

Applications of Flow Cytometry in the Diagnosis of Hematologic Diseases in Brazil: Current Status and Future Prospects

Mariana Alves Rocha, Laila Nur Syafitri, Bambang Wicaksono Hartono

Department of Laboratory Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

ABSTRACT

Contemporary flow cytometric analysis is a dependable and effective method for examining people with haematological malignancies. In the majority of cases with leukaemia and lymphoma involving the blood and marrow, it is the most significant single inquiry. When cancer cells are present in intricate mixes of normal cells, as is often the case in the bone marrow and lymph nodes, flow cytometry is very useful in detecting them. The sensitivity of detection has risen due to advancements in reagents and equipment, and flow cytometry is now used to monitor patients following therapy in cases where extremely low amounts of residual illness may exist. This enables more accurate therapy administration and treatment result prediction. The quality of the sample is the most significant issue that might restrict the use of flow cytometry. Thus, it is essential to place samples in the appropriate bottles and get them to the lab on time. Using a laser beam in a fluid stream, single cells may have several phenotypic features measured and examined using a laser-based analytical method called flow cytometry. Cell components such as surface proteins are labelled throughout the experiment using fluorescent tags that are often affixed to antibodies. The detector located close to the laser beam detects the wavelengths of light that the fluorescently tagged cells release as they move through the beam. It is possible to investigate the identification of individual cells using their fluorescence and light-scattering characteristics.

Keywords: Flow Cytometry (FC), Diagnosis (DD), Hematologic Disease (HD), Brazil (B)

INTRODUCTION

Flow cytometry refers to a special laboratory technique used to differentiate among different cells based on physical and chemical properties. The other term is hematologic diseases, which refer to main blood disorders that may affect the production or function of blood cells such as red blood cells, White blood cells, and platelets. In this introduction, we will discuss how flow cytometry can be used to diagnose hematologic diseases in Brazil [1]. There are various ways of using flow cytometry to diagnose hematologic diseases in Brazilian healthcare sectors. The first and foremost application of Flow Cytometry in diagnosing Hematologic Diseases is the immunophenotyping of leukaemia cells. This immunophenotyping of leukaemia is a complex process of recognizing and characterizing antigens on the surface of leukaemia cells by using antibodies or flow cytometry. There are a variety of benefits to this technique, which are described below. The first and foremost benefit of this technique is that it can be used for early diagnosis of leukaemia [2]. This technique can easily identify the specific subtypes of leukemia. The other benefit is that it can monitor to monitorial diseases. It can also be used to detect leukemia cells in the bone marrow. The diagnosis of leukaemia cells by immunophenotyping consists of a few important steps. The first step is sample collection from bone marrow, tissue, or blood. Then, cell preparation is done using cell staining or cell fixation. The third step, which is the most important, is the flow cytometry analysis. The fourth and final step is the data interpretation from these samples. Another important blood disease is anemia which can also be detected using flow cytometry[3]. As we know, there are different types of anaemia, such as iron deficiency anaemia, vitamin deficiency anaemia, anaemia of chronic disease, sickle cell anaemia, thalassemia, aplastic anaemia, and others. Various flow cytometry biomarkers are used to

diagnose anemia. For example, CD71 can be used to diagnose iron deficiency anemia. The Flow Cytometry biomarker CD 105 can be used to diagnose erythropoiesis. Different flow cytometry techniques are used to diagnose these blood disorders. These Techniques are forward and side scatter analysis for cell size and complexity, fluorescence analysis for antigens expression, cell sorting for cell population, multipara metric analysis for phenotype, and others[4]. The other important hematologic disorder is lymphomas. This blood disorder can be described as a group of cancerous cells that originate from lymph nodes. This is due to abnormal production of lymphocytes in the body, and then these lymphocytes accumulate in lymph nodes, causing lymphomas. There are many types of lymphomas, such as non-Hodgkin lymphoma, Hodgkin lymphoma, T cell lymphomas, and B cell lymphomas. All These types of lymphomas can easily be detected by flow cytometry. For example, important biomarkers used for B cell lymphomas are CD19, CD20, CD23, and CD22. There are also specific biomarkers that are used to diagnose T-cell lymphomas. These biomarkers are CD3, CD4, CD8, CD45RO and others. The important biomarkers for non-Hodgkin lymphoma are CD 10, CD5, and CD 23[5]. All of these are important aspects of the current status of Flow Cytometry in Brazil. However, we also have to discuss the prospects of flow cytometry. The first prospect related to flow cytometry is an aim to expand easy access to Flow Cytometry in small healthcare sectors and clinics. The next prospect related to flow cytometry in Brazil is the standardization of the main reagents used. The third prospect is that there are a variety of efforts to combine flow cytometry with molecular diagnostics to provide accurate and swift results in less effort[6]. The next prospect is that there should be a spread of awareness in healthcare sectors as well as the use of flow cytometry for the diagnosis

