PERFORMANCE EVALUATION OF GLASS SLIDE MORPHOLOGY, WHOLE SLIDE IMAGE AND FLOW CYTOMETRY IN DIAGNOSIS OF ACUTE LEUKEMIA BY TYPES

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By

Hamisa Iddy

Dissertation Submitted in (Partial) fulfillment of the requirements for the Degree of Master of Medicine (Haematology and Blood Transfusion) of

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October, 2019

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation titled: **‘‘Performance evaluation of glass slide morphology, whole slide image and flow cytometry in diagnosis of acute leukemia by types’’,** in (partial) fulfilment for the degree of Master of Medicine (Haematology and Blood Transfusion) of Muhimbili University of Health and Allied Sciences.

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DECLARATION AND COPYRIGHT

I, **Dr. Hamisa Iddy**, declare that this **dissertation** is my original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature……..... **Date**…….

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DEDICATION

I would like to dedicate this work to my lovely family; my parents, my beloved husband Dr. Juma Magogo, my beautiful children (Anisa, Najma and Nabil) and my sister Amina Iddy for a supporting me throughout.

ABSTRACT

Background

Acute leukemia (AL) is a life-threatening disease which require early diagnosis and treatment. In Tanzania, it is a challenge to make early and accurate diagnosis, in part due to shortage of haematologists and limited resources (i.e. availability of immunophenotyping). Diagnosis of AL in adults is made through glass slide morphology (GSM) interpretation only. Whole slide image (WSI) can be used at health facilities where there are no hematologists for early diagnosis. However, it's efficacy in diagnosis of Acute Leukemia is not well established.

Aim

To determine the clinical and laboratory characteristics of confirmed cases of AL and to determine performance evaluation of glass slide morphology, whole slide imaging and flow cytometry in diagnosis of acute leukemia by types.

Methods

This was a descriptive, hospital based, cross-sectional study involving 115 patients with clinical and laboratory suspicion of acute leukemia from January 2019 to May 2019. Structured questionnaires were used to obtain socio-demographic and clinical data. Blood and bone marrow aspirates were collected from all participants for investigations. Romanowsky staining technique was used for peripheral smear and staining bone marrow slides. Flow cytometry (from peripheral blood or bone marrow aspirate) was performed as per European Group for the Immunological Characterization of Leukemia (EGIL) criteria. SPSS v.20.00 statistical software was used for data analysis. Chi square test was employed for categorical data while Mood's median test was used to compare numerical data. Performance characteristics of the diagnostic tests were calculated by cross tabulation and compared in proportions.

Results

A total of 115 individuals with features highly suggestive of acute leukemia were enrolled. Fifty one were diagnosed to have AL by GSM of the bone marrow aspirate, and out of these, 47/51 (92%) were confirmed to have acute leukemia by flow cytometry. Symptoms of anaemia were found in 41/47(87.2%) of patients that had been confirmed to have acute leukemia by flow cytometry, followed by fever 36/47 (76.6%) and bleeding 18/47 (38.3%). Organomegaly was a common sign in ALL; 35.7% and 21.4% of participants with ALL had splenomegaly and hepatomegaly respectively, in contrast to only 10.5% and 0% in AML. Among these patients with AL, 89% were found to have haemoglobin below 10g/dl and 39/47 (83.0%) had deranged white cell count. Monocytosis was reported in 22/47 (46.8%) by automated blood count machine. These were found to be blasts in peripheral smear. GSM was able to diagnose accurately 47/51(92%) of acute leukemia cases and gave the basis for diagnosis of acute leukemia. Both GSM and WSI showed reduced accuracy in diagnosis of acute leukemia by types. The concordance rate for GSM with flow cytometry in the diagnosis of AML and ALL was 57.9 and 81.4%, respectively. The concordance rate of WSI with flow cytometry in diagnosis of AML and ALL was 56.3% and 68.4%, respectively.

Conclusion

The clinical presentation of AL is due to bone marrow failure; anaemia, bleeding due to thrombocytopenia, and infections (fever) related to neutropenia. Majority of patients with acute leukemia had low hemoglobin level, leukocytosis, thrombocytopenia and very high LDH. Morphology with good staining quality gives the basis of diagnosis of acute leukemia. GSM and WSI had low concordance when compared to flow cytometry in diagnosis of acute leukemia by types

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LIST OF ABBREVIATIONS

AL	-	Acute Leukemia
ALC	-	Absolute Lymphocyte Counts
ALL	-	Acute Lymphocytic Leukemia
AMC	-	Absolute Monocyte Counts
AML	-	Acute Myeloid Leukemia
ANC	-	Absolute Neutrophil Counts
APML	-	Acute Promyelocytic Leukemia
ASH	-	American Society of Hematology
BMA	-	Bone Marrow Aspiration
CAP	-	College of American Pathologist
CBC	-	Complete Blood Count
CD	-	Cluster Differentiation
CPL	-	Central Pathology Laboratory
DIC	-	Disseminated Intravascular Coagulopathy
DPA	-	Digital Pathology Association
EDTA	-	Ethylenediamine Tetraacetic Acid
FAB	-	French American British classification
GSM	-	Glass Slide Morphology

Hb	-	Haemoglobin
HEFCE	-	Higher Education Council for England
LDH	-	Lactate Dehydrogenase
MNH	-	Muhimbili National Hospital
MUHAS	-	Muhimbili University of Health and Allied Sciences
RBCs	-	Red Blood Cells
SMI	-	Small Molecular Inhibitors
WBCs	-	White Blood Cells
WHO	-	World Health Organization
WSI	-	Whole Slide Image

DEFINITION OF TERMS

Acute Leukemia - blood disorder characterized by proliferation of immature white blood cells (myeloid or lymphoid blasts) accounting for more than 20% of the nucleated cells in the bone marrow. Classified in two broad categories based on the lineage of blasts; Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL). [French American British (FAB) Classification].

Glass Slide Morphology is conventional microscopic examination of bone marrow aspirate stained by Rowanoswsky technique, which gives details of developing cells, proportion of different cell lines and presence of foreign cell if any in the marrow.

Whole Slide Image is defined as digitized glass slide that has been created on a slide scanner. The digitized glass slide represents a high-resolution replica of the original glass that can then be manipulated by software to mimic microscope review and diagnosis. It is also known as a virtual slide. [Digital Pathology Association (DPA)].

CHAPTER ONE

1.0 INTRODUCTION

Acute leukemia (AL) is a clonal disorder of immature haematopoietic stem cells of myeloid and/or lymphoid cells which results from either increased rate of proliferation, reduced apoptosis or a block in cellular differentiation. The disorder leads to accumulation of immature cells (known as blasts) in the bone marrow which may infiltrate other organs (1). Based on clinical, morphological, immune-phenotypical, genetic and molecular characteristics; AL is divided into two(2) major types; acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL)(2).

The incidence of acute leukemia varies with age, subtype and geography. In the USA cancer registry (2011) (3), AML is the most common acute leukemia in adults, with an incidence of 17.5 per 100,000 and median age at diagnosis of 65 years. Whereas, ALL is most common acute leukemia in children, with an incidence of approximately 3.4 cases per 100,000 (4).

In South Africa (5), acute leukemia has been reported to be the second common haematological malignancy, comprising 17% of all haematological malignancies .

In a study done in Tanzania (6), among 112 children diagnosed with acute leukemia in 2013, 72% had ALL and 22% had AML. However, no published data exists for acute leukemia in adults in Tanzania.

The exact causative mechanism of AL is unknown (7). However, chromosomal rearrangements such as; translocations and inversions are postulated to result in cell differentiation/maturation arrest. The maturation arrest leads to accumulation of an immature clonal cell population mediated by chemokines (8–10).

Management of AL constitutes definitive care, supportive care (administration of blood products such as packed red blood cell and platelets concentrates and antibiotics) and in some patients, palliative chemotherapy (e.g. low dose cytarabine for AML). Until recently, the long term survival rates for patients with AL globally was only achieved after the employment of intensive chemotherapy or allogeneic Haematopoietic Stem Cell Transplantation (SCT)(11–14). The outcome after these regimens, in sub-Saharan Africa and Tanzania has been dismal

due to the lack of adequate supportive care. Recently, ground-breaking studies have demonstrated the use of targeted Small Molecule Inhibitors (SMI) such as; venetoclax in combination with low dose cytarabine to have cure in over 50% of patients with AML (15). The use of SMIs in AML therapy opens the prospect significant improvement of cure rates in low and middle income countries.

