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Laboratory Diagnosis of Acute Leukemia in Kenya: The Gaps and Opportunities

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Abstract

Acute leukemia (AL) is a malignant disease of the bone marrow in which hematopoietic precursors are arrested in an early stage of development. The diagnosis of leukemia and lymphomas, beyond morphology, is limited in low-resource countries including Kenya. Morphological diagnosis includes Cytological and Histological assessment of blood, bone marrow aspirates and tissues on suspected Acute leukemia patients. The World Health Organization (WHO, 2016) international guidelines on Acute leukemia diagnosis recommend that cytogenetic analysis, appropriate molecular genetics, Fluorescent in situ Hybridization (FISH) testing, and flow cytometric immuno-phenotyping should be done in addition to a morphologic assessment of Acute Leukemia. In facilities where resources are relatively available, immunophenotypic and genetic features have resulted not only in providing a more accurate leukemia diagnosis but also in identifying antigens or genes that can then be targeted for therapy. This article will look at the gaps in the diagnosis of Acute leukemia in low-resource settings like Kenya and opportunities available to improve diagnosis.

Keywords

Acute Leukemia, Complete Blood Counts, Peripheral Blood Film, Bone Marrow Aspirates, Flow Cytometry, Cytogenetics, Molecular Tests

1. Introduction

1.1. Epidemiology of Hematological Disorders in Kenya

According to WHO data published in 2020, the incidence of all leukemias is 1579 with 1159 deaths in Kenya [1]. Hematological malignancies account for 10% of all cancers [2]. A review of data collected by Eldoret Cancer Registry

(Hospital and population-based data) on hematological malignancies treated at Moi Teaching and Referral Hospital (MRTH) over a 10-year period (2008-2017), showed that there was a total of 2433 cases of Hematological malignancies out of 19,157 cancers documented in the same period of time. This represents about 12.7% of all cancers recorded in the same period. The commonest hematological malignancy was Non-Hodgins Lymphoma (NHL) accounting for 840 (33%) cases, followed by Acute lymphoblastic Leukemia (ALL) 396 (16%), Chronic Myeloid (CML) 284 (12%) and Acute Myeloid Leukemia (AML) 271 (11%) among others. This data is limited to patients majorly from western Kenya who seek treatment at MTRH and may not be representative of Kenyan Data [3].

1.2. WHO Recommendations on Laboratory Diagnosis of Acute Leukemia

The 3rd edition of the World Health Organization (WHO) (2001) classification of Acute Leukemia formally introduced the requirement for immunophenotyping and cytogenetic studies among other studies for the diagnosis of AL. The 4th edition of the WHO classification, published in 2008, added cytogenetic disease groups for AML and ALL, as a result, introduced the category of mixed-phenotype acute leukemia (MPAL), and included provisional entities of AML that were based on gene mutation patterns [4]. The 2016 WHO classification of hematologic malignancies (Revised 4th Edition), categorizes AL into entities by a combination of morphologic, immunophenotypic, and molecular genetic/epigenetic changes [5]. Some gene mutations and cytogenetic abnormalities in the revised 4th edition include: Improved characterization and standardization of morphological features aiding in the differentiation of disease groups, particularly of the BCR-ABL myeloproliferative neoplasms (MPNs), which has increased the reliability and reproducibility of diagnoses, although not disease-defining, offer significant prognostic information. These genetic and epigenetic changes in AL may be detected by individual, often PCR or reverse-transcriptase PCR-based, assays; by gene panels using NGS methods; or by looking at the entire genome of a given sample. The latter approaches are more comprehensive and are becoming increasingly available because of major advances in molecular genetic testing technology [6].

1.3. Gaps in Laboratory Diagnostics of Acute Leukemia in Kenya

In resource restrained settings including Kenya, diagnosis of acute leukemia falls short of the recommended WHO guidelines. There are a limited number of Laboratory Health care personnel trained to make early diagnosis of acute leukemia. There are a limited number of clinical or hem pathologists (less than 20%) and technicians dedicated to diagnosis of acute leukemia against the more the 50 million population of Kenya. Reagents and stains used for processing Peripheral blood films, Bone marrow aspirates and tissue are available in few County and National Referral Hospitals and supply may be erratic. There are also cost implications of these diagnostics tests. Some of the tests like immunophenotyping

and molecular tests are considered advanced tests and not paid for by the National Insurance fund. This makes their application, availability and accessibility limited in resource restricted settings. The cytogenetics and molecular tests are barely available to the general public but are available in few private laboratories and hence accessible to only those with good insurance cover or those who can afford out of pocket payments. Next generation sequencing platforms are found in few Research labs, among them is Kenya Medical Research Institute in Kenya (KEMRI). They are mainly used as research platforms, not accessible for diagnostics to the patients in public health care system.

