figure 9 - Frequency distribution for QTcF Interval observations.

b) Heart Rate and RR Interval

For Heart Rate, 68 subjects' assessments were used to determine the reference range for this parameter. The lowest value obtained was 46bpm and the highest value was 90bpm. The reference interval was 44-79 bpm, although as we can confirm from the maximum value obtained, values outside this range are not considered pathologic and subjects with results superior to the ones defined by the range can still be admitted to Phase I trials, if consented by the medical evaluation.

For RR Interval, 20 subjects' assessments were used to determine the reference range, resulting in a range of 832-1261ms. The minimum value was 882ms and the maximum was 1250ms.

Figures 10 and 11 present the frequency distribution for Heart Rate and RR Interval.

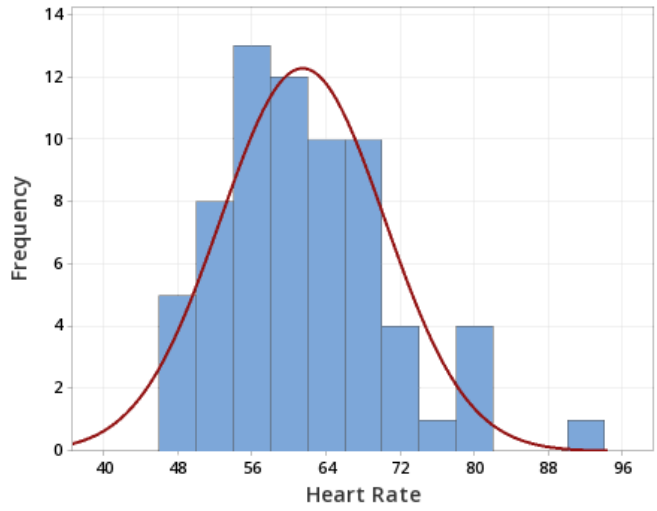

Figure 10 - Frequency distribution for Heart Rate observations.

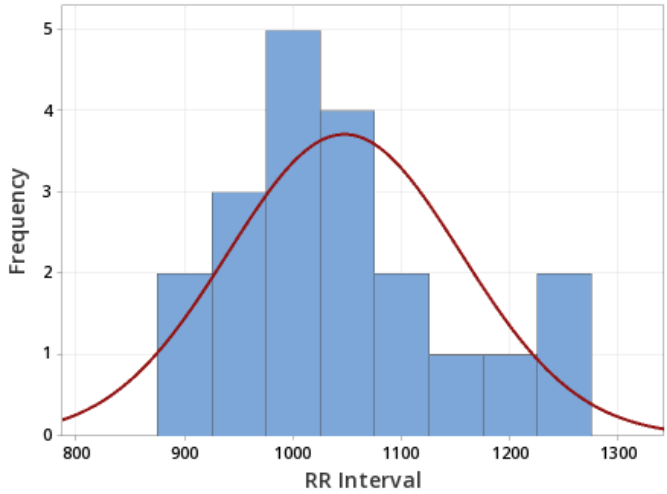

Figure 11 - Frequency distribution for RR Interval observations.

c) PR Interval and QRS Complex

To calculate the reference range for PR Interval and QRS Complex, 118 subjects had their ECGs assessed and the ranges that were obtained were 121-193ms and 71-107ms, respectively. PR Interval minimum value was 114ms and maximum was 200ms and for QRS Complex, 66 and 122 were the highest and lowest assessments.

The frequency distributions for both parameters can be seen below, in Figures 12 e 13.

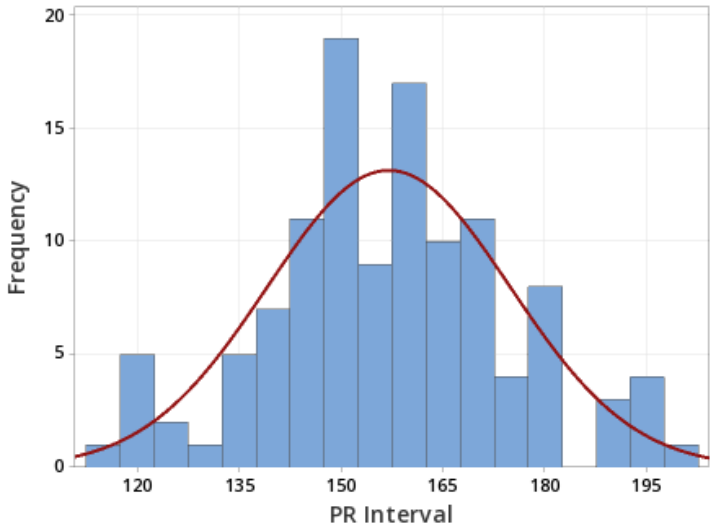

Figure 12 - Frequency distribution for PR Interval observations.