Early and correct diagnosis is vital in the management of AL. Flow cytometry analysis is an important adjunct to morphology in diagnosis of acute leukemia by types (16,17). In view of the pitfalls in FAB (French American British) classification which was based on morphology, the WHO classification stressed the importance of flow cytometry and defined the myeloid and lymphoid malignancies by the antigenic features of the neoplastic cells (18). Tanzania, has only 16 hematologists, half of them are stationed at MNH. This necessitates majority of patients to be referred to MNH for diagnosis and treatment. In addition, at MNH, the diagnosis of AL in adults is based on GSM alone, which may not be sufficient to classify the type of acute leukemia. Identification of the type of AL is crucial in selection of the correct treatment modality and prognosis of the patient. On the other hand, Whole Slide Imaging (WSI) involves the digitization/ scanning of entire glass slides, cropping images for morphological review. WSI has been reported to be useful in diagnosis, joint academic sessions (telepathology), as well as in research purposes (19). Therefore, the WSI systems can be installed in remote health facilities, where there is no access to haematopathology services, and be used to scan images, that will be sent to MNH for review. Studies demonstrating the efficacy of WSI in histopathology exist, however, more studies need to be conducted to assess its utility in haematology (20).

1.1 Literature Review

Clinical characteristics of acute leukemia

Clinical presentations of acute leukemia may vary according to the type and subtype of acute leukemia. They can be categorized broadly into symptoms/signs suggestive of bone marrow failure, and organ infiltration, such as; splenomegaly and constitutional symptoms such as weight loss, anorexia etc. However, common symptoms are those due to bone marrow failure. They include recurrent infections due to abnormal leukocytes, symptoms of anemia, as well as abnormal bleeding tendencies or massive bleeding post minor trauma due to a deficiency and malfunction of platelets (7,21). In a study done in Pakistan, fever was the most common symptom in AL (77%), followed by pallor (33%) and bleeding disorder (23%) (22). Another study done by Kulsoom et al (23) showed similar findings; where fever was the most common presentation in AML occurring in 71.9% followed by generalized weakness 45% and bleeding tendencies 19%. Features of organ infiltration are more common in childhood leukemia, especially ALL. In a meta-analysis done in University of Oxford, by Clarke et al (21) hepatomegaly and splenomegaly was present in 64% and 61% of all children with AL, respectively. Lymphadenopathy was present in 41%. Central Nervous system involvement is generally less common in acute leukemia, and mostly occurs in ALL, at frequency of about 10% (24). Bone pain has been reported in 25 – 40% of cases with acute leukemia (21,25). The blast infiltration that is commonly observed in AML may present as gum hypertrophy, and rarely as skin infiltration known as leukemia cutis, which in this case reflects a poor prognosis (26).

Patients in African countries including Tanzania, have been observed to present with similar clinical symptoms and signs, though published data is limited. A study done in Uganda (27) , reported that majority of AL patients presented with B-symptoms (96%), followed by bleeding tendencies and bone pains, in 54% and 36%, respectively.

Laboratory features

Deranged laboratory parameters are commonly seen in patients with AL. Complete blood count (CBC) at presentation commonly shows elevated leukocyte count (though in some

cases, it may be normal or reduced). Moderate and severe anemia with hemoglobin level in the range of <7 - 8g/dl is a common finding in patients with AL (28,29). Thrombocytopenia with platelets counts < 100,000/L has been reported in about 75% of all cases of childhood ALL(30) . The study done by Kaketopo et al (31) showed leukocyte range of 0.4 to 256K/uL, low heamoglobin (Hb) with mean value of 8.3g/dl (3.6 to 13.2g/dl) and thrombocytopenia of 65k/ul (1 to 192 K/uL). Another study in Kenya (32) showed similar findings in which mean Hb level is reported to be 7.5g/dl, leukocyte 20 K/uL and platelets of 55 K/uL. These deranged laboratory parameters are a reflection of infiltration of the bone marrow by blast cells (31,33-34). Blasts are commonly seen in the peripheral blood smears (unless there is severe pancytopenia). Astudy done by Kulsoom et al (23) in Pakistan showed 61.7% of the patients with acute leukemia have blasts in there peripheral smear. A derangement of lactate dehydrogenase (LDH) and uric acid is also commonly seen with a mean of 1520 IU/L and 5mg/dl respectively (31).

In Tanzania, there is limited documentation of laboratory parameters during the presentation of acute leukemia. Therefore,this study aims to describe the common laboratory presentation in AL patients.

Diagnosis of Acute Leukemia

Flow cytometry

Flow cytometry provides immune-phenotypical assessment of acute leukemia, and is able to distinguish between myeloid and lymphoid leukemia, and their subtypes more accurately. Advances in flow cytometry technology and availability of new monoclonal antibodies have resulted in improvements in diagnosis and classification of acute leukemia (35). A study done by Manju et al (36) showed, glass slide morphology together with immune-phenotying has higher specificity (92%) in diagnosing AML compared to diagnosis of ALL (56%). When flow cytometry is used in addition to morphology in diagnosis of acute leukemia, it aids in rendering a definitive diagnosis in cases where morphology has been inconclusive in classifying subtype of acute leukemia. In a study by Belurkar et al (37) , immune-phenotying of leukemia blasts assisted in reaching definitive diagnosis in 16% of cases whom couldn’t be

confirmed by morphology. It was also able to identify correct lineage of blasts in 22% of partially concordant cases, and 4% of non-concordant cases, preventing misdiagnosis. Flow cytometry is highly recommended as an adjunct investigation by American Society of Hematology (ASH) and College of American Pathologists (CAP) for differentiation of myeloid and lymphoid leukemia (16).

In Africa, several studies have described (32,38–40) the diagnostic and prognostic utility of flow cytometry in patients with acute leukemia. A study done in Uganda (41) showed the use of flow cytometry allowed for better classification of acute leukemia by types. It also enabled the implementation of risk-adapted therapy to children with ALL. This subsequently improved 1-year overall survival from 30% to 70%. Another study done in India, showed that immune-phenotying was of greater help in providing diagnosis in ALL than it was in AML, where it was only useful in confirmation of the morphological diagnosis (36).

Limitations of flow cytometry

Flow cytometers are complex instruments. Clearly, adequate operator training is essential in order to acquire reliable results. (42).

Interpretatively, the combined report is based on the principle whereby a final diagnosis can only be issued when the morphological, flow cytometry, immune-histochemical, molecular and cytogenetic data are integrated into a comprehensive final report. If there are discrepancies then the whole case has to be reviewed and discussed in a wider forum (42).

In Tanzania, flow cytometry is not routinely used in diagnosis of acute leukemia in adults. It is vertical program for children and does not cater for adults. Therefore, diagnosis largely relies on GSM. The full accuracy of GSM compared to flow cytometry in diagnosis of acute leukemia by types is yet to be determined.

Glass slide morphology (GSM)

The role of Glass Slide Morphology (GSM) is central in diagnosis of acute leukemia. Previously, the French America British (FAB) classification was based on cytological and cytochemical characteristics only (17). The new WHO guideline has revised the diagnostic

criteria for diagnosis of AL with GSM to improve diagnosis of AL by types (18). To make a diagnosis of acute leukemia by GSM, a good bone marrow aspirate and a good quality smear for accurate results are needed (43). ASH together with CAP also highly recommend that GSM be used in the diagnosis of acute leukemia (16). A survey conducted by CAP on investigations that were used to diagnose acute leukemia showed that 100% of respondents use GSM in the diagnosis of acute leukemia (44).

Whole slide image (WSI)

Whole slide imaging (WSI) also referred to as virtual microscopy, encompasses the digitization or scanning of entire glass slides without cropping of the digital images for observation by humans or automated image analysis. It is useful for diagnosis, and telepathology sessions and for research purposes(19). Mobile Whole Slide image is an affordable system consisting of a standard light microscope, a connected computer for capturing the slide through the microscope, and a printed adaptor, configured to receive and position the camera on the eyepiece of the microscope. Study done by Mulay (45) in India, showed that mWSI offers excellent diagnostic accuracy with the concordance of 95% in the diagnosis when compared to glass slide morphology.

Comparison of GSM, WSI and flow cytometry

Using WSI scanning devices, a number of studies have shown a perfect inter-observer, as well as intra-observer agreement between the diagnosis obtained with WSI and GSM. Thus, WSI can also be used to make a diagnosis based on morphology(20). Studies (46–49) have also showed good diagnostic concordance between WSI and GSM with error rate approximately 1% to 5%. CAP recommends at least 60 routine cases per application to assess intra-observer diagnosis concordance between WSI and GSM viewed at least two weeks apart (50).One study (51), showed concordance rate of 97.1% between WSI and GSM on breast morphology. The discrepancies were mostly due to inferior image quality and lack of user experience with WSI (50). A study done in Japan (52) showed sensitivity and specificity of GSM in diagnosis of acute leukemia using flow cytometry as gold standard is 72% and 94% respectively.