This article will look at the gaps in diagnosis of acute leukemia in low resource settings like Kenya and opportunities available to improve diagnosis.

2. Laboratory Diagnosis of Acute Leukemia

Comprehensive diagnosis of Acute Leukemia incorporates clinical features, morphology of malignant cells in peripheral blood film and bone marrow aspirate, immunophenotyping, cytogenetics, and molecular genetics to be able to define disease entities of clinical significance.

2.1. Complete Blood Counts (CBC) and Peripheral Blood Films

The blood count is one of the most widely used tests in medicine. Complete Blood Count (CBC) is a measure of bone marrow function. It is usually the first indication of an underlying hematological malignancy. Some blood count features are diagnostic and others may give an indication of a bone marrow defect. The marrow produces red blood cells, white blood cells and platelets [7]. In Kenya, most of the levels 4, 5 and 6 hospitals (Level 6 being the most advanced in terms of availability of tests and expertise) will have a hematology analyzer to run the complete blood counts. Some lower level facilities have the capacity to do Hemoglobin levels only.

A cytopenia reflects poor marrow function or peripheral destruction of the involved cell. Most patients with leukemia have more than 1 cell line affected at diagnosis. When a patient has bicytopenia (reduction of cells in 2 cell lines) or pancytopenia (reduction in cells in 3 cell lines) the diagnosis of leukemia should be more strongly suspected. (Jaime-Pérez *et al.*, 2019). Acute leukemia may also present with leukopenia, normal white blood cell count or leukocytosis due to peripheral circulation of blasts [8]. Hyperleukocytosis can be seen in any patient with leukemia and is generally defined as a WBC count of greater than 100,000/μL (or $>100 \times 10^9$/L). This is more common in those with AML, T-cell ALL, and infantile leukemia. A large number of blasts can cause leukocytosis, which precipitates decreased tissue perfusion. This is a medical emergency causing neurologic and pulmonary sequelae. Patients with AML have more problems with leukocytosis at lower WBC counts because AML blasts are larger and stickier. Those with ALL generally only develop signs of leukocytosis with extremely high WBC counts [9]. Patients presenting with progressive leukocytosis and not responding to antibiotics need further evaluation to rule out leukemic process.

Kenya like other LMIC according to world bank rankings, has few clinical hematologists or hematopathologists based at mainly Level 5 and Level 6 Hospitals. The technical staff in the lower-level facilities do not routinely prepare nor evaluate the peripheral blood films due to either limitation of capacity to perform the test or interpret of results. Some of the challenges faced by these facilities include consistency of running the CBC's once the analyzer is broken down with no back up or may run out of reagents. It may take some time before new reagents are acquired due to long procurement procedures or inadequacy of funds. Usually, initiation of a PBF is often a clinical request by the attending clinician on account of a clinical suspicion or less frequently initiated by the laboratory. This is where the gap is and where delay can occur when it comes to early detection of acute leukemia and other hematological disorders in low resource settings. The reason for this is that the initial technicians and technologists training in college is not translated to practice except in very few facilities. The clinicians/physicians in some instances fail to request for the Peripheral blood films and decide to treat the patient based on the clinical signs and symptoms. Other times, the test may be requested for but is limited by lack of reagents to stain the films.

One of the solutions to this gap is to have laboratory technologists in a good number of facilities empowered through training to reflexly process a peripheral blood film the moment they detect a cytopenia or cytosis of either red cell, white cells or platelets. They should also be trained to able to interpret the PBF and even suggest other ancillary tests to be done to diagnose acute leukemia. Molyneux *et al.* observed that delayed presentation, diagnosis, advanced disease, co-morbidities and underlying malnutrition were the challenges to effective treatment of childhood cancers in Africa [10]

