ADDIS ABABA UNIVERSITY, MEDICAL FACULTY

DEPARTMENT OF PATHOLOGY

PREVALENCE OF ACUTE LEUKEMIA AND ASSOCIATION BETWEEN BONE MARROW ASPIRATION AND FLOW CYTOMETRY IN THE DIAGNOSIS OF ACUTE LEUKEMIA IN TIKUR ANBESSA SPECIALIZED HOSPITAL, DEPARTMENT OF PATHOLOGY

INVESTIGATOR – ELIZABETH HAILEMELEKOT SIRAK, MD, PATHOLOGY RESIDENT

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DANIEL HAILU (MD, PEDIATRIC HEMATO-ONCOLOGIST)

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A THESIS PAPER SUBMITTED TO AAU, MEDICAL FACULTY, DEPARTMENT OF PATHOLOGY IN PARTIAL FULFILMENT OF THE REQUERMNETS FOR CERTIFICATE OF SPECIALITY PROGRAM IN PATHOLOGY

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Approval sheet

Addis Ababa University

College of health sciences, school of medicine

Department of pathology

I the undersigned pathology resident, declare that I have submitted my original proposal on a title; prevalence of acute leukemia and association between bone marrow aspiration and flow cytometry in the diagnosis of acute leukemia in tikuranbessaspecializied hospital, department of pathology, for the examination.

Submitted by:

Dr Elizabeth Hailemelekot

Name of resident **signature** **date**

This proposal work has been submitted for examination with my approval as an advisor.

Approved by:

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Advisors name **signature** **date**

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Advisors name signature date

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List of Abbreviations and Acronyms

AAU	Addis Ababa University
AL	Acute Leukemia
ALL	Acute lymphoblastic (lymphocytic) leukemia
AML	Acute myeloid (myeloblastic/myelogenous) leukemia
AOR	Adjusted odds ratio
ASIR	Age-StandardizedIncidence Rate
ASMR	Age Standardized Mortality Rate
BM	Bone marrow
BMA	Bone Marrow Aspiration
CBC	Complete blood count
CCC	Cancer Care Center
CD	Cluster of differentiation
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
F	Female
FAB	French-American-British group
FC	Flow cytometry
GBD	Global Burden of Disease
GDP	Gross Domestic Product
HCT	Hematocrit
HDI	HumanDevelopment Index
HGB	Hemoglobin
HLA – DR	Human leukocyte antigen-D related
ICSH	International Council For Standardization In Heamatology
IHC	Immunohistochemistry
KCMC	Kilimanjaro Christian Medical Centre
M	Male
MPO	Myeloperoxidase
OR	Odds ratio
PBS	Peripheral blood smear
RICK	Radio-Isotope Center Khartoum
TdT	Terminal DeoxynucleotidylTransferase
TASH	TikurAnbessa Specialized Hospital
WBC	White Blood Cell
WHO	World Health Organization

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Abstract

Background*: Acute leukemia is a disease resulting from the neoplastic proliferation of haemopoieticor lymphoid cells. It results from mutation of a single stem cell, and usually there is a series of genetic alterations rather than a single event. Epidemiologically each type of leukemia has a different distribution.*

Objectives*: The objective of this study is to assess the prevalence of acute leukemia and describe the association between bone marrow aspiration and flow cytometry in patients diagnosed to have acute leukemia.*

Methods*: A cross sectional hospital based study design was conducted on 135 pathology reports of patients who had been diagnosed with acute leukemia by bone marrow aspiration during the period from January 1, 2018 to December 31, 2019 G.C at TikurAnbessa Specialized Hospital, Addis Ababa, Ethiopia. The data was utilized from bone marrow request papers and from patient chart review using a standardized data collection form. Different statistical analyses was used including frequencies of variables, descriptive statistics including mean and standard deviation and fisher exact test for determination of statistical significanceusing IBM SPSS for Widows 20.0.*

Results: *Over all, most of acute leukemia cases had ALL (56.5%) with male predominance (2.4:1). The commonest clinical presentation were symptoms of anemia (64.3%) followed by fever (57.4%) and majority of the patients had organomegaly and lymphadenopathy (67% and 68% respectively).In this study most of the patients had leukocytosis with anemia and thrombocytopenia (59.1%, 93.9% and 91.9%) respectively.The peripheral morphologies we receive had 'fair' smear quality(60.9%) and 40.2% cellular bone marrow yield. The result of flow cytometry in this study showed B-Cell ALL predominates (45%). The associational studies showed children are more likey to have ALL than AML(AOR: 22.54; 95%CI: 2.90, 174.84, P= 0.001) and significant association of fever (AOR: 4.84; 95% CI: 1.28, 18.27; P=0.02)and bone pain(AOR: 9.28; 95%CI: 1.31, 65.47; P=0.025) with ALL.Fisher's exact test did not show significant association between flow cytometry and bone marrow aspiration.*

Recommendation: *Further prospective studies are needed to assess the association between flow cytometry and bone marrow aspiration.*

Key words: Acute leukemia, department of pathology, TikurAnbessa Specialized Hospital, Ethiopia

1. Introduction

1.1. Background

Leukemia is a disease resulting from the neoplastic proliferation of haemopoietic or lymphoid cells. It results from mutation of a single stem cell, and usually there is a series of genetic alterations rather than a single event. (1)
Leukemias are broadly divided into: acute leukemias (AL), and chronic leukemias (1)The four major subtypes of leukemia described by most cancer registries include acute lymphoblastic leukemia(ALL), acute myeloid leukemia(AML), chronic lymphocytic leukemia(CLL) and chronic myelogenous leukemia(CML). (2).
Acute leukemia can be classified in many ways

(1) French-American-British (FAB);

    This classification system originally distinguished different leukemia types by morphologic features and cytochemical studies. (3)

(2) World Health Organization (WHO) Classification of Acute Leukemias;

    It incorporates clinical features, morphology, immunophenotyping, cytogenetics, and molecular genetics to define disease entities of clinical significance.(4)

Acute leukemias are characterized by a defect in maturation, leading to an imbalance between proliferation and maturation. Since cells continue to proliferate and fail to mature, there is continues leukemic clonal expansion and predominance of immature cells. (1)
The clinical manifestations of the leukemias are due, directly or indirectly, to the proliferation of leukaemic cells and their infiltration into normal tissues.
Epidemiologically each type of leukemia has a different distribution. More specifically, AML is the most common type of leukemia in adults and more than half of the cases are in individuals over the age of 60. ALL is a cancer that mostly affects children, making it unique compared with other types of leukemia (that is AML, CLL and CML). However, 40% of ALL cases do occur in adults who have a worse prognosis (5)
According to Global Cancer Statistics 0f 2018, GLOBOCAN estimated that there will be 18.1 million new cases and 9.6 million cancer deaths worldwide in 2018 from which New Cases and Deaths for leukemia in 2018 was 437,033 (2.4%) and 309,006 (3.2%) respectively.(6) while in Ethiopia, from a total of 67,573 new cancer cases 4,464 (6.6%) were leukemia.(7) and population based data registry 2015 showed in Ethiopia leukemia was the leading cancer in pediatric age group. (33)

