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Detecting Abnormalities in Blood Cells by Using Different Modern Techniques: A Comprehensive Review

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ABSTRACT

Blood is the life maintaining fluid that flows through the body's blood vessels Arteries, Veins and Capillaries. Blood cell abnormalities, encompassing alterations in the size, shape, count, color, texture or functionality of red blood cells (RBCs), white blood cells (WBCs), platelets and Plasma are critical indicators of various hematological and systemic diseases. Accurate and early detection of these abnormalities plays a significant role for timely diagnosis, prognosis, and treatment of conditions such as Anemia, Leukemia, Thalassemia, Thrombocytopenia, Hemophilia, Polycythemia vera, Plasma cell myeloma and other infections. In recent years, advancements in modern diagnostic techniques have significantly enhanced our ability to identify and characterize these abnormalities with high Accuracy, Sensitivity and Precision. This review highlights contemporary methodologies employed in detecting blood cell abnormalities, emphasizing their principles, applications and clinical relevance. Fully Automated complete blood count (CBC) analyzers, Artificial intelligence and machine learning techniques and Single cell sequencing technology have revolutionized routine hematology by providing rapid and quantitative as well as qualitative insights into blood cell parameters without much necessity of Hematologists. Modern blood cell analysis techniques have significantly advanced, combining automation, precision, and novel technologies to enhance diagnostic capabilities. Automated hematology analyzers employ methods like flow cytometry, impedance technology, and optical scattering for efficient cell counting and classification. Advanced tools, such as microfluidics, Raman spectroscopy, and single-cell RNA sequencing, enable detailed molecular and cellular profiling. Emerging approaches like imaging flow cytometry, laser-induced breakdown spectroscopy, and mass cytometry further improve the detection of rare and abnormal cells. Integrating artificial intelligence and machine learning has streamlined blood smear analysis and enhanced anomaly detection. These innovations provide faster, more accurate results, paving the way for improved diagnostics and personalized medicine.

The Primary purpose of this Literature Review is to assist readers in understanding the whole body of available different modern techniques in determination of abnormal blood cells.

Keywords: *Red Blood Cells, White Blood Cells, Platelets, Convolutional neural networks (CNN), Thalassemia, Thrombocytopenia, Hemophilia, Hematologists.*

1. INTRODUCTION

Blood cell analysis is a cornerstone of hematology, playing a vital role in diagnosing and managing various diseases, including anemia, infections, and hematological malignancies. Detecting abnormalities in blood cells involves identifying deviations in their morphology, count or functionality, which are indicative of underlying health conditions. Traditionally, manual microscopic examination of blood smears has been the primary method for detecting such abnormalities [1-4]. While effective, this approach is time intensive, prone to inter observer variability and dependent on skilled personnel.

In recent years, technological advancements have revolutionized the field, introducing modern techniques that offer improved accuracy, efficiency and reproducibility. In this review paper there are **10 different methods,** approaches and techniques are analyzed. These include the **1) Microscopy based Techniques** [1-12] in which various Microscopy method is used. **2) Flow Cytometry** [13-30] in which blood cells are tagged with fluorescent makers specific to intracellular proteins and other different approach has been used. **3) Automated Hematological Analyzer** [31-45], here Semi and fully

automated analyzers analyses physical properties like size, granularity and biochemical markers in blood samples. It counts Red blood Cells (RBC's) White blood cells (WBC's) and Platelets. It also detects conditions like Anemia, Thrombocytopenia and Leukemia based on cell counts and size variations. **4) Molecular Techniques** [46-56] Polymerase Chain Reaction (PCR), Mutation in genes, disorders like chronic myeloid leukemia, thalassemia, sickle cell anemia, Next-generation Sequencing (NGS), Comprehensive analysis of genetic mutations and other methods are discussed. **5) Imaging Techniques** [57-66], Scanning electron microscopy (SEM), Transmission Electron Microscopy (TEM), High resolution image of cellular ultra structure, detecting structural abnormalities in platelets and RBC's, Confocal Microscopy techniques are discussed. **6) Artificial intelligence and Machine Learning** [67-71], automated image analysis, Machine learning algorithms, tools like convolutional neural networks (CNN) techniques and Predictive analysis techniques are analyzed. **7) Mass Spectroscopy technique** [72-76] measurement of Molecular and ionized components of blood, hemoglobin variations like sickle cell trait, and biomarkers of hematological malignancies are discussed. **8) Cytogenetic and FISH Analysis technique** [77-84], chromosomal abnormalities like translocations, deletions are analyzed in addition to that identifying specific DNA sequences using fluorescent probe techniques are discussed. **9) Single-Cell sequencing** [85-92], genomic and transcriptomic analysis of individual cells and its applications in evolution of Leukemia And detecting heterogeneity in blood cancers are analyzed, its Accuracy, Precision and Sensitivity are high compared to all other existing techniques discussed in this paper. And Lastly **10) Proteomics and Metabolomics technique** [93-95] Protein expression patterns in abnormal blood cells and metabolic alterations in disorders like anemia and leukemia disorders are discussed.

This review explores the various modern techniques used in detecting blood cell abnormalities. It provides an overview of their principles, applications, and limitations, highlighting how they are transforming clinical diagnostics and paving the way for more precise and personalized healthcare.

2. METHODS

2.1. Microscopy based Techniques.

Microscopic examination of blood cells is essential for diagnosing hematological disorders such as anemia, leukemia, and infections. It involves preparing a blood smear, staining it with dyes like Wright's or Giemsa to enhance visibility, and examining it under a light microscope to assess the structure, size, and appearance of blood cells [1,2]. Abnormalities in red blood cells, white blood cells, and platelets, such as sickle cells, macrocytes, or atypical lymphocytes, can indicate specific conditions. Advanced techniques like phase contrast, fluorescence, and electron microscopy offer detailed insights, aiding in the detection and diagnosis of blood disorders [3,4,5].

Microscopic examination of blood smears is a fundamental method for detecting blood cell abnormalities. The process involves preparing a blood smear, staining it, and examining it under a microscope. Using this method, blood disorders like anemia, leukemia, and infections can be diagnosed by observing changes in the morphology, size, and structure of red and white blood cells. Advanced techniques like phase contrast, fluorescence, and electron microscopy enhance the ability to detect specific cellular abnormalities and provide more detailed insights into blood conditions [6,7,8].

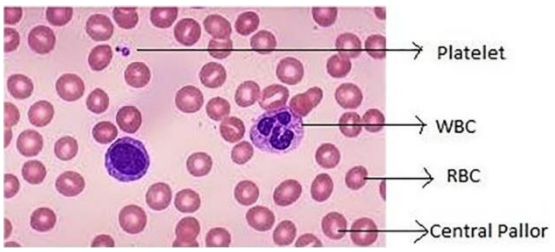

Fig.1- Types of Normal and Abnormal Blood cells

Approaches

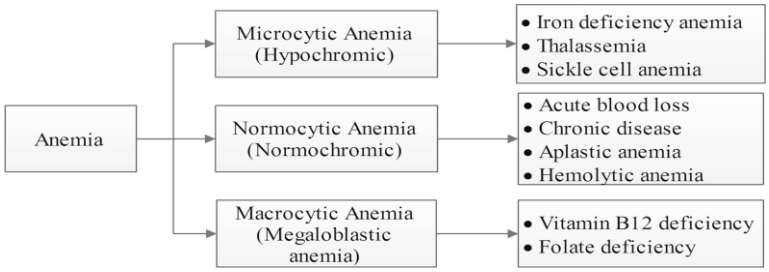

Fig.2- Types of Normal and Abnormal Blood cells

Microscopic examination of blood cells plays a crucial role in diagnosing anemia by analyzing the size and morphology of red blood cells (RBCs) which has shown in Fig-1[9]. In **microcytic anemia**, RBCs appear smaller than normal (microcytes), commonly due to iron deficiency or thalassemia. **Normocytic anemia** involves RBCs of normal size but reduced in number, often linked to acute blood loss or chronic diseases. In **macrocytic anemia**, RBCs are larger than normal (macrocytes), typically caused by vitamin B12 or folate deficiency. By preparing and staining a blood smear, the microscopic method enables the identification of these abnormalities, aiding in the accurate classification and diagnosis of anemia shown in **Fig-2** [10,11,12].

