Cytology and Hematology: A Review of the Fundamental Principles

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Abstract Since ancient times, human beings have been looking for alternative methods to detect and diagnose diseases, mainly by assessing patients' body fluids. The creation of the microscope contributed to the development of modern techniques of clinical analysis, as this instrument allowed the visualization of structures invisible to the naked eye and opened the door to various types of scientific research. Based on this, it is essential to develop studies that synthesize the fundamental information on hematology and cytology, in order to support future research as well as assist in the academic training of professionals in the medical fields.

Keywords Blood cells · Hematology · Blood count · Hematological devices · Cellular biology

1 Introduction

The cells are described as functional and structural units defined as the smallest part of a living organism. The cells that make up a living organism are all the same, with the same genetic load; however, due to the natural process of cell differentiation, they present variations in shape and performance according to the function they perform

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in the organism. This division of tasks between cells is a strategy for saving energy and increasing efficiency in carrying out biological functions [1].

The cells have the same biological function that are grouped together and form a tissue, which in turn form the organs (brain, lungs, intestines, heart). A grouping of organs creates a system (neurological system, respiratory system, digestive system, cardiovascular system). The set of systems originates organisms, such as plants, insects, parasites, and even the human body. This entire process must take place in an extremely precise and harmonic way. Otherwise, the changes will be so drastic that they are incompatible with life [2].

Cells are organisms susceptible to changes in the environment in which they are. According to Darwin's theory, over time, there may be climate changes and/or shortages of nutrients, consequently, such factors stimulate physiological changes to organisms [3]. In this way, only the organisms that are able to adapt are able to survive [2].

The human body has about 100 trillion cells, consisting mainly of carbon, oxygen, hydrogen, calcium, phosphorus, and nitrogen [1, 4]. About 70% of the human organism is formed by hydrogen and oxygen, which are responsible for the formation of water. The other 30% are responsible for the constitution of carbon-based compounds, which are called organic compounds [5, 6].

Cell classification is done in two distinct types: prokaryotes and eukaryotes. The prokaryotic cells (from the Greek, pro = first; carium = nucleus) are small (1–5 μm), have a cell wall, responsible for mechanical protection, and have genetic material dispersed by the cytoplasm, that is, they do not present—they have a nuclear envelope [2, 7]. These cells include bacteria and blue algae, being formed by only a single cell, that is, they are classified as unicellular [2, 7, 8].

Eukaryotic cells (from Greek, *eu* = *true; carium* = *nucleus*) have more complex genetic material, being formed by DNA and RNA, which are organized in the cytoplasm through the cell envelope. Eukaryotic cells are responsible for the formation of more complex organisms, that is, those formed by more than one cell (multicellular organisms). Among these organisms, it is possible to mention: vertebrate and invertebrate animals and plants. In addition, the cytoplasm of eukaryotic cells has compartments that have different molecules responsible for performing specialized functions [2, 7].

These cells have a more complex structure, being composed of several structures such as cell membranes, nuclear envelope, nucleus, cytoplasm, peroxisomes, lysosomes, endoplasmic reticulum, mitochondria, Golgi complex, cytoskeleton, plasma membrane, and cytoplasmic deposit [2, 7]. However, such discoveries could only be made through the invention of the optical microscope.

Thus, the objective of the present study is to carry out a bibliographic review regarding cytology and hematology, in order to easily explain the synthesis of blood cells as well as their biological functions.

2 Methodology

The present study is based on the collection of information from 27 bibliographic sources in the medical literature that addresses the subject of cytology and hematology. These sources were chosen because they have an emphasis on blood cell synthesis.

3 Results and Discussion

The first notion of microscopy appeared around the year 1595, through the works of the Dutchman Zacharias Janssen, a glasses manufacturer who, through the union of two lenses on the same axis, noticed the presence of small structures in objects. Later, in the year 1665, Robert Hooke, using an ocular lens and an objective lens, built the first microscope. This work originated in the book Micrographia, where different living beings observed under microscopy have been described [4].

For 50 years, the Dutchman Anton van Leeuwenhoek, burned lenses and built high-quality microscopes for his time. In this way, Leeuwenhoek was the first to describe the various forms of bacteria in a drop of water from a lake, with his findings constantly being sent to the Royal Society of London. Later, through the scraping of his own teeth and water from washing a pepper, Leeuwenhoek described the presence of bacterial structures. The Royal Society, after analyzing the letters, sent Robert Hooke, who confirmed the veracity of the observations made by him [4].