Another study done by Al-Mawali et al (53) showed the sensitivity of GSM to be 95% in diagnosis of acute leukemia.

The performance characteristics of WSI and GSM in diagnosis of acute leukemia is yet to be established in Tanzania. This study intends to evaluate the performance of WSI and GSM in diagnosing acute leukemia by types, using flow cytometry analysis as an adjunct investigation.

1.2 Problem statement

The clinical presentation of acute leukemia mimics that of many infectious diseases. This poses a challenge in early diagnosis (54). Flow cytometry is an adjunct investigation for differentiating between myeloid and lymphoid acute leukemia(16)**.** At MNH, diagnosis of acute leukemia in adult patients is made solely by assessment of bone marrow aspirate via glass slide morphology. This limits the possibility of accurately diagnosing the different types of leukemia. Furthermore, even with glass slide morphology, due to the limited number of hematologists in the country, it is difficult to make a prompt diagnosis of acute leukemia, as the patient has to be referred to MNH resulting in delays. The use of WSI in diagnosis of acute leukemia may help in making early diagnosis, early referral and early intervention in patients from regional health facilities where there are no hematologists(50). These are all problems that the finding of this study will address.

1.3 Rationale

There is limited literature on acute leukemia in Africa. Also, there are no studies done in Tanzania to determine how best to diagnose acute leukemia with limited resources. . In this study, three different diagnostic tools were used. The use of flow cytometry as adjunct tool for diagnosis of acute leukemia by type will aid to assess the accuracy of GSM in diagnosis. Knowing the accuracy of these tests will help to determine if they are reliable and can be used to diagnose the different types of acute leukemia. WSI has been extensively studied in histopathology but not in hematology where smears rather than histological tissue are used. WSI has not been studied at all in this setting.

This is one of the first studied to be done on acute leukemia that includes adults in Tanzania. This study will form a basis for further extensive studies to be done on acute leukemia in Tanzania.

1.4 Research Questions

This study aimed to answer the following questions;

1. What are the clinical and laboratory characteristics of patients diagnosed with acute leukemia?
2. What is the concordance between GSM, WSI and Flow cytometry in diagnosis of acute leukemia into ALL and AML?

1.5 Objectives

1.5.1 Broad objective

To determine concordance between glass slide morphology (GSM), whole slide image (WSI) and flow cytometry in diagnosis of acute leukemia by types among patients with features highly suggestive of acute leukemia undergoing bone marrow aspiration.

1.5.2 Specific objectives

1. To describe clinical characteristics of patients diagnosed with acute leukemia by flow cytometry.
2. To describe the laboratory characteristics of patients diagnosed with acute leukemia by flow cytometry.
3. To determine concordance between GSM, WSI and flow cytometry in the diagnosis of acute lymphoblastic leukemia and acute myeloid leukemia.

CHAPTER TWO

2.0 METHODOLOGY

2.1 Study design

This was cross sectional, hospital-based study using quantitative approach.

2.2 Study duration

The study was conducted for a period of five months, from January 2019 to May 2019.

2.3 Study area

The study was conducted at Muhimbili National Hospital (MNH). This is a tertiary referral hospital that offers super specialized medical care and interventions to patients. It is located in Dar es salaam, which is the largest commercial city in Tanzania with a population of 4,364,541 and annual growth rate of 5.6% according to the year 2012 population and housing census report by Tanzania National Bureau of Statistics. The hospital receives patients from other health facilities across the country, including those with hematological disorders. There is limited information on acute leukemia in the general population but hospital statistics show that up to 250 patients with clinical features highly suggestive of AL underwent BMA in 2017, in the department of hematology at MNH. Out of these, 72 were diagnosed to have AL by GSM. A pilot study done from September 2017 to November 2017 (period of 3 months) showed that a total of 81 patients with clinical features highly suggestive of acute leukemia underwent BMA of whom 31 were diagnosed with AL by GSM. At the MNH pediatric unit in 2017, samples from a total of 95 children (referral from regional hospitals, Bugando Medical center, Kilimanjaro Christian Medical Center, Mbeya zonal hospital and Mnazi Mmoja) with features highly suggestive of acute leukemia underwent flow cytometry of which 64 were diagnosed with acute leukemia. The hematology laboratory is equipped with two flow cytometry machines for the diagnosis of acute leukemia by type and a static whole slide scanner. These machines are used for paediatric patients as part of a vertical program which

does not cover the adult patients. They are therefore not routinely used for diagnosis of AL in adults.

2.4 Study population

All patients with clinical and laboratory features of acute leukemia, who had Bone Marrow Aspiration (BMA) done during the study period. Both in and out-patients, from all departments were enrolled.

2.4.1 Inclusion criteria

1. All patients with clinical and or/ laboratory features, highly suggestive of AL, who had BMA taken.

Clinical features of AL include; symptoms of recurrent fever, bleeding tendencies and symptoms of anemia and signs of fever, petechial or bruises, pallor and lymphadenopathy

Laboratory features of AL include; complete blood count (CBC) with any cytopenia(s) with/without leukocytosis or normal leukocyte counts.

2. Patients who consented/assented to participate in the study.

2.4.2 Exclusion criteria

1. Patients with cytopenia and no blast seen at peripheral smear and dry tap from BMA.
2. Patients who declined consent.

2.5 Sample size

According to Israel (1992), the sample size is calculated using the formula:

$$n = \frac{Z^2 * P (1 - P)}{E^2}$$

And $n = n_1 + n_2$

Where;

P_1 = 0.92 (the specificity of morphology in diagnosis of AML using flow cytometry as adjunct investigation by Manju et al, 2009 (36).

P_2 = 0.56 (Specificity of morphology in diagnosis of ALL using flow cytometry as adjunct investigation by Manju et al (36).

Z = Confidence level (Z = 1.96 for 95%)

E = Margin of error 2% (0.02)

$$n_1 = \frac{1.96^2 * 0.92(1 - 0.92)}{0.02^2}$$

$$\therefore n_1 = 707$$

$$n_2 = \frac{1.96^2 * 0.56(1 - 0.56)}{0.02^2}$$

$$n_2 = 2366$$

$$n = 707 + 2366$$

$$n = 3073$$

Since the study was conducted on a finite population, the population (N) of patients with clinical and laboratory features highly suggestive of acute leukemia is therefore, 120 (Total number of patients with features highly suggestive of acute leukemia from MNH data in past five months). Also, since the study took five months, a final sample estimate was adjusted for a finite correlation factor using the formula:

$n_c = n/(1+n/N)$

Where;

n_c = Adjusted sample size

n = Unadjusted sample size= 3073 patients

N = Population=135 patients

$n_c = 3073/(1+3073/120)$

∴ n = 116 patients with features highly suggestive of acute leukemia.

Therefore, the sample size was 116 patients with clinical and laboratory features highly suggestive of acute leukemia.

2.6 Study variables

For the purpose of this study the following variables were used:

1. Dependent variables

- AML
- ALL.

2. Independent variables

- **Social demographic characteristics:**
  - Age
  - Gender
  - Residence
  - Occupation
- **Clinical parameters**

  Duration of symptoms

  Recurrent fever

  Symptoms of anaemia

  Bleeding tendencies

  Lymphadenopathy

  Splenomegaly

  Hepatomegaly

- **Laboratory parameters**

  CBC and peripheral smear,

  Serum uric acid,

  LDH

  GSM

  WSI

  Flow cytometry

2.7 Enrolment of study participants

Enrolment was done at the Central Pathology Laboratory (CPL) and the Paediatrics complex for all patients with clinical and laboratory features suggestive of acute leukemia who underwent BMA and fitted the inclusion criteria. Bone marrow aspirations were done on Mondays, Wednesdays (paediatrics) and Thursdays.

2.8 Collection of social demographic and Clinical Information

Recruitment and data collection was initiated after receiving the ethical clearance from MUHAS IRB and permission to conduct the study was granted by MNH. . Socio-demographic and clinical data were collected by using a questionnaire. All patients were examined for relevant physical signs and vital signs were recorded. Data collection was done on the day of BMA collection.

2.9 Sample collection

Peripheral blood

For each study participant, 5mls of venous blood was drawn for laboratory test. Blood was collected into sterile vacutainers containing EDTA anticoagulant for complete blood count, peripheral smear and flow cytometry for patients with blasts in the peripheral circulation, Blood collection for serum uric acid, LDH the blood was collected in a vacutainer with no anticoagulant (red top). CBC was run within two hours of collection on 3700 Celldyn machine. A peripheral smear was prepared and fixed within two hours of blood collection. Peripheral blood film was stained using leishman stain.