of hematologic disorders. This technique can provide effective, accurate, and timely results. The next prospect is that it can be used to monitor the treatment response in the body. As we know, this technique is based on identifying specific antigens, so when treatment is provided, the body's response to the provided treatment can easily be understood by flow cytometry. The other prospect is that many rare and undiscovered hematologic disorders have to be understood shortly. So, these rare and complex cases of hematologic disorders can easily be solved by flow cytometry in Brazil [7]. The next important prospect of using flow cytometry in the diagnosis of hematologic diseases is that it can be accurately and effectively used for research purposes such as stem cell analysis. However, there are some important challenges to the use of flow cytometry in Brazil's healthcare sectors. The first and foremost challenge is limited availability of such systems, making it difficult to use and operate. The other important challenge to the use of Flow Cytometry in the diagnosis of hematologic diseases is that the equipment used in flow cytometry has a high cost, which acts as an obstacle to the proper adoption of such systems in the healthcare sectors of Brazil [8]. The next challenge to the use of flow cytometry in Brazil is the stringent need for quality control and standardization for accurate results. Such advanced technologies are rarely available in urban areas, so there is no question of their use in rural areas [9]. However, some important research initiatives are taken by Brazilian research departments, such as the National Institute of Science and Technology in stem cells and cell therapy, the Brazilian Hematology research network, the Flow Cytometry research group, and others. Along with this, there are some international initiatives in the aspect of flow cytometry [10].

Research Objective

The main objective of this research is to understand the main applications of Flow Cytometry in the diagnosis of Hematologic Diseases in Brazil. This study effectively explains the current status and prospects of Flow Cytometry in Brazil. The research study determines the Applications of Flow Cytometry in the Diagnosis of Hematologic Diseases in Brazil. The research paper is divided into five specific chapters. The first section represents the introduction also explains the objective of the research. The second section represents a literature review, and the third portion describes the methods and implications of research. The fourth section describes the results, and its descriptions also the last portion summarizes the overall research study and presents recommendations about the topic.

LITERATURE REVIEW

Researchers claim that ALL is a disease characterized by its ability to cause malignancy and genetic defects. The dysfunction of the hematopoietic system or improper functioning is the main cause of ALL. Using biomarkers for disease diagnosis and malignancy plays a crucial role in clinical processes [11]. Studies reveal that various diagnostic techniques like ELISA and IPMA are used to detect the agent responsible for causing PPE. The diagnostic test IFAT detects the PEE-causing agent as *lawsonia intracellular*. Of all these diagnostic test methods, the most critical one is FCAT.

The flow cytometry test is special as it uses antibodies to detect disease-causing agents [12]. Studies conclude that about one-fourth of the world's population faces the TBI problem. This disease is caused by a bacterial infection that causes severe infections and results in another TBI type, LTBI. The diagnostic test used to identify the bacteria

