UNIVERSIDADE D
COIMBRA

Gabriela Vaz Ramires

Selection Criteria in Clinical Trials in Healthy Volunteers: Hematology and Biochemistry Parameters

Dissertação no âmbito do mestrado em Biotecnologia Farmacêutica orientada pelo Professor Doutor Sérgio Paulo Magalhães Simões e pelo Professor Doutor José Luís de Almeida e apresentada Faculdade de Farmácia da Universidade de Coimbra.

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"Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no oceano. Mas o mar seria menor se lhe faltasse uma gota."

- Madre Teresa de Calcutá

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À minha família, em especial à minha mãe, pelo apoio incondicional que me dão.

Abstract

Criteria selection in clinical trials are divided in inclusion criteria and exclusion criteria. There are several parameters evaluated to ensure that all subjects included in a study meet all the requirements for the drug under study, whether healthy (phase I trials) or not (phase II and III trials), in order to accomplish two major objectives: subject's safety and data reliability.

Within the selection criteria, the analytical parameters play a very important role. In all clinical trials, hematology and biochemistry are always evaluated, namely a standard profile of parameters, adapted or not according to the study drug.

Reference or normal values or ranges are defined for each analyte by the laboratory, attending to the quantification method used. The fact that a parameter is outside the normal range does not necessarily mean that the individual is not healthy. The clinical significance of each abnormality must be assessed in order to evaluate the potential impact on the subject safety.

The aim of this work is to reflect on reference intervals in safety laboratory tests used for screening evaluation of study subjects. It will be used a healthy subjects database from a Portuguese Clinical Research Organization referring to clinical trials performed in the year 2020.

It was realized that reference intervals cannot be faced as fixed and inflexible and that in a healthy population it is possible to find laboratory abnormalities, and this doesn't necessarily mean absence of a healthy status.

Keywords: Clinical trials; Reference values; Phase I studies; Laboratory parameters

Resumo

Os critérios de seleção em ensaios clínicos estão divididos em critérios de inclusão e critérios de exclusão. Existem vários parâmetros avaliados para garantir que todos os sujeitos incluídos num ensaio cumprem todos os requerimentos para o fármaco em estudo, sejam saudáveis (ensaios de fase I) ou não (ensaios de fase II e III), com o propósito de cumprir dois objetivos principais: a segurança do individuo e a confiança dos dados.

Dentro dos critérios de seleção, os parâmetros analíticos têm um papel muito importante. Em todos os ensaios clínicos, a hematologia e a bioquímica são sempre avaliadas, nomeadamente um perfil padrão de parâmetros, adaptados ou não de acordo com o fármaco em estudo.

Valores ou intervalos de referência ou normais são definidos para cada analito pelo laboratório, atendendo ao método de quantificação utilizado. O fato de um parâmetro estar fora do valor normal não significa necessariamente que o indivíduo não seja saudável. A significância clínica de cada anormalidade deve ser avaliada para determinar o impacto na segurança do participante.

O objetivo deste trabalho é refletir sobre os intervalos de referência utilizados nas análises de segurança realizadas na triagem de participantes de ensaios clínicos. Será utilizada uma base de dados de uma Clinical Research Organization portuguesa, com dados de indivíduos saudáveis que participaram em ensaios clínicos no ano 2020.

Compreendeu-se que os intervalos de referência não podem ser encarados como fixos e inflexíveis e que em uma população saudável é possível encontrar anormalidades laboratoriais, o que não significa necessariamente ausência do estado de saúde.

Palavras-chave: Ensaios Clínicos; Valores de referência; Estudos de fase I; Análises clínicas

Table of Contents

List of Figures

List of Tables

List of Acronyms

ADME- Absorption, Distribution, Metabolism and Excretion

AE- Adverse Event

ALP- Alkaline Phosphatase

ALT- Alanine Aminotransferase

AST- Aspartate Aminotransferase

CEIC- Comissão de Ética para a Investigação Clínica

CK- Creatin Phosphokinase

CR- Clinically relevant

CRO- Clinical Research Organization

CSP- Clinical Study Protocol

CTCAE- Common Terminology Criteria for Adverse Events

eGFR- Glomerular Filtration Rate

EMA- European Medicines Agency

FDA- US Food and Drug Administration

FN- False Negative

FP- False Negative

GCP- Good Clinical Practices

GGT- Gamma-Glutamyl Transferase

IND- Investigational New Drug

INFARMED- Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.

LD- Lactic Dehydrogenase

MCH- Mean Corpuscular Hemoglobin

MCHC- Mean Corpuscular Hemoglobin Concentration

MCV- Mean Corpuscular Volume

MSD- Most Successful Dose

NCR- Not clinically relevant

NDA- New Drug Application

NK- Natural Killers

NPV- Negative Predictive Value

PK- Pharmacokinetic

PPV- Positive Predictive Value

PSA- Prostate Specific Antigen

RCB- Red Blood Cells

RU- Relevance unknown

TN- True Negative

TP- True Positive

ULN- Upper Limit of the Normal Range

WBC- Total White Blood Cells

WHO- World Health Organization

1- Introduction

For a new drug to obtain marketing authorization, it is necessary to go through an extensive development process that is divided into the discovery process, pre-clinical development, and clinical development. This process can take an average of 10 to 15 years and costs approximately 1.8 billion dollars. During this time, efficacy, safety, therapeutic dose, and the best route of administration are studied. (Sinha *et al.*, 2017; Lansdowne, 2020)

This whole process is highly regulated by different authorities worldwide. In Portugal the competent regulatory entity is INFARMED, in Europe the European Medicines Agency (EMA) and, in the United States of America, the Food and Drug Administration (FDA). These entities are responsible for regulating the entire process of development of a new drug, ensuring that it follows the Good Clinical Practice (GCP) guidelines. (Sinha *et al.*, 2017; Lansdowne, 2020)

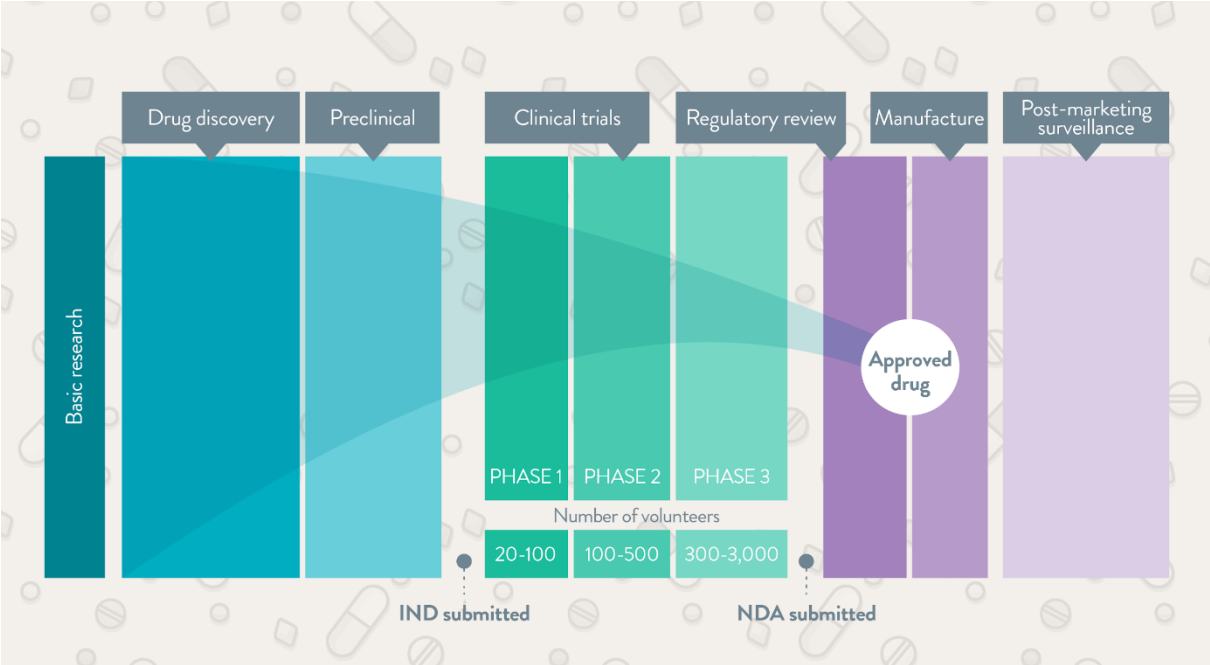

***Figure 1**- Overview of the drug discovery, development, and approval process. Adapted from: Lansdowne, 2020.*

1.1- Discovery and Pre-Clinical Development

The process of developing a new drug begins with the identification of a disease in need for treatment. A molecular target with pharmacological potential is selected and validated. Subsequently, *in vitro* assays are performed and are followed by a high throughput screening of compounds libraries against the target molecule in order to identify hits. These hits are optimized to become lead compounds that have the desired potency and selectivity. (Sinha *et al.*, 2017)