Bone Marrow Aspiration

BMA was done on all study participants and samples were processed for GSM, WSI and flow cytometry (for those with no blast in peripheral smear). The BMAs were done under aseptic technique and local anesthesia. Collections were done from the posterior superior iliac spine. Bone marrow aspiration slide for GSM was prepared within 24 hours of collection. . Flow

cytometry was done by an experienced senior laboratory scientist together with principle investigator within 24 hours of blood collection

2.10 Laboratory procedures

Glass Slide Morphology (GSM)

Staining

Using the Rowanoswsky technique (55)**:** a thin film was smeared on clean glass slide, and left to air dry. The film was then fixed in alcohol for 20 minutes. It then was stained with Leishman stain for 3 minutes, followed by addition of buffered water and left to stain for 20 minutes. The film was then washed with running water and left to air dry, ready for microscopic examination for GSM and scanning for WSI.

Interpretation of Morphology

The smeared slides were placed under a light microscope and blasts were characterized and counted. Blasts with less distinct nucleoli, more condensed chromatin and absence of cytoplasmic granules were counted as lymphoblasts. The blasts with more prominent nucleoli, less condensed nuclear chromatin, and presence of cytoplasmic granules with or without aura rods were counted as myeloblasts. Acute leukemia was diagnosed if blast count was equal or above 20% of all nucleated cells in the myelogram.

Whole Slide Imaging (WSI)

Staining

The same glass slide used for GSM was used for WSI.

Reading

The stained glass slide was put on a slide holder and placed on robotic scanning table for viewing on a screen. Images were captured and saved on Alexapath mobile scanner (ADA1) server software in the computer attached to the scanner. A total of 20 images were acquired per patient and subjected to review.

Quality assessment of morphology

Both GSM and WSI were rated subjectively according to reviewer experience using a scale of 1–5, reporting general satisfaction with the practical aspects of image assessment. 1 was least favorable and 5 was most favorable. Features observed include field size, pattern of cell, important morphological features (hematopoietic cells maturation lines and infiltration of non-hematopoietic cells in the marrow). The score considered to be poor if it was less than 3 and good when it was 3 and above.

Interpretation of GSM and WSI was done by the same haematologist. To minimize bias, the patient's identity was concealed and following GSM interpretation, an interval of two weeks was allowed to elapse before interpretation of WSI.

Flow Cytometry

Flow cytometry was done either on peripheral blood (for those patients with blasts seen in the peripheral smear) or on the bone marrow aspirate (for those patients who had no blasts seen on peripheral smear. The sample was immunophenoted using Multi parametric, eight-colour flow cytometer (Beckman Coulter, FACS Canto). Panels of monoclonal antibodies from the European Group for the Immunological Characterization of Leukemia (EGIL) criteria were used (56). Cell suspensions were stained with multiple panels of three monoclonal antibodies and a two-step strategy was used. The samples were then labeled with fluorescein isithiocyanate (FITC), Phycoerythrin (PE) and Peridin Chlorophyll Protein Complex (PerCP). Cell suspensions was also stain with panels of identically conjugated isotype controls for the antibodies of each panel. Leukemic samples were considered positive for a particular antigen if 20% or more of leukemic cells reacted with a particular monoclonal antibody. The panels of antibodies included the following: CD34, HLA-DR, CD117, CD13, CD14, CD33, CD19, CD10, CD20, CD2, CD3, CD5, CD7, CD45 and CD64.

A case was considered B ALL if it expressed (CD19+, CD20+, CD34+ and HLADR+). The T-cell ALL were considered when the markers for T-cell were positive (CD2+, CD3+, CD5+ CD7+, CD34+). Myeloid cases were considered AML if expressed progenitor antigens (HLA-DR, CD34, CD117), as well as myeloid antigens (CD13, CD33, CD14, CD15).

2.11 Validity of the study

The structured questionnaire used was pre-tested for its reliability and validity by pilot testing prior to commencement of the study. The GSM and WSI were reported the by principle investigator and counter checked by an experienced hematologist. Daily calibration of flow cytometry machine was done before the samples were run. Positive and negative controls were done parallel to the patients' samples.

2.12 Data management and statistical analysis

Data from the questionnaire was entered on a Microsoft Excel spread sheet with appropriate skips and checks. Once data entry was completed, the database were exported into SPSS v.20.0 statistical software for further data cleaning and analysis.

To analyse objective 1; clinical characteristics of ALL and AML patients were summarized by frequencies and proportions, and chi square test was used for comparison. P value of less than 0.05 was considered to be statistically significant.

For objective 2; laboratory characteristics of ALL and AML patients were summarized by median and mood median. The T- test was also used for comparison.

For objective 3; comparison of GSM, WSI and flow cytometry were calculated by crosstabs and compared as proportions.

2.13 Ethical clearance

Ethical clearance was sought from the Research and Publications Committee of Muhimbili University of Health and Allied Sciences (MUHAS). The permission to conduct this study was sought from authority of MNH. A formal written informed consent, in Swahili, was sought from the participants before recruitment. Patients were informed about the purpose of the study`

Non-consenting patients and those not eligible for the study were attended by the investigator as part of the haematology team. As the study entails collecting venous blood and bone marrow aspiration aseptic technique were employed to avoid contaminations. Patients names were not used, information collected on questionnaires were entered into computer using identification numbers to maintain confidentiality.

2.14 Dissemination Plan

Findings from this study will be disseminated to the MNH, MUHAS library, Ministry of Health and social welfare, University Research Seminars, Local and International Conferences, and as publication, to scientific journals.

CHAPTER THREE

3.0 RESULTS

A total of 261 patients had BMA done at MNH during the study period. Of these, 115 patients with clinical and laboratory features suggestive of acute leukemia were enrolled into the study after obtaining their consent or assent. Among these (115), 51 were found to have AL by GSM. Thirty four (29.6%) of all slides were scored as of poor quality. WSI and Flow cytometry was done on these 51 patients and these three investigations were compared for their concordance. The clinical and laboratory features of patient's with a final diagnosis of acute leukemia (as confirmed by flow cytometry) were described.