Human eyes are capable of detecting variations in intensity and in the length of light waves. This human capacity has been expanded through advances in the field of microscopy, which have allowed for increased resolution as well as the use of techniques that compensate for the transparency of cells. Cellular components are generally transparent, with the exception of some cytoplasmic pigments capable of absorbing certain wavelengths of light. Living cells have low absorption of these wavelengths, as they have a high concentration of water, so even when dehydrated this problem persists. To compensate for this limitation, dyes are used that selectively stain the different cellular structures. These dyes are made up of compounds capable of absorbing specific wavelengths [8, 9].

The resolution limit of the microscope is responsible for providing the details observed in the images generated from a sample. In this way, it has no relation to the capacity of increasing the size of the analyzed image. Thus, it is determined by the shortest distance between two points [3]. It is important to note that the increased capacity is valid only when accompanied by a parallel increase in the equipment's resolving power. Thus, the resolution limit is dependent on the ocular and objective lenses, which are unable to add details. Thus, ocular lenses are totally dependent on objective lenses, as they are responsible for increasing the size of the image (Fig. 1) [3, 9].

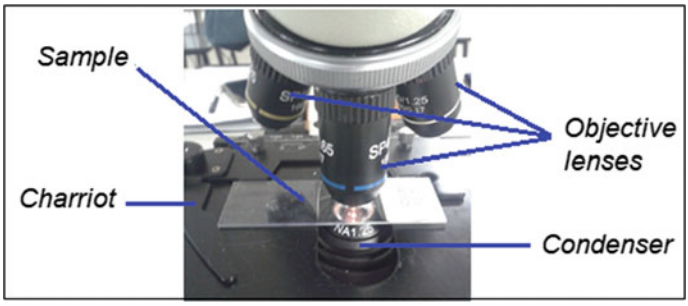

Fig. 1 Microscope structure—sample analysis example

In Eq. 1, it is possible to note the instrument's resolution limit (RL) of the objective lens. In this context, the microscope resolution limit is totally dependent on the numerical aperture (NP) coming from the objective lenses, and it is also necessary to consider the length of light applied to the sample.

$$\mathrm{NP} = k \times \lambda AN \tag{1}$$

where k is a constant estimated in seconds, being numerically represented by 0.61 and λ (lamina) is the wavelength of the light used. Due to the greater sensitivity of human eyes to the wavelength of the yellow–green band, the value of 0.55 μm is used in the calculation of the resolution limit. Through this formula, it is notable that the resolution limit is directly proportional to the wavelength of the light used, and it is also inversely proportional to the numerical aperture of the objective lens [2, 3, 9].

The creation of the first microscope opened the door to the development of techniques used in the areas of clinical analysis. Clinical analyses date back to the age of Hippocrates, the father of medicine. At that time, the color, odor, taste, and texture of biological fluids were important tools for patients' clinical diagnoses [2, 8].

Later, with the creation of the microscope, it was possible to correlate the physical characteristics of biological fluids with their microscopic constitution of the analyzed samples. In other words, it was understood that the human organism is formed by cells and can be attacked by fungi, bacteria, and parasites. The characterization of the morphology and after of the physiology of blood cells was essential (and still is) for the conclusion of several types of human pathologies [2, 8].

Human blood represents approximately 7% of a person's total body weight, with its constitution corresponding to 55% of the plasma and 45% of blood cells. Among the diverse functions performed by the blood, it is possible to mention: basic acid balance, excretion of metabolites, transport of gases and nutrients to organs and tissues, distribution of hormones, osmotic balance, and temperature regulation [2, 7–9].

The function of transporting substances such as plasma proteins, water, amino acids, glucose, hormones, immunoglobulins (IG), albumin, components of the coagulation cascade, among others, is the responsibility of the plasma. Plasma is the liquid part of the blood, disregarding any and all types of cells. Plasma is of great importance for coagulation analysis, while serum is characterized by being a plasma

with the absence of coagulation factors. The serum is widely used for biochemical tests, such as glucose measurement, renal markers, liver chewers, pregnancy tests, hepatitis, HIV, among others [2, 8, 9].

The solid portion of the blood is made up of blood cells, which are called erythrocytes, leukocytes, and platelets. The main function of these elements is homeostasis of the body, tissue repair, blood clotting, and defense of the body against various types of etiological agents, which are usually: viruses, bacteria, and intestinal parasites. However, the discovery of all blood elements as well as their respective functions is the result of the invention of instruments capable of increasing the size of cells and ethylene agents hundreds of times [7–9].