In pre-clinical development, toxicological and safety studies are carried out in order to obtain the maximum safe concentration, to verify possible adverse events and target organs. Studies on the manufacturing process are also conducted (formulation, formulation development control, stability studies) in order to obtain the best cost-benefit ratio. At this moment, clinical protocols are prepared to the conduction of clinical studies. (Singh, 2017a)

1.2- Clinical Development

The process of clinical development of new drugs is conducted in humans, with the objective of discovering or verifying the clinical, pharmacological or pharmacodynamic effects, adverse effects, and the absorption, distribution, metabolism and excretion (ADME) process. The main objective is to evaluate the safety and efficacy of the drug. (Portugal, 2014)

The clinical development is divided into 4 sequential phases: (Singh, 2017b)

- Phase I: In most cases subjects are healthy subjects, but sometimes patients with a disease may be required. Usually, they involve a smaller number of subjects, 20 to 100. It is evaluated the best administration route, frequency, dose and side effects. These trials focus mainly on pharmacokinetic (PK) and safety. (Mahan, 2014)
- Phase II: It is determined the most successful dose (MSD), the dose with no toxicity but with a therapeutic response. Phase II studies evaluate efficacy and treatment benefit but is not presumed to have any therapeutic effect. These studies include 100 to 300 subjects. (Mahan, 2014)
- Phase III: This phase is the most expensive and time-consuming and is the "pre-marketing phase". Usually, 100 to 3000 subjects are recruited. It is compared the efficacy of the study treatment with the standard treatment. (Mahan, 2014)
- Phase IV: After marketing authorization, treatment still needs to be on surveillance because not all safety or efficacy issues have been determined. New clinical indications may be established, or the drug can be removed from the market or restrict to certain indications. (Mahan, 2014)

Clinical studies can be divided into observational studies (without intervention) and interventional studies (with intervention). (Vale, 2007) In observational studies, subjects are not subjected to any intervention, they play a passive role. In interventional studies, subjects are subjected to treatment. (Vale, 2009)

1.3- Selection Criteria

The study population is defined according to selection criteria stated in the clinical study protocol (CSP). Selection criteria are divided in exclusion and inclusion criteria.

Inclusion criteria define the main characteristics of the target population that allow to answer the research question. Exclusion criteria indicate subsets of individuals who are not eligible for the study because of characteristics that may interfere with the success of follow-up efforts, the quality of the data, the acceptability of treatment, or that would put the subject at a high risk of adverse events. (Hulley *et al.*, 2007)

Selection criteria are based on characteristics such as age, gender, the type and stage of a disease, previous medical history, concomitant medication, for example. (United States of America, 2019)

Any subject before participating in a clinical trial goes through a screening process. In this process medical history, physical examination, vital signs, 12-lead electrocardiogram, clinical laboratory tests and others as needed are performed. (Ramey *et al*., 1998)

1.4- Regulatory entities

Any new medicine or treatment that gets to the market is regulated by national/international entities.

The US Food and Drug Administration (FDA) has several guidelines that need to be followed for the new medicine to be approved in the United States of America. These cover every aspect of the clinical study from the Study Design, Informed Consent, FDA Inspections, Reporting Adverse Events or Data Monitoring. (United States of America, 2020)

Although in the European Union the authorisation and approval of clinical trials occurs at a national level, the European Medicines Agency (EMA) assures that the investigation is done under GCP and has several guidelines that apply to the European Economic Area countries. It also manages the database of clinical trials in the European Union. (EMA- Clinical Trials in Human Medicines)

At a national level, INFARMED (Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.) is the regulatory entity in charge. Medicines for human use are regulated by the law n° 21/2014 of April 16 and by the EMA regulations and guidelines. (Portugal, INFARMED)

A clinical trial also needs approval from an Ethics Committee. Each country has an ethics committee that protects the interests of research participants. The committee reviews the application documents and issues an opinion if the research is ethical and fair. (United Kingdom, 2020) In Portugal the research ethics committee is CEIC (*Comissão de Ética para a Investigação Clinica*).

2- Healthy Subject

The concepts of health and disease are difficult to define because they can include value judgments and because there are many metaphors associated with these concepts. (Boyd, 2000) Since the concept of healthy is not unique, it is also important to try to understand what the concept of disease is, and whether they are opposite.

Within each of these concepts it is also interesting to define the concept of a healthy individual (and the concept of a sick person) and try to understand if this concept and definition can also be applied from an analytical point of view.

According to the World Health Organization (WHO), health is defined as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (WHO, 1946), the Textbook of Pharmaceutical Medicine adds that a healthy individual does not require regular or frequent medication. (Griffi *et al*., 2013)

The Royal College of Physicians defines a healthy subject 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". (Hoffenberg *et al*., 1986)

According to Professor Marshall Marinker, there are three stages to the presence of unhealth. The first stage is disease, which is defined by a pathological process that results in a biological variation. There is objectivity at this stage; the disease may be seen, touched, measured, or smelled. The second stage is illness. It is a personal feeling that can be accompanied by disease; however, the disease may go unnoticed. The third stage is sickness. It is defined as the way disease is shown to the public. It's a social role, where the person is classified as sick. Sickness based only on illness is an uncertain status. People with different diseases have different status of sickness. (Marinker, 1975)

Usually, these three concepts overlap (Figure 2). The person identifies themself as ill based on self-reported symptoms, the physician diagnoses the disease, and if the disease somehow affects the life of the patient, the patient is classified as sick. (Wikman, 2005)

This doesn't always happen, either because the ill person doesn't visit the physician to diagnose the disease or because not all illnesses and diseases lead to sickness. However, most of the illnesses and diseases do not lead to a sickness absence since they don't reduce the capacity of the patient. (Wikman, 2005)

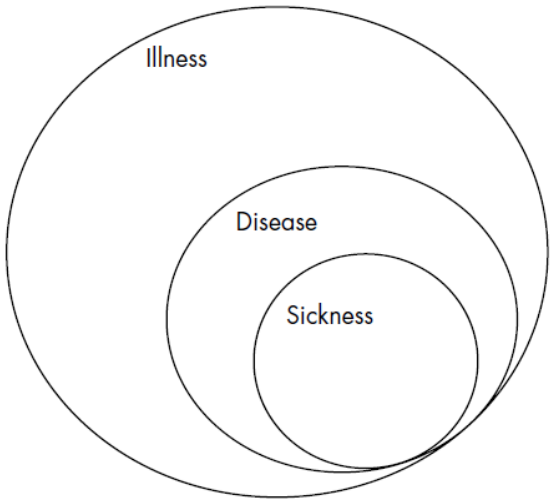

Figure 2- *Relation between Illness, Disease and Sickness. Adapted from: Wikman, 2005.*

To diagnose the disease, the physician should perform a clinical history interview (anamnesis), conduct a physical exam, perform diagnostic testing, and refer to or consulting with other clinicians. (Balogh *et al.*, 2015)

2.1- Healthy Subject from an Analytical Point of View

To characterize a subject as healthy from an analytical point of view it is necessary to understand the concept of sensitivity and specificity of a test.