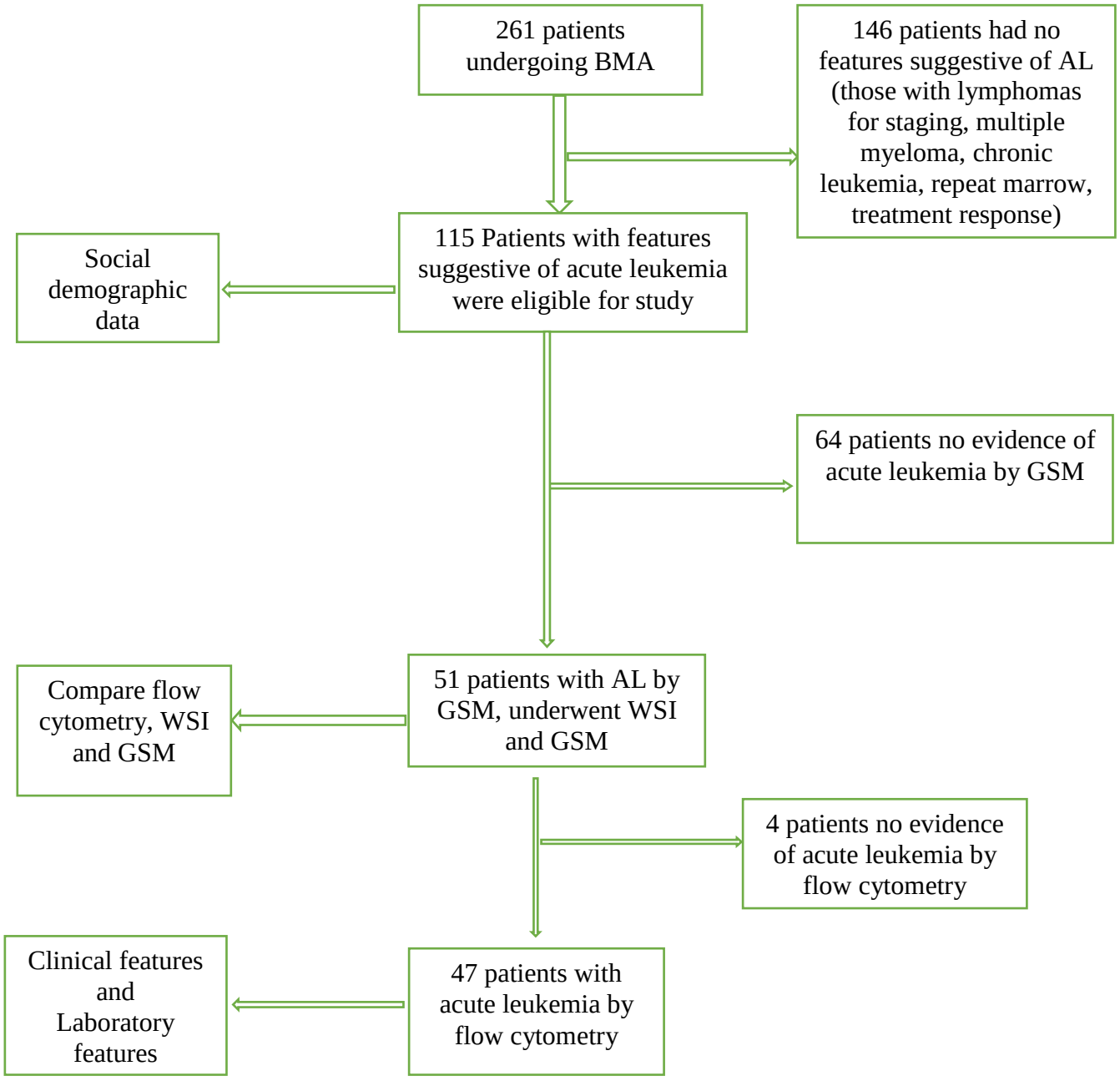


Figure 1: Flow chart

Table 1 below show that the majority of patients (74 (64.1%)) were adults, and only 9 (7.9%) patients were above 65 years. Fifty eight out of 115 patients were male. The male to female ratio was 1:1. Fifty-three (46.5%) patients were from the coastal zone. By comparison, the lake zone was comprised of only 4 (2.6%) patients. The majority of patients (58 (50.9%)) had primary education and only 7 (6.1%) had no formal education. Forty (35.1%) were self-employed and 25 (21.9%) were students. Twelve children were below the age of going to school.

Table 1: Social demographic characteristics of patients with features highly suggestive of acute leukemia (n=115)

Variable	Acute Leukemia (n=47) Frequency (%)	No Acute Leukemia (n=68) Frequency (%)	P value
Age Groups			
< 18	16 (34.0)	16 (23.9)	0.29
18-65	29 (61.7)	45 (65.7)	
>65	2 (4.3)	7 (10.4)	
Sex			
Male	19 (40.4)	39 (56.7)	0.09
Female	28 (59.6)	29 (43.3)	
Residence (Zone)			
Central	7 (14.9)	12 (16.4)	0.28
Coastal	23 (48.8)	30 (44.8)	
Lake	2 (4.3)	2 (1.5)	
Northern	9 (19.2)	6 (8.9)	
South Highland	2 (4.3)	8 (11.9)	
Zanzibar	4 (8.5)	11 (16.5)	
Level of Education			
No formal education	2 (4.3)	5 (7.5)	0.41
Primary education	22 (46.8)	36 (53.7)	
Higher education	14 (29.8)	20 (29.8)	
Others	9 (19.1)	7 (8.9)	
Employment status			
Employed	6 (12.8)	13 (17.9)	0.59
Unemployed	6 (12.8)	13 (19.4)	
Self-employed	16 (34.0)	24 (35.8)	
Student	13 (27.6)	12 (17.9)	
Others	6 (12.8)	6 (8.9)	

Fifty one (44.3%) patients were diagnosed with acute leukemia by GSM. Among them 47 (92.1%) patients had AL by flow cytometry.

Table 2 below show that, for AML, the majority (16 (84.2%)) were adults, while for ALL, the majority were children (15 (53.6%)) statistically significant (p=0.001). Forty-three patients (91.5%) presented with a duration of symptoms of less than 6 months. The majority of patients presented with anaemia and fever (87.2% and 76.6%, respectively). Jaundice, CNS manifestation, lymphadenopathy, splenomegaly and hepatomegaly were uncommon in patients with AML.

Table 2: Clinical characteristics of patients with acute leukemia by types (n=47)

Variable	AML (n = 19) Frequency (%)	ALL (n=28) Frequency (%)	Total (n=47) Frequency (%)	P value
Age				
<18	1(5.3)	15(53.6)	16(34.0)	
18-65	16(84.2)	13(46.4)	29(61.7)	0.001
>65	2(10.5)	0(0)	2(4.3)	
Duration of Symptoms				
< 6months	17 (89.5)	26 (92.9)	43 (91.5)	0.68
> 6 months	2 (10.5)	2 (7.1)	4 (8.5)	
Symptoms				
Anaemia	16 (84.2)	25 (89.3)	41 (87.2)	0.9
Fever	15 (79.0)	21 (75.0)	36 (76.6)	0.75
Bleeding	9 (47.4)	9 (32.1)	18 (38.3)	0.29
B symptoms	3 (15.8)	9 (32.1)	12 (25.5)	0.35
Signs				
Pallor	17 (89.5)	23 (82.1)	40 (85.1)	0.78
Jaundice	0 (0)	4 (14.3)	4 (8.5)	0.23
Petechia	9 (47.4)	5 (17.9)	14 (29.8)	0.03
Gingival hypertrophy	2 (10.5)	0 (0)	2 (4.3)	0.08
CNS manifestations	0 (0)	3 (10.7)	3 (6.4)	0.39
Lymphadenopathy	4 (21.0)	12 (42.9)	16 (34.0)	0.12
Splenomegaly	2 (10.5)	10 (35.7)	12 (25.5)	0.05
Hepatomegaly	0 (0)	6 (21.4)	6 (12.8)	0.03

FBC and peripheral smear were performed on all patients together with LDH and uric acid as shown in table 3 below. The median Hb level was 7.5g/dl (3.5-13.3g/dl) and 5.9g/dl (2.9-13.1) for AML and ALL, respectively. Approximately two thirds (27) of patients with AL had severe anaemia (Table 3). Only five (10.6%) patients with acute leukemia had hemoglobin above 10g/dl (figure 2a). An abnormal leukocyte count was found in 83% (39) of participants, of which 46.8% had leukocytosis and 36.2% had leukopenia. Monocytosis was present in 42.6% (20) of participants reflecting the peripheral blasts which were found in 63.8% of patients. For 5 (10.6%) patients with AL no WBC differential was generated by the automated cell counter. More than 80% of participants (41) presented with thrombocytopenia (figure 2b).

Table 3: Laboratory features of patients with acute leukemia by types diagnosed by flow cytometry (n=47)

Variable	AML (n = 19) Median (Range) n(%)	ALL (n=28) Median (Range) n(%)	Total (n=47) Median (Range)	P value
Hemoglobin (g/dl)	7.5 (3.5 – 13.3)	5.9 (2.9 -13.1)	6.43 (2.9- 13.3)	0.28
Non anaemic	1 (5.3%)	1 (3.6%)	2 (4.3%)	
Mild anaemia	1 (5.3%)	2 (7.1%)	3 (6.4%)	
Mod anaemia	9 (47.4)	6 (21.4%)	15 (31.9%)	
Severe anaemia	8 (42.0)	19 (67.9%)	27 (57.4%)	
WBC x 10^9/L	12.9 (0.8 – 318.0)	4.8 (0.5 – 240.0)	11.6 (0.5- 318.0)	0.81
AMC x 10^9/L	3.3 (0.0 – 298.0)	0.5 (0.0 – 30.0)	0.9 (0.0- 298.0)	0.18
Platelets x 10^9/L	31 (12.0 – 192.0)	35 (5.0 – 178.0)	33.0 (5.0- 192.0)	0.93
Lactate dehydrogenase	720.0 (126.0 – 1646.0)	567.0 (130.0 - 3374.0)	586.0 (126.2 – 3374.0)	0.20
Uric Acid	0.25 (0.1 – 0.65)	0.39 (0.20 – 0.9)	0.3 (0.1- 0.9)	0.06
Peripheral blast	13 (76.5)	17 (60.7)	30 (63.8)	0.32
Bone marrow blast	90.0 (5.0-90.0)	90.0 (0.0-100.0)	90.0 (0.0-100.0)	0.69