2.2. Flow Cytometry Technique

Flow cytometry (FC) is a powerful methodology for the characterization of complex phenotypes in cellular populations, as well as the quantification of cellular processes such as proliferation, cell death and cell differentiation [13]. The rationale of FC is based on the analysis of the spectral characteristics of cells in a homogeneous liquid mixture. A standard flow cytometer, in which FC analysis is performed, can be divided into three distinct systems [14,15] **hydraulic system, an optical system, and an electronics system**. The hydraulic system performs hydrodynamic focusing in order for cells to pass sequentially, as single events, through an interrogation point. At this point, as part of the optical system, a laser source is used for excitation-specific fluorophores (that have been added in a preanalytical step), [16,17] while the emitted fluorescence from each cell is passed through detection filters. Third, the electronics system stores fluorescence signals in a digital format. These characteristics make flow cytometry invaluable in several scientific fields such as hematology, immunology, and oncology, among others.

Here we have two basic methods which will give good accuracy, precision, sensitivity and expected results in Flow Cytometry. A. Imaging flow cytometry B. Detecting Acutye myloid lukemia (AML) using Flow Cytometry Method (FCM).

A.Imagingflowcytometry(ImFC): It stands as an innovative and technologically advanced [17] offshoot of conventional flow cytometry (FC), distinguished by new modifications and improvements to existing techniques. Using a camera, ImFC is able to provide granular and high-quality visual information about the detected cells. More specifically, it provides broad information about them orphology, or structural characteristics, of the cell. This includes a detailed analysis of the cell size, shape, internal structure, and the distribution of specific components or markers within the cell. Thus, the combination of imaging technologies with flow cytometry represents a major advance in cellular analysis. This way, users have a supplementary source of information to validate their fluorescence data and provide a comprehensive and accurate characterization of cell populations. The principles and key applications of ImFC are presented in below Figure-3.

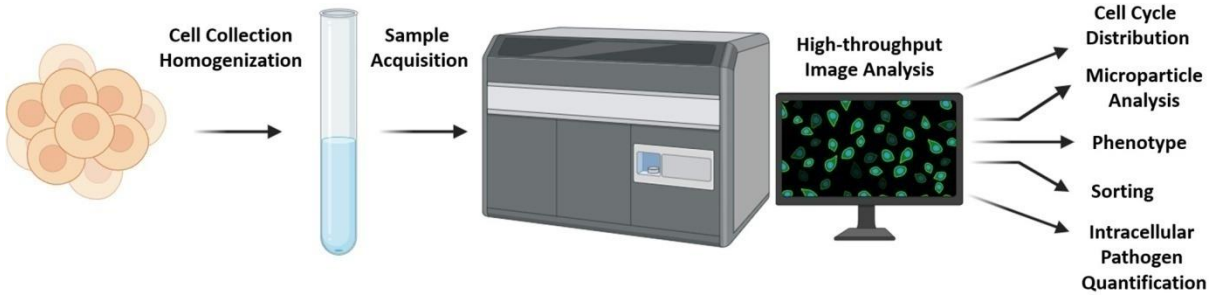

Fig.3- workflow for imaging Flow Cytometry (ImFC)

Above Figure.3, analyzes the workflow for imaging flow cytometry (ImFC), a technology that combines flow cytometry and microscopy to enable high-throughput analysis of cells. Cells are collected and homogenized to ensure a consistent sample containing single cells, which is crucial for accurate flow cytometry. Cell suspension is then loaded into the ImFC instrument (an Image stream MK II is depicted for reference), where individual cells are isolated and aligned for imaging. As cells pass through the ImFC device, they are imaged at high speed, capturing detailed fluorescence and bright field images for each cell. The collected images are analyzed to extract multiple data points. The technology has several applications, including assessment of cell cycle stages based on nuclear DNA content and morphology; detection and characterization of small particles like exosomes or microvesicles that may be present with the cells; morphological and phenotypic profiling of cells, including size, shape, and internal complexity; sorting capabilities to physically separate and collect cells based on the analysis (not available in the depicted cytometer); and quantitative analysis of pathogens inside cells, which is vital for studying infections and immune responses. ImFC workflow enables rapid and detailed cellular analysis, facilitating advanced research in cell biology, immunology, and related fields [18,19,20].

In the evolving landscape of cytometric technologies [21,22], ImFC emerges as a cornerstone advancement, distinguishing itself by its application versatility, particularly in the study of non-adherent cells, and its unmatched capability in detecting rare cell populations. As such, [19-23] ImFC has the advantages of both flow cytometry and microscopy. First, it is advantageous over typical FC in a manner that provides structural information apart from the spectral properties of cells.

ImFC's ability to capture high resolution images of cells in flow enables the visualization of cellular morphology, the organization of intracellular components, and the spatial relationships between different cell markers [23, 24, 25, 26]. This is a significant advancement over conventional FC, which is largely limited to measuring fluorescence intensity without providing any contextual imagery of the cellular structures producing these signals. By integrating structural with spectral data, ImFC facilitates a more understanding of cellular behavior, phenotypic variations, and complex biological processes [27,28,29,30].

2.3. Automated Analyzers Technique

Automated analyzers have revolutionized the way we diagnose and assess blood related disorders, [31,32] detecting abnormalities in red blood cells (RBCs), white blood cells (WBCs), and platelets. These analyzers provide precise and accurate measurements and can perform a wide range of tests on blood samples. They offer fast and accurate results that are crucial for the diagnosis of various blood disorders such as anemia, leukemia, and thrombocytopenia. Below are detailed approaches for determining abnormal blood cells using automated analyzers [33,34,35].

Automated hematology analyzers provide precise and efficient analysis of blood samples, utilizing impedance (Coulter Principle) and optical analysis for cell counting, size, and morphology. They play a critical role in diagnosing conditions like anemia, leukocytosis, and thrombocytopenia. For anemia, parameters such as RBC count, MCV, MCH, MCHC, and RDW are analyzed to classify it as microcytic, normocytic, or macrocytic [36,37]. WBC abnormalities are assessed through total counts, differential analysis, and advanced techniques like flow cytometry for detailed cell subtype differentiation [38]. Platelet abnormalities, including counts and size variations (MPV, PDW), are evaluated for disorders like thrombocytosis or thrombocytopenia [39,40].

Advanced features, including reticulocyte counts and nucleated RBC detection, enhance diagnostic capabilities. For parasitic infections, such as Trypanosoma brucei detection, specialized analyzers like the XN-31 demonstrate accuracy comparable to microscopy, with a detection limit around 20 parasites/µL. This showcases the adaptability of automated systems for both routine and specialized hematological assessments, the same in Regular microscopic examination of thick blood films are shown in below Fig-4 [41,42,43,44,45]

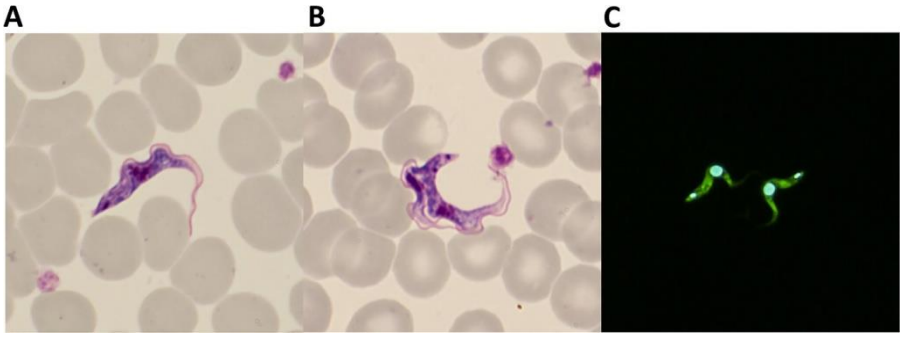

Fig.4- Regular microscopic examination of thick blood films

2.4. Molecular Techniques

Molecular techniques have [46] significantly advanced our ability to diagnose blood disorders by detecting abnormalities in blood cells at the genetic, transcriptional and proteomic levels. These approaches allow for more precise diagnosis, monitoring of disease progression and identification of underlying causes of blood disorders that are not detectable through conventional methods. Below, I'll outline the main molecular techniques used to determine abnormal blood cells in detail [47,48, 49].