White blood cell (WBC) count with differential usually gives important clues for the diagnosis of acute leukemia followed by peripheral blood smear (PBS) examination (8).
PBS with various diagnostic approaches including examination of the bone marrow (BM) aspirate and trephine biopsy together with relevant investigations such as cytochemistry, immunophenotypic analysis, cytogenetic and molecular genetic techniques, as well as biochemical and microbiological test results is essential for the diagnosis of BM disorders.(9)

1.2. Statement of the Problem

The incidence of cancer is increasing worldwide. GLOBOCAN estimated, in 2012 leukemia accounts for more than 352,000 new cases and 265,000 deaths and in 2018 the number of new cases increased into 437,033 and number of deaths to 309,006. (6, 11, 12)
Based on GLOBOCAN 2012 in Ethiopia estimated number of new cancer cases was 60,960 and hematologic malignancy accounts for 6916. These numbers increased in 2018 to 67,573 new cases and 4,464 leukemia cases. (13, 14)

According to the Global Burden of Disease (GBD) database the incidence of AML has increased gradually in the past 28 years by 87.3% and particularly in Eastern sub-Saharan Africa by 3.71% (1.64×10^3in 1990 to 4.01×10^3in 2017). (15)And a ten year forecast study to determine global incidence and prevalence of acute lymphoblastic leukemia stated that there were a total of 53,000 cases in 2016 worldwide and expected to increase to 56,000 cases by 2020. (16)

WHO Europe, 2009 stated that leukemia accounts for 30% of all childhood cancers in pediatric age group in western countries. A study done in south central Asia,2012 and estimates of cancer incidence in Ethiopia showed leukemia is most common cancer type in this age group. (33, 43, 44)

Since recent advances in molecular biology have shown that various subtypes of AML and ALL behave differently WHO highly recommends additional evaluation of the leukemic blasts by molecular analysis and flow cytometry. The combination of morphology, staining, molecular analysis, and flow cytometry not only differentiate ALL from AML, but also categorize the subtypes of acute leukemia (19)

1.3. Significance of the Study

There are few studies done in our country on correlation between different diagnostic modalities of acute leukemia and no recent prevalence study done in TASH. Furthermore, we are having problems with the quality of samples in practice and very limited availability of flow cytometery. This paper will addresses the gap between morphological and flow cytometry diagnosis and show how much difficulty we are facing to diagnose subtypes of acute leukemia because of varies diagnostic challenges like poor sample quality (including sample adequacy, smear and staining quality)and unavailability of flow cytometry. (18)It can be said that there is a limited knowledge with regards to the epidemiology, risk factors and correlation for these patients in the Ethiopian context. So, this study can be used as a baseline study for further studies.

2. Literature Review

Definition

By definition, leukemia is 'a progressive malignant disease of the blood forming organs, characterized by distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow.' (20). It has marked variations in incidence according to socio-economic status and by geography, with high incidence in northern and eastern Africa, western Asia, and parts of Latin America. (21)

Leukemia manifests itself either in immature (precursor) cells or in mature cells, giving rise to its acute or chronic form and a distinctive age-driven prognosis.(21) The four major subtypes of leukemia described by most cancer registries include acute lymphoblastic leukemia(ALL), acute myeloid leukemia(AML), chronic lymphocytic leukemia(CLL) and chronic myelogenous leukemia(CML). (2).

Classification

Acute leukemia can be classified in many ways based on four methods, that is: morphology, staining, molecular analysis and flow cytometer. (19). The two widely accepted methods are French-American-British (FAB) classification for leukemias which is based on morphology and simple cytochemical stains, and remains effective enough, divides AML into 8 subtypes (M0 to M7) and ALL into 3 subtypes (L1 to L3).This classification uses 30% myeloblast in blood and/or bone marrow as a cutoff to diagnose AML. (22,3).

Table 1, Morphologic Classification of Acute Lymphocytic Leukemia (19)

Morphologic Classification	
FAB Type	Features of Blasts
L1	Small cells with scant cytoplasm; nucleoli indistinct and visible
L2	Large, heterogeneous cells with moderately abundant cytoplasm; clefting and indentation of nucleus; and prominent nucleoli
L3	Large cells with moderately abundant cytoplasm; regular, oval to round nucleus; prominent nucleoli; prominent cytoplasmicbasophilia and cytoplasmic vacuoles

Table 2, French-American-British (FAB) Classification of Acute Myelogenous Leukemia (19)

FAB Type	
M0	AML with no Romanowsky or cytochemmical evidence of differentiation
M1	Myeloblastic leukemia with little maturation
M2	Myeloblastic leukemia with maturation
M3	Acute Promyelocytic Leukemia
M3h	APL, hypergranular variant
M3v	APL, microgranular variant
M4	Acute myelomonocytic leukemia (AMML)
M4eo	AMML with dysplastic marrow eosinophis
M5	Acute monoblastic leukemia (AMoL)
M5a	AMoL, poorly differentiated
M5b	AMoL, Differentiated
M6	Erythroleukemia
M6a	AML with erythroid dysplasia
M6b	Erythroleukemia
M7	Acute megakaryoblastic leukemia (AMkL)

the World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues is based on the principles initially defined in the Revised European-American classification of lymphoid neoplasms(REAL), proposed by the International Lymphoma Study Group(ILSG).The REAL classification is based on the principle that a classification is a list of ‘‘real’’ disease entities, which are defined by a combination of morphology, immunophenotype, genetic features, and clinical features – to define the disease. There is no single gold-standard by which all diseases are defined in the WHO classification. (23). Since 2001, WHO editions are increasingly based on genetic criteria and continues to define specific acute myeloid leukemia (AML) disease entities by focusing on significant cytogenetic and molecular genetics subgroups.(24, 4)

In 1997, the World Health Organization proposed a composite classification of ALL in attempt to account for morphology and cytogenetic profile of the leukemic blasts and identified three types of ALL: B lymphoblastic, T lymphoblastic and Burkitt-cell Leukemia. Later revised in 2008, Burkitt-cell leukemia was eliminated as it is no longer seen as a separate entity from

Burkitt lymphoma, and B-lymphoblastic leukemia was divided into two subtypes: B-ALL with recurrent genetic abnormalities and B-ALL not otherwise specified. B-ALL with recurrent genetic abnormalities is further delineated based on the specific chromosomal rearrangement present. In adults, B-cell ALL accounts for ~ 75% of cases while T-cell ALL comprises the remaining cases. (25)
In the WHO classification, the blast threshold for the diagnosis of AML is reduced from 30% to 20% blasts in the blood or marrow. In addition, patients with the clonal, recurring cytogenetic abnormalities(8;21)(q22;q22), inv(16)(p13q22) or t(16;16)(p13;q22),and t(15;17)(q22;q12) should be considered to have AML regardless of the blast percentage.(26).