3.1 Hematopoiesis

The synthesis of human blood, uterine life begins at the same time. During the embryonic phase, the first blood cells are synthesized by the yolk sac. Later, when the fetus begins to develop, the synthesis of blood elements becomes a liver and splenic function. When the individual reaches adulthood, the bone marrow takes on the task of producing blood cells. This is because this region is composed of hematopoietic tissue with controlled and orchestrated conditions for the formation of each precursor cell. It is important to note that any of the blood cells come from the stem cell, which resides inside the bone marrow [10–13].

The entire process of blood cell formation is called hematopoiesis and occurs within the bone marrow from a mother cell also known as a stem cell. The main characteristic of this cell is to be of the pluripotent type, that is, it has the capacity to originate different blood cell lines, be they erythrocytes, leukocytes, or platelets (Fig. 2). However, for these syntheses, several coordinated actions are necessary that generate orchestrated responses that culminate in processes of duplication, differentiation, and maturation. It is important to note that many sanguineous cells end their maturation process in the bloodstream or in lymphoid organs [14, 15].

The stem cell is a cell capable of self-duplication, giving rise to daughter cells. The daughter cells are the Colony Forming Units (CFU), as they have the genetic information necessary for the formation of a cell series. The hematopoiesis process is characterized by the participation of regulatory and stimulating factors such as cytokines, erythropoietin (regulator of erythropoiesis), thrombopoietin (regulator of thrombocytopoiesis), Granulocyte Colony-Stimulating Factor (G-CSF), Monocyte Colony-Stimulating Factor (M-CSF), Macrogaph Colony-Stimulating Factor (M-CSF), IL-3 (multiple colony-stimulating factors) and interleukins IL-2, IL-4, and IL-6 [12, 13, 16].

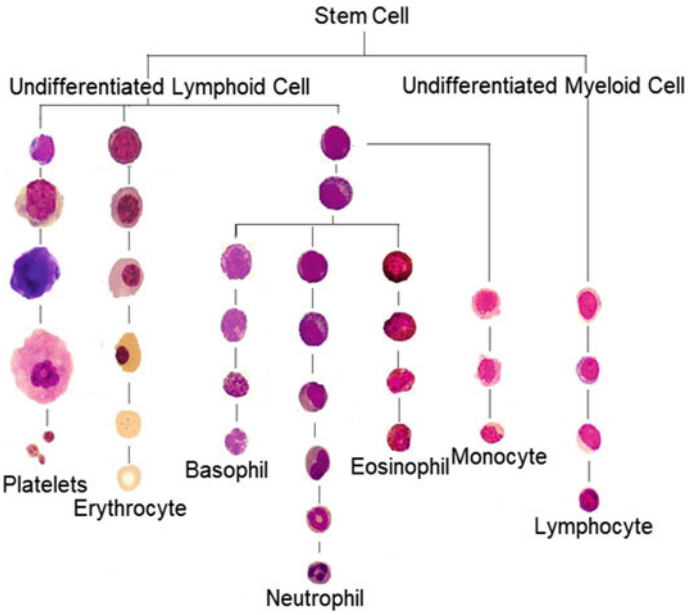

Fig. 2 Hematopoiesis

During the embryonic phase, the yolk sac is responsible for the formation of the first blood cells; later, during fetal development, this function is transferred to the liver and spleen. During adulthood, the inside of the bone marrow consists of hematopoietic tissue (or hematopoiesis), which resides inside long bones and in the axial skeleton. Inside the bone marrow, there is a network of venous sinusoids around an arteriole and central veins, which permeate the developing cells. All blood cells are derived from bone marrow stem cells. Thus, the bone marrow presents a suitable microenvironment for the development of hematopoietic cells and for the proliferation of primitive cells and progenitor cells [10–12].

Cytokines are glycoproteins that act in low concentrations on re-receptors, producing signals that control the cell cycle, maturation and cell functions. Generally, they are responsible for the regulation of more than one cell line and show an additive and synergistic effect with other growth factors, modulating the expression of regulatory genes producing cytokines [12, 13].