responsible for TBI is TST. This laboratory test identifies the disease, and then proper TBI therapies are provided to the patients for full recovery [13]. Studies claim that diagnostic and clinical trial methods for detecting the presence of promyelocytic leukaemia have been greatly advanced. The Brazilian Society for Hematology Pathology provides insight into the complexity associated with leukemia-related disorders and also predicts the use of salvage therapy for intervention purposes [14]. Studies suggest that CLL is a disease characterized as choric due to ROR 1 expression. The expression patterns of ROR 1 are identified using immuno-phototyping. this prototyping technique is one of the applications of flow cytometry [15]. Studies show that Libyan patient's clinical examination predicts that ALL is an acute disorder. This disorder increases the chances of cancer cell development in patients. The technique used for acute ALL detection is flow cytometry. In medical centers, using flow cytometry provides information about the morphological future of ALL patients [16]. Scholar predicts that the chronicity of Chagas disorders increases due to the high toxic effects of this disease. New and effective drug development is crucial for treating the toxicity associated with Chagas disease. different assay techniques have developed active compounds for drug development against Chagas disorder [17]. Studies claim that flow cytometry methodology was developed during the 1950s. but in the present time, this technology is used as a gold standard technology that provides information related to cellular activities assisted with specific diseases. LSC is a modernized form of a flow cytometer as it thoroughly scans the target area and then provides authentic information [18]. Studies predict that Brazilian clinicians have made great efforts to photo-ty the leukemia disease. flow cytometry-based leukaemia tests are performed to profile the phenotypic and genotypic characteristics associated with the disease. After profiling the disease, related treatment therapies are developed [19]. Studies elaborate that the main system of the body that saves it from any foreign attack is the immune system. but in some cases, the disease condition overpowers the immune system and causes severity. The flow cytometry technique is used in laboratories to identify the pathology of different disorders that impact the immune system. flow cytometry is specialized to determine the influx of Ca ions in the blood of a disease patient [20]. Researchers claim that iPSC is used to produce RBCs. these RBCs mimic the actual RBCs and thus produce blood cells in SCD patients. The second use of iPSC was blood cell regents. these reagents act effectively for diagnostic purposes against various diseases. DNA array technology developed through advanced medical technology provides detailed information about the erythrocyte antigens [21]. Studies show that T cell neoplasm is a disease that is diagnosed through the use of TRBC 1 using clinical-based flow cytometry technique [22]. Studies suggest that millions of people globally are affected by managing the complexity caused by this disease. It is possible using modern preventive strategies. Biomarkers are used to track the changes that occur during the CKD condition and to target the exact cause of the disease. AI and machine technology enable clinicians to predict the chances of improvement in CKD disease conditions when drug supplements are provided to the patients [23]. scholars explain that improvement in the treatment methods of ALL is because of the early identification of risk factors. in children with ALL clinicians [refer to optimized conventional therapies based treatment methods [24]. Studies show that the effectiveness of western blotting and florescence technology fades as improved intracellular protein abundance technology is developed to detect different abnormalities. Protein analysis by flow cytometry provides improved results for multiple [protein identification. only a large-scale

technology is acceptable in clinical labs for large protein determination in most cases[25]. Scholarly studies conclude that bacterial cells are identified using a more prominent technology, flow cytometry. The majority of foodborne diseases are detected through an antibiofilm monitoring system. developing natural compounds in laboratories for food-borne patients significantly lowers the number of cases of food-borne disease [26]. Studies show that the population already suffering from any vulnerable disease tends to get TBM. The patients at high risk of HIV are more prone to TBM. With technological advancement, the disease management methods for TBM have been revolutionized. Laboratory clinicians use PCR tests to make reports regarding the condition of TBM patients [27]. Studies claim that new reports have been made regarding bioanalysis through research papers. many professional biotechnology and pharmaceutical companies have discussed different concepts and approaches in white research papers regarding the effectiveness of bioanalysis. bioanalysis allows for assay development and disease identification The development of different technology-based imaging techniques is due to bioanalysis[28] Studies point out the ability of bacteriophages to cause disease and spread from one population to another The technique made for the analysis of bacteriophage structure is bioassay. Bacteria-based assay technology is an emerging technology that predicts the mode of microbial action. by assessing the mode of spread of microbial disease, it becomes easy to develop medications for its treatment[29].

Studies claim that in thrombosis, the ability of blood cells to flow normally becomes disturbed, resulting in blood clots. The anticoagulation ability of blood cells is reduced greatly due to disease complexity. Moreover, the prediction of the genetic factor for inducing thrombosis is possible using AI-based techniques in diagnostic procedures. Based technology enables laboratory clinicians to deeply understand the nature of any complex human disease caused by fungus or bacteria[30].

Methodology

The research study determines the Applications of Flow Cytometry in diagnosing Hematologic Diseases in Brazil. The research was based on primary data analysis to determine the data used smart PLS software and generate results that including descriptive and correlation. The research study also explains the smart PLS Algorithm model between them. Hematologic Diseases are those related to blood disorders such as anaemia, leukaemia, thalassemia, and others. The increasing level of these hematologic diseases has worried the whole world. There is a need for such techniques that can be useful for the early diagnosis of these hematologic diseases in Brazil. With advancements in technology, the technique of flow cytometry was developed, which can be effectively used for the diagnosis of these hematologic diseases. Following are the important implications of using Flow Cytometry for diagnosis of hematologic Diseases in Brazil:

Immunophenotyping of Leukaemia Cells, Diagnosis of Other Blood Disorders, Monitoring of Stem Cell Transplantation, Detection of Residual Diseases