Molecular techniques play a critical role in diagnosing, monitoring, and treating hematologic disorders by identifying genetic mutations, chromosomal abnormalities, and abnormal gene expression. PCR and its variants (qPCR and RT-PCR) [50,51] are used for detecting mutations, gene fusions, and pathogen presence in blood disorders like leukemia, lymphoma, and hemoglobinopathies. Next-Generation Sequencing (NGS), including WGS, WES, and targeted gene panels, provides high-resolution mutation profiling for diseases like AML, MDS, and hereditary anemias. Techniques like FISH and CMA detect chromosomal abnormalities and CNVs with precision, while proteomics and mass spectrometry identify abnormal proteins in conditions such as hemophilia and leukemia [52, 53]. Southern blotting, though less common, detects large gene rearrangements. In MDS and AML, genomic technologies like NGS enable mutation profiling and MRD detection, aiding risk stratification and treatment decisions. Advanced bioinformatics ensures accurate analysis of sequencing data, enhancing diagnostic accuracy and monitoring relapse. These methods collectively revolutionize personalized treatment and prognosis in hematologic malignancies [54].

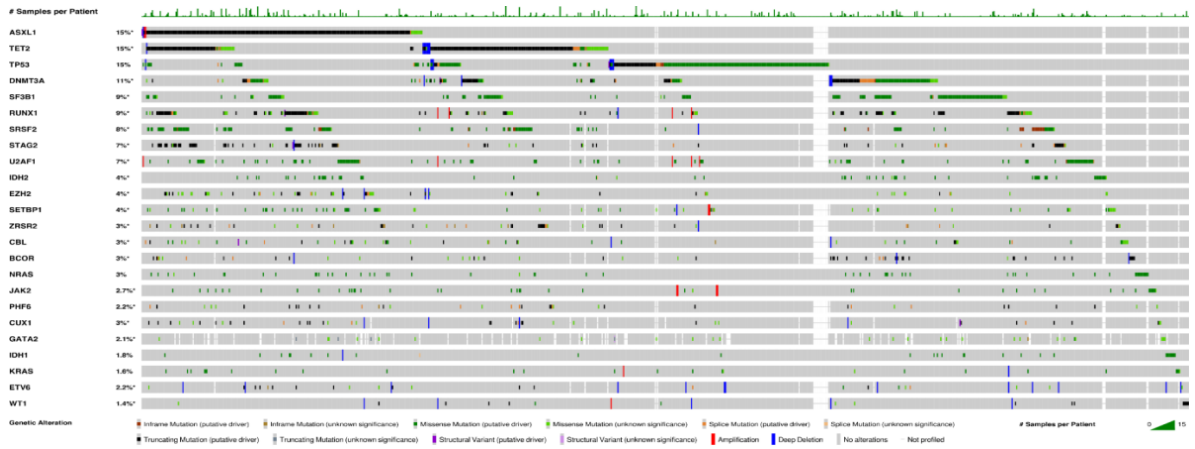

Fig.5- The mutation landscape of MDS is well represented in the AACR Project GENIE Cohort v15.0-public dataset.

Acute myeloid leukemia (AML) consists of a group of hematologic neoplasms characterized by abnormal differentiation and proliferation of myeloid progenitor cells. Acute myeloid leukemia is associated with poor outcome due to the lack of efficient therapies and early diagnostic tools. The existing gold standard diagnostic tools are based on bone marrow biopsy. These biopsies, apart from being very invasive, painful, and costly, have low sensitivity. Despite the progress uncovering the molecular pathogenesis of AML, the development of novel detection strategies is still poorly explored. This is particularly important for patients that check the criteria for complete remission after treatment, since they can relapse through the persistence of some leukemic stem cells.

Chromosome analysis Chromosome analysis is a genome-wide assay capable of revealing all clonal and microscopically detectable abnormalities present in leukemic cells, albeit with a rather low resolution estimated to be the 5–10 Mb or slightly more sometimes. Chromosome analysis evaluates metaphase chromosomes obtained from various viable, dividing cells from bone marrow, blood, lymphoid tissue, or other tumor containing tissue using staining (banding) techniques, shown in Fig-5 [55, 56].

2.5. Imaging Techniques

Medical Imaging (MI) plays [57] a crucial role in healthcare, including disease diagnosis, treatment, and continuous monitoring. The integration of non-invasive techniques such as X-ray, Positron Emission Tomography (PET) scan, Computed Tomography (CT) scan, Magnetic Resonance Imaging (MRI), and Ultrasound has significantly enhanced medical treatment. MI enables visualization of internal structures without invasive procedures, aiding in the diagnosis of various diseases. The introduction of Medical Image Processing (MIP) has further improved disease prediction, detection, analysis, and evaluation. MIP data is utilized in Machine Learning (ML) and Deep Learning (DL) models to develop intelligent systems that enhance medical assistance and better recognition, because human interpretation of medical images is error prone and exhaustive. However, [58,59,60] accuracy is crucial for the provision of high-quality healthcare. This has motivated various works on MI using MIP therefore, this paper emphasizes how these imaging modalities can be used to analyze, model, and manipulate data in order to achieve maximum treatment outcome. Moreover, a comprehensive literature survey is conducted to provide a detailed analysis of the working principles, benefits, and limitations of diverse imaging modalities. It explores state-of-the-art methodologies rooted in MI approaches and highlights potential future developments, challenges, trends, observations and significant improvements in the field [61].

The healthcare system is a public institution that provides medical facilities with the help of various medical resources and support systems for diagnosis and detection of diseases in a target population. Since its inception, the system has undergone changes for the betterment and welfare of the society. Today, the healthcare system is highly advanced and involves minimal human interpretation.

One such significant impact is the Medical Image Processing (MIP) or Medical Imaging (MI). MIP can assist medical experts through its potential to examine the anatomy, tissues, and pathological samples and diagnose a disease with the help of energy sources such as light, electrons, lasers, radiations, sound waves, radio nuclides, and nuclear magnetic resonance. Medical Imaging Informatics (MII) is concerned with aspects of medical imaging, including preprocessing, storing, evaluation, access, and comprehension. Thus, the purpose of MII is to enhance medical service with clarity, accuracy, efficiency, and consistency. Moreover, the World Health Organization (WHO) reports that India has a low doctor-to patient ratio of 1:1,000. The constraints of resources and lack of availability of expert opinions have made the process of detection for a huge number of targeted populations cumbersome. The dependency on traditional methods for diagnosis and detection of diseases has reduced because of the challenges associated with it such as huge time consumption, tiring detection process,

unreliable process of detection, high error rate etc. which has caused incurable diseases, delayed treatments and deaths [62].

This review incorporates a systematic approach used to search for relevant literature in multiple databases. The fundamental phases of this review include planning, searching, faltering primary studies, information extraction, and information synthesis. The planning phase defines the review goals, scope, and protocols. Scope identification of this review aims to identify various studies related to MI modalities using ML and DL techniques [63].

Medical Imaging (MI) plays a crucial role in non-invasive disease diagnosis, treatment, and monitoring, using modalities like X-ray, PET, CT, MRI, and Ultrasound. Medical Image Processing (MIP) enhances prediction and detection by integrating imaging data into Machine Learning (ML) and Deep Learning (DL) models, improving accuracy and efficiency over traditional methods. Techniques like Optical Coherence Tomography Angiography (OCTA) assess retinal parameters such as Linear Vessel Density (LVD), Perfusion Vessel Density (PVD), and Foveal Avascular Zone (FAZ), providing valuable insights into hematologic diseases. Automated systems like CellaVision® DM96 streamline blood smear analysis by automating cell classification and RBC morphology assessment. These advancements enable better disease modeling, data manipulation, and personalized healthcare [64].