Normal ranges: found in appendix V**.**

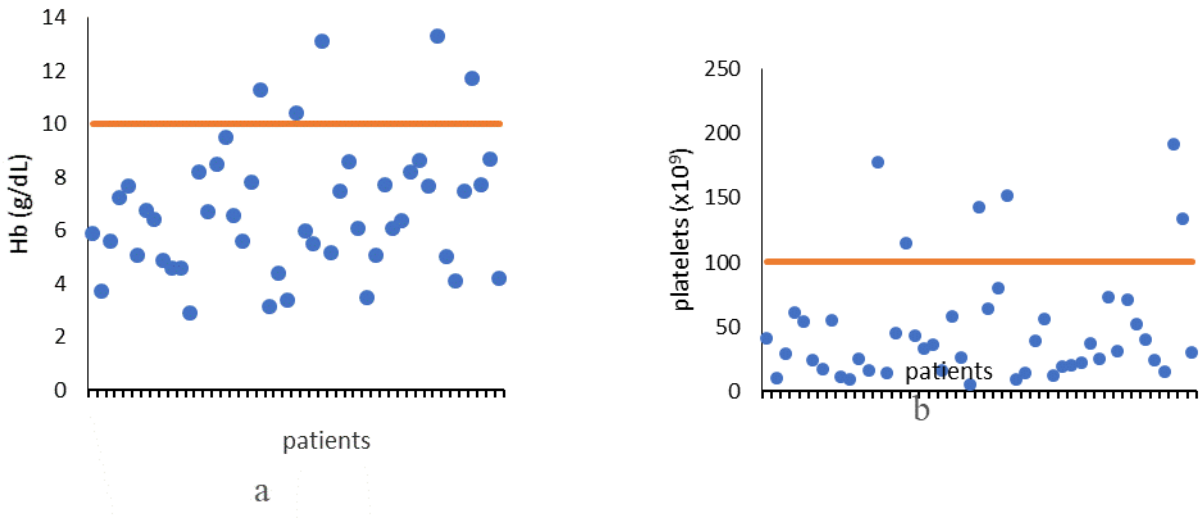


Figure 2: Level of haemoglobin and platelets in patients with acute leukemia (n=47)

Fifty-one patients (44.3%) among all patients with features highly suggestive of acute leukemia were diagnosed with acute leukemia by GSM and 47/51 of them (92.2%) were confirmed to have AL by flow cytometry.

Nineteen cases were classified by GSM as AML. Following confirmation with flow cytometry, it was found that 11 cases were AML, 4 were ALL and there was no evidence of acute leukemia in the remaining 4. This gives GSM a concordance rate of 57.9% with flow cytometry in the diagnosis of AML (Table 4). Of the 27 cases diagnosed as ALL by GSM, flow cytometry confirmed the presence of ALL in 22 and AML in 5, giving GSM a concordance rate of 81.4% with flow cytometry in the diagnosis of ALL GSM was unable to classify 5 cases of AL which were classified as AML (3 cases) and ALL (2cases) by flow cytometry

Table 4: Comparison of GSM and flow cytometry in diagnosis of acute leukemia by types (n-51)

GSM diagnosis	Cases (n=51)	Flow cytometry / final diagnosis	Cases (n=51)	Concordance (%)
AML	19	AML	11	57.9
		ALL	4	
		No AL	4	
ALL	27	AML	5	81.4
		ALL	22	
		No AL	0	
AL unspecified	5	AML	3	
		ALL	2	
		No AL	0	

WSI was able diagnose 42 cases as acute leukemia out of 51 cases diagnosed by GSM giving it a concordance rate of 82.4%.

Diagnosis made by WSI classified 16 cases as AML. Flow cytometry confirmed 9 of these to be AML and 7 to be ALL, giving WSIa concordance rate of 56.3% with flow cytometry in the diagnosis of AML. Nineteen cases were classified by WSI as ALL. Flow cytometry confirmed ALL in 13 of these, AML in 5 and in 1 there was no evidence of acute leukemia. This gives it a concordance rate of 68.4% with flow cytometry in the diagnosis of ALL. In four cases diagnosed with AL (3 unspecified and 1 ALL) by WSI, there was no evidence of AL when flow cytometry was done. WSI was unable to classify 7 cases of AL which were classified as AML (2 cases), ALL (2 cases) and no AL (3 cases) by flow cytometry. Six cases which were classified as no AL by WSI were found to have AL by flow cytometry.

Table 5: Comparison of WSI and flow cytometry in diagnosis of acute leukemia by types (n-51)

WSI diagnosis	Cases (n=51)	Flow cytometry / final diagnosis	Cases (n=51)	Concordance (%)
AML	16	AML	9	56.3
		ALL	7	
		No AL	0	
ALL	19	AML	5	68.4
		ALL	13	
		No AL	1	
AL unspecified	7	AML	2	
		ALL	2	
		No AL	3	
No AL	9	AML	2	33.3
		ALL	4	
		No AL	3	

CHAPTER FOUR

4.0 DISCUSSION

This study describes the demographic, clinical and laboratory characteristics of patients with AL and performance evaluation of GSM, WSI and flow cytometry in the diagnosis of acute leukemia among patients with features highly suggestive of acute leukemia at the largest referral hospital in Tanzania.

In this study, the majority of patients with features highly suggestive of acute leukemia were below 65 years and only 9 patients (7.9%) were above 65 years of age. This could be attributed to the fact that Tanzania has an overall younger population with life expectancy of approximately 60 years. Other studies (7,57,58) in high-income countries with a longer life expectancy than in Tanzania showed that the incidence of AL is distributed as two peaks: one in childhood below 5 years of age consisting mostly of ALL and a second peak in adults above 65 years of age .

This study confirms an equal male to female ratio at 1.02:1 similar to another study in sub Saharan Africa (59). By comparison, studies in the USA (60,61) found a male predominance in the incidence of AL.

The majority of patients with AL (about 90%) experienced a duration of symptoms of less than 6 months similar to other studies (14,62). This is because the disease is acute in nature and symptoms present faster lead to early hospital visit. Symptoms of anemia were the commonest clinical presentation in both AML and ALL occurring in 41 (87.2%) of patients with AL followed by fever in 36 (76.6%) and bleeding tendencies in 18 (38.3%). All these symptoms were due to the replacement of the bone marrow by the blasts resulting in a decrease in the production of normal blood cells (7). Other studies (23,32) show that fever is the commonest symptom followed by anemia and bleeding tendencies . The lower frequency of fever at presentation could be due to fact that patients present with fever were treated as other common infectious diseases like malaria and most of patients who were referred to

heamatology unit were those with symptoms of anaemia. CNS involvement was only observed in ALL (n=3; 10.7%). Majority of patients with CNS involvement, are usually asmptomatic and diagnosed following a routine lumbar puncture. In the present study, results for lumbar puncture were not assessed and diagnosis of CNS involvement was made clinically. It is therefore possible that this was under diagnosed.

Pallor was the commonest sign in all patients with AL which was about 85%. Petechial and gingival hypertrophy were common in AML. Gingival hypertrophy was due to blast infiltration and more common in the monocytic type of acute leukemia (63). Although lymphadenopathy and splenomegaly were not common in AML they occured in 4 (21%) and 2 patients (10.5%), respectively similar to other studies done in Kenya and USA (32)(64). It is possible that patients could have had coexisting viral and mycobacterial infections presenting with adenopathy. This also emphasizes the importance of including granulocytic sarcoma in the differential diagnosis of patients with lymphadenopathy. Common signs in ALL included lymphadenopathy 12 (42.9%), splenomegaly 10 (35.7%) and hepatomegaly 6 (21.4%). This is similar to other studies (9,21). Jaundice was observed in 4 (14.3%) ALL patients, this could be due to hemolytic anaemia or hepatic infiltration which are both more common in lymphoproliferative malignancy (65).

Hemoglobin levels, WBCs and platelets counts were altered due to the fact that bone marrow is mostly populated by leukemic blasts. Despite the fact that about 80% of patients had a history of blood transfusion (BT) prior to diagnosis, only 10% had Hb level above 10g/dl similar to other studies (23,32). The high ratio of patients with BT before diagnosis suggest delay in diagnosis as well as risk of alloimunization which could interfere with the treatment, especially when there is a need of bone marrow transplant. About half of patients presented with leucocytosis and “monocytosis” by automated machine which revealed to be blasts on peripheral smear. Blast are large mononuclear cells with high nuclear cytoplasmic ratio. More than half of the patients had more than 90% of blast in the bone marrow at time of diagnosis. High blast count show aggressive disease, late presentation and poor outcome (66). About one quarter of the patients had very high LDH more than five times normal and 30% had high uric

acid. This show the high cell turnover for AL. This is similar to the previous studies done by Kulsoom et al (23).

This study was undertaken to see how the diagnosis of acute leukemia by morphology (GSM and WSI) correlated with the flow cytometry in diagnosis of acute leukaemia by types. The performance characteristics of GSM for the diagnosis of AL was above 90%. This means that morphology has good utility for the initial diagnosis of acute leukemia (44). This was due to the high percentage of blast count in the BMA at time of diagnosis as the vast majority of patients had blast counts of 90% or more.