The term immunophenotyping refers to a special laboratory technique in which antigens that are present on the surface of cells are characterized. This is the most important clinical implication of using Flow Cytometry to diagnose blood disorders. The whole procedure of immunophenotyping consists of three important steps. The first

important step of immunophenotyping is the binding of antibodies in which antibody is bonded to antigens to form a complex. The second step of immunophenotyping follows the procedure of flow cytometry, in which cell surfaces are analyzed deeply. It has been observed that which antigen is attached to the antibody. The third step is the data interpretation, in which it is observed that whichever blood disorder is present in the sample. The other important implication of using Flow Cytometry in the diagnosis of Hematologic Diseases is identifying other blood disorders such as anaemia, thalassemia, and others. As we know, in every case of blood disorder, there is some specific kind of antigen in the body. If there is an accurate identification of this antigen in the sample. For this purpose, Flow Cytometry can be proven a very effective option because, in this Technique, there is an analysis of each component of the cell, making it possible to analyze antigens in blood cells. The other important implication of flow cytometry for diagnosing of hematologic diseases in Brazil ring of stem cell transplantation in the body. As we know, bone marrow's main function is producing blood cells. If there is any abnormality in the production of normal blood cells, it can also give rise to blood disorders. So, for diagnosis and treatment of blood disorders, it is also mandatory to monitor stem cells. In some cases, there is a fault in stem cells, so there is a need to transplant these unhealthy stem cells with healthy stem cells. After the transplantation of these stem cells, there is a need to monitor whether these cells are working accurately or not. For this purpose, Flow Cytometry can effectively monitor the transplantation of these stem cells. The other important implication of using flow cytometry for the diagnosis of hematologic diseases is the detection of residual diseases. The word residual diseases refer to the presence of remaining cancerous cells in the body, which can again lead to disease progression. Flow Cytometry can detect the presence of these cancerous cells in the body so that better and more effective treatment can be provided. In this way, Flow Cytometry is also helpful in preventing disease progression after treatment. The important implications of Flow Cytometry convince us that it is an emerging and beneficial technique of the laboratory for many reasons.

Investigation of New Biomarkers for Hematologic Disease Diagnosis, Development of Novel Therapeutic Strategies, and Analysis of Immune Responses

As we know, the Flow Cytometry procedure is based on using biomarkers to identify antigens in blood disorders. The efficiency of this biomarker is measured by its ability to detect antigens in less time. The technique of Flow Cytometry is helpful in the development of new biomarkers that can perform their function in a better way. Such Biomarkers are designed with the help of Flow Cytometry, which can be used to detect multiple antigens at a single time by single use. In this way, we can say that the technique of Flow Cytometry is also helpful in the research perspective of healthcare sectors as well. The other important implication of flow cytometry for the diagnosis of hematologic diseases is the development of novel therapeutic strategies for the treatment of blood disorders. As we know science and technology are making progress day by day and this progress is also utilized in healthcare sectors as well. By data interpretation of Flow Cytometry, there are efforts to development of such novel therapeutic Strategies that can be proven less costly and more effective for the treatment of Hematologic disorders. The other important implication of using Flow Cytometry in the diagnosis of Hematologic Diseases is the aspect of studying the immune responses in the body. Medical science has proved that whenever a disease arises in the body, it means

that the body's immune system has failed in combating such disease. In other words, disease-causing antigens are not coped up by antibodies of immune systems. Because Flow Cytometry is based on the analysis of antigens it can also be used to analyze the efficiency of the immune system of the body to response to such disease-causing antigens in the body.

Implication Related to Future Directions of Flow Cytometry

Although flow cytometry has gained much importance in Brazilian healthcare sectors, there are some Future Prospects related

to Flow Cytometry in Brazil as well. The Brazilian healthcare sectors are working to make affordability and accessibility of Flow Cytometry possible in all areas of the country. It is because of the uses and importance of Flow Cytometry in the diagnosis of blood disorders. The other prospect related to Flow Cytometry in Brazil is the aspect of providing proper training to healthcare professionals. As we know there is the use of High-cost instruments in flow cytometry so there must be proper training of healthcare professionals to perform these procedures carefully. The other prospect related to flow cytometry is that there is a need for expansion in the application of Flow Cytometry as well.