Epidemiology

According to World Health Statistics posted by WHO, in 2016, an estimated 41 million deaths occurred due to non-communicable diseases (NCDs), accounting for 71% of the overall total of 57 million deaths and of which 9.0 million deaths (22%) were due to cancer (10).
According to Global Cancer Statistics 0f 2018, GLOBOCAN estimates that there will be 18.1 million new cases and 9.6 million cancer deaths worldwide in 2018. New Cases and Deaths for leukemia in 2018 was 437,033 (2.4%) and 309,006 (3.2%) respectively.(6)

Population-based study conducted in Five Continents, 184 countries to evaluate the epidemiological patterns of leukemia stated that more than 350, 000 new leukemia cases were estimated in 2012.The highest leukemia incidence rates by region among men were in Australia and New Zealand (age-standardized rates 11・3 per 100 000),northern America (10・5 per 100 000), and western Europe(9・6 per 100 000), and the lowest in the sub-Saharan African sub-regions namely western Africa (1.4 per 100 000)and middle Africa (2.6 per 100 000). Rates were generally higher in males than females with elevated rates in the same sub-regions. The highest incidence rates in specific countries in each of the continents were: Canada (9・5 per 100 00), Australia (9・4 per 100 000), Ireland (9・4 per 100 000), Ecuador (6・5 per 100 000), Singapore(6・0 per 100 000), and Egypt (5・9 per 100 000).Rates were generally higher in males than females with an overall male to female ratio of 1·4. In children, acute lymphoblastic leukemia was the main subtype in all studied countries in both sexes, and characterized by a bimodal age-specific pattern, rates of acute lymphoblastic leukemia remained relatively high among adults in selected South American, Caribbean, Asian, and African populations.(21)
Worldwide ecologic study done to assess the correlation between Age-Standardized Incidence Rate (ASIR) and Age Standardized Mortality Rate (ASMR) of leukemia with Human Development Index (HDI) showed in 2012, there were 351965 cases of leukemia of which 200676 cases occurred in males and151289 in females (Sex Ratio= 1.32). Among all cases, 130469 cases occurred in countries with very high Human Development Index (HDI), 58981 cases in countries with high HDI, 136378 cases in countries with moderate HDI, and 26004 cases in countries with low HDI. Five countries with the highest number of leukemia were China with 65778 cases, the United States with 39658 cases, India with 32532 cases, Russia with 11773 cases, and Germany with 11038cases. It also stated that ASIR and ASMR of leukemia were 4.7 and 3.4 per 100,000 people, respectively. Countries with the highest ASIR were Mauritius (12), Cyprus (9.5), Canada (9.5), Ireland (9.4), and Australia (9.4). Also, countries with the highest

ASMR were State of Palestine (7.7), Iraq (6.5), Mauritius (6), Syrian Arab Republic (5.7), and Ethiopia (5.4). There was a statistically significant and positive correlation between HDI and ASIR of leukemia. (27).

The 2018 global snapshot of leukemia incidence showed that rates for acute myeloid leukemia were highest in Australia for men (2.8 per 100,000) and Austria for women (2.2), with the United States near the top for both men (2.6) and women (1.9). The lowest rates occurred in Cuba and Egypt for men (0.9 per 100,000) and Cuba for women (0.4). (28)

The Global Burden of Disease (GBD) database from the statistical data of 354 diseases in 195 countries showed that the incidence case of AML was increased gradually in the past 28 years (from 63.84×10^3 in 1990to 119.57×10^3cases in 2017, increasing by 87.3%).Of this, Male accounts 33.60×10^3in 1990 to 66.79×10^3 in 2017 and Female 30.24×10^3 in 1990 to 52.79×10^3in 2017. Eastern sub-Saharan Africa showed 1.64×10^3in 1990 to 4.01×10^3in 2017. Countries with low socio-demographic index showed 6.55×10^3in 1990 to 13.34×10^3in 2017. (15).

A ten year forecast to determine global incidence and prevalence of acute lymphoblastic leukemia for 45 countries done in 2020 estimated ALL incidences to range from 0.4 to 2 per 100,000 in Asia-Pacific and South American countries, respectively; while prevalence will range from 0.37 to 1.6 per 100,000 in these regions. There were a total of 53,000 cases in 2016 worldwide and with incorporation with other data this number should increase to 56,000 cases by 2020. Most of these cases are in the Asia Pacific region, representing 55% of the worldwide total. (16)

The integrative literature review on clinical and epidemiological aspects of leukemias (2015 - 2016) showed that most studies reported childhood leukemia, and the most incident subtype is ALL. It also stated that Gender distribution in Brazil showed M: F – 1.2 :1.(42)

According to WHO fact sheet on the incidence of childhood leukemia, 2009 leukemia is most common childhood malignancy which accounts for 30% of all cancer diagnosis in patients less than 15 years old in industrialized countries. (43)

A descriptive cross sectional study done in south-center Asia, 2012 showed that hematologic malignancy is the most common type of cancer in infants and it accounts for 3.2% of total cancer cases in less than 14 years. (44)

A ten year cross sectional retrospective epidemiological study on 601 reported cases of leukemia registered at the Zimbabwe National Cancer Registry showed 601(1.4%) were diagnosed with leukemia . Of which 353 (59%) males and 248 (41%) females had leukemia. Children (< 14 years) and the elderly (>75 years) were the most and least affected age groups respectively. Of all, acute myeloid leukemia was found in 147 (24%) patients, acute lymphoblastic leukemia 106 (18%) patients and unclassified leukemia 103 (17%). The rest was occupied by chronic leukemias. ALL was predominantly found in children, AML in children and young adults, CML in the middle aged, CLL in the elderly and unclassified leukemia in children and young adults. (29)

A cross-sectional analysis of all hematological malignancies from the cancer registry of Cancer Care Center (CCC), Kilimanjaro Christian Medical Centre (KCMC), Northern Tanzania from December 2016 until May 2019 showed that out of 209, 15 of them were acute myeloid

leukemia while acute lymphoblastic leukemia accounts for 17. The rest was occupied by other hematologic malignancies.(30)

A hospital based retrospective descriptive study of 2535 diagnosed leukemia cases from 2009 till 2013 done at Radio-Isotope Center Khartoum (RICK), Sudan reveled that male gender represent 58.1% of cases while Females 41.9% with male to female ratio 1.3:1. Among all leukemia cases 6.7% (n=338) were children aged under 20 years old. The combined median age for all leukemia is 40.3 years. AML was the second most common case seen in 24.8% of patients preceded by CML with the mean age of 35.1 year. The least frequent case seen was ALL (21.1%) with the mean age 19.3.The frequency of the type of leukemia among both sex, is dominated by CML among male 28.6% followed by CLL 24.6, then AML 23.9% while the least common is ALL 22.9%. In contrast to female CML represent 37.4% of all leukemia cases followed by AML 26.1% then ALL 18.9% while the least frequent leukemia is CLL 17.6%.(31)

A retrospective analysis done at university college hospital, Ibadan, Nigeria between 2003 and 2008 showed that a total of 50 acute leukemia cases seen with mean age of 20.4 years and of which 28(56%) were AML and 22(44%) were ALL. Sex distribution of both ALL and AML were M:F- 2.1:1 (41)

A 3 year retrospective study (from September 2010 to August 2013)was carried out among all children aged below 15 years old admitted into the pediatric wards of Gondar University Hospital, Northwest Ethiopia showed that total of 71 cancer cases were diagnosed and admitted to the pediatrics ward during the study period. More than two-third of the study subjects 50 (70.4 %) were males. The mean age of study subjects was 7 ± 4 year where majority 26 (36.6 %) of the study subjects were ≥10 years. Of all, 43 (60.6 %) were hematological malignancy followed by Wilms tumor 13 (18.3 %), Neuroblastoma 5 (7 %). (32)