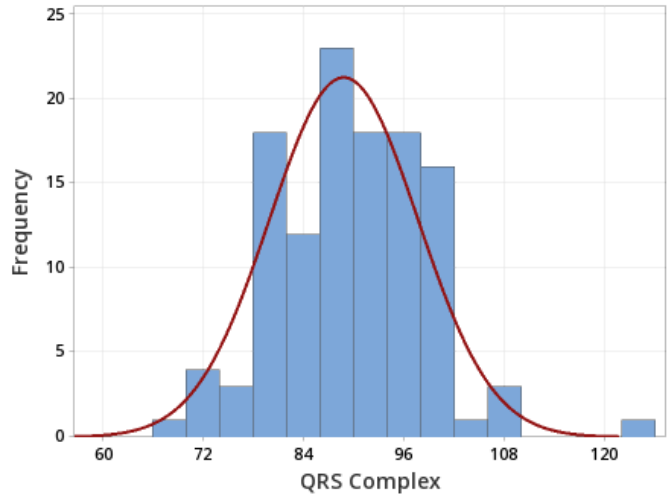

Figure 13 - Frequency distribution for QRS Complex observations.

II. Reference Ranges Compilation

This report presents ECG parameters distribution and defines reference limits using the values obtained in one phase I Unit. Each subject was eligible after the screening process, providing a term of comparison with other phase I units.

The detailed results of the screening reference ranges obtained from this population are presented in Table 8.

Table 8 - Healthy subjects' population: screening reference values for ECG Parameters.

ECG Parameter	Unit	Number of subjects	Mean	Standard Deviation	Minimum	Maximum	Reference Range
QT	ms	20	407.1	24.35	354	450	358–455
QTcB	ms	20	398.65	22.42	354	434	354–444
QTcF	ms	144	400.06	21.03	354	448	358–442
HR	bpm	68	61.46	8.84	46	90	44–79
PR	ms	118	156.98	17.89	114	200	121–193
QRS	ms	118	88.81	8.85	66	122	71–107
RR	ms	20	1047.15	107.37	882	1250	832–1261

III. Reports from Literature

Olbertz *et al.* analyzed ECGs from all healthy subjects accepted into Phase I clinical trials in Celerion's proprietary ClinQuick database, conducted from 2005 to 2014, to establish reference ranges for HR, PR, QRS, QTcB and QTcF.

The analysis population included 39,303 complete tracings and the normal ranges defined for this team for ECG intervals can be seen in Table 9. [66]

Table 9 - Observed normal ranges and suggested inclusion criteria for Healthy Subjects. [66]

ECG Parameters	Observed Normal Range		Suggested Healthy Subject Inclusion Criteria
	Low	High	
QT (ms)	333	450	≤ 450
QTcB (ms)	350	446	≤ 450
QTcF (ms)	351	438	≤ 450
HR (bpm)	47	88	50-100
PR (ms)	120	196	≤ 200
QRS (ms)	72	112	≤ 110

This is the largest database of its kind in healthy subjects, including a wide ethnically diverse population, and provides a scientific basis for establishing normal ranges applicable to the screening of participants to Phase I trials. These findings can only be used for early clinical trials assuming no prior evidence of cardiac pathology. [66]

IV. Comparison of Reference Ranges

As both reference ranges were obtained across a population of subjects enrolled in Phase I trials, it would be interesting to ascertain for discrepancies or similarities in the healthy population.

Olbertz *et al.* used a much broader population, however, the ranges obtained in this study are particularly similar and can be included in the suggested inclusion criteria defined by Olbertz *et al.* (except for QT interval, which was above the suggested IC).