Descriptive Statistical Analysis

Table 1: Results of Descriptive Statistical Analysis

Name	No.	Mean	Median	Scale Min	Scale Max	Standard Deviation	Excess Kurtosis	Skewness	Cramér-Von Mises P Value
FC1	1	1.500	1.000	1.000	3.000	0.608	-0.260	0.825	0.000
FC2	2	1.520	1.000	1.000	3.000	0.640	-0.262	0.867	0.000
FC3	3	1.500	1.000	1.000	4.000	0.728	1.707	1.443	0.000
FC4	4	1.540	1.000	1.000	3.000	0.639	-0.361	0.794	0.000
DD1	5	1.840	2.000	1.000	4.000	0.758	-0.156	0.568	0.000
DD2	6	1.720	2.000	1.000	4.000	0.749	0.261	0.818	0.000
HD1	7	1.680	2.000	1.000	3.000	0.676	-0.744	0.507	0.000
HD2	8	1.720	2.000	1.000	4.000	0.801	-0.230	0.804	0.000
HD3	9	1.980	2.000	1.000	4.000	0.969	-0.760	0.585	0.000

The results of table 1 mentioned above illustrate the descriptive statistical analysis and show the skewness value, mean values, median rates, lowest and maximum values, as well as the standard deviation rate. Additionally, the total probability value for each variable—dependent and independent—is included in the result. The results in Table 1 depict FC1, 2, 3, and 4, which are all regarded as independent variables. The mean values of these variables are 1.500, 1.520, 1.500, and 1.540, respectively, indicating a positive average value. 60%, 64%, 72%, and 63% of the standard deviation rate vary from the mean

values. The total probability value of the result is 0.000, indicating a 100% degree of significance between them.

The skewness values between them are 82%, 86%, 1.443, and 79% positive skewness rate. The mean values of the DD1,2 mediator variables are 1.840 and 1.720, respectively, indicating a positive average rate. The 75% and 74% deviations from the mean are indicated by the standard deviation. The research indicates that the maximum value is 4.000, and the minimum rate is 1.000 (Figure 1).

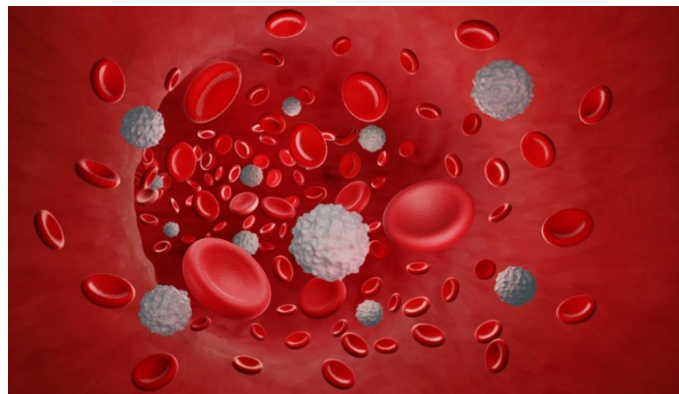


Figure 1: Flow Cytometry

The characteristics or features of cells that are frequently examined by flow cytometry shown in figure 1 include viability, size, volume, and cell cycle. The method was initially used to calculate the volume of cells in a sample in 1950. Today, nevertheless, it is frequently employed in biotechnology and pharmaceutical labs for a broad range of tasks, including protein abundance analysis, cell sorting, evaluating the purity of a cell population, and confirming the monoclonality of a cell line. In this article, you will learn further about the flow cytometry principles, instrumentation, applications and advancements in technology.

Principles of Flow Cytometry

For both qualitative and quantitative examination of the characteristics of individual cells, flow cytometry is an ideal

experiment. The principles of light scattering, excitation, and emission underpin the operation of flow cytometry. When fluorescently tagged cell components are exposed to a laser beam, they get stimulated and emit various wavelengths of light. Cellular characteristics are analyzed by the use of fluorescence. Cells are analyzed using the following parameters: Visible light scatter: The determination of the forward direction size is aided by the forward scatter. The side scatter at 90 degrees provides a measure of a cell's granularity and complexity. Fluorescence: Cells can shine due to fluorescently attached antibodies, reporter genes coupled to fluorescent proteins like GFP, or dyes like propidium iodide. The targeted cells are first fixed with formaldehyde, which immobilizes proteins while preserving transient signals. The fixed cells are then treated with a detergent to render them permeable, which allows antibodies to flow across the intercellular space. A certain

concentration of formaldehyde and detergent is necessary for effective antigen detection, depending on the kind of target epitopes. After this is finished, the blood cells may be quantified using the sample and sheath fluid flow through. We can classify cells using this technique based on their size and complexity, and it can even help us understand the differentiation/stage of fluctuating cells.