Sensitivity refers to a test's capacity to correctly identify patients who have a disease and specificity refers to a test's capacity to correctly identify those who do not have the disease. To calculate these parameters, the number of true positives, true negatives, false positives, and false negatives is necessary. (Swift, 2020)

The number of true positives refers to the person who has the disease and tested positive and the true negative is a person who does not have the disease and tested negative. The false positive is someone who does not has the disease and tested positive, and the false negative is someone who does have the disease and tested negative. (Swift, 2020)

Therefore:

- Sensitivity = number of true positives / (number of true positives + number of false negatives) (Swift, 2020)
- Specificity = number of true negatives / (number of true negatives + number of false positives) (Swift, 2020)

Another point to consider is the relationship between predictive values and the prevalence of a disease. The positive predictive value (PPV) is the fraction of people who tested positive and actually have the disease in question. Negative predictive value (NPV) is the fraction of people who tested negative and who do not have the disease. (Carvajal, 2010)

- PPV = number of true positives / (number of true positives + number of false positives) (Carvajal, 2010)
- NPV = number of true negatives / (number of false negatives + number of true negatives) (Carvajal, 2010)

Although sensitivity and specificity are only related to the test and not to the prevalence of a disease, the predictive values are highly related to disease prevalence. Therefore, the higher the prevalence of a disease, the higher the PPV will be. (Carvajal, 2010)

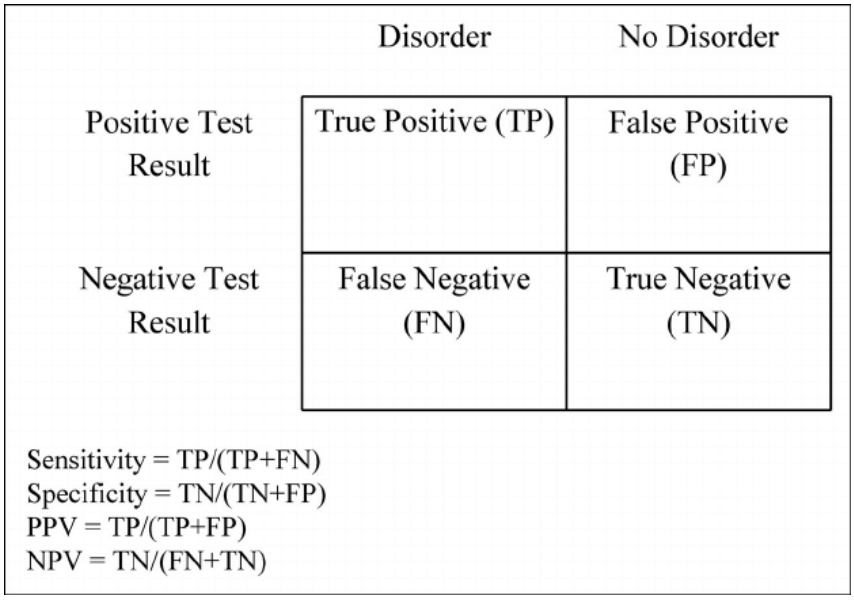

Figure 3- *Calculation of sensitivity, specificity, and positive and negative predictive. Adapted from: Carvajal, D.; Rowe, P., 2010.*

The ideal test would be 100% diagnostic in terms of sensitivity and specificity, and each test involves a trade-off between sensitivity and specificity, with a cutoff value that maximizes sensitivity at the expense of specificity. (Wians, 2009)

In Figure 4 it is possible to see how false negatives or false positives can be overlapped, showing that PSA (Prostate Specific Antigen) testing isn't a perfect test in what concerns either sensitivity or specificity. (Wians, 2009)

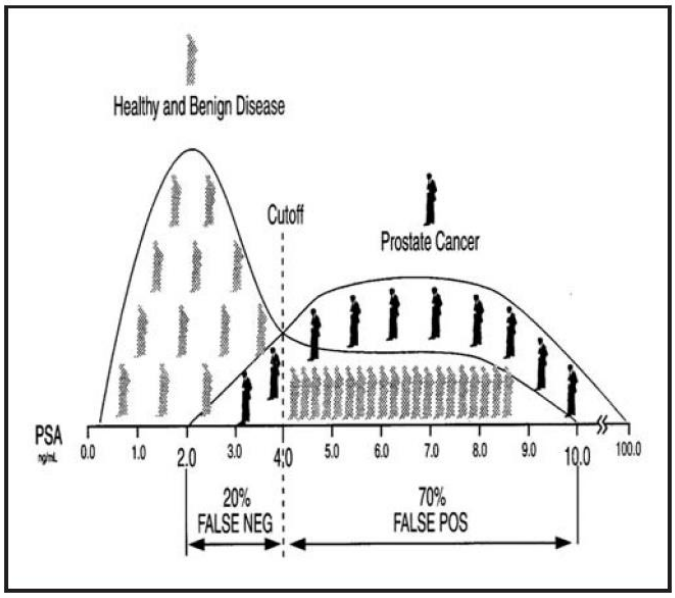

***Figure 4**- Example of an analyte (PSA) showing false negative, false positive and the cut-off value. Adapted from: Wians, 2009.*

70% of the PSA tests performed are positive, although the patient does not have the disease, that is, they are false positives. (Wians, 2009)

Disease is the combination of several parameters that require a diagnosis by the physician. Most of the times, an isolated abnormality isn't enough to define an illness, with exception for pathognomonic features (a sign or symptom specifically characteristic or indicative of a particular disease or condition). The same apply to the laboratory tests: a value outside of the reference range, by itself and in isolation, does not mean a pathology.

Nevertheless, reference intervals must be used by the physician when analyzing lab results. Always interpreting these results in a broader picture of all the data collected during the clinical evaluation. (Jones *et al*., 2008)

3- Analytical Parameters

Reference intervals for analytical parameters are determined from a population of healthy individuals where 95% of the values will be between the lower limit and the upper limit. Values outside the reference values will be classified as abnormally low or abnormally high and all values between them as "normal". Since the population used to define the reference values is made up of healthy individuals, it means that 5% of these healthy individuals will still have values outside these limits, where 2.5% will be abnormally low and 2.5% abnormally high (Figure 5). (Wians, 2009; Higgins *et al*., 2020)

For this reason, the concept of normal ranges and normal values has been substituted by reference ranges and reference values, defined by the reference limits. (Lewis, *et al*., 2006)

The definition on normal laboratory values can be interpreted in different ways. A normal concentration of an analyte can be (Boyd, 2010):

1- the most representative concentration determined by the mean; (Boyd, 2010)
2- the most common concentration defined by an interval; (Boyd, 2010)
3- analyte concentrations associated with the best performance; (Boyd, 2010)
4- a consensus approved analyte concentration or; (Boyd, 2010)
5- the ideal concentration. (Boyd, 2010)

In medicine, physicians usually prefer the definitions associated with the interval or the performance. (Boyd, 2010)

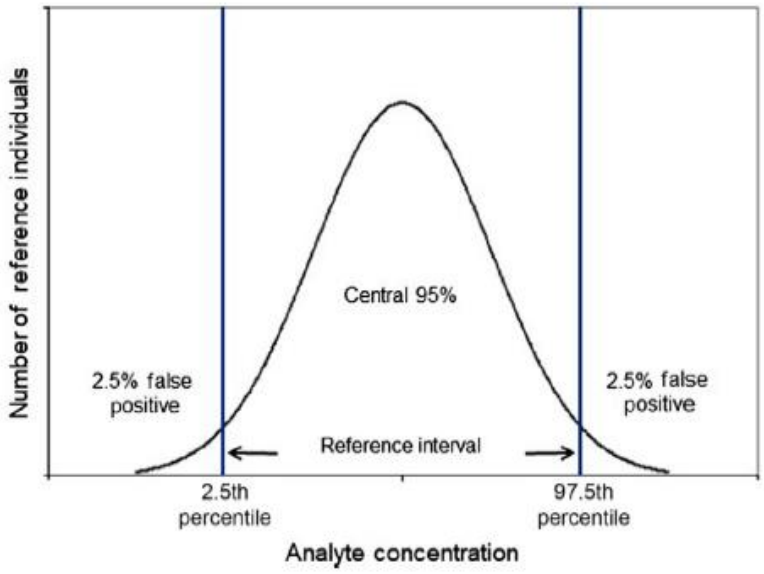

***Figure 5**- Normal distribution of an analyte concentration. Adapted from: Higgins et al., 2020.*

In some cases, only the lower or the upper limit may be considered, in other cases 99% of the healthy population is used to establish the upper limit. An example of an analyte where 99% of population is used to establish the upper limit is the cardiac troponin. (Higgins *et al*., 2020)

Reference values should be used as a guidance and should take in consideration gender, age, fasting, exercise, ethnic background, or race since values for some analytes vary. (Basten, 2011) For example, one analyte that should take age in consideration is alkaline phosphatase (an enzyme produced by osteoblasts) it is expected to be higher in 10 to 12 years-old than in younger or older population. (Wians, 2009)

3.1- Classification of Analytical Parameters

Since 5% of the normal values are outside the reference ranges, but occur in healthy individuals, this means that these values may or may not be considered clinically relevant. However, there is no guideline to identify which value up to (or down to) should be considered "not clinically relevant" (NCR) or from what value it is considered "clinically relevant" (CR). That is, this decision is on the physician discretion who interprets the totality of the results and of other data and performs an integrated clinical evaluation.