Table 10 - Comparison of reference ranges defined on this assay with the ones established by Olbertz *et al.* [66]

ECG Parameter	**Olbertz et al.**	**Reference Ranges define by this Assay**
QT	333 - 450	358 - 455
QTcB	350 - 446	354 - 444
QTcF	351 - 438	358 - 442
HR	47 - 88	44 - 79
PR	120 - 196	121 - 193
QRS	72 - 112	71 - 107
RR	-	832 - 1261

5.4. Morphological Abnormalities on Baseline ECGs

Specific thresholds are required in Phase I trials to identify a normal value and an abnormality. Such thresholds must be dependent on reference range limits as well as a deviation from baseline. Minor abnormalities with no pathological relevance are common in healthy subjects. [54][5]

The clinical significance of an abnormality depends on the physician's evaluation of that data along with the whole other clinical information.

5.4.1 Reports from literature

Besides the work provided by Olbertz et al., there are other two large studies on ECG intervals in healthy volunteers:

I. Dmitrienko *et al.,* looked at the ECG intervals and heart rate reference levels in baseline ECG recordings from 13,039 clinical trial participants. However, they did not look into morphological ECG abnormalities. [65]

II. Mason *et al.* published a report on drug-free ECGs from 79,743 subjects aiming to identify reference ranges for ECG intervals. In this study, were omitted 8455 (15.5%) of the subjects due to morphological ECG abnormalities. [67][68]

As there was lack of evidence in the literature on morphological abnormalities, Hingorani et al. investigated the occurrence of morphological anomalies in baseline ECGs in 3978 healthy subjects participating in clinical trials. [68]

ECG data was analyzed from 62 phase I studies performed globally by different pharmaceutical companies between 2005-2009 and subjects were rigorously screened for all studies using a combination of history, physical examination, and laboratory tests to exclude any co-morbidities.

They excluded subjects with a history of long QT syndrome or other cardiac dysfunctions and the research only included subjects with normal resting blood pressure and resting heart rate.

Table 11 - Inclusion range used on a population of 3978 HS to detect morphological anomalies in baseline ECGs. [68]

Vital Sign	Reference Range
Systolic Blood Pressure	>90 mmHg and <140 mmHg
Diastolic Blood Pressure	>60 mmHg and <90 mmHg
Heart rate	>50 bpm and <100 bpm

Their results revealed that morphological abnormalities in ECG were frequent: 1015 (25.5%) of the 3978 subjects had an abnormal ECG with a higher frequency in males (731 subjects, 29.3%) than females (284 subjects, 19.2%).

The 10 most common abnormalities can be seen in Table 12. The team found that the most prevalent abnormality was sinus bradycardia (heart rate <50 bpm), observed in 312 (7.8%) of the subjects although it may occur due to diurnal variations, during sleep, at rest and it is also common in individuals that practice regular exercise. [68]

Table 12 - The 10 most common abnormalities in baseline ECGs of 3978 healthy subjects participating in 62 Phase I studies. [68]

Morphological Abnormalities	All Subjects (n=3978)	%
Sinus Bradycardia	312	7.8
Right Axis Deviation	130	3.3
Non-Specific T Wave Changes	98	2.5
Intraventricular Conduction Delay (IVCD)	90	2.3
Prolonged QT	90	2.3
First Degree A-V Block	88	2.2
Ectopic Atrial Rhythm	84	2.1
Short PR Interval	48	1.2
Left Ventricular Hypertrophy By Voltage Only	46	1.2
Left Axis Deviation	30	0.8

This study proved that morphological ECG abnormalities were common and when these are found during regular health check-ups, it's important to notice that they can occur in healthy subjects, without pathological significance. [68]

To check whether these morphological abnormalities remain or if some of them were only temporary and vanish over time, Hingonari et al. analyzed 16,472 ECGs from 19 Phase I studies, recorded at multiple timepoints from 420 healthy subjects, demonstrating that temporary ECG changes occur spontaneously in the presence of placebo and that those abnormalities would revert to normal during the trial in 45% of the subjects.

The results revealed that 8.1% of the subjects had sinus bradycardia at baseline, which only persisted throughout the trial in 2.1% of subjects, underlining that this abnormality is not uncommon and may occur due to physiological changes or food intake. [69]

Taken together, these findings indicate that when referring to screening parameters, all changes must be carefully interpreted as they may not reflect disease or adverse reactions caused by an IMP. As a result, assessing safety signals in a clinical trial remains a complex medical decision that requires the clinical judgment of a highly qualified and experienced physician. [29]

5.4.2 Reports from a Phase I Unit Database

ECG data pooled from 16 phase I studies conducted at a Portuguese CRO, during the year 2020, was used to analyze the measurements range and the type of abnormalities that were more frequent. The objective was to obtain data of ECG findings in a healthy cohort and to compare those data with reports from other authors.