Correlation Coefficient

Table 2: Results of Correlation Coefficient

	FC1	FC2	FC3	FC4	DD1	DD2	HD1	HD2	HD3
FC1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FC2	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FC3	0.068	-0.043	1.000	0.000	0.000	0.000	0.000	0.000	0.000
FC4	0.077	0.243	-0.236	1.000	0.000	0.000	0.000	0.000	0.000
DD1	-0.087	-0.117	0.217	-0.152	1.000	0.000	0.000	0.000	0.000
DD2	0.219	0.262	0.147	0.149	0.132	1.000	0.000	0.000	0.000
HD1	-0.146	-0.216	0.244	-0.155	0.095	0.021	1.000	0.000	0.000
HD2	0.205	0.011	-0.034	-0.095	-0.206	0.436	0.093	1.000	0.000
HD3	0.458	0.081	0.128	-0.015	-0.004	0.102	0.021	0.044	1.000

The above results of table 2 describes correlation coefficient analysis overall result shows a positive also negative correlation between them. A laboratory test called flow cytometry is used to examine the properties of cells or particles. A sample of cells or particles is first suspended in liquid before the procedure begins. In order to identify (or "tag") particular proteins on the outside or inside of cells, a lab worker applies fluorescent antibody dyes to the cells. Fluorescence-activated cell sorting (FACS) is the term for this process. Next, the cells are introduced into a flow cytometer apparatus. About 10,000 cells may be analyzed and processed by a computer in less than a minute. The following uses of flow cytometry in medical settings: 1. Counting cells. 2. Sorting cells. 3. Identifying the properties and functions of cells. 4. Diagnosis and management of malignancies of the bone marrow and blood. 5. Identifying biomarkers, which are quantifiable substances whose presence may be used to predict therapy response or cancer behaviors. 6. Immunophenotyping: a method of cell type classification.

Pathologists analyze the flow cytometry data, which are performed by lab personnel. They require a sample of your blood, bone marrow, tissue, or other bodily fluid in order to perform the test. A sample is taken and sent to the pathology lab for testing by the healthcare professional (such as your oncologist or primary care physician) who ordered the test.

Smart PLS Algorithm Model

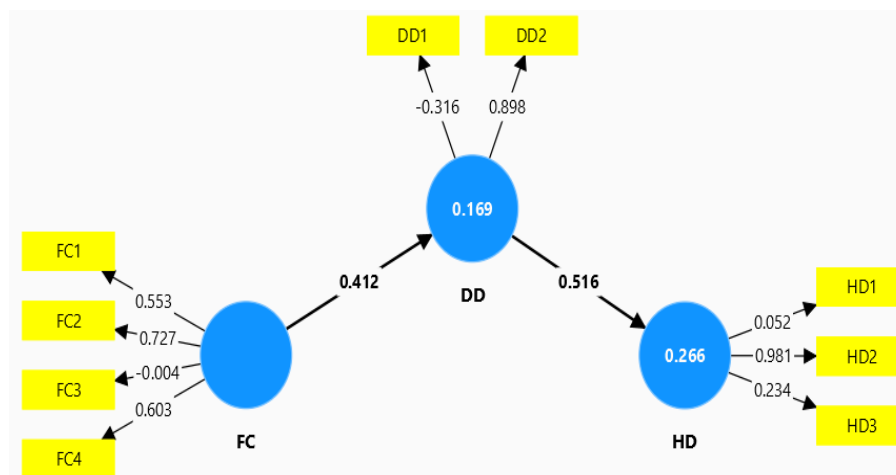


Figure 2: Smart PLS Algorithm Model

Fluorescence-activated cell sorting (FACS) is another aspect of flow cytometry. We were introduced to this particular type of flow cytometry in the 1960s. With this method, one may sort a large number of living cells using a heterogeneous combination by using the light scattering properties of fluorescence. Since every cell has unique light scattering properties, this is done one cell at a time and works well.

The above results shown in figure 2 graph present that smart PLS Algorithm relation between the FC, DD and HD. The FC represent that 55%, 72%, -0.004, 0.603 shows that 60% positive link with them. the FC describe 41% significant and positive relation with DD. According to the graph the HD describe that 51% positive and significant relation with DD.

Conclusion

Every year, leukaemia and lymphoma are diagnosed in hundreds of thousands of people. Flow cytometry is being used for immunophenotyping of leukaemias and lymphomas in order to avoid the radiation associated with CT scans. This may be done by classifying the different cell surface indicators that are present in all hematolymphoid neoplasms, such as CD markers. Further

investigation reveals that the kind of leukaemia may be systematically determined from the CD markers detected by the flow cytometer. For instance, if the markers CD19/20/22 are positive, we may be certain that the malignancy is connected to the immune system's B cells. Hodgkin's lymphoma is the diagnosis if both CD5 and CD23 are positive. Mantle cell lymphoma is the diagnosis if CD22 is positive and CD23 is negative, and so forth.