There may be limitations or pitfalls in interpretation of automated complete blood counts. Some of the causes of these are: 1) specimen characteristics that interfere with the measurement of one or more CBC parameters; 2) abnormal cells and/or cellular phenomena that mimic other abnormal or normal cells and therefore are misidentified and miscounted. Interferents generating inaccurate CBC results include lipemia, hemolysis, hyperbilirubinemia, RBC agglutinins, WBC agglutinins, PLT agglutinins, hyperproteinemia/paraproteinemia, cry proteinemia, microorganisms (marked bacteremia, fungemia, and possibly, malaria), hyperglycemia, dilution with intravenous (IV) fluid infusion(s), adipose tissue fragments/fat globules, fibrin clumps, and small clots. Abnormal cells and/or cellular phenomena that may adversely affect one or more CBC parameters include RBC fragments/schistocytes including micro spherocytes, extremely microcytic RBCs, lysis-resistant RBCs (e.g., RBCs containing Hb C), hyperleukocytosis, giant PLTs, cytoplasmic fragments of leukemic cells, PLT satellitosis, nucleated RBCs (NRBCs), megakaryocytes, and non-hematopoietic cells/carcinoma cells [11].

In preparation of peripheral blood films, to ensure accurate and reliable results, pre-analytical variables that can affect the quality of film must be con-

trolled. These include patient preparation and consent, blood sampling technique, transport to the laboratory, and sample preservation. Commonly, blood is obtained from peripheral veins and stored in an EDTA anticoagulant bottle. Blood to anticoagulant ratio should be in the right proportion, a standard tube has a limit of 4 ml of blood. When using capillary blood excess tissue fluid affects the distribution of the cellular elements of blood. Samples should be sent to the laboratory as soon as possible. Samples are best analysed within 2 hours of blood collection. Delay in preparation of blood smear may allow for the degeneration of the cellular elements of blood and may result in pseudo-thrombocytopenia (falsely reduced platelet count) due to the formation of platelet aggregates. Slide preparation is done by trained personnel preferably a medical laboratory technologist, who can ensure quality slides for microscopy. Laboratory assistants can also be trained in the art of slide preparation [12].

2.2. Bone Marrow Aspirate and Trephine Biopsy

A bone marrow aspiration (BMA) is a procedure that draws liquid part of the bone marrow. Bone marrow is the spongy tissue inside of the bones that produces blood cells. A trephine biopsy is a sample of the solid, spongy part of the bone marrow. Bone marrow aspirate smear gives cytological detail, and trephine biopsy provides information about marrow cellularity, architecture, cellular distribution, and extent of fibrosis. Indications of performing BMA and Trephine biopsies include but are not limited to definitive diagnosis of hematologic disorders such as leukemia, multiple myeloma, lymphoma, unexplained anemia, and myelodysplastic syndrome, fever of unknown origin, atypical fungal and parasitic disorders such as histoplasmosis, leishmaniasis, cryptococcus, granulomatous inflammation like disseminated tuberculosis and metastatic disease [13].

When acute leukemia is suspected clinically or on PBF findings, the treating physician typically recommends a bone marrow evaluation for further morphologic assessment. American Society of Hematology-College of American Pathologists (ASH-CAP) guidelines recommend that the treating clinician or pathologist should obtain a fresh bone marrow aspirate for all patients suspected of acute leukemia, a portion of which should be used to make bone marrow aspirate smears for morphologic evaluation. The pathologist should evaluate an adequate bone marrow trephine core biopsy, bone marrow trephine touch preparations, and/or marrow clots, in conjunction with bone marrow aspirates [6].

A bone marrow aspirate is necessary to definitively diagnose leukemia. The diagnosis can also be made from peripheral blood if a sufficient number of blasts are present. The diagnosis of acute leukemia requires the presence of ≥20% blasts in the peripheral blood or bone marrow. The 2016 WHO criteria defined AML as an entity with any evidence of extramedullary myeloblasts (myeloid sarcoma), or presence of AML defining genetic abnormalities PMLRARA, RUNX1: RUNX1T1, and CBFB: MYH11 irrespective of blast percentage (Arber

et al., 2016). Typically, both bone marrow aspiration and bone marrow trephine biopsy are performed in levels 5 and 6 in Kenya. The advantage of having both the procedures done together is because some disease processes like aplastic anemia, different phases of myeloproliferative neoplasm (MPN), multiple myeloma, tubercular granulomas which may mimic Acute leukemia at clinical presentation are best assessed on Trephine Biopsies [14].