This research included only healthy subjects that met all eligibility criteria at screening. For each ECG, HR, PR interval, QRS duration, QT interval, QTcB interval, QTcF interval and RR interval were measured.

The unit follows the reference values defined by SOP but outside of this set are clinical trials in which the protocol or other specific trial documents define reference values other than those defined by SOP. (Table 13)

Table 13 - ECG reference ranges used by the Phase I Unit.

Parameter	Criteria
Heart Rate	60 to 100
RR Interval	600 to 1000
PR Interval	120 to 200
QRS Complex	80 to 100
QTc Interval	Men: 350 to 440
	Women: 350 to 460

The investigator must verify that the values are within the range of reference values defined by SOP or by the protocol or other specific documents of the study. If outside that range, the investigator classifies the abnormality as clinically relevant or not.

In this analysis were included 526 subjects, with 276 (52%) of them presenting Abnormal NCR results and 250 (48%) presenting normal results.

A total of 335 abnormalities was seen. On these 276 subjects with Abnormal NCR results, 62.3% had Sinus Bradycardia, 18.8% Incomplete right bundle branch block and 13.8% Short QRS.

Table 14 - Abnormalities in baseline ECGs of 526 Healthy Subjects participating in 16 Phase I studies.

Morphological abnormalities	**All Subjects (n=526)**	**%**
Sinus bradycardia	172	62.3
Incomplete right bundle branch block	52	18.8
Short QRS	38	13.8
Intraventricular conduction defect	15	5.5
Short PR	14	5.1
Sinus arrhythmia	9	3.3
Left fascicular block	7	2.5
First Degree A-V Block	7	2.5
Non-specific repolarization changes	4	1.5
Ventricular extrasystole	3	1.1
Right axis deviation	3	1.1
Sinus tachycardia	2	0.7
Complete right bundle branch block	2	0.7
Left axis deviation	2	0.7
Short QT	2	0.7
Long QT	2	0.7
Ectopic atrial rhythm	1	0.4

All parameters must be within normal ranges as specified in the protocol.

According to Breithaupt-groegler *et al*., if the heart rate meets the inclusion criteria and the first-degree AV is not interpreted as a sign of cardiac dysfunction/disease, AV block appears to be acceptable. [5] The same happens to the other ECG abnormalities, as they were all classified as NCR.

In conclusion, reports from Hingorani *et al*. [68] and analysis of this 2020 Database demonstrated that non-clinically relevant ECG abnormalities are common in healthy individuals, and do not represent a risk for participation in phase I trials. Some of these so-called abnormalities are just variants of normal.

VI. CONCLUSION

The selection of healthy participants is one of the pillars for phase I trials outcomes. Healthy participants are required for volunteer safety, data quality, and study results reliability. To assess what is considered abnormal or unexpected, it is important to establish what the predicted (normal, reference) values of vital signs and ECG parameters are and mean.

To discriminate between a regular variation from normal and a serious abnormality, appropriate thresholds are required. This study has determined a reference range for vital signs and ECG parameters in a population of healthy subjects participating in 16 Phase I trials and analyzed ECGs morphological abnormalities in the same healthy cohort, which provides a larger population compared with individual studies and may enable discussion on clinical decision-making based on observational data.

As safety parameter deviations from reference intervals are not always pathologic nor represent any additional risk (meaning are not clinically significant), the prevalence of values outside these normal ranges may be analyzed before considering it clinically relevant. Its potential causes can be related to several characteristics of healthy subjects such as food intake, physical activity or inactivity, sex, age, BMI and physiological alterations.

Considering the causes of variability, the likelihood of a given parameter exceeding a predetermined threshold or the presence of morphological ECG abnormalities will be determined by the parameter itself, as well as the frequency and interval of observations. Outside the interval of what is considered "normal", there are also healthy individuals.

The limitations of this assay are that subjects were not chosen at random from the population, as phase I trials often have fewer older participants. However, the rigorous clinical examination at screening that all of them underwent ensures that these subjects are actually healthy, which is a great advantage for this study.

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