An analytical parameter classified as NCR means that the abnormality in question does not appear to be relevant from a clinical point of view, that is, it means that it doesn't imply the presence of a disease nor an increased risk that prevents participation in the clinical study. When doubts remain about the clinical significance of a determined parameter, the physician may decide to perform a repetition or request an additional measurement to correctly assess the impact of the abnormality in the subject's safety.

The assessment of the clinical significance of a finding also depends on the study drug. Certain changes may not impact the subject's safety with a particular drug but can be harmful when the drug may act on the organ/system to which that analyte refers to. Thus, a parameter abnormality maybe classified as NCR in one study and as CR in another.

The presence of a laboratory abnormality by itself does not mean necessarily an unhealthy status or an increased risk. The definition of eligibility criteria based on laboratory parameters must attend to this fact.

Not all parameters are equally important in the safety of a phase I subject. It depends on several factors and require a directed evaluation.

4- Hematology Parameters

The three main types of blood cells are white blood cells (WBCs), red blood cells (RBCs), and platelets (thrombocytes).

The usually analyzed hematological parameters are:

- Total white blood cells (WBC)
- Lymphocytes
- Neutrophils
- Basophils
- Eosinophils
- Monocytes
- Red blood cells (RCB)
- Hemoglobin
- Hematocrit
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Platelets

4.1- White blood cells

White blood cells are divided into two groups, granulocytes and agranulocytes, yet they all come from the same progenitor, the myeloid cell. Eosinophils, basophils, and neutrophils are granulocytic cells, while monocytes and lymphocytes are agranulocytic cells. As part of the immune system, this cell group's primary role is to defend the body. (Gordon-Smith, 2009)

Granulocyte cells are characterized by the presence of granules in their cytoplasm and can change shape and migrate across blood vessel walls in response to signals of infection and inflammation. (Gordon-Smith, 2009)

Eosinophils are mostly found in the extracellular space and their main function is protection against parasitic organisms. (Gordon-Smith, 2009) Eosinophilia, an increase in the number of eosinophils, can lead to tissue damage when wrongly activated. Eosinophilia can be caused by allergies, myeloid leukemia, lymphomas, rheumatoid arthritis, or celiac disease. (Kaushansky *et al*., 2010)

Basophils are the least common granulocyte cells in human blood. They have a high affinity for the immunoglobulin E receptor. Since basophil levels are usually already low, even in healthy individuals, it is difficult to determine when basophilopenia (a decreased basophil value) occurs. However, it has been identified in association with urticaria and anaphylaxis. Basophilopenia also occurs in pathologies associated with eosinophilopenia. They may also decrease after pharmacological administration of thyroid hormones. Basophilia, an increase in the number of basophils, is associated with various conditions such as allergies or inflammation, diabetes mellitus, iron deficiency, or carcinomas. (Kaushansky *et al*., 2010)

Neutrophils circulate in the bloodstream for 6 to 10 hours and the number of circulating cells increases depending on stimuli. These stimuli can be physical exercise or the presence of inflammation. (Gordon-Smith, 2009) A neutropenia is characterized by a decrease in neutrophils, but several factors such as age, sex, and even race must be considered. Severe neutropenia is a predisposing factor for infections. On the other hand, neutrophilia is characterized by an increase in neutrophils. In healthy individuals the number of neutrophils follows a diurnal pattern of variation, with an increase at the end of the day. The number of neutrophils also increases after meals, with an upright posture, and with emotional stimuli, but these changes are not sufficient to cause neutrophilia. (Kaushansky *et al*., 2010)

Monocytes and lymphocytes do not have granules in their cytoplasm. Monocytes play a direct role in sepsis and are relatively resistant to viral infections. A metabolic, microbial, or environmental stimulus is required for the activation of these cells. An increase in monocytes, monocytosis, can be induced through exercise or can be a sign of chronic idiopathic monocytosis or various monocytic leukemias. A monocytopenia, decrease in the number of monocytes, may be a sign of aplastic anemia or the monoMAC (monocytopenia and mycobacterial infection) syndrome. (Kaushansky *et al*., 2010)

Lymphocytes divide into different functional types and subtypes, depending on the organ where they developed and their function. The most important cell types are T, B, and Natural Killer (NK) lymphocytes. T-lymphocytes are responsible for cell-mediated cytotoxic reactions and also produce cytotoxins that regulate the immune response and assist B cells. B-lymphocytes capture, internalize and present antigens to T-cells and are precursors of immunoglobulins. NK cells are responsible for innate immunity against infectious agents. The pathologies associated with lymphocytes are divided into "primary disorders", associated with intrinsic defects, and "acquired disorders" resulting from viral infections, bacterial infections, or from drugs. When the lymphocyte count is lower than expected it is called

lymphocytopenia, and when it is increased it is called lymphocytosis. (Kaushansky *et al*., 2010)

4.2- Red Blood Cells

Red blood cells are responsible for transporting oxygen to all tissues and removing carbon dioxide. In adults, the number of circulating red blood cells is at a stable value unless disturbed by some pathology or environmental factors. Pathologies associated with this disturbance are anemia and polycythemia. Anemias are characterized by a decrease in the number of red cells in circulation, while polycythemias by an increase in red blood cells. Changes in the number of red blood cells can be clinically detected by the concentration of hemoglobin or hematocrit. (Kaushansky *et al*., 2010)

Hemoglobin is a tetramer, present inside red blood cells, and is mostly responsible for oxygen transport. (Steinberg, 2017) The levels of this protein vary with age. (Kaushansky *et al*., 2010)

Hematocrit is the fraction of blood volume, expressed as a percentage, occupied by red blood cells. (Kaushansky *et al*., 2010)

Mean Cell Volume (MCV) determines the size of red blood cells and is used to guide the diagnosis of patients with anemia. If it is a microcytic anemia, it may indicate iron deficiency or thalassemia, and if it is a macrocytic anemia, it may indicate a vitamin B12 or folic acid deficiency. (Kaushansky *et al*., 2010)

Mean Cell Hemoglobin (MCH) is the parameter that determines the amount of hemoglobin per red blood cell and its value increases or decreases in conjunction with MCV, since they reflect similar diagnostic information. (Kaushansky *et al*., 2010)

Mean Cell Hemoglobin Concentration (MCHC) correlates the amount of hemoglobin with cell size. (Gordon-Smith, 2009)

Since these last three analytical parameters represent average amounts, they may not detect abnormalities in samples with different cell types. (Kaushansky *et al*., 2010)

4.3- Platelets

Platelets are anucleate cells adapted to adhere to damaged blood vessels, to aggregate with each other, and to facilitate thrombin formation. (Kaushansky *et al*., 2010)

Thrombocytopenia, a decrease in the number of platelets, can be classified as severe, moderate, or mild depending on the amount of platelets present. The decrease in the number of platelets can be due to a decrease in platelet production (hereditary or acquired: nutritional, alcohol intake, aplastic anemia, drugs, pregnancy) or to an increase in platelet destruction (immune, thrombotic microangiopathic, and drug-induced or pregnancy). (Kaushansky *et al*., 2010)

The main causes of thrombocytosis, high platelet numbers, are high blood loss, acute infection or inflammation, response to exercise, iron deficiency, chronic inflammation (e.g. rheumatoid arthritis), response to some drugs, or hemolytic anemia. (Kaushansky *et al*., 2010)

5- Biochemistry Parameters

The usually analyzed biochemistry parameters are:

- Sodium
- Potassium
- Chloride
- Calcium
- Magnesium
- Glucose
- Direct bilirubin
- Indirect bilirubin
- Total bilirubin
- Uric acid
- Creatinine
- Urea
- Glomerular Filtration Rate (eGFR)
- Total protein
- Albumin
- Alkaline phosphatase (ALP)
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Gamma-glutamyl transferase (GGT)
- Lactic dehydrogenase (LD)
- Creatin phosphokinase (CK)
- Lipase
- Total cholesterol
- Triglycerides

Creatinine, alanine and aspartate aminotransferase, alkaline phosphatase and bilirubin are the ones that may be more important because they refer to organ systems more frequently and adversely affected by drugs. (Sibille *et al*, 1999)

5.1- Electrolytes

Sodium is a cation responsible for maintaining fluid balance in circulation. Sodium blood levels are regulated by the kidneys through excretion and reabsorption. When outside the normal range can be a sign of dehydration, Cushing's syndrome, diabetes insipidus, diarrhea, vomiting, Addison's disease, renal disease. (Reed, 2021)

Potassium is an intracellular cation responsible for muscle contraction and maintenance of normal heart rate. When outside the normal range can be a sign of circulatory failure, renal disease, vomiting, diarrhea, diuretic use and some cancers. (Reed, 2021)