On estimates of Cancer Incidence in Ethiopia in 2015 Using Population-Based Registry Data For 2015, Memire S. stated that estimated that new Leukemia cases in2015 will be men 1386 and Women 1886, of a total of 3,707 cases occurred in the pediatric age group, leukemia being the commonest cancer (29%), followed by NHL, Wilms tumor, and retinoblastoma. Acute leukemia accounted for 89% (of which91% was acute lymphocytic leukemia and 9%was acute myeloid leukemia) of all the leukemia cases in children.(33)

A retrospective cross-sectional study conducted from January 01 to April 30, 2017to trend and Projection of Childhood cancer incidence in Addis Ababa, Ethiopia showed the overall incidence rate of childhood cancer from 2012- 2016 was 88.47 cases per million. The most common childhood cancers were leukemias followed by lymphomas. From leukemias, myeloproliferativediseases, and myelodysplastic diseases the most common was Lymphoid leukemias with 75.76%, followed by Acute myeloidleukemias (13.13%), (34)

From January 1982 to December 1992 eighty-two consecutive cases of adult acute leukemia cases were admitted to the TikurAnbessa Specialized Hospital, a teaching and referral hospital in Addis Ababa, Ethiopia. In the study period AML and ALL occurred in 53.7% and 46.3%, respectively with male to female ratio was 1.6:1.Of all cases eligible for evaluation treated with chemotherapeutic agents, only 38.4% of ALL and 6.2% of AML achieved complete remission. Twenty-seven patients with ALL died from one day to 84 (median 1.0) months after diagnosis. Ten are lost to follow-up from two weeks to 36 (median 2.5) months, one is still alive 40 months after diagnosis. Thirty-nine of the AML patients died from one day to nine (median 0.3) months

after diagnosis. Five are lost to follow-up from two weeks to two and a half (median 2.0) months. The causes of death were sepsis and bleeding, separately or in combination.(17).
A prospective, correlational study conducted to assess the correlation between morphology, cytochemistry, and flow cytometry in the diagnosis of acute leukemia at TikurAnbessaSpecialized Hospital, Addis Ababa, Ethiopia, from May2017 to March 2018 showed that a total of 40 study participants were diagnosed with acute leukemia based on morphology, of which, 26 participants (65%) were male and 14 (35%) were female and majority (72.5%) were from rural areas. Ten participants (25%) were in the age group from 0 to 15 years, 19 participants (47.5%) were in the age group 16 to 30 years, 6 participants (15%) were of age group 31 to 45 years and 5 participants (12.5%) were above 45 years. Based on BM morphology of total 40 cases ALL accounts for 15(37.5%), AML for 21 (52.5%) and 4(10%) cases remained unclassified. Flow cytometry classify similar cases as, 14(35%) as ALL (B lineage ALL in 7 (17.5%) cases and T lineage ALL in 7 (17.5%) cases), 25(62.5%) as AML and 1(2.5%) case as Bi-lineage. (18)

Risk Factors

A 10 year forecast study to estimate global incidence and prevalence of ALL stated that increasing age and male sex were the non-modifiable risk factors with the largest effects. To account for additional risk factors such as the increasing adoption of Western lifestyles characterized by dietary changes and more sedentary lifestyles, the proxy measure of forecast gross domestic product (GDP) were used. Prevalence was also estimated as a cumulative incidence over the preceding 12-month period with adjustments for disease-specific and competing-cause mortality. (16)

Clinical Features

A retrospective study done on 47 newly diagnosed patients with acute leukemia attending the Hematology Oncology Unit, Mansoura University Hospital, Egypt showed most of the patients (87.0%) were pale and presented with symptoms of anemia, such as easy fatigability, dizziness and palpitations. Out of the 47 patients, 12 (25.5%) patients were feverish (temperature 38.8^0C) upon presentation. Thirteen patients (27.7%) have presented with bleeding tendency. The same number of patients presented with bone pains. CNS manifestations were encountered in 7 (14.9%) patients. Lymphadenopathy predominated in patients with acute leukemia (80.9%). Splenomegaly was more pronounced in ALL than AML cases (66.6 and 23.1%, respectively). (35)
Shubhnita G. stated that most common clinical presentation in comparative analysis between bone marrow biopsy and bone marrow aspration in patients with a diagnosis of leukemia from Jan 2015 – 2016 at Gectanjali medical collage and hospital, India was pallor, followed by fever, fatigue hepatosplenomegaly, bleeding tendencies, backpain and pathological fracture. (36)
According to Shamebo M. study done at TikurAnbesa Specialized Hospital from January 1982 to December 1992 the commonest symptoms were anaemia, fever and bleeding tendencies. The commonest signs were pallor, fever, sternal tenderness and purpura. Splenomegaly was more commonly seen in ALL patients. The haematological findings were anaemia (mean Hgb 6.35

g%), leucocytosis (mean WBC count 88,507/mm3) and thrombocytopenia (mean platelet count 31,700/mm3). (17)

Diagnosis

WBC count with differential usually gives important clues for the diagnosis of acute leukemia and chronic lymphoid or myeloid disorders as well as for the presence of leukopenia and neutropenia. Furthermore, in abnormal count the type of WBC affected: neutrophils, lymphocytes, monocytes, eosinophils, or basophils should be confirmed by the human eye (i.e.,peripheral blood smear [PBS] examination) before it is acted on. (8)

According to ICSH guidelines Examination of the bone marrow (BM) aspirate and trephine biopsy is essential for the diagnosis of BM disorders. A comprehensive diagnosis of a BM disorder often requires the integration of various diagnostic approaches. These include peripheral blood (PB)counts and smear evaluation, BM aspirate smear, particle clot section, BM trephine biopsy and imprint morphology, together with results of other relevant investigations such as cytochemistry, immunophenotypic analysis, cytogenetic and molecular genetics techniques, as well as biochemical and microbiological test results, as appropriate. The final interpretation should be in the context of clinical and preliminary diagnostic finding.(9)

Italsostatedthatintheabsenceofparticles,megakaryocytesorotherhaemopoieticprecursors,thesample shouldbereportedasa'bloodtap'orperipheralblood.Intheabsenceofparticles,butinthepresenceofmega karyocytesorotherprecursorcells,thesampleshouldbereportedasadilutedBMsampleandaqualitative evaluationcanbeperformed.Inthepresenceofparticleswithabsentorveryreducedcellularity,onlyaqua litativedescriptionshouldbeprovided.(9)

On similar guidelines, it stated that the length of the trephine bone marrow biopsy from an adult should be at least 2 cm. A shorter core(e.g. 1 cm) may sometimes contain sufficient diagnostic information.(9)While according to recent (2017) edition of WHO Classification of Tumors of hematopoietic and Lymphoid Tissue to take adequate specimen, it must be at a right angle from the cortical bone, and be ~ 1.5 cm in length (to enable evaluation of ~ 10 partially preserved intertrabecular areas. (26)