In of Kenya, less than 20 Level 4 and 5 public Hospitals are able to routinely perform bone marrow aspirates and trephine biopsies. Unpublished survey by Ampath Myeloma programme on training on BMA procedure and slides staining at 8 county Hospitals in Western Kenya revealed that some challenges in routinely performing BMAs included; difficult processes in obtaining BMA needles and reagents for staining slides, patients being unable to pay for the procedure, challenges in obtaining trephine biopsies during the procedure and limited number of pathologists to evaluate and report on the slides. A study by Sayed *et al.* to address the issue of shortage of pathologists to perform Bone Marrow aspirates demonstrated that a model of task-shifting diagnostic procedures and skills from pathologists to Medical officers, Clinical officers and other medical personnel can address some of the challenges associated with the shortage of pathologists to perform Bone Marrow test [15]. The training of medical officers and clinical officers ensured sharing and shifting of technical skills (Bone marrow aspirate procedure and Fine needle aspirate), this was found to have a positive influence on turnaround time to diagnosis of both benign ad malignant diseases. The drawback is that skills take time to acquire and so several retraining sessions may need to be done, which has time and cost implications. There is also the aspect of increasing the workload on the staff who already have other duties. Staff transfer also has an impact that may require training of new staff in the unit.

Some facilities in Kenya perform aspiration alone and not trephine biopsy and the reason for this is that they do not have adequate training on performing a trephine biopsy or they do not have histopathology services within their Health facilities to process the trephine biopsies. The challenge of performing Bone marrow aspirate without accompanying trephine biopsy comes about when the procedure yields a hemodiluted aspirate, a dry tap or diagnostic uncertainty where Immunohistochemistry is required to make a conclusive diagnosis. A biopsy is always indicated in pancytopenic patients especially where there are no circulating blasts in the peripheral blood and yet Acute Leukemia is suspected (Percival *et al.*, 2017). Further analyses required by guidelines (WHO, 2016) to be performed on the bone marrow samples include flow cytometry, metaphase cytogenetics, fluorescence in situ hybridization (FISH), and molecular analyses. This combined information if collated at the time of Acute Leukemia diagnosis is useful in prognostication for patients as well as in recommendations for individual patients on whether to proceed to investigational induction chemotherapy and/or allogeneic hematopoietic cell transplant (HCT).

2.3. Flow Cytometry Immunophenotyping in Acute Leukemia

Flow cytometry measures optical and fluorescence characteristics of single cells. Physical properties, such as size (represented by forward angle light scatter) and internal complexity (represented by right-angle scatter) can resolve certain cell populations. Fluorescent dyes may bind or intercalate with different cellular components such as DNA or RNA. Additionally, antibodies conjugated to fluorescent dyes can bind specific proteins on cell membranes or inside cells. When labeled cells are passed by a light source, the fluorescent molecules are excited to a higher energy state. Upon returning to their resting states, the fluorochromes emit light energy at higher wavelengths. The use of multiple fluorochromes, each with similar excitation wavelengths and different emission wavelengths, allows several cell properties to be measured simultaneously. Commonly used dyes include propidium iodide, phycoerythrin, and fluorescein, although many other dyes are available.

Flow cytometry immunophenotyping has developed over time to include more fluorochromes allowing more monoclonal antibodies assessment. One of the earliest platforms used for CD3 and CD4 Monitoring was the 4 color BD FACS caliber. The Becton Dickinson FACS Calibur Flow Cytometer offers a unique approach to flow cytometry, allowing users to perform both cell analysis and cell sorting in a single system, providing flexibility for a variety of research and clinical applications. The BD FACS Calibur is a fully integrated multiparameter system that has the performance and sensitivity to ensure objective and reproducible results through the use of dual-laser technology, interbeam compensation, and an alignment-free optical design. The fluorochromes FITC, PE, PerCP, PerCP Cy5.5 and APC are used in this platform [16]. Some of the limitations of this platform include: cost of running adequate controls, the (lack of appropriate) compensation, sorting strategies, or even the description of the methods used Other factors include choice of reagents, the preparation and eventual storage of the cells under analysis, the overall experimental plan and, last but not least, data analyses and interpretation [17].

CytoFLEX is the newest cytometer offered by Beckman Coulter Inc. that uses up to 3 lasers and 13 colors for detection in a compact system. To demonstrate the ability of its multicolor detection, the standard multicolor panel design principles were followed to develop a 14 marker, 13 color experiment. The cocktail design process also considered the desired immunophenotypic markers, the available fluorochromes, and the use of well-defined control populations to identify the classic TBNK populations. Data is collected from normal, whole blood stained with single color reagents and lysed with VersaLyseTM Lysing Solution. Data is analyzed using the CytExpert software and is presented with special emphasis on the optimization of flow analysis and compensation visualization [18].