Chloride is an extracellular anion which variation in concentrations usually mirrors sodium concentrations variation. When outside the normal range, it can be a sign of dehydration, low blood sodium and vomiting. (Reed, 2021)

Calcium is a mineral important for bone formation, blood clotting and in nerve and muscle function. Usually in very little concentrations. When outside the normal range, it can be a sign of hyperparathyroidism or hypoparathyroidism, some cancers, excess or deficiency of vitamin D, chronic kidney disease and pancreatitis. (Reed, 2021)

Magnesium is a mineral important for enzymes converting energy for muscle functions and in bone structure. When outside the normal range, it can be a sign of renal disease, severe dehydration, malabsorption, pancreatitis, diarrhea and alcoholism. (Reed, 2021)

5.2- Small Molecules

5.2.1- Nutrition

Glucose is a source of energy for many tissues. It is regulated by hormones. When outside the normal range, it can be a sign of diabetes, Cushing's disease, stress, insulin excess, starvation, adrenal insufficiency. (Reed, 2021)

5.2.2- Waste Products

Total Bilirubin is a breakdown product of hemoglobin that is excreted by the liver. Is present in the blood in two different forms, conjugated and unconjugated. When outside the normal range, it can be a sign of hepatitis, cirrhosis, hemolytic diseases, obstruction of biliary or hepatic ducts. (Reed, 2021)

Direct or conjugated Bilirubin has the carboxyl groups esterified, is a water-soluble compound that reacts directly with diazo dyes. When outside the normal range, it can be a sign of obstruction of biliary or hepatic ducts, hereditary conditions like Dubin-Johnson syndrome. (Reed, 2021)

Indirect or unconjugated Bilirubin has free carboxyl groups, is fat-soluble and reacts with diazo dyes in the presence of activators. When outside the normal range, it can be a sign of hereditary conditions like Gilbert's disease and Crigler-Najjar syndrome. (Reed, 2021)

Uric Acid is a product resulting from the breakdown of purines and is excreted by the kidneys. When outside the normal range, it can be a sign of gout, kidney disease, leukemia. (Reed, 2021)

Creatinine is a product resulting from the muscle breakdown of creatine. Is excreted into urine. When outside the normal range, it can be a sign of kidney disfunction, the disfunction can be caused by drug toxicity, uncontrolled diabetes, or inadequate blood flow. (Reed, 2021)

Glomerular filtration rate (eGFR) is a parameter calculated based on the creatinine levels. It indicates the glomerular function and is influenced by muscle mass, age, gender, and race. When outside the normal values, it is an indicator for kidney diseases. (Waad-Allah S. *et al*., 2012)

Urea is produced by the liver as a major product of metabolism of nitrogen-containing substances and is excreted by the kidneys regulating their function. (Weiner *et al*., 2015; Baum *et al*., 1975)

5.3- Proteins

5.3.1- General and Transport

Total Protein measures the serum or plasma proteins, mostly albumin and globulins. Maintains circulatory system oncotic pressure. When outside the normal range can be a sign of dehydration, infections, some myelomas and lymphomas, liver disease, malnutrition. (Reed, 2021)

Albumin is the major protein in blood, made in the liver. Acts as a transport protein of many substances. When outside the normal range can be a sign of dehydration, infections, malignancy, starvation, burns, kidney disease and liver disease. (Reed, 2021)

5.3.2- Enzymes

Alkaline Phosphatase (ALP) is an enzyme found in bone, intestine, kidney, and liver. When outside the normal range can be a sign of liver disease, bone disease and periods of bone growth, low phosphatase, hypothyroidism, pernicious anemia. (Reed, 2021)

Aspartate Aminotransferase (AST) is an enzyme present in liver, heart, and skeletal muscle. When outside the normal range can be a sign of liver disease, heart attack and trauma. (Reed, 2021)

Alanine Aminotransferase (ALT) is an enzyme mostly found in liver. When outside the normal range can be a sign of hepatitis, cirrhosis, Reye's syndrome, monitor drug induced liver damage. (Reed, 2021)

Gamma-Glutamyl Transferase (GGT) is present in liver. When outside the normal range can be a sign of any liver disorder, including biliary obstruction, alcoholic liver disease. (Reed, 2021)

Lactic Dehydrogenase (LD) is present in tissues like heart, lung, liver, kidney, and skeletal muscle. When outside the normal range can be a sign of heart attack, liver disease, lung disease and trauma. (Reed, 2021)

Creatine Phosphatase (CK) is present in different tissues, in different forms. When outside the normal range can be a sign of muscle damage, extreme exercise, trauma and low muscle mass. (Reed, 2021)

5.4- Lipids and Lipoproteins

Total Cholesterol is a steroid lipid made by the liver. Used for production of steroid hormones and cell walls. When outside the normal range can be a sign of hypothyroidism, uncontrolled diabetes, kidney disease, liver disease, starvation and anemia. (Reed, 2021)

Triglycerides acts in transport and storage in adipose tissue. When outside the normal range can be a sign of hypothyroidism, alcoholism, liver disease, uncontrolled diabetes. (Reed, 2021)

Lipase is a hydrolytic enzyme, that's responsible for removing triglycerides from the circulation. Lipase levels that are lower than normal could indicate hypothyroidism or diabetes. (Eckle, 1989)

6- Analysis of a Population of Healthy Volunteers

The aim of this dissertation is to make the exercise to establish reference intervals based on analytical values from Phase I trial subjects, in order to compare with the standard reference values from the laboratory. Being so, it's required a statistical analysis of the available data from healthy subjects.

The data used belong to a Portuguese Clinical Research Organization and are from the year 2020, where 1784 screening consultations were performed. From these subjects, 966 participants were female and 814 were male. The participants age ranged from 18 to 65 years, with a mean age of 31.2 years.

To analyze these data, the classifications (Normal, NCR and CR) of each parameter were registered and their frequency was calculated, the mean and standard deviation were also calculated, to calculate reference intervals. For these reference values it was taken in account that some parameters results are different between men and women and reference values were calculated for each sex.

Subsequently, the calculated reference intervals were compared with those used by the laboratory performing the analysis of the CRO samples and with the values presented by Sibille, 1999.

In Sibille's analysis of 927 male subjects from phase I trials, an interval was also calculated based on the values presented in the screening analyses of these subjects.

In tables 1 and 3, it is possible to see the classification of each parameter and in tables 2 and 4 it is possible to see their frequencies. In most of the parameters the classifications are "Normal" in more than 90% of the analyses, except for the parameter's monocytes, direct bilirubin, total bilirubin, eGFR (both in men and women), ALP, CK (both in men and women) and total cholesterol.

The most important parameters for subject participation are in general hemoglobin, eosinophils, lymphocytes, monocytes, platelets, creatinine, AST, ALT, and bilirubin (Sibille, 1999), and of these parameters only bilirubin has a "normal" classification of less than 90%, but it remains the classification that is most often obtained.

The most frequent abnormalities are NCR, meaning no impact on the safety of the subject. eGFR is the only parameter where NCR classification has a higher frequency than Normal classification.

In the year 2020 no abnormalities considered clinically relevant were found, and even those that required a repetition for clarification (initially classified as relevance unknown: RU) and final classification have a very low frequency that never exceeds 8.4% of the analyses performed.

Since different protocols ask for different analytical parameters to ensure the safety of the subject it is to be expected that not all parameters will have the same number of subjects. This also happens because it can be requested a repetition analysis whenever it is deemed necessary by the physician

As can be seen, only magnesium and urea in women have a Normal frequency of 100%. Of all the analyses performed, only 129 subjects (2.01% women and 4.32% men) had all normal hematological values and 213 (5.99% women and 5.94% men) had all normal biochemistry values. Of these, only 31 (1.07% women and 0.67% men) had all values of all hematology and biochemistry parameters inside the reference intervals, although all were healthy.

Once calculated the reference intervals, a comparison was performed in order to evaluate if they were similar and if they were within the reference intervals in use by the laboratory. Tables 5 and 6 show the mean and standard deviation values for each parameter, which were used to calculate the reference intervals, as well as the minimum and maximum values obtained in the subjects' analyses.