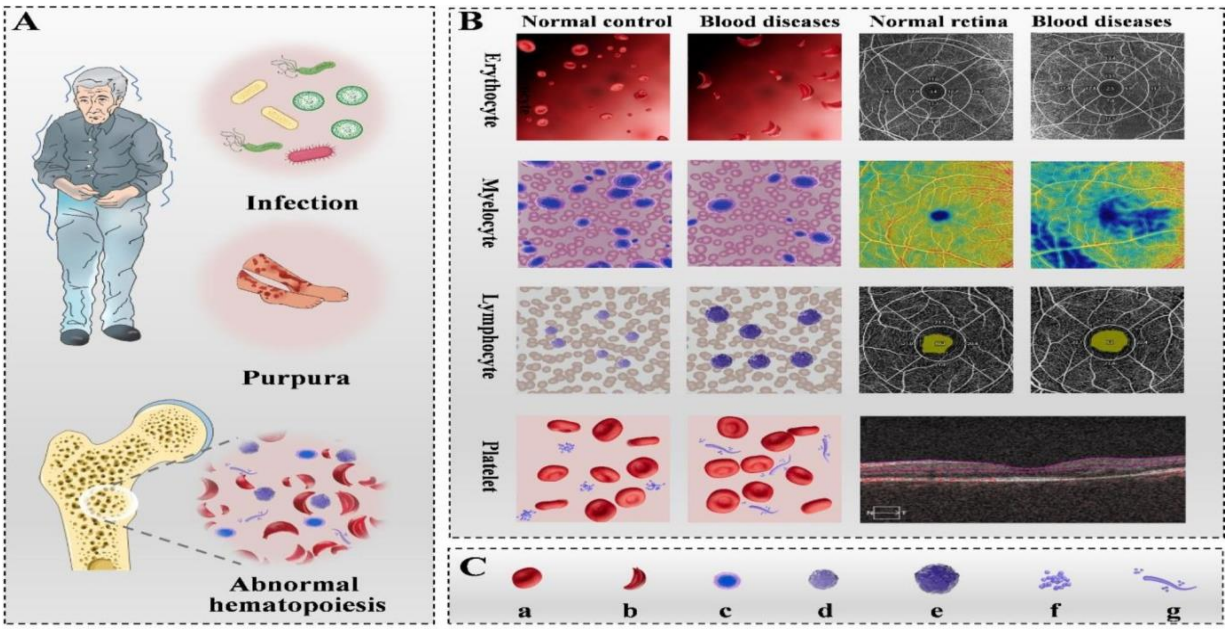

Fig.6- shows an overview of the study

(**A**)Subject Enrollment: The diagnosis of blood diseases was based on the patients' clinical manifestations and confirmed through blood and bone marrow data. (**B**) Data Collection: Subjects were categorized into different groups based on their diagnoses and laboratory disease indicators. Each patient and healthy control underwent a comprehensive ophthalmic examination using slit-lamp and Optical Coherence Tomography Angiography (OCTA). Parameters including Linear Vessel Density (LVD), Perfusion Vessel Density (PVD), and Foveal Avascular Zone (FAZ) were recorded for subsequent analysis. (**C**) Diagram of blood cell composition, a, normal erythrocyte; b, sickle-shaped erythrocyte; c, Normal leukocyte; d, Normal lymphocyte; e, abnormal lymphocytes. The cell body is larger than normal lymphocytes, the chromatin may be darker, and sometimes vacuoles may appear; f, normal platelets; g, deformation of platelets. OCTA, optical coherence tomography angiography; LVD, linear vessel density; PVD, perfusion vessel density. FAZ, foveal avascular zone.

The primary outcome measures were the differences in vessel density of the retina in patients with hematological diseases measured by OCTA. Secondary outcomes were the differences in FAZ and the analysis of the correlation between retinal parameters and hematology test data, which has shown in above **Fig-6** [65,66].

2.6. Artificial Intelligence & Machine Learning Technique

Artificial intelligence (AI) stems from the data generated mainly since the beginning of the fourth industrial revolution, which has progressively changed how people live, interact, and work [67]. Automated systems, meant to emulate human cognitive capabilities, deploy supervised applications to perform repetitive tasks more accurately and efficiently, saving time and effort for high volume workloads. In medicine, AI has become a valuable tool for improving patient outcomes, particularly in diagnostics, where image and text-based systems supported by machine learning (ML) and deep learning (DL) technologies are reaching remarkable clinical results. The COVID-19 pandemic is the paramount example of how AI applications enable new screening tools and achieve early diagnosis by measuring disease severity, progression, and mortality

prediction through the interpretation of routine blood tests. For instance, a recent meta-analysis from Li et al. demonstrated that computational methods based on multi-center clinical datasets could generate more accurate COVID-19 diagnosis, stratify patients into clusters of severity and discriminate them from Influenza with 97.9% specificity. John McCarthy first outlined the concept of AI in 1956 during the Dartmouth conference, on which several scientists discussed the concept of "thinking machines" in different areas such as abstraction, creativity, computational theory, natural language processing, and neural networks. Since then, progress slowed and remained stationary until 2012, when an Image Net-DL-Algorithm triggered significant attention for the technology, with high-accuracy performance classification metrics that disrupted the current state-of-the-art. AI is defined as a computer science subdivision that aims to automatically understand and create intelligent systems based on high amounts of data in medicine, the inequities and deficiencies that arose from the global COVID-19 pandemic catalyzed a boost in AI applications. Therefore, it aims to deliver effective, high-quality care, leveraging increasing clinical world data to democratize and decentralize health into patient care. The transformation of a patient's blood analysis into a probability state to epitomize a likely diagnosis is already a reality.

Here, we [68] focus on routine blood analysis as a proxy for determining pathological states supported by AI algorithms. We offer a comprehensive description of the ML pipeline with contextualization on the learning strategies (machine, reinforcement, deep, and federated learning), model development (application, preprocessing, modelling, and validation), and clinic deployment. We summarize the pathologies based on general health parameters (summarized according to their function and associated causes of variation), their inherent classification performance, and principal findings associated with model development and selected blood parameters. Finally, we discuss challenges related to clinic deployment and suggest future research directions for the development of models.

The RBC dataset used in this study comprises 7,108 images across eight classes: Elliptocytes, Dacrocytes, Acanthocytes, Stomatocytes, Spherocytes, Hypochromic, Codocytes, and Normal RBCs, excluding the Pencil class due to limited data. Deep learning models VGG16, ResNet50, and Inception V3 were implemented using Tensor Flow and Keras with pre-trained ImageNet weights for faster convergence. These architectures were modified to include a dense layer with 1024 neurons, a dropout layer (50%) to prevent over fitting, and a SoftMax output layer for classification. The Adam optimizer was used with a learning rate of 0.00001. Grad CAM, a technique for explainable AI, generated heat maps to highlight key regions influencing the model's predictions, aiding interpretability. Federated Learning (FL) was employed to maintain data security and privacy by leveraging distributed data sources while addressing challenges like heterogeneity, communication overhead, and security. The FL framework used lightweight models to optimize client server communication and performance shown in below Fig-7 [69, 70].