Using GSM in the diagnosis of AML had a concordance of about 57% while for ALL it was about 80% concordant with flow cytometry as adjunct investigation. The concodance of AML was low compaired to other studies which show about 90% concodence (34,36). Morphologically, the presence of Auer rods and granulation is diagnostic of AML. However, frequently the monoblastic AML poses difficulty in confident diagnosis of AML due to their deviation from the above mentioned characteristics which can cause one to miss the diagnosis (36). In this study cytochemistry was not performed, this could be the cause of reduced concordance of GSM in diagnosis of acute leukemia by types. Furthermore, the poor quality of slides observed in over 30% of cases may have contributed to the reduced concordance. The two main factors influencing slide quality were absence of cytoplasmic granules due to poor quality staining technique and also hemodilution of the bone marrow aspirate which lead to inadequate samples for morphology assessment as explain in the study done by Burthem et al (67).

The reduced accuracy of AML versus ALL differentiation shows that there is a need for immunophenotyping in differentiating between these types of acute leukemia (12,16,35,68,69) especially when patients present earlier in the disease course with low blast count in the marrow. It also illustrates the importance of improving the staining quality.

WSI had a lower concordance in diagnosis of AML and ALL of about 56% and 68% respectively. The low concordance rate could be due to low quality of the staining which lead to poor quality or/and lack of experience for reviewers as this is new technology at our setting. These two were the main sources of false results in WSI (70).

This study showed that where morphological diagnosis of AL by types was not possible, flow cytometry confirmed diagnosis of either ALL or AML in 5 cases for GSM and 9 cases for WSI. Similar improvement of sub classification was found in the study done by Sengar et al (36) in which flow cytometry confirmed diagnosis of ALL in 88% (37/42). Of the 37 patients found with ALL, GSM was unable to subclassify 30 cases (81%). Similarly, in the present study, flow cytometry was able to rule out acute leukemia in 4 cases which were diagnosed as acute leukemia by GSM and WSI.

WSI and GSM showed an inter-observer concordance of 82.4% for the diagnosis of AL. This is different with other studies which show a discordance of only 1%-5% (20,46,47,50,70). The discrepancies in diagnoses between GSM and WSI could be attributed to poor staining, poor image quality including poor image resolution on high magnification secondary to loss of focus (20).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study show acute leukemia mostly affects people below the age of 60 years. The common clinical presentation is due to bone marrow failure; anaemia, bleeding due to thrombocytopenia, and infections (fever) related to neutropenia. Organomegaly was a common sign in ALL. Majority of patients with acute leukemia had low hemoglobin level, leukocytosis, thrombocytopenia and very high LDH. Morphology with good staining quality gives the basis of diagnosis of acute leukemia. GSM and WSI had low concordance when compared to flow cytometry in diagnosis of acute leukemia by types, thus flow cytometry is an important adjunct investigation in diagnosis of acute leukemia by types.

5.2 Recommendation

1. Since the concordance of GSM and WSI is relatively high in initial diagnosis of acute leukemia, WSI can be used in peripheral hospitals where there is no haematologist to acquire images and send for haematology review so as to have an early diagnosis.
2. Quality of smear for morphology must be improved by improving reagent used and training technician on staining protocols.
3. Flow cytometry is recommended to be done to all patients diagnosed with acute leukemia by GSM and/or WSI for accurate classification of acute leukemia by types.

5.3 Limitations

Poor staining for some slides lead to misdiagnosis of acute leukemia by types and could be the cause of low concordance.

Loss of focus on high magnification in WSI.

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APPENDICES

Appendix I: Questionnaire

PROFORMA FOR ALL STUDY Study No |___|___|___|

REGISTRATION

- Date (DD-MM-YY) ……|___|___|-|___|___|-|___|___|
- Telephone number 1)…… 2)…….

DEMOGRAPHIC HISTORY

- Hospital ID Number ……|___|___|___|___|___|___|___|___|___|
- Age (Years) ……|___|___|-|___|___|-|___|___|
- Sex (F/M) ……..|___|
- Place of origin …… |_____|
- Current residence …… |_____|
- Level of education ……..|_____|
- Occupation ……. |_____|

HISTORY (Y for Yes, N for N)

- Duration of symptoms (weeks) ……..|_____|
- Easy fatigue ……..|___|
- Dizziness ……....|___|
- Awareness of heart beats …….....|___|
- Fever ……....|___|
- Bleeding tendencies ……. |___|
- Lymph node enlargement ……..... |___|
- Bruising ……. |_____|
- Weight loss ……|____|
- Others (specify) ……....|_____|

- Number of blood transfusion received ……. |_____|
- Number of platelets packs received ……. |_____|
- History of radiation exposure ……|_____|
- History of pesticide/ chemical use ……|_____|
- History of myeloproliferative disorder ……|_____|if yes mention…….

PHYSICAL EXAMINATION

- Pulse rate……...|_____|
- SBP……|_____|
- DBP……...|_____|
- Height……|_____|
- Weight ……..|_____|
- Pallor (N/Y)……....|___|
- Jaundice (N/Y)……|___|
- Petechiae (N/Y) ……...|___|
- Ecchymosis (N/Y) ……|_____|
- leukemia cuties (N/Y) …… |_____|
- Chloroma (N/Y)……|_____|
- CNS involvement (N/Y) ……...| |
- Gingival hypertrophy (N/Y)……|_____|
- Testicular hypertrophy (N/Y) ……|_____|
- Lymphadenopathy(N/Y) …….|___|
- Liver palpable (N/Y) …….|___|
- Spleen palpable (N/Y) ……...|___|
- Others (specify)……..

INVESTIGATIONS

- Hb……|_____|
- MCV ……..|_____|
- WBC …….|_____|
- ANC……|_____|
- ALC ……|_____|
- AMC …….
- ABC……..
- PLT ……|_____|
- Peripheral smear (Y-blast seen, N-No blast seen)……
- HIV (1- positive, 2- negative) ……..|_____|
- Serum Uric acid level (1- low, 2- normal, 3-high)……..|_____|
- LDH (1- normal, 2-high)……..|_____|
- GSM % blast…….
- GSM ddx(1- AML, 2- ALL, 3 –APML, 4 – AL not specified, 5- no AL)…….|_____|
- WSI % blast…….
- WSI ddx(1- AML, 2- ALL, 3 –APML, 4 – AL not specified, 5- no AL) ……...|_____|

FLOW CYTOMETRY ANALYSIS

Early markers

- HLA-DR ……|_____|
- CD 34 …..|_____|
- CD10…….
- CD 117…….
- CD45……..

B-Lymphocyte markers

- CD 19……..
- CD 20…….

T-Lymphocyte markers.

- CD 2……..
- CD 3……..
- CD 5 ……
- CD 7 ……

Myeloid Markers

- CD 13 …….
- CD 33 ……
- CD 15 ……
- CD 14 ……..
- CD 64 ……

Diagnosis (1- AML, 2- T-ALL, 3-B-ALL, 4 – AL, Mixed type, 5- no AL)

……..|_____|

- Treatment option (1- only supportive, 2- palliative chemotherapy, 3- intensive chemotherapy, 4- bone marrow transplant) …….|_____|
- Outcome after 6 months (1-alive , 2- death) …….||

Appendix II: Consent form (English Version)

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES (MUHAS)

PERFORMANCE EVALUATION OF GLASS SLIDE MORPHOLOGY, WHOLE SLIDE IMAGE AND FLOW CYTOMETRY IN DIAGNOSIS OF ACUTE LEUKEMIA BY TYPES

Consent to participate in the study of evaluation of performance characteristics of glass slide morphology and whole slide image for diagnosis of acute leukaemia among adults doing bone marrow aspiration at MHN

Greetings!

My Name is Dr. Hamisa Iddy, a resident doctor in the Department of Haematology and Blood Transfusion at MUHAS. I am conducting a study aiming to find out the performance characteristics of glass slide morphology and whole slide image for diagnosis of acute leukaemia among adults doing bone marrow aspiration. I am requesting your participation.

How to participate

Patients who will be ready to participate will sign a consent form to approve his/her willingness. Short interview and examination will be done and sample for investigation which is venous blood and Bone Marrow Aspiration will be taken.

Confidentiality

Information obtained from you will be confidential and will be of help in this study and better care for patients with acute leukemia.

Costs

You will not be required to pay anything for your participation.

Voluntary participation & rights to withdraw

Your participation is voluntary and you have the right to withdraw from participating in our study at any time. Whatever your decision may be, it will not affect in any way your rights to care and treatment.