Despite the fact that the device was created many years ago, contemporary research has managed to apply the flow cytometer's methodology to diverse types of examination. After an overview of these aspects of the important implications of using Flow Cytometry for the diagnosis of hematologic diseases, we can say that Flow Cytometry has played its role very well in the detection and treatment of blood disorders in Brazil.

References

- [1] G. Janossy, "The changing pattern of "smart" flow cytometry (S-FC) to assist the cost-effective diagnosis of HIV, tuberculosis, and leukemias in resource-restricted conditions," *Biotechnology Journal: Healthcare Nutrition Technology*, vol. 3, no. 1, pp. 32-42, 2008.
- [2] K. A. d. S. Silva, F. Spagnol, M. G. Farias, A. P. Alegretti, M. B. Michalowski, and L. E. Daudt, "Influence of minimal residual disease by multiparametric flow cytometry at day 15 of induction in risk stratification of children with B-cell acute lymphoblastic leukemia treated at a referral hospital in southern Brazil," *Hematology, Transfusion and Cell Therapy*, vol. 42, no. 4, pp. 348-355, 2020.
- [3] A. Ruiz-Argüelles, L. Rivadeneyra-Espinoza, R. E. Duque, and A. Orfao, "Report on the second Latin American consensus conference for flow cytometric immunophenotyping of hematological malignancies," ed: Wiley Online Library, 2006.
- [4] M. S. P. de Oliveira, S. Koifman, G. M. Vasconcelos, M. Emerenciano, C. de Oliveira Novaes, and B. C. S. G. o. I. A. Leukemia, "Development and perspective of current Brazilian studies on the epidemiology of childhood leukemia," *Blood Cells, Molecules, and Diseases*, vol. 42, no. 2, pp. 121-125, 2009.
- [5] C. S. Matos *et al.*, "Applicability of an optimized non-conventional flow cytometry method to detect anti-Trypanosoma cruzi immunoglobulin G for the serological diagnosis and cure assessment following chemotherapeutic treatment of Chagas disease," *Journal of immunological methods*, vol. 369, no. 1-2, pp. 22-32, 2011.
- [6] M. R. Madkaikar *et al.*, "Application of flow cytometry in primary immunodeficiencies: experience from India," *Frontiers in immunology*, vol. 10, p. 1248, 2019.
- [7] C. Pedreira *et al.*, "From big flow cytometry datasets to smart diagnostic strategies: The EuroFlow approach," *Journal of immunological methods*, vol. 475, p. 112631, 2019.
- [8] A. M. de Figueiredo, J. C. Glória, Y. O. Chaves, W. L. L. Neves, and L. A. M. Mariúba, "Diagnostic applications of microsphere-based flow cytometry: A review," *Experimental Biology and Medicine*, vol. 247, no. 20, pp. 1852-1861, 2022.
- [9] L. C. Bento *et al.*, "The use of flow cytometry in myelodysplastic syndromes: a review," *Frontiers in oncology*, vol. 7, p. 270, 2017.
- [10] C. E. Pedreira, E. S. Costa, Q. Lecomte, J. J. van Dongen, and A. Orfao, "Overview of clinical flow cytometry data analysis: recent advances and future challenges," *Trends in biotechnology*, vol. 31, no. 7, pp. 415-425, 2013.
- [11] C. D. Álvarez-Zúñiga, I. Garza-Veloz, J. Martínez-Rendón, M. Ureño-Segura, I. Delgado-Enciso, and M. L. Martínez-Fierro, "Circulating Biomarkers Associated with the Diagnosis and Prognosis of B-Cell Progenitor Acute Lymphoblastic Leukemia," *Cancers*, vol. 15, no. 16, p. 4186, 2023.
- [12] D. Z. Baldasso *et al.*, "Development and validation of a flow cytometry antibody test for Lawsonia intracellularis," *Frontiers in Immunology*, vol. 14, p. 1145072, 2023.
- [13] K. L. Chin, L. Anibarro, M. E. Sarmiento, and A. Acosta, "Challenges and the Way forward in Diagnosis and Treatment of Tuberculosis Infection," *Tropical medicine and infectious disease*, vol. 8, no. 2, p. 89, 2023.