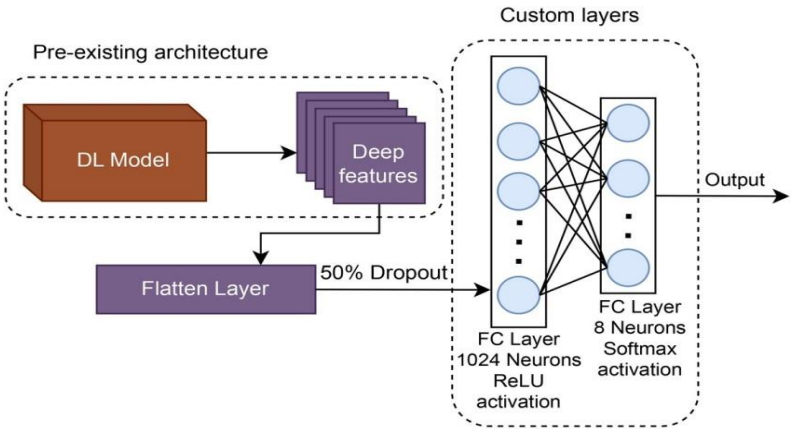

Fig.7- Architecture of the used deep learning models

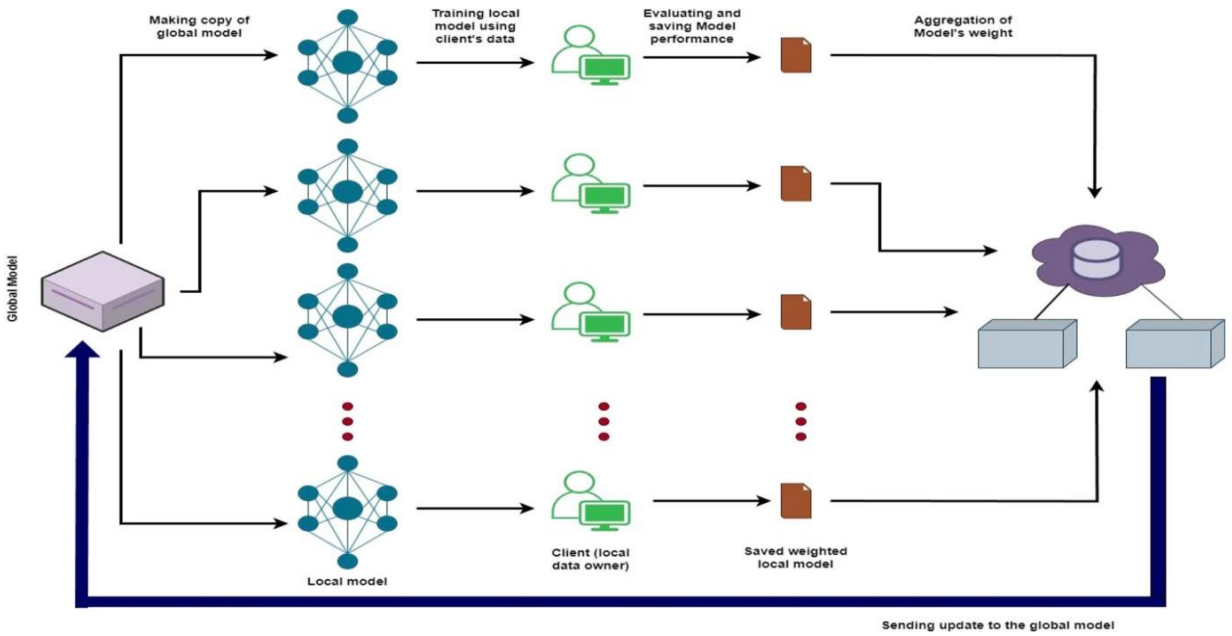

Fig.8- Architecture of federated learning (FL) environment.

The expected security and privacy in medical data prognosis are maintained by the FL method. However, despite the promising aspects of FL, there are some critical challenges to be addressed. Unlike traditional learning systems, where all data are kept in a single system leveraging a single model, FL framework utilizes multiple data sources spread across multiple devices. Thus, an FL framework needs to with stand heterogeneity across different systems and data counts. In addition, it also needs to ensure that the network overhead during the client-server communication does not bottleneck the entire system. In order to keep the communication overhead minimal and to ensure smooth participation of clients with low-end configuration, often lightweight models with a limited number of parameters are used. In addition, making attempt of FL framework secured from attacking and poor lyper forming clients is a good practice. The architecture of the FL framework that we used is shown in above Fig- 8 [71].

2.7. Mass Spectroscopy Technique

Mass spectrometry (MS) is an advanced analytical technique that is increasingly being used in the field of hematology to detect and characterize abnormal blood cells. MS offers high sensitivity, specificity, and the ability to analyze complex protein mixtures, making it invaluable for identifying protein biomarkers, detecting post-translational modifications and profiling the proteome of blood cells. In the context of hematological disorders, MS can provide insight into abnormalities at the protein and peptide levels, which are often key to understanding disease mechanisms, diagnosing conditions and monitoring treatment progress. Here are the detailed approaches for determining abnormal blood cells using mass spectrometry techniques [72].

Negative Oligoclonal, alternate band Positive for original clone (not CR) Not done

Subject number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
IFE at Baseline	AL	AL	AL	AL	AL	AL	AL	AL	AL	GL	GL	GL	GL	GL	GL	GL	L	L	L	L	L	L	L+ GK	DL	N*	N*	N*	N*	GK	GK	GK	GK	N#
FLC clone at CR																																	
SIFE at CR																									GK								
MASS-FIX at CR					GK			GK	AL	L^						L^	L^								GK				GK	GK^			
ESI-TOF at CR		AL		AL				GK	AL	L^						GL^	L^									L^				GK^			

Fig.9 – Grading of complete response using various techniques. Any SIFE with a sub sequent isotope discordant with base line represents oligoclonal banding and unrelated to the original clone. *L by MASS-FIX; #AK by MASS-FIX; different mass, so considered negative.

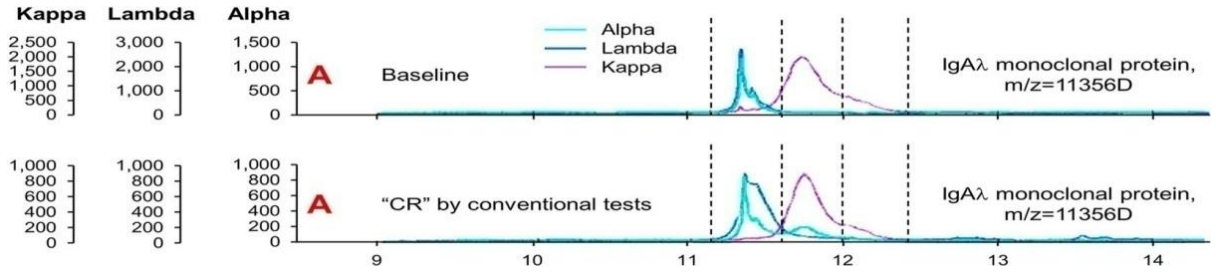

Fig.10- Example of positive MASS FIX in patient otherwise deemed to be in complete response.

Here Author has taken total 33 patients, those patients met the criteria of CR by blood and bone marrow and their baseline characteristics are taken. Median age was 56 years. 55% were male and all were Caucasian. No test performed perfectly at baseline with the exception of the MASS-FIX due to inclusion requirements. The positive baseline results for the other assays are, shown in Supplementary Fig.15.SIFE is85%; UIFE is79%andabnormal FLC ratio is 84%. Five SIFE negative patients were positive by MASS FIX and ESI-TOF another SIFE negative was found to have a monoclonal λ by ESI-TOF and UIFE and four SIFE negatives had abnormal FLC ratios. Apart from disagreements between positive and negative, isotype discrepancies were seen between SIFE and MASS-FIX in only one instance: IgG λ by SIFE, but free λ by MASS-FIX; ESI-TOF detected the IgG λ. No isotype discrepancies were observed between FLC and MASS FIX apart from the disagreements in four cases in which MASS-FIX was positive and FLC negative. There were eight disagreements in positive or negative calls between FLC and SIFE [73, 74].

At CR assessment, by dentition all patients had negative SIFE, negative UIFE, normal FLC ratio and a negative bone marrow by six color flow cytometry. By MASS FIX and ESI-TOF respectively, two and four patients were found to have their original monoclonal protein detected at CR determination (Figs. 9 and10). Another eight had monoclonal proteins that did not coincide with their original protein at CR measurement, consistent with transient post-therapy oligoclonal banding.

Hence, a total 12% (4 of 33) of patients who were thought to be in CR by high resolution bone marrow flow cytometry, SIFE, UIFE, and FLC were found to have residual disease by mass spectrometric techniques of the blood [75,76].

2.8. Cytogenetic and Fluorescence in Situ Hybridization (FISH) technique

Cytogenetic and Fluorescence in Situ Hybridization (FISH) techniques are pivotal for analyzing chromosomal abnormalities and gene mutations in blood cells. These approaches offer critical insights into the genetic basis of hematologic disorders, helping diagnose conditions such as leukemia, anemia, and myelodysplastic syndromes (MDS), among others. Below, I'll provide a detailed explanation of the **cytogenetic** and **FISH** analysis techniques used to determine abnormal blood cells [77].

Cytogenetic analysis techniques, including karyotyping, chromosome banding, fluorescence in situ hybridization (FISH), and comparative genomic hybridization (CMA), are essential for studying chromosomal abnormalities in blood disorders. **Karyotyping** is a conventional method that visualizes the complete set of chromosomes to detect large-scale changes like aneuploidy, translocations, deletions, and inversions. Blood cells are cultured, arrested in metaphase, stained (e.g., Giemsa), and analysed microscopically to identify abnormalities associated with leukaemia, myelodysplastic syndromes (MDS), and other conditions. **Chromosome banding**, such as G-banding, provides more detailed structural analysis by creating unique banding patterns, helping detect chromosomal translocations (e.g., t(9;22) in CML), deletions (e.g., del(5q) in MDS), and inversions. **FISH** is a high-resolution technique that uses fluorescent probes to target specific chromosomal regions, enabling the detection of translocations (e.g., BCR-ABL1 in CML, PML-RARα in APL), deletions (e.g., del(17p) in CLL), and aneuploidy (e.g., trisomy 21 in Down syndrome) [78,79]. FISH is faster, more sensitive, and allows quantification of abnormal cells compared to karyotyping. **CMA** provides genome-wide analysis for detecting copy number variations (CNVs) and uniparental disomy (UPD) without the need for cell culture, making it applicable to fixed tissues. Although highly automated and precise, CMA cannot detect balanced structural abnormalities or single-cell-level changes. Together, these techniques play a vital role in diagnosing and monitoring hematologic disorders, offering critical insights for prognosis and treatment decisions [80, 81, 82, 83].