ICSH recommendations for the standardization of nomenclatureand grading of peripheral blood cell morphological features stated that qualitative and/or quantitative information of peripheral morphology (PM) and are an essential part of the diagnostic work-up. Worldwide, there is a marked variation in blood film evaluation, reporting practices and morphology terminology. Abnormal morphologic findings are reported in various ways: (i)a simple description, (ii) the use of terms such as presenter absent, (iii) a semi-quantitative determination, mild (+), moderate (++), marked (+++), (iv) a quantitative percentage of the morphological abnormalities. (37)

A study for evaluation of different diagnostic approaches in acute leukemia in Egypt, was carried out on 47 newly diagnosed patients with acute leukemia attending the Hematology Oncology Unit, Mansoura University Hospital showed ALL cases, 18/21 patients (85.7%) were positive for both CD19 and CD79a, 10/21 (47.6%) wereCD10 positive, 5/21 (23.8%) were SmIg positive and3/21 (14.3%) were CD3 and CD7 positive. In addition, there was an aberrant expression of CD33in one patient (4.8%).In AML cases, all patients were CD33 and MPO positive, 25/26 (96.2%) were CD13 positive, 8/26(30.8%) were CD34 positive, 9/26 (34.6%) wereCD14

positive and one case (3.8%) aberrantly expressed CD19. On the other hand, five cases (19.2%) were negative for HLA-DR.(36)

The previously mentioned prospective, correlational study conducted at TikurAnbessa Specialized Hospital, Addis Ababa, Ethiopiashowed that of a total of 40 cases flow cytometricimmunophenotypingidentify all AML - 25(62.5%), cases expressed cMPO while CD117, CD33, CD13, and TdT were present in 84%, 80%, 72%, and 20% respectively. Aberrant expression of T cell antigens (CD4 and CD7) was observed in 12(48%) and 5(20%) cases, respectively. And also the expression of B cell antigens (CD19 and cCD79a) was observed in 2(8%) of AML cases, while the progenitor cell markers CD34, and HLA-DR were expressed in 17 (68%)each. In T lineage ALL - 7 (17.5%), cCD3, and CD7 were expressed in all cases, followed by CD8, TdT, CD4, CD3, and HLA-DRin 3(42.9%), 3(42.9%), 2 (28.6%), 2 (28.6%), and 2(28.6%) cases, respectively. The expression of myeloid-associated antigens (CD33, CD117, CD13, and cMPO) was observed in 1(14.3%), 1(14.3%), 2(28.6%) and 3(42.9%) cases, respectively. Progenitor cell markers CD34was expressed in 3(42.9%) cases. In B lineage ALL which accounts for 7 (17.5%), CD19, CD10, cCD79a and TdT were expressed in all cases. Myeloid-specific markers CD33 and cMPO were present in 1(14.3%) and 2(28.6%) subjects, respectively. The T and monocyte marker CD14 was observed in 2(28.6%) of cases. Finally, the progenitor cell marker CD34 was expressed in 4(57.1%) of cases. The HLA-DR marker is a progenitor marker for the myeloid lineage, but is expressed by all immature and mature B cells and B cell ALL. In Bilineage leukemia, one cell population was positive for T-cell markers(CD3 and CD4) and the other was positive for AML markers(CD33, CD13, and MPO).(18)

Correlation

A comparative analysis between bone marrow biopsy and bone marrow aspiration in patients with a diagnosis of leukemia from jan 2015 – 2016 at Gectanjali medical college and hospital, India showed a total of 30 patients of which 7 of them were AML while 4 of them were ALL. The rest of the cases where categorized as chronic hematologic malignancy. A maximum number of cases were aged between 20 – 40 years (40%) followed by 0 – 20 years (33.34%). For 2 cases the bone marrow aspiration was dry tap & inadequate. (36).

A retrospective review that was conducted at King Hussein Medical Center, Jordan between January and December 2012 showed that from a total of random 500 reports of bone marrow aspirates and biopsy reports AML were one of the most commonly correlated disease with the diagnostic findings of bone marrow aspirate - 26, biopsy - 28, and percentage of correlation 92.8% followed by ITP and CML.(38)

On similar retrospective review Dr. M. Aljadayeh stated that Out of the 500 cases, aspirate was diagnostic in 381 cases (76.2%) while biopsy was diagnostic in 459 cases (98.8%), the sensitivity for aspirate and biopsy were respectively 78.3%, 99.1%, specificity 96.2%, 97.1%, positive predictive value 99%, 99% , and negative predictive value 57%, 96%. The majority (76.2%) of the cases showed positive correlation between the bone marrow aspirate and biopsy findings. (38)

A study in a Tertiary Institute of North India, from 2013–2014 showed out of 130 cases, 30 cases were excluded due to inadequacy of BMA, BMB, or both. Inadequate material was obtained more in BMA 70% (21/30) as compared to BMB 20% (6/30), whereas, 10% (3/30) cases were inadequate on both. Dry tap 66.6% (16/21) was the most common reason for failed aspiration followed by hemodilution (25%). Out of these 16cases with dry tap2 cases (12.5%) were acute leukemia. On bone marrow examination, the most common malignant disorder was acute leukemia (21%) in our study. Out of 21 cases of acute leukemia, aspirates were diagnostic in 14 (8 acute myeloid leukemia, 6 acute lymphocytic leukemia) cases. However, in 5 cases, aspirate smears were unable in exact typing of acute leukemia, the definite typing was achieved by BMB and these cases were further categorized as 2 cases of AML (2/5) and 3 cases of ALL (3/5)and further sub-typing of ALL cases was done with the use of immunohistochemistry (IHC).(39)
A three year retrospective study was done in Kathmandu Model Hospital, Nepal, from July 2010 to June 2013 and showed from a total of 95 cases only 89 were biopsied. A total of 75 cases were diagnosed on BMA cytology with acute leukemia were diagnosed as the second common diagnosis (N= 9; 12%) following erythroid hyperplasia. And 88 cases were diagnosed by bone marrow biopsy of which ALL accounts for 7(7.95%) and AML for 5 (5.68%) **(**40**)**

The previously mentioned prospective, correlational study between morphology and flowcytometery done at TikurAnbessa Specialized Hospital showed that of a total 40 acute leukemia cases, there were nine discrepancies between flow cytometry and morphology result. Two cases of T-ALL were defined by flow cytometry which had been classified as AML by morphology and Six cases of AML were diagnosed by flow cytometry which had been classified as ALL (2) and AL (4) by morphology. Moreover, one Bi-lineage case was found based on flow cytometry which was ALL based on morphology. A complete concordance between morphologic and flow cytometric diagnosis was seen in 31 (77.5%) cases, of which 12/15 (80%) were ALL and 19/21 (90.5%) were AML. (18)

3. Objectives

General

The general aim of this study was:

a. To assess the prevalence of acute leukemia and describe the associationbetween bone marrow aspiration and flow cytometery in patients diagnosed to have acute leukemia.