Risks

Blood sample will be drawn from your arm and BMA from iliac crest. You will feel some pain when the needle pierces your skin for drawing this blood (on the arm) and during bone marrow aspiration procedure. Bleeding can be prolonged even through not frequent. The skin on your arm and at place of taking MBA will be thoroughly cleaned prior to prevent infections.

Benefits

Your participation in this study will help you know the accurate diagnosis and type of acute leukemia you have thus determining type of treatment to be given as well as prognosis of your disease. We hope that the information from this research will be useful in contributing to improve the quality of care in patients with acute leukemia.

Contact persons

If you have any inquiries about this study, please do not hesitate to contact:

Dr. Hamisa Iddy
Principal Investigator
Muhimbili University of Health and Allied Sciences (MUHAS)
Department of Haematology and Blood Transfusion
P.O. Box 65001 Dar es Salaam.
Tel. 0717 456 222

I will be grateful if you willingly agree to participate in this study.

I _____Have understood the above information and my questions have been answered by the investigator to my satisfaction. I willingly agree to take part in this research.

Signature of the participant/guardian ……

Date …….

Signature of the investigator ……

Appendix III: Fomu ya makubaliano ya kushiriki katika utafiti

PERFORMANCE EVALUATION OF GLASS SLIDE MORPHOLOGY, WHOLE SLIDE IMAGE AND FLOW CYTOMETRY IN DIAGNOSIS OF ACUTE LEUKEMIA BY TYPES.

Habari! Mimi ni Dk Hamisa Iddy ni Daktari mwanafunzi wa shahada ya Uzamili katika Chuo Kikuu Cha Sayansi Za Tiba cha Muhimbili. Nafanya utafiti kuhusu ugonjwa wa Acute leukemia hapa Muhimbili hospitali.
Ninaomba ushirikiano wako.

Nia ya Utafiti;
Dhumuni ni kujua jinsi ufanisi wa vipimo vinavyotumika kugundua ugonjwa wa saratini ya damu (Acute leukemia) ambovyo ni kusomarojo ya mfupa kwa njia ya darubini, kusomarojo ya mfupa kwa njia ya computa na kwa kuangalia mtiririko wa seli.

Jinsi ya Kushiriki:
Mgonjwa ambaye yuko tayari kushiriki ataweka sahihi yake ,ilikuonyesha utayari. Yatafuata maswali machache ya Utangulizi, kasha vipimo vya damu, ya mshipa na rojo ya mfupa vitachukuliwa.

Usiri:
Taarifa ya magonjwa yako hazitatangazwa kwa yoyote zaidi ya mtafiti. Matokeo ya utafiti kwa ujumla yatasaidia kuboresha huduma ya tiba kwa kwa wagonjwa wa saratani ya damu ya Acute leukemia.

Gharama
Hutatakiwa kulipa gharama yoyote kwa kushirikik wako.

Utayari wa kushiriki au kujitoa:
Kushiriki kwako nihiyari na waweza kujitoa. Lakini haitakunyima haki ya kupata tiba zingine.

Athari

Damu kwa ajili ya vipimo itatolewa kwenye mkono na rojo ya mfupa itatolewa kiunoni. Unaweza kupata maumivu wakati wa kutoa damu na kutoa rojo ya mfupa. Pia unaweza kutoka dawa kwa muda mrefu baada ya vipimo. Ili kuepuka kusababisha maambukizi, mara zote ngozi ya koita safishwa vema na dawa kabla ya kuchomwa sindano yoyote.

Faida

Kushiriki kwako katika utafiti huu, kutakusaidia kujua ufanisi wa vipimo vya rojo ya mfupa vinavyosomwa kwa darubini na kwa kompyuta ukilinganisha na vya mtiririko wa seli hivyo kusaidia kujua aina ya saratani yako damu na matibabu stahiki.

Ni tumaini letu kuwa utafiti huu utasidia kuboresha uelewa na huduma ya uchunguzi kwa wagonjwa wa saratani ya damu nchini kwetu na penginepo. Nitakushukuru kwa kushiriki kwako utafiti huu. Ahsante.

Iwapo utakuwa na swali lolote kuhusu utafiti huu wasiliana na

Dr. Hamisa Iddy,

Chuo kikuu Cha Afya Na Sayansi za Tiba Muhimbili;

Idara ya Tiba; S.L.P 65001 Dar es Salaam.

Simu0717 456 222.

Endapo utakuwa na swali lolote kuhusu haki zako kama mshiriki katika utafiti huu wasiliana na:

Prof ;

Mwenyekitii wa Kamati ya Tafitina Matoleo Chuoni.

Chuo Kikuu Cha Afya na Sayansi Shirikishi Muhimbili;

S.L.P 65001 Dar Es Salaam .

Simu 2151489.

Mimi……nimeelezwa/ nimesoma yaliyomo katika fomu hii na nimeelewa maana yake. Nakubali kushiriki katika utafiti huu.

Sahihi……..(Mshiriki) Tarehe……..

Appendix IV: Assent form (7-18 years)

Habari! Mimi ni Dk Hamisa Iddy ni Daktari mwanafunzi wa shahada ya Uzamili katika Chuo Kikuu Cha Sayansi Za Tiba cha Muhimbili. Nafanya utafiti kuhusu ugonjwa wa Acute leukemia hapa Muhimbili hospitali.
Ninaomba ushirikiano wako.

Nia yaUtafiti;
Dhumuni ni kujua jinsi ufanisi wa vipimo vinavyotumika kugundua ugonjwa wa saratini ya damu (Acute leukemia) ambovyo ni kusomarojo ya mfupa kwa njia ya darubini, kusomarojo ya mfupa kwa njia ya computa na kwa kuangalia mtiririko wa seli.

Jinsi ya Kushiriki:
Mgonjwa ambaye yuko tayari kushiriki ataweka sahihi yake ,ilikuonyesha utayari. Yatafuata maswali machache ya Utangulizi, kasha vipimo vya damu, ya mshipa na rojo ya mfupa vitachukuliwa.

Usiri:
Taarifa ya magonjwa yako hazitatangazwa kwa yoyote zaidi ya mtafiti. Matokeo ya utafiti kwa ujumla yatasaidia kuboresha huduma ya tiba kwa kwa wagonjwa wa saratani ya damu ya Acute leukemia.

Gharama
Hutatakiwa kulipa gharama yoyote kwa kushirikik wako.

Utayari wa kushiriki au kujitoa:
Kushiriki kwako nihiyari na waweza kujitoa. Lakini haitakunyima haki ya kupata tiba zingine.

Athari

Damu kwa ajili ya vipimo itatolewa kwenye mkono na rojo ya mfupa itatolewa kiunoni. Unaweza kupata maumivu wakati wa kutoa damu na kutoa rojo ya mfupa. Pia unaweza kutoka dawa kwa muda mrefu baada ya vipimo. Ili kuepuka kusababisha maambukizi, mara zote ngozi ya koita safishwa vema na dawa kabla ya kuchomwa sindano yoyote.

Faida

Kushiriki kwako katika utafiti huu, kutakusaidia kujua ufanisi wa vipimo vya rojo ya mfupa vinavyosomwa kwa darubini na kwa kompyuta ukilinganisha na vya mtiririko wa seli hivyo kusaidia kujua aina ya saratani yako damu na matibabu stahiki.

Ni tumaini letu kuwa utafiti huu utasidia kuboresha uelewa na huduma ya uchunguzi kwa wagonjwa wa saratani ya damu nchini kwetu na penginepo. Nitakushukuru kwa kushiriki kwako utafiti huu. Ahsante.

Kama umeamua unataka kuwa sehemu ya utafiti huu, tafadhali andika jina lako hapo chini.

Mimi, _____, nakubali kuwa sehemu utafiti.

_____ _____

(Sahihi/ Dole gumba) **(Andika Tarehe)**

Appendix V: Normal ranges for laboratory parameters

Parameter	Unit	Normal Range
Hemoglobin (Hb)	g/dl	12 - 16
Mild anaemia	g/dl	11.9 -10
Moderate anaemia	g/dl	9.9 - 7
Severe anaemia	g/dl	< 7
White blood cell (WBC)	x 10^9/L	4 - 10
Absolute neutrophil count (ANC)	x 10^9/L	2 – 6.9
Absolute lymphocyte count (ALC)	x 10^9/L	0.6 – 3.4
Absolute monocyte count (AMC)	x 10^9/L	0 – 0.9
Absolute basophil count (ABC)	x 10^9/L	0 - 2
Absolute eosinophil count (AEC)	x 10^9/L	0 – 0.7
Platelets	x 10^9/L	150 - 410
Lactate dehydrogenase	U/L	125 - 220
Uric acid	Mmol / L	0.15 – 0.35
Bone marrow blast count	%	0 - 20