- [14] L. L. de Figueiredo-Pontes *et al.*, "Diagnosis and management of acute promyelocytic leukemia: Brazilian consensus guidelines 2024 on behalf of the Brazilian Association of Hematology, Hemotherapy and Cellular Therapy," *Hematology, Transfusion and Cell Therapy*, 2024.
- [15] F. A. de Sousa *et al.*, "ROR1 expression in mature B lymphoid neoplasms by flow cytometry," *Cytometry Part B: Clinical Cytometry*, vol. 106, no. 1, pp. 74-81, 2024.
- [16] A. S. Elbnnani *et al.*, "Flow cytometric detection of leukemic blasts in Libyan pediatric patients with acute lymphoblastic leukemia," *Libyan Journal of Medicine*, vol. 19, no. 1, p. 2319895, 2024.
- [17] M. E. A. Lopes, J. M. Ribeiro, A. Teixeira-Carvalho, S. M. Murta, and E. M. Souza-Fagundes, "A functional assay using human whole blood and flow cytometry analysis to evaluate cytotoxicity and immunomodulatory effect of anti-Trypanosoma cruzi drugs," *Experimental Parasitology*, vol. 247, p. 108490, 2023.
- [18] G. Ribeiro and D. Lenz, "Laser scanning cytometry: The future of conventional flow cytometry is past," *Instrumentation Science & Technology*, pp. 1-10, 2023.
- [19] M. M. d. Santos, A. S. d. Santos, H. H. d. M. Santos, L. d. S. Santos, R. J. M. Nascimento, and A. J. L. Torres, "Immunophenotypic characterization of acute leukemias in Bahia, Brazil," *Einstein (São Paulo)*, vol. 21, p. eAO0117, 2023.
- [20] C. Brun *et al.*, "A bibliometric analysis: Ca2+ fluxes and inflammatory phenotyping by flow cytometry in peripheral blood mononuclear cells," *Frontiers in Immunology*, vol. 14, p. 1272809, 2023.
- [21] L. F. Catelli *et al.*, "Highly Defined Induced Pluripotent Stem Cell Lines Mimic Donor Red Blood Cell Antigen Profiles for Therapeutic and Diagnostic Use," *Cellular Reprogramming*, vol. 26, no. 3, pp. 107-115, 2024.
- [22] K. A. Devitt *et al.*, "TRBC1 in flow cytometry: Assay development, validation, and reporting considerations," *Cytometry Part B: Clinical Cytometry*, 2024.
- [23] K.-U. Eckardt *et al.*, "Trends and perspectives for improving quality of chronic kidney disease care: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference," *Kidney international*, vol. 104, no. 5, pp. 888-903, 2023.
- [24] H. Hayashi, A. Makimoto, and Y. Yuza, "Treatment of Pediatric Acute Lymphoblastic Leukemia: A Historical Perspective," *Cancers*, vol. 16, no. 4, p. 723, 2024.
- [25] S. M. Manohar, "Shedding Light on Intracellular Proteins using Flow Cytometry," *Cell Biochemistry and Biophysics*, pp. 1-15, 2024.
- [26] V. Poscente, L. Di Gregorio, M. Costanzo, R. Bernini, and A. Bevivino, "Flow cytometry: Unravelling the real antimicrobial and antibiofilm efficacy of natural bioactive compounds," *Journal of Microbiological Methods*, vol. 222, p. 106956, 2024.
- [27] Y. Shi *et al.*, "The diagnosis of tuberculous meningitis: advancements in new technologies and machine learning algorithms," *Frontiers in Microbiology*, vol. 14, p. 1290746, 2023.

- [28] G. Sumner *et al.*, "2022 White Paper on Recent Issues in Bioanalysis: Enzyme Assay Validation, BAV for Primary End Points, Vaccine Functional Assays, Cytometry in Tissue, LBA in Rare Matrices, Complex NAb Assays, Spectral Cytometry, Endogenous Analytes, Extracellular Vesicles Part 2—Recommendations on Biomarkers/CDx, Flow Cytometry, Ligand-Binding Assays Development & Validation; Emerging Technologies; Critical Reagents Deep Characterization," *Bioanalysis*, vol. 15, no. 15, pp. 861-903, 2023.
- [29] B. Wahid and M. S. Tiwana, "Bacteriophage-based bioassays: an expected paradigm shift in microbial diagnostics," *Future Microbiology*, pp. 1-14, 2024.
- [30] R. Wang, L. V. Tang, and Y. Hu, "Genetic factors, risk prediction and AI application of thrombotic diseases," *Experimental Hematology & Oncology*, vol. 13, no. 1, p. 89, 2024.



This is an open-access article distributed under the terms of the Creative Commons Attribution License.