Specific

The specific objectives of this study were:

a. To describe the prevalence, clinical presentation and associated factors of acute leukemia
b. To determine the degree of concordance between flow cytometry and morphology in the diagnosis of acute leukemia.
c. To assess the adequacy of the sample including the quality of the slides sent for BMA for the diagnosis acute leukemia

4. Materials and Methods

4.1. Study Area

The study area is pathology unit, TikurAnbessa Specialized Hospital, School of Medicine, College of Health sciences; Addis Ababa, Ethiopia is the largest referral hospital in the country. It was established in 1964 with over 700 beds. The hospital is now the main teaching center for both undergraduate and postgraduate medical students, dentists, nurses, midwives, pharmacists, medical laboratory technologists, radiology technologists and others. It is also an institution where specialized clinical services, that are not available in other public or private institutions, provided to the whole nation. Currently TASH has more-than 200 doctors, 379 nurses' and115 other health professionals.

Pathological practice started in the hospital since 1965. Currently the department has a total of eleven attending pathologists and 22 pathology residents. The department now provides Fine Needle Aspiration Cytology (FNAC), histopathology, cyto-pathology, bone marrow aspiration, medical autopsy and with limited resources, flow cytometry services.

4.2. Study Design

A hospital based retrospective cross-sectional study with analysis of clinical records of all acute leukemia patients during the study period was used.

4.3. Source Population

All patients who had bone marrow aspiration during the period from January 1, 2018 to December 31, 2019 G.C

4.4. Study Population

All patients who had been diagnosed with acute leukemia by bone marrow aspiration during the period from January 1, 2018 to December 31, 2019 G.C

4.5. Sample Size

Assuming this study was presented with 95% level of confidence (z=1.96), 5 % precision (d= 0.05) and taking proportion 0.066(the proportion of leukemia patients among all cancer patients in Ethiopia which is 6.6 % according to Globocan 2018 (6)), we used the following formula to get the minimum sample size:

$$n = \frac{Z^2 p(1-p)}{d^2} = \frac{1.96^2 * 0.066(1-0.066)^2}{0.05^2} = 95$$

Where,

- n: minimum sample size
- Z: the value at 95 % confidence level
- p: the expected prevalence of leukemia
- d: level of precision

We added 20% considering the missing and incomplete data making the minimum sample size 114.

4.6. Sampling Method

We selected the study participants from the hard copy of patients result at the pathology department using simple random sampling with computer generated random numbers. Those acute leukemia patients with flowcytometry results were included in the study by purposive sampling.

4.7. Inclusion/Exclusion Criteria

Inclusion Criteria

- All patients (male and female) with a diagnosis of acute leukemiaby BMA during the study period at the hospital

Exclusion Criteria

- Those patients with missing medical records.
- Those patients with two or more variables missing.

4.8. Data collection

Data was collected by the pathology residents from patient records using a standardized data collection checklist (Annex 1). Patients' charts were identified through the log book at the pathology department.

4.9. Operational Definitions

- **Acute leukemia**: a patient with greater than or equal to 25% blasts in the BMA
- Yield of bone marrow aspiration, quality of peripheral morphology and adequacy of bone marrow will be used according to the pathologist's description.
    - Yield of bone marrow aspiration will be described as highly diluted, diluted, cellular or hypercellular.
    - Peripheral morphology smear quality will be described as poor, fair, good, very good or excellent.
    - Adequacy of bone marrow biopsy will be described as adequate (optimal), suboptimal or inconclusive.
- Pediatric age: patients who are less than 15 years.

4.10. Variables

Independent Variables

a. Background characteristics
    a. Age
    b. Sex
    c. Residence
b. Clinical data
    a. Presenting feature/chief compliant
    b. Presence of organomegaly
    c. Lab values at presentation (Baseline Characteristics)
        i. CBC: WBC, HGB, HCT, platelet count
c. Sample
    a. Yield of bone marrow aspiration
    b. Quality of peripheral morphology
    c. Length of bone marrow biopsy

Dependent Variable

a. Diagnostic modalities
    a. Bone marrow aspiration (blast percentage)
    b. Bone marrow biopsy (cellularity according to the stated age)
    c. Flow cytometery(CD markers)
b. Correlation of BMA, BMB and flow cytometery

4.11. Data Management, Processing and Analysis

Data was entered and checked for any errors using EPI INFO and any error was corrected. Different statistical analyses were used including frequencies of variables, descriptive statistics including mean, median and standard deviation. Comparison between groups for measure of association was performed by chi-square and fisher exact test. Multivariate analysis was done by binary logistic regression and p-value <0.05 was taken to represent statistical significance. These were done using IBM SPSS for Widows 20.0.

4.12. Ethical Considerations

Before getting into the data collection process, ethical clearance to conduct this study was obtained from the Department of Pathology and DRPC of the Department of Pediatrics and Child Health with Publications Committee of AAU, Medical Faculty after submission of the study proposal. And all information obtained in the process of this research was confidential. The patients' names and card numbers was anonymous.

4.13. Dissemination of Findings

After the completion of this research, the findings will be submitted to AAU, medical faculty, Department of Pathology and pediatrics and child health and after reviewing it will be published.

5. Result

5.1 Socio-demographic characteristics

Among the total acute leukemia patients from 2018-2019, 115 were included in this study. Thirty three (29.2%) of the patients age was 6-15 years with mean ±SD (18.11±15.30) and majority (70.2%) were males. Regarding residence, nearly half (47.8%) of the patients were came from Oromia followed by SNNPR (13.9%) and Addis Ababa (12.2%) respectively.

Table 3 - Socio-demographic characteristics of the patients, TikurAnbessa Specialized Hospital, 2018-2019.

Variable			Frequency	Percentage
Socio- demographic characteristics	Age	0 – 5	27	23.9
		6 – 10	25	22.1
		11 -15	15	13.3
		16 - 25	26	23.0
		26 - 35	9	8.0
		36 - 45	7	6.2
		46 - 55	1	0.9
		56 - 65	2	1.8
		> 66	1	0.9
	Sex	Male	80	70.2
		Female	34	29.8
	Residence	Amhara	23	20
		Oromia	55	47.8
		SNNP	16	13.9

		Addis ababa	14	12.2
		Other (Tigray and Somalia)	4	3.4

5.2 Clinical features

Among the total 115 AL patients, 66(57.4%) had fever as a presenting sign and majority 74 (64.3%) had symptoms of anemia while 79(68%) had lymphadenopathy and 77(67%) had organomegaly

Table 4 - Presenting feature/chief compliant, TikurAnbessa Specialized Hospital, 2018-2019

Clinicalfeatures	Frequency	Percentage
Fever	66	57.4
Neckswelling	16	13.9
Bonepain	9	10.3
Weightloss	30	26.1
Nightsweating	22	19.1
Lossofappetite	28	24.3
Symptomsofanemia	74	64.3
Bleeding	42	36.5
Cough	34	29.6
Periorbitalswelling	18	15.7
Abdominalswelling	8	7.0
Scrotalswelling	2	1.7
Organomegaly	77	67.0
Lymphadenopathy	79	68.7

5.3 Laboratory values at presentation (Baseline Characteristics)

Regarding baseline laboratory values majority (59.1%) had white blood cell count more than 10,000cells/dl and most of the patients' (93.9%)hemoglobin level was 2.1-11g/dL.One hundred and two (91.9%) of the patients platelet count was below 150,000/microliter). Regarding peripheral smear quality, in the majority of cases (69.9%) the smears were assessed to be of fair quality while 82(71.3%)hadblastpercentageof>20%andtherest,33(28.7%) had<20%.