The Beckman Coulter flow cytometry platform is available in few Reference laboratories in Kenya. In the Public sector we have it at AMPATH Reference

Laboratory where we are situated and in the private sector, we have the Aga Khan University Hospital, few others are being added in other facilities. One of the studies assessing the performance of this platform was done by Kabera *et al.* this study was whose main objective was Immunophenotyping of Acute leukemia in 132 patients at Aga khan Hospital Nairobi. Bone marrow and blood samples were immunophenotyped by using the multiparametric five-colour flow cytometer (Beckman Coulter FC500), utilizing the panels of monoclonal antibodies using the European Group for the Immunological Characterization of Leukemia (EGIL) criteria. The panels of antibodies included the following: CD34, HLA-DR, CD117, CD13, CD14, CD33, CD11c, CD19, CD10, CD20, cCD3, TdT, CD7, cMPO, sIg, CD15, CD56, CD45. They found out that there were 88 AML, 42 ALL and two cases of biphenotypic leukemia. The commonest overall AML morphological sub-type was AML-M2, 26 (29.5%). Majority of ALL cases were B-cell immunological sub-type (96.6%). There were only 4 cases of T-cell ALL (Kabera *et al.*, 2013).

There are times when morphological diagnosis and Flow cytometry immunophenotyping may not be concordant. These could be due to several reasons including quality of sample collection and processing. A study by Patel *et al.* comparing morphological and flow cytometry of 33 patients diagnosed with Acute leukemia by morphology s as 17 patients had AML and 15 had ALL, one case was inconclusive. Using flow cytometry, they comprised of B-ALL-6 cases, T-ALL-13 cases, AML-10 cases, Biphenotypic-1 case and inconclusive-1 case. There was morphologic and immunophenotyping concordance in 25 out of the 33 cases. The cases that were discordant needed more characterization through Immunohistochemistry or acquiring a new sample [19].

In Kenya and other low resource settings, flow cytometry platform is not easily accessible to most patients who require the test. It is a cost intensive platform that is mainly available in private/commercial laboratories and very few Public Hospitals. Leak *et al.* was looking at Hematological malignancies in Tanzania they used a BD FACS Calibur which had been installed in the main hospital laboratory for CD4 counts of HIV patients to run leukemia and lymphoma diagnostics, this was after the updated guidelines favored use of viral loads over CD counts in monitoring HIV patients [2]. Similarly, at MTRH, Eldoret Kenya, Flow cytometry for CD4 and CD8 counts were being run to monitor HIV patients using the 4 color BD FACS Calibur. Later, this platform was used to run Flow cytometry for patients who were suspected to have acute leukemia with a limited panel adequate for Acute leukemia characterization.

Few years ago, in 2017, Burkitt's Lymphoma for Africa Organization (BLFA) installed a new machine (13 color Cytoflex that comes with lyophilized antibodies in the tubes that enables rapid performance of the test and results analyzed with Kaluza software. The panel includes; CD2CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD 33, CD 34, CD38,56, CD64, CD117, CD123, CD200, HLAD-R, Kappa and Lamdha and serves the Acute leukemia patients in MTRH and wider western region because the sam-

ples can be sent via curio to the lab. The challenge is that this test is very costly approximate cost per sample and not available to many patients without insurance cover. There are ongoing conversations to have the test covered by the National Hospital Insurance Fund, it is yet to bear fruit. Fortunately, the pediatric oncology programme was able to secure a grant in 2022 to cover the costs of flow cytometry for paedtraic patients with suspected acute leukemia which has made the test accessible to this population.

2.4. Flow Cytometry and MRD Testing

MRD assessment may be performed through three different modalities: multiparameter flow-cytometry (MFC), quantitative polymerase chain reaction (qPCR), or next-generation sequencing (NGS). Specimens for MRD assessment can be obtained via peripheral blood or from a small volume of first pull bone marrow to avoid hemodilution. For each leukemia type, MRD is usually assessed after initial treatment, serial testing during therapy, and also during follow-up once therapy has been completed MFC identifies the MRD based on characteristic "leukemia-associated immunophenotypes" (LAIPs) on the residual leukemic cells [20].

The significance of doing MRD testing in acute leukemia management is that based on the MRD results, further therapy can either be intensified or de-intensified to achieve long-term complete remission and reduces the harmful effects of toxic chemotherapies. MRD has emerged as a strong prognostic factor for assigning a particular case of acute leukemia into various risk categories and treatment arms. Another upcoming indication of MRD testing has been its role in the treatment decision making for the patients undergoing stem cell transplant (SCT). Among the ALL patients, MRD estimation before the SCT is an essential indicator of the outcomes, also after SCT, MRD estimation is a good predictor of impending relapse in such patients.