3.1.1 Erythrocytes

In the literature and in the medical routine, erythrocytes receive other nomenclatures such as red blood cells (RBC) and erythrocytes. The main morphological characteristics of these cells are the absence of a cell nucleus, a biconcave disk shape, and formation based on a tetramer, that is, two α chains and two β chains, which carry out the transport of oxygen through the connection of the oxygen molecule to each of the iron molecules [17, 18]. The gas exchange process occurs when the erythrocytes reach the lungs, at that moment there is the formation of oxyhemoglobin (oxygen molecules linked to the iron molecules present inside each red cell). Upon reaching the tissues, this iron–oxygen bond is broken due to the pressure difference. In this way, red blood cells deposit oxygen molecules and remove carbon dioxide from tissues. The binding of carbon dioxide to iron is called carboxyhemoglobin. Subsequently, the dioxide is transported to the lungs or dissolved in the plasma [17–20].

The erythrocytes originate in the bone marrow, being formed through the cell maturation processes of the following cells: proerythroblast, basophilic erythroblast, polychromatic erythroblast, orthochromatic erythroblast, and reticulocyte. By differentiating the pluripotential cell (Stem cell) and erythropoietin stimuli, proerythroblasts are created [13, 19, 20].

The final stages of the maturation process are characterized by the formation of reticulocytes, which are also called young red blood cells. The reticulocytes have mitochondria, an irregular shape, and several remaining organelles inside. The most striking feature of cell maturation is the reticulocytes leaving the bone marrow into the bloodstream, where within 72 h, their cell nucleus expels, giving rise to erythrocytes [13, 20].

3.1.2 Leukocytes

The body's innate immune response and adaptive immune response are the results of the action of leukocytes. Both in the literature and in the medical routine, leukocytes are also called white blood cells (WBC). These cells are classified into two major groups: granulocytes and agranulocytes. Granulocytic leukocytes are defense cells characterized by the presence of granules dispersed in the cytoplasm. These granules exert anti-inflammatory and antimicrobial action. Another morphological characteristic of these cells is the presence of a variable number of lobes. Thus, granulocyte cells are also called polymorphonuclear cells. The leukocytes that fit these characteristics are neutrophils, eosinophils, and basophils. In turn, leukocytes classified as agranulocytes do not have granules visible under optical microscopy generally used in the routine of several clinical analysis laboratories. Another striking morphological feature is the presence of only one lobule. In view of this, these leukocytes are also called monomorphonuclear nomenclature. Agranulocyte leukocytes are lymphocytes and monocytes [17, 18].

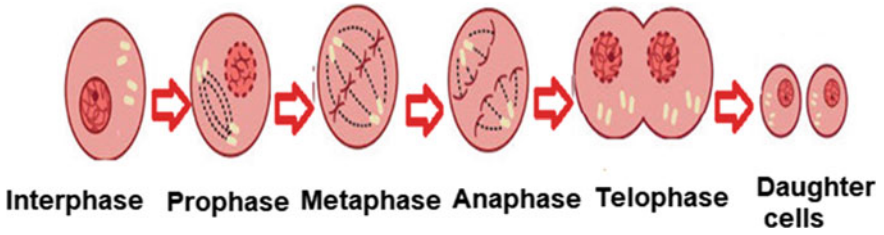

Fig. 3 Mitosis phases

The synthesis of leukocytes occurs during hematopoiesis, where the leukocyte precursor cells of the granulocytic series (neutralphils, eosinophils, and basophils) start the process of producing proteins and cytoplasmic granules. In this context, the primary granules are characterized by blue staining and are responsible for the conversion of precursor cells called myeloblasts to promyelocytes. Subsequently, specific granules appear, which are responsible for the progression to myelocytes of the type: neutrophils, eosinophils, and basophils [19, 21].

The leukocytes resulting from the process of determining the granulocytic lineage are indivisible by mitosis (The phases of mitosis can be seen in Fig. 3). In addition, these cells have a segmented nucleus, the ability to phagocyte particles, the destruction of microorganisms and motility. It is important to note that depending on the leukocyte lineage, when it reaches the maturation process, it has the ability to cross the wall of venules in order to destroy invading agents and rebuild damaged tissues [13, 21].

Neutrophils

After determining the production of the neutrophil lineage, the cell maturation sequence is myeloblast, promyelocyte, myelocyte, metamyelocyte, rod neutrophil, and segmented neutrophil, with the production of neutrophils stimulated by G-CSF. An adult human being produces more than 1×10^{11} neutrophils per day, so neutrophils make up about 70% of blood cells. In addition, after leaving the inside of the bone marrow, each neutrophil survives an average of 6 h in the bloodstream [12, 13, 19].