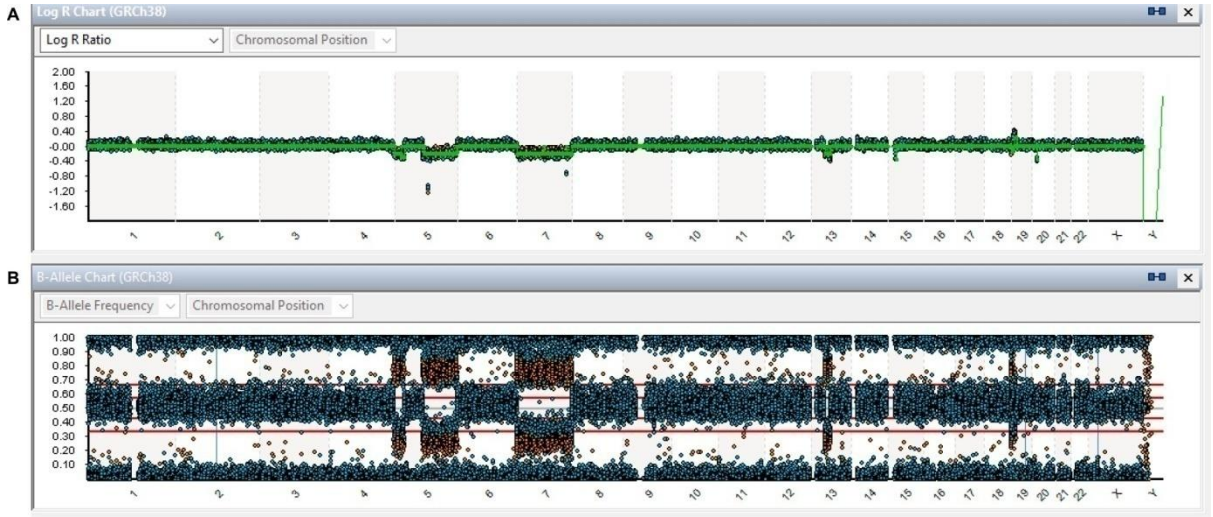

Fig.11- Example of a SNP (Infinium CytoSNP-850 K, Illumina) in myelodysplasia in a female patient in the case of a failed bone marrow karyotype.

A: Log R whole genomes how sad eletion5p15.3p13.3, adeletion5q14.2q35.3, amonosomy7, adeletion13q14.11q21.1, a complex rearrangement of the short arm of chromo some19. B: B-Allele Frequency indicates the proportion of abnormal cells, 80% for all abnormalities in this case. A SNP profile classifies this myelodysplasia as having a very poor prognosis according to R-IPSS [84].

2.9. Single-Cell sequencing Technique

Single cell sequencing: is an advanced technology that enables the analysis of individual cells at the genomic, transcriptomic, and epigenomic levels. This technique provides unprecedented resolution to detect heterogeneity within cell populations, which is particularly important in identifying abnormal blood cells associated with diseases like leukemia, anemia, lymphoma, and myelodysplastic syndromes (MDS). Unlike traditional bulk sequencing, which averages the data from many cells, single-cell sequencing reveals the specific genetic and molecular characteristics of each individual cell, allowing for more precise diagnosis, prognosis, and understanding of disease mechanisms [85,86].

Fig.12 - single cell sequencing

Single-cell sequencing techniques, including single-cell RNA sequencing (scRNA-seq), DNA sequencing (scDNA-seq), and epigenomics (scATAC-seq and scChIP-seq), offer precise tools for identifying and characterizing abnormal blood cells in diseases like leukemia, anemia, and myelodysplastic syndromes. **scRNA-seq** profiles gene expression in individual cells, identifying aberrant transcripts (e.g., BCR-ABL1 in CML) and cellular heterogeneity in tumors. **scDNA-seq** detects mutations, chromosomal abnormalities, and clonal evolution in hematologic malignancies, offering insights into rare genetic variations [87]. **Epigenomic approaches**, such as scATAC-seq and scChIP-seq, study chromatin accessibility and histone modifications, revealing gene regulatory mechanisms in abnormal blood cells, which shown in above Fig-12. Advances in single-cell techniques, such as combinatorial marker sequencing, novel amplification methods, and spatially resolved sequencing, have enhanced detection accuracy, reduced costs, and improved the ability to study clonal evolution and tumor

heterogeneity. Together, these tools enable a deeper understanding of molecular mechanisms, disease progression, and the discovery of biomarkers for improved diagnosis and treatment [88, 89, 90 , 91, 92].***2.10. Proteomics and Metabolomics Techniques***

Blood cell abnormalities, whether morphological, functional, or biochemical is hallmarks of various hematological disorders such as anemia, leukemia, and hemoglobinopathies. This study explores how advanced proteomics and metabolomics techniques provide comprehensive molecular insights into blood cell abnormalities. These approaches enable identification of disease-specific biomarkers, understanding of altered metabolic pathways and improvements in early diagnosis and therapeutic strategies. This review highlights the techniques, their applications, challenges, and future perspectives in blood cell analysis [93].

Blood cell abnormalities, reflecting underlying hematological disorders, require precise detection and understanding for effective diagnosis and treatment. Modern techniques, such as proteomics and metabolomics, have emerged as powerful tools to unravel the molecular and metabolic changes in blood cells. Proteomics enables the large-scale study of proteins using advanced techniques like mass spectrometry (MS), liquid chromatography-mass spectrometry (LC-MS/MS), and protein microarrays, aiding in the detection of altered protein expression, oxidative stress-related proteins in sickle cell disease, and biomarkers in anemia and leukemia. Similarly, metabolomics investigates small molecules and their roles in cellular processes using nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and LC-MS, revealing altered pathways like glycolysis and oxidative stress in diseases such as leukemia and anemia. Integrating these approaches offers a systems biology perspective, linking protein expression to metabolic alterations, enabling early detection, personalized therapies, and biomarker discovery. Despite challenges like high costs, variability in blood samples, and complex data integration, advancements in technology, AI-driven analysis, and multiomics tools hold promise for tailored diagnostics and treatment in hematological diseases, exemplified by studies like mitapivat's impact on the metabolome and proteome in sickle cell disease [94].