A clear advantage of MFC-MRD is the applicability in the majority of AML patients (up to 90%). However, it has a lower sensitivity than that of most other methods, and it is highly dependent on the degree of difference of the leukemic phenotype to normal antigen expression patterns. MFC analyses require a high level of experience and the assay quality remains operator-dependent to some degree, hampering inter-laboratory harmonization and standardization. Subsequently, the recent ELN guidelines recommend MFC-MRD analysis to be carried out only in experienced laboratories until techniques have been further standardized [21].

Minimal residual disease (MRD) testing allows for the accurate detection of very small amounts of leukemic blasts in the bone marrow. The MRD levels are evaluated from patient bone marrow aspirates, which are obtained at multiple independent time points throughout the treatment regimen. Cellular MRD counts have general prognostic value at the cutoff level of 0.01% MRD cells meaning 1 MRD cell in 10,000 cells out of all bone marrow mononuclear cells within a specimen. The prognostic limit of 0.01% is based on the immunohisto-

chemical detection limits of 3 - 4-color flow cytometers. It is not clear the clinical significance of the 0.01% MRD cutoff level is that when a patient has cellular MRD levels ≥ 0.01% in a bone marrow sample at important measurement time points during therapy, the patient will have a significantly higher risk for leukemia relapse than if MRD levels are less than 0.01%. Data also suggest that the higher the MRD value (e.g., MRD > 1%) at the end of the induction phase of chemotherapy, the higher the risk of relapse and the lower the survival rate MRD has been shown to be a strong prognostic indicator across all leukemia subtypes [22]. Historically, remission was defined as having less than 5% blasts in the bone marrow by morphologic analysis alone after initial induction chemotherapy. This has been largely replaced with the use of MRD testing of the bone marrow at the end of induction using flow cytometry. Clinical trials in precursor B-cell ALL showed that MRD was highly prognostic (Campana, 2009).

This test has limited accessibility and affordability on low resource settings.

2.5. Cytogenetic Analysis

Cytogenetic Analysis (Karyotyping). Cytogenetic testing is done using either a bone marrow or a blood sample. The leukemia cells in the sample are allowed to culture in the laboratory and then they are stained prior to examination. The stained sample is examined under a microscope and then photographed to show the arrangement of the chromosomes (the karyotype). The karyotype will show if there are any abnormal changes in the size, shape, structure or number of chromosomes in the leukemia cells. Fluorescence in situ Hybridization (FISH) is a type of cytogenetic laboratory technique that is used to identify and examine specific genes or chromosomes in leukemic cells using specific probes and examined using a fluorescent microscope.

2.6. Molecular Tests

Many molecular tests exist and can be used to detect the molecular changes at gene level in acute leukemia. These tests include, but are not limited to endpoint? Polymerase chain reaction (PCR), real-time PCR, Sanger sequencing, pyrosequencing and next-generation sequencing. These tests are very sensitive laboratory techniques that are used to detect and measure some genetic mutations and chromosomal changes that are too small to be seen with a microscope. Polymerase chain reaction testing essentially increases or "amplifies" small amounts of specific pieces of either RNA (ribonucleic acid) or DNA to make them easier to detect and measure. Molecular PCR can be used to determine the amount of minimal residual disease (MRD), the small amount of cancer cells left in the body after treatment. This testing can be done on a bone marrow or a blood sample [23].

The current ASH-CAP guidelines recommend that for pediatric patients with suspected or confirmed B-ALL, the pathologist or treating clinician should ensure that testing for t (12; 21) (p13; q22); ETV6-RUNX1, t (9; 22) (q34; q11.2); BCR-ABL1, KMT2A (MLL) translocation, iAMP 21, and trisomy 4 and 10 is

performed. In adult patients with suspected or confirmed B-ALL, testing for t (9; 22) (q34; q11.2); BCR-ABL1 is performed, in addition, testing for KMT2A (MLL) translocations may be performed. In pediatric and adult patients with suspected or confirmed AML of any type, testing for FLT3-ITD is performed. The pathologists or treating clinician may order mutational analysis that includes but is not limited to: IDH1, IDH2, TET2, WT1, DNMT3A, and/or TP53 for prognostic and/or therapeutic purposes [6].