Until the metamyelocyte phase, the cells are contained within the bone marrow; however, from the neutrophil rod stage, cells are released into the bloodstream. The rod neutrophils measure about 12 μm, with a horseshoe-shaped nucleus or stick, remaining in this stage until the segmentation of the lobes undergoes. When found in abundance in the bloodstream, these cells indicate the presence of infection and/or acute inflammation [12, 13, 19].

Segmented neutrophils, in turn, are characterized by a spherical shape with an approximate diameter of 12–15 μm, and the nucleus is segmented from 3 to 5 connected lobes. Mature neutrophils have primary and secondary granules; however,

secondary granules are more numerous, about twice as many as primary granules. The primary granules are azurophilic, have positive peroxidase, and measure about 500 nm. These granules are made up of a large amount of lysosomal enzymes (a lysosome, elastase, proteinase 3—and α—antitrypsin), and bactericidal factors are formerly known as cationic proteins (defensins, bactericidal factors and bactericidal protein to increase permeability). In turn, the secondary granules are smaller, measuring about 200 nm and contain lactoferrin, lysozyme, and protein-bound to B12 and other proteins [13, 19].

Classified as phagocytic cells, neutrophils have the primary function of identifying, ingesting, and destroying microorganisms. The host's defense responses consist of sequential stages of recognition and activation of phagocytes and the destruction of microorganisms. Neutrophils spend less than 48 h in the bloodstream before migrating to the tissues, being the first cells to reach the sites of infection and/or inflammation. Thus, through direct contact with protein secretion, and the presence of receptors for antibodies and complements, these cells are able to recruit and mediate other cells of different lineages to the site of inflammation and/or infection. After the defense action, these cells undergo programmed cell death (apoptosis) [12, 21].

Eosinophil

The maturation phases of eosinophils follow a similar line to that of neutrophils, is described through the following stages: eosinophil myeloblast, promyelocyte, myelocyte, and mature eosinophil, which suffer the action of G-CSF, IL-3, and IL-5, responsible for eosinophil maturation) [13, 21].

Mature eosinophils represent about 2–3% of leukocytes and act on innate and acquired immunity, being present in the mucous membranes of the respiratory, gastrointestinal, and genito-urinary tracts, and may increase in number due to the recruitment of these blood cells in inflammatory and/or infectious conditions. Classified as granulocytic cells, eosinophils have cytoplasmic granules that contain enzymes responsible for damaging the cell walls of parasites or acting against allergic responses. These granules contain basic proteins that bind to acidic dyes like eosin [21, 22].

Eosinophilic granules and their myeloid precursors are abundant in peroxidases and lysosomal enzymes; however, eosinophil peroxidase is genetically different from neutrophils, conferring less bactericidal action when compared to neutrophils. Thus, the cellular function of eosinophils is directly linked to the storage of proteins, such as major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin [12, 20].

The larger basic proteins represent more than half of the granule proteins and have cytotoxic activity against parasitic infections. They also induce the release of histamine by basophils and mast cells, this process acts in the negative regulation of allergic and inflammatory processes. The other basic proteins can cause the formation of transmembrane pores capable of causing tissue damage [11–13].

Basophils

Basophils are granulocytic cells that act on innate and acquired immunity. Its granules are filled by various inflammatory and antimicrobial mediators, thus being responsible for the immune responses against allergic reactions. Despite the structural and functional similarities with mast cells, basophils originate from other progenitor cells: promyelocyte, basophilic myelocyte, basophilic metamyelocytes, basophilic, and basophilic rod. The entire process of development and maturation occurs inside the bone marrow, with only the mature basophil being released into the bloodstream [12, 19].

These cells represent less than 1% of the leukocytes in the blood. The granules of the basophils are abundant and dense, capable of preventing complete visualization of the nucleus under an optical microscope and have an affinity for basic dyes. In addition, they are able to synthesize several mediators. Although they are not normally present in the tissues, basophils can be recruited to some inflammatory sites. Like mast cells, basophils express receptors for immunoglobulin G (IgG) and immunoglobulin E (IgE) and can be stimulated by binding the antigen to the IgE on its surface [12, 15].

Monocytes

Monocytes are classified as agranulocytic cells, originating from the following lineage: monoblast, pro monocyte, monocytes, and macrophages. The functions of monocytes are the removal of dead or senescent cells, removal of foreign particles, processing, and presentation of antigens in immunological reactions and participation in acute and chronic inflammatory reactions. They also have an important role in the formation of atheromatous plaque (related to atherosclerosis) and in the destruction of both microorganisms and neoplastic cells and grafts [22, 23].