***Table 1**- Number of subjects per classification (Normal, NCR, CR, RU)- Hematology.*

Parameter		Normal	NCR-Low	NCR-High	CR-Low	CR-High	RU-Low	RU-High	n
WBC		834	7	33	-	-	3	11	888
Lymphocytes		854	6	23	-	-	-	5	888
Neutrophiles		832	13	32	-	-	3	8	888
Basophiles		869	7	12	-	-	-	-	888
Eosinophils		846	15	26	-	-	-	1	887
Monocytes		680	206	1	-	-	1	-	888
RCB-Count		833	23	32	-	-	-	-	888
	Male	344	4	16	-	-	-	-	364
	Female	489	19	16	-	-	-	-	524
Hemoglobin		835	36	16	-	-	1	-	888
	Male	345	8	11	-	-	-	-	364
	Female	490	28	5	-	-	1	-	524
Hematocrits		824	39	25	-	-	-	-	888
	Male	343	4	17	-	-	-	-	364
	Female	481	35	8	-	-	-	-	524
MCV		862	18	8	-	-	-	-	888
MCH		834	47	7	-	-	-	-	888
MCHC		807	81	-	-	-	-	-	888
Platelets		832	11	43	-	-	-	2	888
	Male	341	2	21	-	-	-	-	364
	Female	491	9	22	-	-	-	2	524

***Table 2**- Frequency of each hematological parameter classification.*

Parameter		Normal	NCR-Low	NCR-High	CR-Low	CR-High	RU-Low	RU-High
WBC		93.9	0.8	3.7	-	-	0.3	1.2
Lymphocytes		96.2	0.7	2.6	-	-	-	0.6
Neutrophiles		93.7	1.5	3.6	-	-	0.3	0.9
Basophiles		97.9	0.8	1.4	-	-	-	-
Eosinophils		95.4	1.7	2.9	-	-	-	0.1
Monocytes		76.6	23.2	0.1	-	-	0.1	-
RCB-Count		93.8	2.6	3.6	-	-	-	-
	Male	94.5	1.1	4.4	-	-	-	-
	Female	93.3	3.6	3.1	-	-	-	-
Hemoglobin		94.0	4.1	1.8	-	-	0.1	-
	Male	94.8	2.2	3.0	-	-	-	-
	Female	93.5	5.3	1.0	-	-	0.2	-
Hematocrits		92.8	4.4	2.8	-	-	-	-
	Male	94.2	1.1	4.7	-	-	-	-
	Female	91.8	6.7	1.5	-	-	-	-
MCV		97.1	2.0	0.9	-	-	-	-
MCH		93.9	5.3	0.8	-	-	-	-
MCHC		90.9	9.1	-	-	-	-	-
Platelets		93.7	1.2	4.8	-	-	-	0.2
	Male	93.7	0.5	5.8	-	-	-	-
	Female	93.7	1.7	4.2	-	-	-	0.4

***Table 3**- Number of subjects per classification (Normal, NCR, CR, RU)- Biochemistry.*

Parameter		Normal	NCR-Low	NCR-High	CR-Low	CR-High	RU-Low	RU-High	High	n
Sodium		859	9	-	-	-	-	-	-	868
Potassium		863	2	2	-	-	-	-	1	868
Chloride		393	-	1	-	-	-	-	-	394
Calcium		118	8	5	-	-	-	-	-	131
Magnesium		153	-	-	-	-	-	-	-	153
Glucose		865	1	2	-	-	-	-	-	868
Direct Bilirubin		685	-	84	-	-	-	1	-	770
Indirect Bilirubin		727	-	21	-	-	-	1	-	749
Total Bilirubin		734	91	43	-	-	-	1	-	869
Uric Acid		144	6	4	-	-	-	-	-	154
	Male	71	3	1	-	-	-	-	-	75
	Female	73	3	3	-	-	-	-	-	79
Creatinine		861	17	3	-	-	-	2	-	883
	Male	358	1	1	-	-	-	2	-	362
	Female	503	16	2	-	-	-	-	-	521
Urea		203	-	2	-	-	-	-	-	205
	Male	124	-	2	-	-	-	-	-	126
	Female	79	-	-	-	-	-	-	-	79
eGFR		321	6	474	-	-	4	-	-	805
	Male	143	6	160	-	-	3	-	-	312
	Female	178	-	314	-	-	1	-	-	493
Total Protein		177	-	6	-	-	-	-	-	183
Albumin		810	7	3	-	-	-	-	-	820
ALP		775	84	8	-	-	-	-	-	867
AST		866	-	7	-	-	-	4	-	877
ALT		864	6	3	-	-	3	13	-	889
GGT		828	-	40	-	-	-	2	-	870
	Male	352	-	6	-	-	-	-	-	358
	Female	476	-	34	-	-	-	2	-	512
LDH		754	11	4	-	-	-	-	1	770
CK		647	3	119	-	-	-	45	-	814
	Male	250	-	77	-	-	-	30	-	357
	Female	397	3	42	-	-	-	15	-	457
Lipase		126	-	1	-	-	-	3	-	130
Total Cholesterol		102	-	52	-	-	-	-	-	154
Triglycerides		140	-	14	-	-	-	-	-	154

***Table 4**- Frequency of each biochemistry parameter classification.*

Parameter		Normal	NCR-Low	NCR-High	CR-Low	CR-High	RU-Low	RU-High	High
Sodium		98.96	1.04	-	-	-	-	-	-
Potassium		99.42	0.23	0.23	-	-	-	-	0.12
Chloride		99.75	-	0.25	-	-	-	-	-
Calcium		90.08	6.11	3.82	-	-	-	-	-
Magnesium		100.00	-	-	-	-	-	-	-
Glucose		99.65	0.12	0.23	-	-	-	-	-
Direct Bilirubin		88.96	-	10.91	-	-	-	0.13	-
Indirect Bilirubin		97.06	-	2.80	-	-	-	0.13	-
Total Bilirubin		84.46	10.47	4.95	-	-	-	0.12	-
Uric Acid		93.51	3.90	2.60	-	-	-	-	-
	Male	94.67	4.00	1.33	-	-	-	-	-
	Female	92.41	3.80	3.80	-	-	-	-	-
Creatinine		97.51	1.93	0.34	-	-	-	0.23	-
	Male	98.90	0.28	0.28	-	-	-	0.55	-
	Female	96.55	3.07	0.38	-	-	-	-	-
Urea		99.02	-	0.98	-	-	-	-	-
	Male	98.41	-	1.59	-	-	-	-	-
	Female	100.00	-	-	-	-	-	-	-
eGFR		39.88	0.75	58.88	-	-	0.50	-	-
	Male	45.83	1.92	51.28	-	-	0.96	-	-
	Female	36.11	-	63.69	-	-	0.20	-	-
Total Protein		96.72	-	3.28	-	-	-	-	-
Albumin		98.78	0.85	0.37	-	-	-	-	-
ALP		89.39	9.69	0.92	-	-	-	-	-
AST		98.75	-	0.80	-	-	-	0.46	-
ALT		97.19	0.67	0.34	-	-	0.34	1.46	-
GGT		95.17	-	4.60	-	-	-	0.23	-
	Male	98.32	-	1.68	-	-	-	-	-
	Female	92.97	-	6.64	-	-	-	0.39	-
LDH		97.92	1.43	0.52	-	-	-	-	0.13
CK		79.48	0.37	14.62	-	-	-	5.53	-
	Male	70.03	-	21.57	-	-	-	8.40	-
	Female	86.87	0.66	9.19	-	-	-	3.28	-
Lipase		96.92	-	0.77	-	-	-	2.31	-
Total Cholesterol		66.23	-	33.77	-	-	-	-	-
Triglycerides		90.91	-	9.09	-	-	-	-	-

***Table 5**- Calculated reference values- Hematology.*

Laboratory Parameter	Unit		N° of Subjects	Mean	Standard Deviation	Minimum[1]	Maximum[1]	Reference Values	
								Minimum	Maximum
WBC	x10^9/L		888	6.46	1.72	2.83	13.8	3.023	9.896
Lymphocytes	%		888	1.97	0.54	0.74	4.83	0.883	3.050
Neutrophiles	%		888	3.91	1.37	1.16	10.97	1.176	6.644
Basophiles	%		888	0.03	0.02	0	0.14	0.000[2]	0.071
Eosinophils	%		887	0.16	0.13	0	0.82	0.000[2]	0.412
Monocytes	%		888	0.34	0.11	0.09	0.9	0.109	0.567
RCB-Count	x10^12/L		888	4.73	0.43	3.64	6.12	3.86	5.59
		Male	364	5.06	0.34	4.15	6.12	4.39	5.74
		Female	524	4.49	0.33	3.64	6.05	3.84	5.15
Hemoglobin	g/DL		888	13.80	1.33	10.1	17.8	11.1	16.5
		Male	364	14.98	0.92	12.2	17.8	13.1	16.8
		Female	524	12.98	0.88	10.1	15.5	11.2	14.7
Hematocrits	%		888	41.29	3.53	32.1	53.8	34.2	48.3
		Male	364	44.21	2.65	36.8	53.8	38.9	49.5
		Female	524	39.26	2.49	32.1	46.7	34.3	44.2
MCV	fl		888	87.46	4.28	62.8	101.9	78.9	96.0
MCH	pg		888	29.21	1.67	19.5	34.5	25.9	32.5
MCHC	g/dL		888	33.40	1.08	29.3	36.4	31.2	35.6
Platelets	x10^9/L		888	254.55	56.63	116	478	141	368
		Male	364	235.05	49.73	116	427	136	335
		Female	524	268.09	57.23	132	478	154	383

[1]Minimum and maximum referring to CRO data. [2]These values were originally negative numbers, consider to be zero.