For adult or pediatrics patients with confirmed core binding factor (CBF) AML (AML with t (8; 21) (q22; q22.1)); RUNX1-RUNX1T1 or inv (16) (p13.1; q22)/t (16; 16) (p13.1; q22); CBFB-MYH11, the pathologist or treating clinician should ensure that appropriate mutational analysis for KIT is performed. In suspected APL patients, the pathologist or treating clinician should also ensure that rapid detection of PML-RARA is performed in addition to tests for DIC. For patients other than those with confirmed core binding factor AML, APL, or AML with myelodysplasia-related cytogenetic abnormalities, the pathologist or treating clinician should also ensure that mutational analysis for NPM1, CEBPA, and RUNX1 is also performed. In patients with confirmed mixed phenotype acute leukemia, the pathologist or treating clinician should ensure that testing for t (9; 22) (q34; q11.2); BCR-ABL1, and KMT2A (MLL) translocations is performed [24].

Both the cytogenetic and molecular tests have limited use in low resource settings because of the costs of setting up the platform, lack of trained personnel to running the tests interpret the tests and utility of the results in terms of choice of treatment like bone marrow transplant which is still out of reach for many. Some strides are being made towards reducing these limitations but it will take some time. These tests are accessible in private laboratories in Kenya in collaboration with labs in South Africa or India. At AMPATH Reference Laboratories, a FISH platform was set up in 2018 in collaboration with Pediatric Oncology program and Indiana University. It has been validated for use for T (8:14) for Burkitt's lymphoma diagnosis and BCR ABL test for CML diagnosis. There are also plans to establish a Multiple Myeloma FISH panel starting with t (11; 14) to begin with.

Over the past 20 years, advances in microarray and high-throughput sequencing (HTS) (next generation sequencing) "-omics" technologies have dramatically increased our knowledge of the molecular pathogenesis of hematological malignancies. The future is now in NGS and personalized or precision medicine [25].

3. Conclusion

A lot of milestones have been achieved as far as diagnosis of acute leukemia is concerned. More effort is required in the areas of continuous training of all lab staff cadres on processing of PBF and BMA for morphological diagnosis. There is also need for advocacy to policy makers on resources allocation for consistent supply of reagents, accessibility and affordability of tests like Flow cytometry

immunophenotyping, cytogenetics/FISH and Molecular tests to achieve the required WHO criteria for diagnosis of acute leukemia.

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Conflicts of Interest

None declared.

References

[1] Sung, H., *et al.* (2021) Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA*: *A Cancer Journal for Clinicians*, **71**, 209-249. https://doi.org/10.3322/caac.21660

[2] Leak, S.A., Mmbaga, L.G., Mkwizu, E.W., Mapendo, P.J. and Henke, O. (2020) Hematological Malignancies in East Africa—Which Cancers to Expect and How to Provide Services. *PLOS ONE*, **15**, e0232848. https://doi.org/10.1371/journal.pone.0232848

[3] Lotodo, T., *et al.* (2022) Geographical Distribution of Hematological Malignancies Managed at Moi Teaching and Referral Hospital. *East African Medical Journal*, **99**, Article No. 3. https://www.ajol.info/index.php/eamj/article/view/226457

[4] Jaffe, E., *et al.* (2008) WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues: An Overview. *Critical Values*, **2**, 30-32. https://doi.org/10.1093/criticalvalues/2.2.30

[5] Arber, D.A., *et al.* (2016) The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. *Blood*, **127**, 2391-2405. https://doi.org/10.1182/blood-2016-03-643544

[6] Sojitra, P., Arber, D.A. and George, T.I. (2017) ASH-CAP Guidelines for the Diagnosis of Acute Leukemia. *The Hematologist*, **14**. https://doi.org/10.1182/hem.V14.2.7120

[7] Dean, L. (2005) Blood Groups and Red Cell Antigens. National Center for Biotechnology Information (US), Bethesda. https://www.ncbi.nlm.nih.gov/books/NBK2263/

[8] Riley, L.K. and Rupert, J. (2015) Evaluation of Patients with Leukocytosis. *American Family Physician*, **92**, 1004-1011. https://www.aafp.org/pubs/afp/issues/2015/1201/p1004.html https://doi.org/10.4236/ojn.2015.511107