Table 5 - Baseline laboratory value of patients at TikurAnbessa Specialized Hospital, 2018-2019.

Variables		Frequency	Percentage
WBC(cellspermicroliter)	200-10,000	47	40.9
	10,001-238,300	68	59.1
Hemoglobin (g/dL)	2.1-11	108	93.9
	11-15.0	7	6.1
Platelet (permicroliter)	<150,000	102	91.9
	>=150,000	9	8.1
Peripheral smear quality	Poor	30	26.1
	Fair	70	60.9
	Good	13	11.3
	Very good	1	.9
	Excellent	1	.9
Blast percentage	<20%	33	28.7
	>=20%	82	71.3

Yield of bone marrow aspiration

The graph below show that 45(40.2%) of the BMA yield was cellular followed by diluted (31.3%). However, only 5.4% and 4.5% of the yield was slightly diluted and diluted cellular respectively.

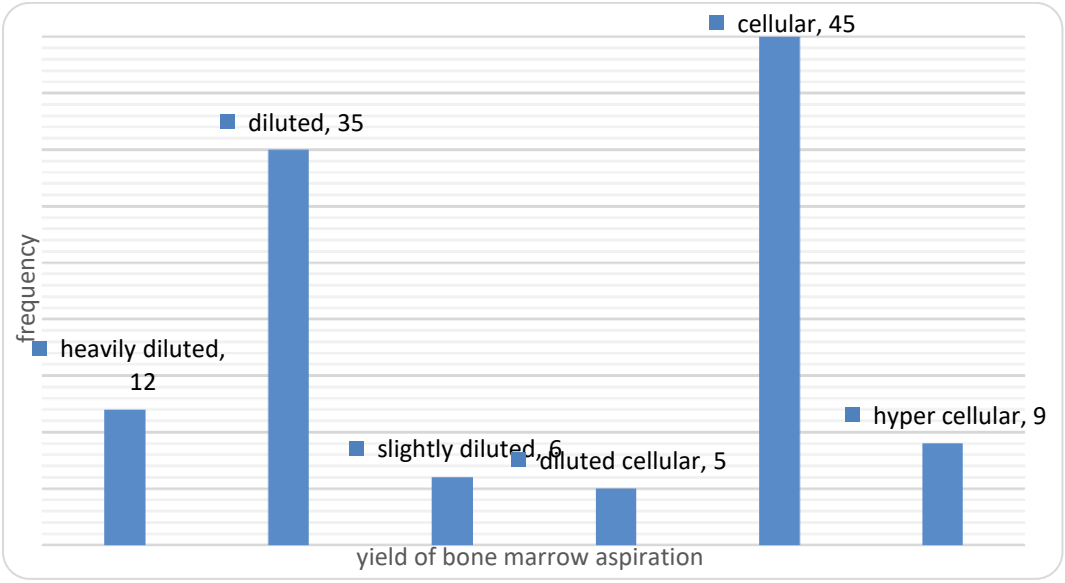

Figure 1: Yield of bone marrow aspiration, TikurAnbessa Specialized Hospital, 2018-2019.

5.4 Dependent Variable

Prevalence of acute leukemia

From a total of 4683 bone marrow aspiration cases sent for a diagnosis in a study period (2018-2019), 155 had acute leukemia by both bone marrow aspiration and peripheral morphology (prevalence of 3.3%) while 46 cases were diagnosed only by peripheral morphology(prevalence of4.3%)

Among the total 115 patients with acute leukemia, over the specified period 65(56.5%) had Acute Lymphocytic Leukemia (ALL), 44(38.3%) had Acute Myeloid Leukemia (AML) and only 6(5.2%) had unclassified acute leukemia.

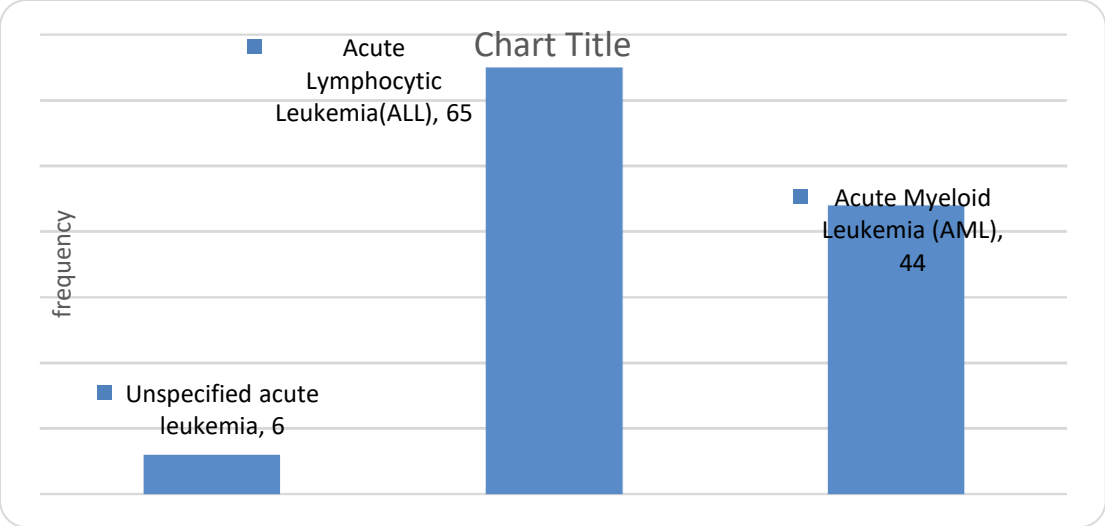

Figure 2: Prevalence of acute leukemia, TikurAnbassa specialized hospital, 2018-2019.

Flowcytometry Final Diagnosis

Among the total of20patients investigated with flow cytometry 10(50%) were diagnosed with ALL.Ofwhich9(45%) of them diagnosed to have B-cell ALL while only one case wasT- cell ALL. From 9(45%)casesdiagnosedtobeAML,4 (20%) caseswasAML,M0. And one cases determined to have bilineage. (Table 6)