Monocytes are characterized by acquiring their functional maturity in the tissues in the form of macrophages. There are two possibilities after the monocytes leave the medulla: either the cells are housed in the marginal compartment of blood vessels (adhered to endothelial cells) or are released into the bloodstream [22, 24].

These cells are the largest leukocytes, measuring 15–20 μm in diameter; however, they are responsible for forming only a small portion of the total blood cell population. The monocyte nucleus is euchromatic being relatively large and irregular, having the characteristic of invagination on one side. Next to this nuclear invagination, this cell contains a prominent Golgi complex and vesicles. Monocytes are actively phagocytic cells, containing numerous lysosomes. They are also highly mobile and with a well-developed cytoskeleton. In turn, the cytoplasm is light in color [23, 24].

Lymphocytes

Lymphocytes are the second most numerous type of leukocytes in adults, while during childhood they are the most numerous type of blood leukocytes. They are formed through the following lineage: undifferentiated lymphoid cell, lymphoblast, pro-lymphocyte, medium lymphocyte, small lymphocyte, and plasmocyte [25, 26].

Most circulating lymphocytes have a small size of 6–8 μm in diameter and increased cytoplasmic volume. Often, this change occurs as a result of antigenic stimulation. These cells are found in extravascular tissues (including lymphoid tissue), being classified as the only leukocyte that returns to the circulation. The lifespan of lymphocytes varies from a few days to many years. Long-lived lymphocytes play an important role in maintaining immune memory [25].

Blood lymphocytes are a heterogeneous collection, mainly of B and T cells, and consist of different subsets and stages of activity and maturity. About 85% of all lymphocytes circulating in the blood are T cells. Primary immunodeficiency diseases can cause molecular damage to T and B lymphocytes. Included as lymphocytes are Natural Killer Cells (NK). NK cells are morphologically similar to T cells [26].

B lymphocytes and T lymphocytes have a rounded nucleus with dense color, surrounded by a sparse cytoplasm, which is hardly visible under optical microscopy. In the electron microscope, few cytoplasmic organelles can be seen, except for a small number of mitochondria, isolated ribosomes, scarce ER profiles, and occasional lysosomes. These cells become mobile when they come into contact with solid surfaces and can pass between endothelial cells to exit or reenter the vascular system. Thus, they migrate widely within the various tissues, including the epithelia [26, 27].

3.1.3 Platelets

Platelets are cytoplasmic fragments of megakaryocytes produced in the bone marrow. Because they are fragments, these cells have no nucleus and are therefore anucleated. They have a discoid shape of about 2-4 μm in diameter and a very complex internal structure divided into four zones: peripheral zone, sol-gel zone, organelle zone, and membrane system. The interior of the platelet is able to communicate with the external environment due to the presence of a channel system known as the open canalicular system. This communication is important, as it guarantees the release of molecules stored in platelets [13, 19, 20].

Platelets have significantly reduced blood circulation time, taking an average of 10 days to remove. These cell fragments are removed from the circulation by the reticuloendothelial cells of the liver and spleen. These cells have important functions for the maintenance of our bodies. When, for example, a lesion occurs in a blood vessel, they clump together, forming a plug, and release substances that ensure that more platelets move to the site. In addition, they participate in the coagulation cascade, releasing important substances that guarantee the formation of a clot. It is worth noting that platelets also have enzymes that contribute to the removal of the clot [13, 19, 20].

4 Conclusions

Cells are the fundamental units of life on Earth, having undergone countless changes since their emergence. These changes allowed the evolution of living beings to the form and function they currently have. This evolution was due to the specialization of cell groups to perform specific functions aimed at ensuring the balance of life of organisms. Among these developments, there was the appearance of blood cells, which are divided into three basic types: erythrocytes, leukocytes, and platelets, which are responsible for the transport of gases throughout the body, defense against antigens, blood clotting, among other functions.

The importance of the classification of blood cell types is that through this knowledge it is possible to detect and carry out treatment of types of blood disease in which blood components are affected such as anemia derivations through red blood cells, immune thrombocytopenic derivations through platelets, leukemia derivations by white blood cells, among other several hematological diseases, such as types of hemoglobinopathies and hereditary coagulopathies, in the same way, that it is possible to detect several types of related disorders and diseases.

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