[9] Bewersdorf, J.P. and Zeidan, A.M. (2020) Hyperleukocytosis and Leukostasis in Acute Myeloid Leukemia: Can a Better Understanding of the Underlying Molecular Pathophysiology Lead to Novel Treatments? *Cells*, **9**, Article No. 2310. https://doi.org/10.3390/cells9102310

[10] Molyneux, E., Scanlan, T., Chagaluka, G. and Renner, L. (2017) Haematological Cancers in African Children: Progress and Challenges. *British Journal of Haematology*, **177**, 971-978. https://doi.org/10.1111/bjh.14617

[11] Gulati, G., Uppal, G. and Gong, J. (2022) Unreliable Automated Complete Blood Count Results: Causes, Recognition, and Resolution. *Annals of Laboratory Medicine*, **42**, 515-530. https://doi.org/10.3343/alm.2022.42.5.515

[12] Adewoyin, A.S. and Nwogoh, B. (2014) Peripheral Blood Film—A Review. *Annals*

of Ibadan Postgraduate Medicine, **12**, 71-79.

[13] Rindy, L.J. and Chambers, A.R. (2020) Bone Marrow Aspiration and Biopsy. StatPearls Publishing, Treasure Island. http://www.ncbi.nlm.nih.gov/books/NBK559232/

[14] Kaur, M., Singh Rana, A.P., Kapoor, S. and Puri, A. (2014) Diagnostic Value of Bone Marrow Aspiration and Biopsy in Routine Hematology Practice. *Journal of Clinical and Diagnostic Research*, **8**, FC13-FC16.

[15] Sayed, S., *et al.* (2018) Task Sharing and Shifting to Provide Pathology Diagnostic Services: The Kenya Fine-Needle Aspiration Biopsy Cytology and Bone Marrow Aspiration and Trephine Biopsy Training Program. *Journal of Global Oncology*, **4**, 1-11. https://doi.org/10.1200/JGO.18.00094

[16] Montante, S. and Brinkman, R.R. (2019) Flow Cytometry Data Analysis: Recent Tools and Algorithms. *International Journal of Laboratory Hematology*, **41**, 56-62. https://doi.org/10.1111/ijlh.13016

[17] Cossarizza, A., *et al.* (2017) Guidelines for the Use of Flow Cytometry and Cell Sorting in Immunological Studies. *European Journal of Immunology*, **47**, 1584-1797.

[18] Lawson, J., Fischer, K. and Tung, J. (2015) A 13-Color Parametric Analysis for TBNK Subset for the CytoFLEX Instrument (TECH3P.935). *The Journal of Immunology*, **194**, 207.5. https://doi.org/10.4049/jimmunol.194.Supp.207.5

[19] Patel, K., *et al.* (2015) Use of Flow Cytometry Immunophenotyping for Diagnosis of Acute Leukemia at Moi Teaching and Referral Hospital, Eldoret, Kenya. *American Scientific Research Journal for Engineering, Technology and Sciences*, **13**, 72-80.

[20] Van Dongen, J.J.M., van der Velden, V.H.J., Brüggemann, M. and Orfao, A. (2015) Minimal Residual Disease Diagnostics in Acute Lymphoblastic Leukemia: Need for Sensitive, Fast, and Standardized Technologies. *Blood*, **125**, 3996-4009. https://doi.org/10.1182/blood-2015-03-580027

[21] Jentzsch, M., Schwind, S., Bach, E., Stasik, S., Thiede, C. and Platzbecker, U. (2019) Clinical Challenges and Consequences of Measurable Residual Disease in Non-APL Acute Myeloid Leukemia. *Cancers*, **11**, Article No. 1625. https://doi.org/10.3390/cancers11111625

[22] Kruse, A., *et al.* (2020) Minimal Residual Disease Detection in Acute Lymphoblastic Leukemia. *International Journal of Molecular Sciences*, **21**, Article No. 1054. https://doi.org/10.3390/ijms21031054

[23] Qin, D. (2021) Molecular Testing for Acute Myeloid Leukemia. *Cancer Biology & Medicine*, **19**, 4-13.

[24] Shimony, S., Stahl, M. and Stone, R.M. (2023) Acute Myeloid Leukemia: 2023 Update on Diagnosis, Risk-Stratification, and Management. *American Journal of Hematology*, **98**, 502-526. https://doi.org/10.1002/ajh.26822

[25] T.R.C. of Pathologists (2021) Genomic Testing for Haematological Malignancies: The Next Generation. https://www.rcpath.org/profession/publications/college-bulletin/april-2021/genomic-testing-for-haematological-malignancies-the-next-generation.html