***Table 6**- Calculated reference values- Biochemistry.*

Laboratory Parameter	Unit		N° of Subjects	Mean	Standard Deviation	Minimum[1]	Maximum[1]	Reference Values	
								Minimum	Maximum
Sodium	mmol/dL		868	139.83	1.96	133	145	136	144
Potassium	mmol/dL		868	4.22	0.30	3.4	5.9	3.6	4.8
Chloride	mmol/L		394	102.54	1.64	98	108	99	106
Calcium	mg/dL		131	9.50	0.53	8.15	10.81	8.44	10.56
Magnesium	mg/dL		153	2.00	0.15	1.7	2.5	1.7	2.3
Glucose	mg/dL		868	87.53	6.49	57	119	75	101
Direct Bilirubin	mg/dL		770	0.17	0.09	0.02	0.8	0[2]	0.35
Indirect Bilirubin	mg/dL		749	0.45	0.28	0.02	2.2	0[2]	1.01
Total Bilirubin	mg/dL		869	0.60	0.35	0.06	2.69	0[2]	1.30
Uric Acid	mg/dL		154	4.6	1.17	2.2	8.7	2.3	6.9
		Male	75	5.22	1.01	2.8	8.7	3.2	7.2
		Female	79	4.02	1.01	2.2	8.1	2.0	6.0
Creatinine	mg/dL		883	0.79	0.15	0.43	1.48	0.5	1.1
		Male	362	0.93	0.12	0.65	1.48	0.7	1.2
		Female	521	0.70	0.09	0.43	1.05	0.5	0.9
Urea	mg/dL		205	30.59	7.69	15	66	15	46
		Male	126	32.60	7.95	19	66	17	48
		Female	79	27.38	6.03	15	45	15	39
eGFR	mL/min/1.73m^2		805	120.73	21.32	72	224	78	163
		Male	312	121.94	20.93	72	192	80	164
		Female	493	119.96	21.55	79	224	77	163
Total Protein	g/dL		183	7.20	0.52	5.9	8.4	6.2	8.2
Albumin	g/dL		820	4.25	0.35	3.1	5.6	3.5	5.0

***Table 6**- Calculated reference values- Biochemistry (cont.).*

Laboratory Parameter	Unit		N° of Subjects	Mean	Standard Deviation	Minimum[1]	Maximum[1]	Reference Values	
								Minimum	Maximum
ALP	U/L		867	65.04	17.17	20	143	31	99
AST	U/L		877	18.17	5.54	2	51	7	29
ALT	U/L		889	24.33	9.75	4	94	5	44
GGT	U/L		870	25.94	12.51	6	135	1	51
		Male	358	29.34	14.35	6	135	1	58
		Female	512	23.56	10.42	<7	121	3	44
LDH	mg/dL		770	166.72	23.27	99	323	120	213
CK	U/L		814	123.27	80.09	26	876	0[2]	283
		Male	357	153.34	82.06	47	851	0[2]	317
		Female	457	99.79	70.15	26	876	0[2]	240
Lipase	U/L		130	34.8	9.43	21	65	16	54
Total Cholesterol	mg/dL		154	185.83	36.20	96	277	113	258
Triglycerides	mg/dL		154	84.19	48.55	20	269	0[2]	181

[1]Minimum and maximum referring to CRO data. [2]These values were originally negative numbers, consider to be zero.

7- Discussion and Conclusions

For the parameters in which there is no difference between men and women, the reference values were compared with those used by the laboratory and with those presented by Sibille, 1999. In cases where the values differ between men and women, only the reference values for men were compared with those of Sibille, 1999. Since we do not have access to the quantification methods used by the laboratory performing the analysis of CRO samples, not all values were compared with those of Sibille, because they present very different values. The values presented from Sibille for the parameter's calcium, glucose, total bilirubin, uric acid, creatinine, total protein, albumin, total cholesterol, and triglycerides were converted to the same units used by the laboratory in order to be able to compare.

The guideline "Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0" defines various degrees of severity of adverse events (AEs), describing, whenever possible, changes in laboratory parameters. With this, it was possible to observe whether or not the changes to the calculated reference values are changes that could jeopardize the safety of the subjects. (United States of America, 2017) Although in this guideline the values presented are specific to changes throughout the study, whether or not related to the drug, we can use these values as a reference about the values that subjects should not present at screening.

Since CTCAE is not a guideline specific for phase I trials, FDA created the guideline "Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" where it is discussed some analytical parameters that can be used in phase I trials. (United States of America, 2007) Although applying to vaccine clinical studies, we can extrapolate to studies with other types of medicinal product.

***Table 7**- Comparison between calculated, CRO laboratory and Sibille reference values- Hematology.*

Laboratory Parameter	Units		Reference Values		CRO laboratory		Sibille	
			Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
WBC	x10^9/L		3.023	9.896	3.400	9.600	3.71	9.55
Lymphocytes	x10^9/L		0.883	3.050	0.950	3.070	1.17	3.33
Neutrophiles	x10^9/L		1.176	6.644	1.560	6.450	1.70	6.50
Basophiles	x10^9/L		0.000	0.071	0.010	0.080	0.01	0.09
Eosinophils	x10^9/L		0.000	0.412	0.030	0.480	0.05	0.48
Monocytes	x10^9/L		0.109	0.567	0.260	0.810	0.12	0.70
RBC-Count	x10^12/L		3.86	5.59	-	-	-	-
		Male	4.39	5.74	4.35	5.65	4.46	5.95
		Female	3.84	5.15	3.92	5.13	-	-
Hemoglobin	g/dL		11.1	16.5	-	-	-	-
		Male	13.1	16.8	13.2	16.6	13.4	17.5
		Female	11.2	14.7	11.6	15.0	-	-
Hematocrits	%		34.2	48.3	-	-	-	-
		Male	38.9	49.5	38.3	48.6	39.8	52.3
		Female	34.3	44.2	35.5	44.9	-	-
MCV	f/L		78.9	96.0	78.2	97.9	81.5	95.5
MCH	ng		25.9	32.5	26.5	32.6	-	-
MCHC	g/L		31.2	35.6	32.0	36.5	-	-
RDW CV	g/dL		11.5	15.5	-	-	-	-
		Male	11.6	15.0	11.8	14.5	-	-
		Female	11.5	15.8	12.2	16.1	-	-
Platelets Count	x10^9/L		141	368	-	-	-	-
		Male	136	335	135	317	153	324
		Female	154	383	157	371	-	-

As for the hematology values, the parameters WBC; Lymphocytes; Basophils; Eosinophils; Monocytes; RCB-Count in women; Hemoglobin in men and women; Hematocrits in women; MCH; MCHC; RDW in men and women; and Platelets in women have the minimum value below the values presented by the laboratory. In cases where the minimum value is above the minimum value presented by the laboratory (Neutrophils; RCB-Count in men; Hematocrits in men; MCV; Platelets in men), it has no relevant significance because they are still within the reference values.

According to the guideline CTCAE, an AE is characterized by a value of less than 3.0 x10^9/L in WBC, less than 0.8 x10^9/L in lymphocytes and less than 75 x10^9/L in platelets. (United States of America, 2017) All other parameters are not defined. With this, it turns out that none of the changes are significant, and a subject with these values can be included if there is no information on the contrary described in the study protocol.

According to the guideline "Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials", a laboratory abnormality is characterized by a value of less than 2.5 x10^9/L in WBC, less than 0.75 x10^9/L in lymphocytes, less than 13.5 g/dL in male hemoglobin, less than 12.0 g/dL in female hemoglobin, and less than 125 x10^9/L in platelets. (United States of America, 2007) The parameters hemoglobin in male and female are below the recommendations, however in hemoglobin both recommendations values are inside the laboratory reference values which means that this recommendation cannot be used.