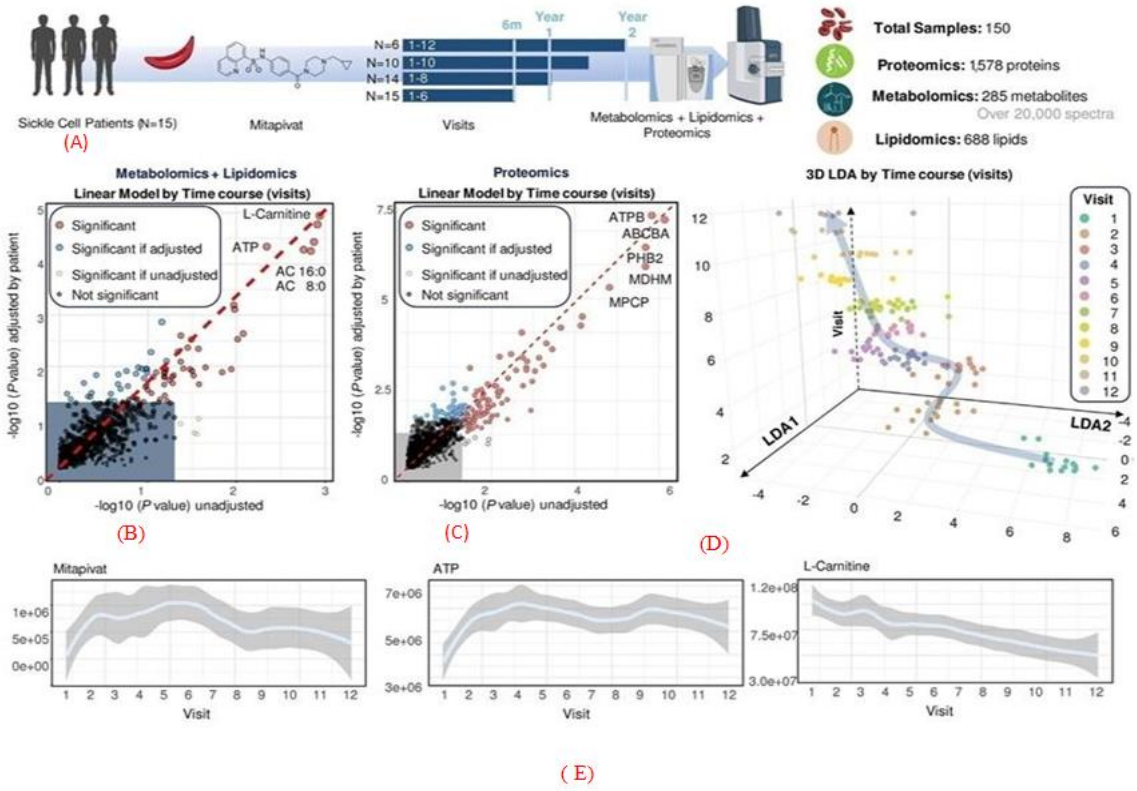

Fig.13- Alterations of the metabolome in sickle red blood cells from patients on treatment with mitapivat.

(A) Overview of the clinical study. Fifteen sickle cell patients (SS genotype) were enrolled in this clinical trial, with all 15patientsbeing treated for 6 months, 14 for a whole year and six for up to2 years (visit 12).Red blood cell (RBC) samples underwent multiomics characterization. (B,C)Linear model analysis of metabolomics and lipidomics data (B) or proteomics data(C) identified molecules associated with the treatments, either unadjusted (x-axis) or adjusted by patient-specific responses (y-axis). Highlighted metabolites (B) or proteins (C) represent the variables with the highest weights across linear discriminant analysis 1 (LDA1). (D) Line plots of mitapivat, ATP and carnitine, the very drug being administered, along

with the levels of the top metabolites affected by the treatment. In light blue, median meta bolite levels across all samples, while range intervals are shown in light gray. Data are shown as peak area abundance (arbitrary unit on the y axis), while the x axis represents visits 1-12. (E) LDA identified two major components (LDA1 and LDA2 –x and y, respectively) discriminating samples across visits (z axis) [95].

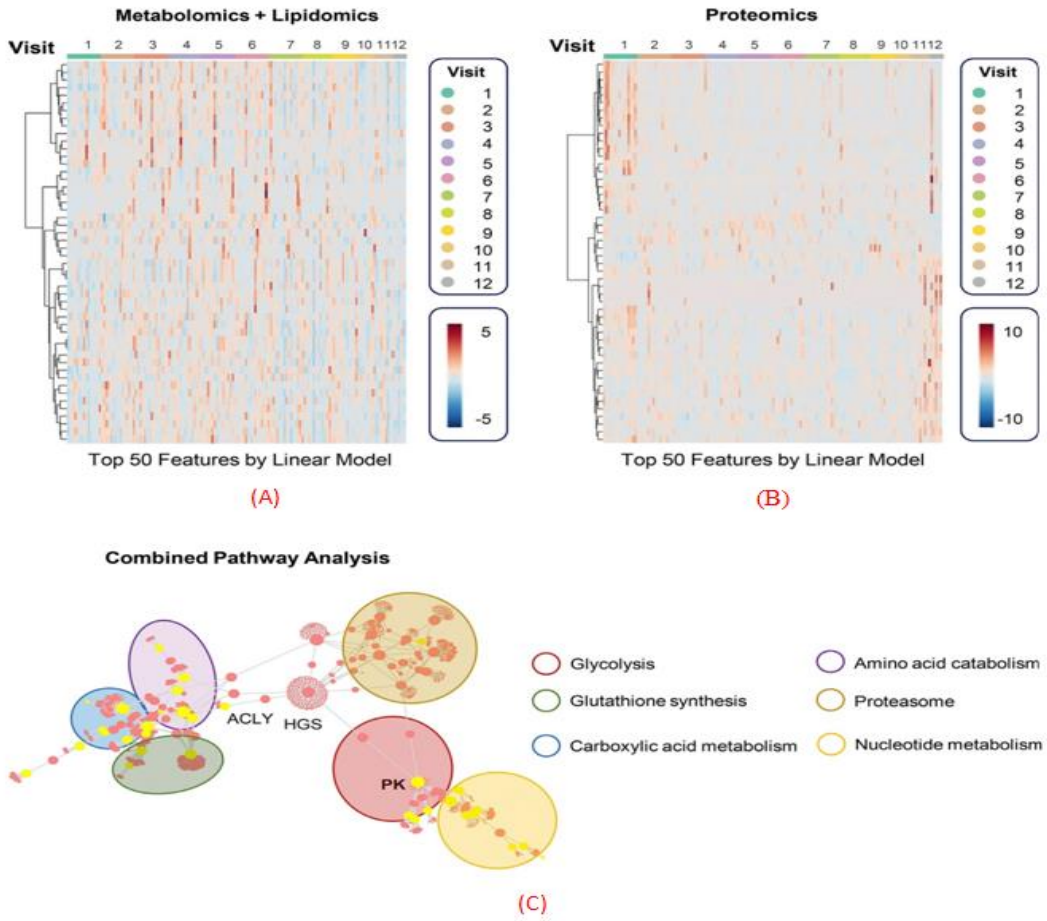

Fig 14- Heatmap and network analysis of top metabolites and lipids or proteins affected by mitapivat treatment in sickle red blood cells.

(A, B) The top 50 metabolites/lipids (A) and proteins (B) (based on linear discriminant analysis) affected by mitapivat treatment are shown as a function of time (visits).(C) Merged data from these analyses were uploaded to Omicsnet to perform combined pathway analyses.

3. RESULTS AND DISCUSSION

Here is the detailed table including **Accuracy (%), Precision (%), Sensitivity (%)**, overall results, pros, and cons for each technique used in determining abnormal blood cells:

S. No	Technique	Accuracy (%)	Precision (%)	Sensitivity (%)	Overall Result	Pros	Cons
1	Microscopy-based Techniques	70-80%	65-75%	60-70%	Good for basic morphological assessment.	Cost-effective, widely available, simple to use.	Operator-dependent, low sensitivity for subtle abnormalities.
2	Flow Cytometry	90-95%	90-95%	85-95%	Excellent for cell marker-based analysis.	Rapid, multi-parameter analysis, high-throughput.	Expensive, requires trained personnel, complex data interpretation.
3	Automated Analyzers	85-92%	85-90%	80-88%	Fast and reproducible results.	High speed, consistent results,	Limited to predefined parameters, less

						useful for routine diagnostics.	effective for rare abnormalities.
4	Molecular Techniques	95-99%	95-98%	90-95%	Accurate for genetic and molecular changes.	Detects genetic abnormalities, high precision, identifies mutations.	Time-consuming, expensive, requires specialized labs.
5	Imaging Techniques	85-90%	80-88%	75-85%	Useful for spatial analysis of cells.	Provides visual and spatial insights, non-invasive.	Resolution-dependent, may miss molecular-level changes.
6	AI & Machine Learning	95-99%	95-99%	95-99%	Rapid and highly accurate results.	Fast, automates analysis, identifies patterns in large datasets.	Requires large training datasets, initial implementation is complex.
7	Mass Spectrometry Techniques	90-95%	90-95%	85-92%	Effective for molecular-level analysis.	Detects proteins and metabolites accurately, sensitive and versatile.	Costly instruments, time-consuming sample preparation.
8	Cytogenetics & FISH	95-99%	95-98%	90-95%	Gold standard for chromosomal analysis.	Accurate for detecting chromosomal abnormalities, highly reliable.	Labor-intensive, expensive, requires skilled professionals.
9	Single-Cell Sequencing Techniques	97-99%	95-98%	95-99%	Uncovers cellular heterogeneity.	Detects rare abnormal cells, high sensitivity at single-cell resolution.	Costly, requires complex data analysis, not widely available.
10	Proteomics and Metabolomics Techniques	95-98%	93-97%	90-95%	Deep molecular-level insights.	Identifies biomarkers, provides comprehensive molecular data.	Expensive, complex analysis, requires advanced instrumentation.