Regarding the maximum values presented for the hematological parameters, and when compared with the values used by the laboratory, it can be seen that in the parameter's Lymphocytes; Basophils; Eosinophils; Monocytes; Hemoglobin in women; Hematocrits in women; MCV; MCH; MCHC and RDW in women have the maximum value below the maximum value presented by the laboratory. This difference is not relevant since these values remain within the reference values. The parameters WBC; Neutrophils; RCB Count in men and women; Hemoglobin in men; Hematocrits in men; RDW CV in men; and Platelets in men and women have maximum values higher than the laboratory values.

According to the guideline CTCAE, an AE is characterized by an increase of 100 x10^9/L in WBC and an increase of 0.2 g/dL in hemoglobin relative to the baseline values. (United States of America, 2017) Thus, as this is a value that differs between subjects, it is not possible to make this comparison.

According to the guideline "Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials", an abnormality is characterized by a value above 10.8 x10^9/L in WBC. (United States of America, 2007) The change in the calculated upper WBC value is not a laboratory abnormality.

When compared to the reference values presented by Sibille, all parameters have the lower value below Sibille's minimum value. As seen earlier, this difference is not significant because none of the values would be considered AE. On the other hand, the WBC and MCV parameters have the maximum value higher than Sibille's maximum value.

***Table 8**- Comparison between calculated, CRO laboratory and Sibille reference values- Biochemistry.*

Laboratory Parameter	Units		Reference Values		CRO laboratory		Sibille	
			Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Sodium	mmol/L		136	144	136	145	138	146
Potassium	mmol/L		3.6	4.8	3.5	5.1	3.50	4.90
Chloride	mmol/L		99	106	98	107	97	108
Calcium	mg/dL		8.44	10.56	8.70	10.40	8.74	10.1
Magnesium	mg/dL		1.7	2.3	1.6	2.6	-	-
Glucose	mg/dL		75	101	60	100	68.4	106.2
Direct Bilirubin	mg/dL		0.00	0.35	-	<0.30	-	-
Indirect Bilirubin	mg/dL		0.00	1.01	0	1.1	-	-
Total Bilirubin	mg/dL		0.00	1.30	0.30	1.20	0.29	1.81
Uric Acid	mg/dL		2.3	6.9	-	-	-	-
		Male	3.2	7.2	3.5	7.2	3.85	7.77
		Female	2.0	6.0	2.6	6.0	-	-
Creatinine	mg/dL		0.5	1.1	-	-	-	-
		Male	0.7	1.2	0.6	1.2	0.88	1.28
		Female	0.5	0.9	0.5	1.1	-	-
Urea	mg/dL		15	46	-	-	-	-
		Male	17	48	17	51	-	-
		Female	15	39	13	45	-	-
eGFR	mL/min/ 1.73m2		78	163	-	-	-	-
		Male	80	164	90	120	-	-
		Female	77	163	80	110	-	-
Total Protein	g/dL		6.2	8.2	5.7	8.2	6.4	8.0
Albumin	g/dL		3.5	5.0	3.5	5.2	4.26	5.41
ALP	U/L		31	99	46	116	-	-
AST	U/L		7	29	-	<34	-	-
ALT	U/L		5	44	10	49	-	-
GGT	U/L		1	51	-	-	-	-
		Male	1	58	-	<73	-	-
		Female	3	44	-	<38	-	-
LDH	U/L		120	213	120	246	-	-
CPK	U/L		0	283	-	-	-	-
		Male	0	317	46	171	-	-
		Female	0	240	34	145	-	-
Lipase	U/L		16	54	12	53	-	-
Total Cholesterol	mg/dL		113	258	-	<200	122.39	254.44
Triglycerides	mg/dL		0	181	-	<150	36.28	169.03

Regarding the biochemistry values, the parameters Calcium; Total Bilirubin; Uric Acid in men and women; eGFR in men and women; ALP; ALT; and CPK in men and women have the minimum values below the laboratory values. The parameters Potassium; Chloride; Magnesium; Glucose; Creatinine in men; Urea in women; Total protein; and Lipase have the minimum value above the reference values, which does not represent a problem because they are within the reference values. There are also some parameters that have the minimum value equal to the laboratory's, such as Sodium; Indirect Bilirubin; Creatinine in women; Urea in men; and Albumin. In cases where the laboratory does not present a minimum value, the parameters AST; GGT; Total Cholesterol; and Triglycerides only present minimum values calculated to guarantee that they were not higher than the maximum value.

According to the guideline CTCAE, an AE is characterized by a calcium value of less than 8.0 mg/dL and an eGFR value of less than 60 ml/min/1.73m2. (United States of America, 2017) Thus, it can be seen that in neither of these parameters the difference is significant for the safety of the subject.

According to the guideline "Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials", a laboratory abnormality is characterized by a value bellow 8.4 mg/dL in calcium, which means that the alteration is not relevant for subject´s eligibility to a clinical trial. (United States of America, 2007)

The parameters Calcium; Glucose; Direct Bilirubin; Total Bilirubin; eGRF in men and women; GGT in women; CPK in men and women; Lipase; Total Cholesterol; and Triglycerides present the maximum value higher than the maximum value defined by the laboratory. The parameters with which the maximum value is within the laboratory's reference values, are Sodium; Potassium; Chlorides; Magnesium; Indirect Bilirubin; Creatinine in women; Urea in men and women; Albumin; ALP; AST; ALT; GGT in men; and LDH. The parameters that have the maximum values equal to those in the laboratory are Uric Acid in men and women; Creatinine in men; and Total Protein.

According to the guideline CTCAE, an AE is characterized by a value higher than 11.5 mg/dL in calcium, 300 mg/dL in total cholesterol, and higher than 150 mg/dL in triglycerides. About glucose, ALT and CPK values, the changes are taking into account the baseline, which is not possible to obtain in this work. (United States of America, 2017) Within these parameters, calcium and triglycerides have a maximum value higher than normal, which

means that in this cases the inclusion of the subjects should take into account the drug being studied and the changes that are expected to occur.

According to the guideline "Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials", a laboratory abnormality is characterized by a value higher than 10.5 mg/dL in calcium, higher than 1.1 x ULN (upper limit of the normal range) in bilirubin, higher than 1.25 x ULN in CPK, higher than 1.1 x ULN in lipase and, higher than 201 mg/dL in total cholesterol. (United States of America, 2007) The calcium value and the total cholesterol value are above the recommendations, and comparing the laboratory upper value with the recommendations, only CPK has the calculated value above the recommendation value. These three parameters should be taken into consideration when evaluating the enrollment of a subject.

When compared to the Sibille reference values, it is observed that the parameters Sodium; Calcium; Total Bilirubin; Creatinine in men; Total Protein; Albumin; Total Cholesterol; and Triglycerides present the minimum value lower than the Sibille value. Again, this difference is not significant, because none of the changes are considered AE. As for the maximum values, Calcium; Total Protein; and Triglycerides show a value higher than the maximum Sibille value, calcium and triglycerides remain the parameters to be taken into consideration.

After this analysis, it was possible to verify that despite the differences, the study population has laboratory results that do not appear to be relevant either for the safety of the subjects or to the study objectives, even though they may be outside of the "normal" range defined by the laboratory.

Although no major differences between men and women are expected in the parameters for which there are no separate reference values, a problem with this analysis is the fact that Sibille's study only contained male subjects and that these values were also used for comparison.

It is important to note that not all the parameters had the same number of subjects which makes the comparison in the parameters with smaller samples, more likely to show a value out of reality.

The purpose of this dissertation was to reflect on the reference intervals used for the screening and inclusion/exclusion of subjects in phase I clinical trials. It was realized that reference intervals cannot be faced as fixed and inflexible. As said before, a single value outside the reference range does not mean a pathology. With this the calcium calculated

upper limit, the CPK calculated upper limit, the total cholesterol calculated upper limit and, the triglycerides calculated upper limit, may not be a reason, by themselves, to exclude a subject from a clinical trial.

This analysis was performed in a population of healthy individuals. Nevertheless, not all analytical parameters were within the interval classified as normal. Also, the reference intervals have some differences according to the laboratory and, when calculated, according to the healthy population used. Being so, it is possible to conclude that in a healthy population it is possible to find laboratory abnormalities, and this doesn't necessarily mean absence of a healthy status. In order not to exclude healthy participants because of a punctual not relevant abnormality, it is important to define which range of abnormalities are allowed, never jeopardizing the safety of the participants or the study results, and always respecting the physician interpretation of all data.

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