Table 1: Comparison of different modern techniques

The detailed Graph including **Accuracy (%), Precision (%), Sensitivity (%)**, overall results, pros, and cons for each technique used to detect abnormal blood cells:

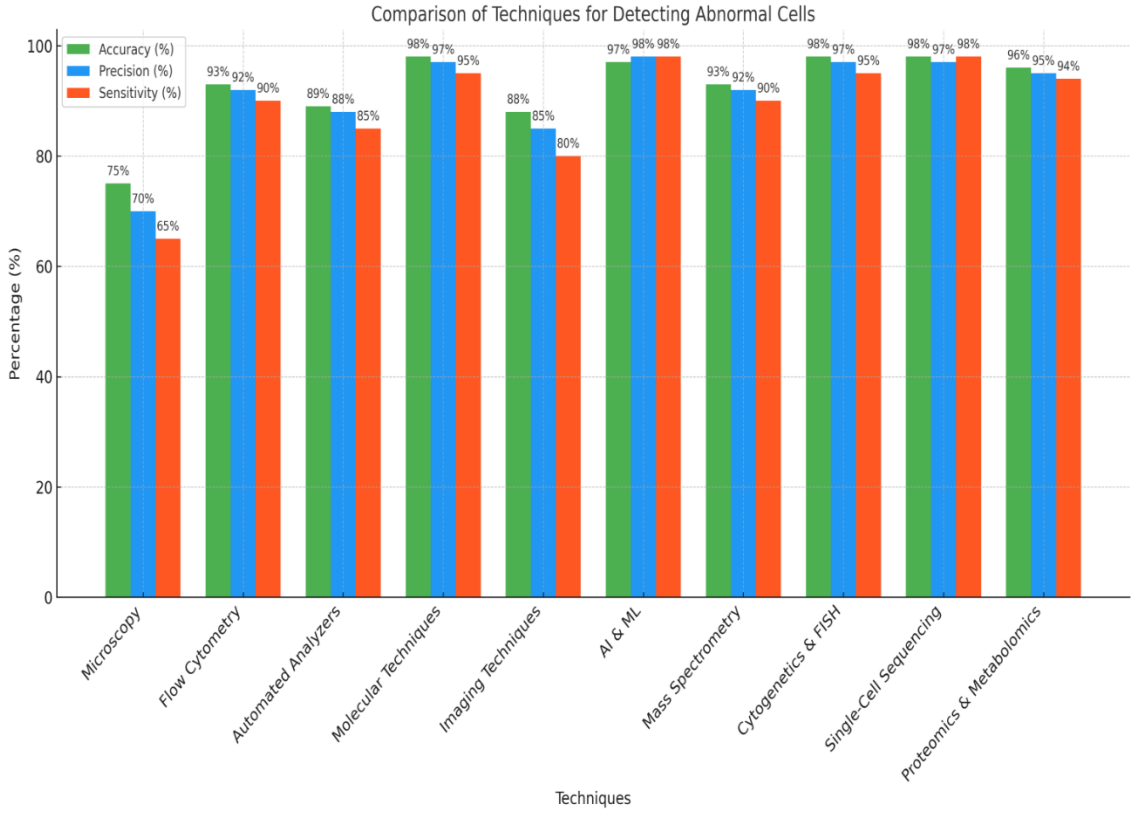

Fig.15- Comparison of Techniques for Detecting Abnormal Cells

4. CONCLUSION

The techniques for detecting abnormal blood cells vary significantly in their capabilities, applications and limitations. **Microscopy-Based Techniques** are suits for basic morphological assessment in resource-limited settings. But it has some Limitations like Operator-dependent, lower accuracy and sensitivity compared to modern techniques. **Flow Cytometry** suitable for Identifying specific cell populations and surface markers, its Advantages are High accuracy, sensitivity, and throughput and its Limitations are requiring expensive instruments and skilled personnel. **Automated Analyzers** are best for Routine clinical diagnostics for quick and reproducible results. And its Limitations are Predefined parameters may miss rare or subtle abnormalities. In **Molecular Techniques** suits for detecting genetic mutations and molecular abnormalities with near-perfect accuracy, some of its advantages are High precision and reliability and Limitations are time-consuming and costly. **Imaging Techniques** Provide spatial insights into abnormal cells and its Limitations are Moderate sensitivity and resolution dependent.**AI & Machine Learning** best suits for Large-scale automated analysis with unmatched accuracy and speed, and its advantages are Excellent in pattern recognition and handling complex datasets. Some of its Limitations includes, it enquires substantial data and advanced computational resources. In **Mass Spectrometry** Technique Molecular-level detection of proteins and metabolites findings are resulted. Its advantages are highly sensitive and versatile for proteomics/metabolomics. Its Limitations are high cost and complex sample preparation.

Cytogenetics & FISH Method is good for Chromosomal analysis and detecting genetic abnormalities. Its advantages are Gold standard for diagnosing chromosomal aberrations in cancers. And some Limitations are Labor-intensive and costly. Whereas in **Single-Cell Sequencing** gives best Accuracy, Precision and Sensitivity among all modern techniques in determining abnormal blood cells. It suits for Unveiling heterogeneity at the single-cell level and rare abnormal cells. It's some advantages are extremely high sensitivity and resolution, and some Limitations are it has high-cost operative and data analysis complexity, and limited accessibility. **Proteomics and Metabolomics Techniques suits for** Comprehensive molecular insights into abnormal cells. Its advantages are identifying novel biomarkers and disease pathways and some Limitations requires sophisticated instrumentation and advanced analysis methods.

Some Key Recommendations are For Routine Diagnostics: Automated Analyzers and Flow Cytometry are preferred due to their speed and reliability, For High-Resolution Molecular Analysis, Molecular Techniques, Single-Cell Sequencing, and Proteomics/Metabolomics techniques are used. For Large-Scale Automation AI & Machine Learning provides unmatched accuracy and efficiency. For Chromosomal Abnormalities Cytogenetics and FISH remain the gold standard combining techniques (e.g., AI with Flow Cytometry or Proteomics) can further enhance diagnostic accuracy and sensitivity. The choice

of technique depends on the clinical context, available resources, and the required depth of analysis.

Acknowledgements: The Findings herein reflect the work and are solely the responsibility of the authors.

Abbreviation

1. **ACD** - Anemia of Chronic Disease
2. **AML** - Acute Myeloid Leukemia
3. **CBC** - Complete Blood Count
4. **CNN** - Convolutional Neural Networks
5. **DIC** - Disseminated Intravascular Coagulation
6. **EBV** - Epstein-Barr Virus
7. **FISH** - Fluorescence In Situ Hybridization
8. **FAZ** - Foveal Avascular Zone
9. **IGC** - Immature Granulocyte Count
10. **IRF** - Immature Reticulocyte Fraction
11. **ImFC** - Imaging Flow Cytometry
12. **LDH** - Lactate Dehydrogenase
13. **LVD** - Linear Vessel Density
14. **MALDI** - Matrix-Assisted Laser Desorption/Ionization
15. **MCH** - Mean Corpuscular Hemoglobin
16. **MCHC** - Mean Corpuscular Hemoglobin Concentration
17. **MCV** - Mean Corpuscular Volume
18. **MIP** - Medical Image Processing
19. **MII** - Medical Imaging Informatics
20. **MPV** - Mean Platelet Volume
21. **MDS** - Myelodysplastic Neoplasms
22. **NGS** - Next-Generation Sequencing
23. **OCTA** - Optical Coherence Tomography Angiography
24. **PDW** - Platelet Distribution Width
25. **PCR** - Polymerase Chain Reaction
26. **PTMs** - Post-Translational Modifications
27. **RBCs** - Red Blood Cells
28. **RDW** - Red Cell Distribution Width
29. **SEM** - Scanning Electron Microscopy
30. **sTfR** - Serum Transferrin Receptor
31. **TIBC** - Total Iron-Binding Capacity
32. **TEM** - Transmission Electron Microscopy
33. **WBCs** - White Blood Cells

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