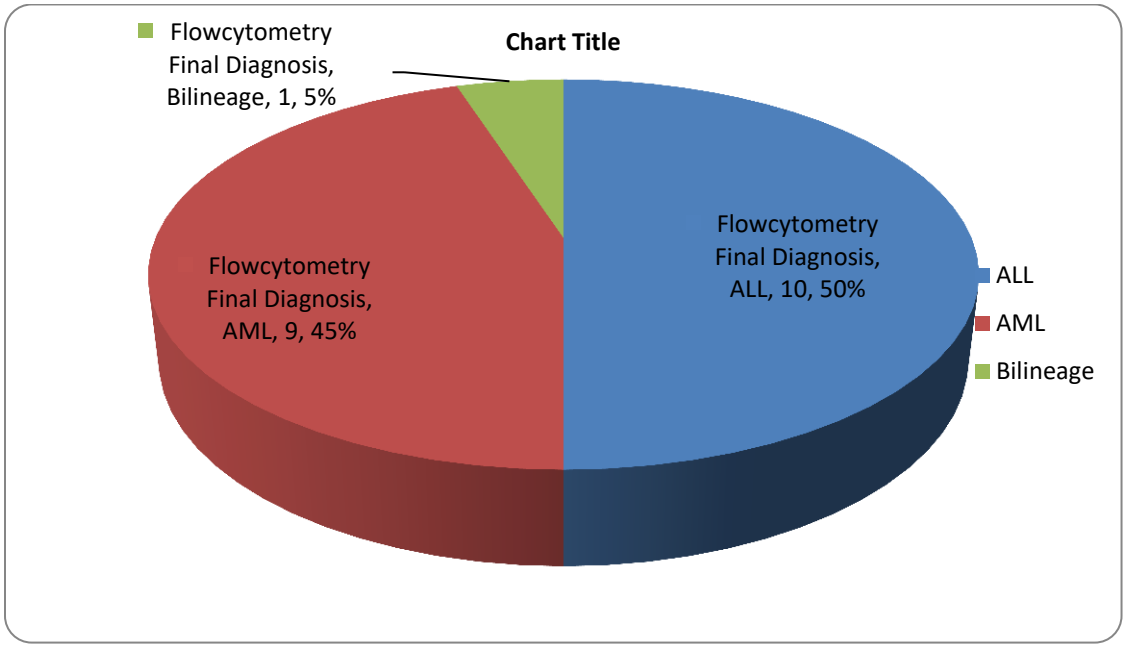

Figure 3: Flow cytometry final diagnosis, TikurAnbessa Specialized Hospital, 2018-2019

Table 6. Results of flow cytometry in acute leukemia, TikurAnbassa specialized hospital, 2018-2019.

Flow cytometry diagnosis	Frequency	Percent
B-cell ALL	9	45
T- cell ALL	1	5
AML,M0	4	20
AML,M1	1	5
AML,M5	1	5
Biphenotype	1	5
AML	2	10
Myeloid phenotype	1	5
Total	20	100

From a total of 6 (30%) cases diagnosed as AL by BMA, 3 (15%) of themwereALL while the rest wasAML. One case diagnosed as AML by BMAwasbilineage in flow cytometry. Two cases which were signed out as AML by BMA wereALL in flow cytometry examination.

Table 7 – Crosstabulation of flow cytometry and bone marrow aspiration diagnosis, Tikur Anbesa Hospital , 2018-2019

		BMA			
		AL	AML	ALL	Total
Flow cytometry	ALL	3	2	5	10
	AML	3	6	0	9
	Bilineage	0	1	0	1
Total		6	9	5	23

5.5 Factors associated with BMA diagnosis in Acute leukemia

To assess the relationship between acute leukemia and associated factors, both bivariate and multivariate binary logistic regression was conducted. The assumption and model fitness (HomerandLemshow) was checked and fulfilled. The dependent variable is coded 0 and 1. Thus AML is coded 0 and ALL is coded 1. Each of the important factor was checked in bivariate model and these variable with p value less than 0.2 in bivariatelogistic regression model were entered into multivariate model to adjust for every variable. Age (COR: 12.28; 95%CI: 3.51,42.91; P=0.001), sex (COR: 1.20; 95%CI: 0.53,2.69;P=0.081), fever(COR: 5.02 ; 95%CI:2.25, 11.16; P=0.001), bone pain(COR: 0.23; 95%CI:0.04,1.20; P=0.083), night sweating(COR: 1.77;

95%CI: 0.74, 4.24; P=0.195), symptoms of anemia(COR:0.46; 95%CI: 0.20, 1.03; P=0.060), organomegaly(COR:7.00; 95%CI: 2.91, 16.79; P=0.001), lymphadenopathy(COR:5.95; 95%CI:2.48,14.26; P=0.001), abdominal swelling (COR:5.66; 95%CI:1.55, 20.63; P=0.009), WBC(COR:2.26 ; 95%CI: 1.04, 4.91; P=0.039) and hemoglobin of the patients(COR:0.50; 95%CI:0.09,2.69; P=0.042) were entered into multivariate logistic regression model for further analysis based on the preset criteria.

In multivariate model, age of the patients was significantly associated with BMA diagnosis. Thus children (<15years) are more likely to be diagnosed ALL than AML (AOR: 22.54; 95%CI: 2.90, 174.84, P= 0.001). Also, patients with fever had 4 times more likely to be ALL than AML (AOR: 4.84; 95% CI: 1.28, 18.27; P=0.02). Patients with acute leukemia who had bone pain were 9 times more likely to be diagnosed ALL rather than AML after controlling for other factors. (AOR: 9.28; 95%CI: 1.31, 65.47; P=0.025). Even though organomegaly and lymphadenopathy were not significantly different in both class of acute leukemia, abdominal swelling was less likely among ALL (AOR: 0.04 95%CI: 0.004, 0.41; P=0.007). However, sex, anemia symptom, night sweating, WBC and hemoglobin level were not significantly different among both AML and ALL.

Table 8- Factors associated with BMA diagnosis in acute leukemia, TikurAnbessaSpeciaizied Hospital, 2018-2019

Variables	**Acute leukemia**		**COR,95%CI**	**AOR,95%CI**
	AML	**ALL**		
Age (years)				
<15	14	43	12.28(3.51,42.91)**	22.54(2.90,174.84)**
15-35	20	17	3.40 (0.95, 12.13)	5.19 (0.69,38.78)
>35	16	4	1	1
Sex				
Male	34	46	1.20 (0.53,2.69)	0.93(0.27, 3.15)
Female	16	18	1	1
Fever				
Yes	18	48	5.02 (2.25,11.16)**	4.84 (1.28,18.27)*
No	32	17	1	1
Bone pain				
Yes	3	6	0.23 (0.04,1.20)	9.28 (1.31, 65.47)*
No	44	63	1	1

Night sweeting				
Yes	7	15	1.77 (0.74,4.24)	0.60 (0.132, 2.74
No	43	50	1	1
Symptom of anemia				
Yes	37	37	1.77 (.74, 4.24)	0.42 (0.11,1.60)
No	13	28	1	1
Organomegaly				
Yes	22	55	7.00 (2.91,16.79)**	3.20 0(.989, 10.37)
No	28	10	1	1
Lymphadenopathy				
Yes	24	55	5.95 (2.48,14.26)**	2.87 (0.89,9.25)
No	26	10	1	1
Abdominal swelling				
Yes	3	17	5.66 (1.55, 20.63)**	0.04 (0.004, 0.41)
No	47	47	1	1
WBC count				
200-10000	15	32	2.26 (1.04, 4.91)*	0.30 (1.82, 0.577)
10001-238300	35	33	1	1
Hemoglobin				
2.1-11	48	60	0.50 (0.09,2.69)*	0.64(0.06, 6.13)
11-15.0	2	5		1

*Note: * P: 0.001-0.005; ** p <0.001, COR: Crude odds ratio; AOR: Adjusted odds ratio; CI: confidence interval*

5.6 Association between BMA diagnosis and flow cytometry

Since number of sample or cases under the variable flow cytometry is very small, bivariable and multivariable logistic regression were not employed because the variable was not fulfilling the assumptions for the respective analyses. Hence, Fisher Exact Test, a more robust test, was done to assess whether there is association with the dependent variable. Consequently, the value was insignificant with p value (0.057). Therefore, there was no statistically significant association between the BMA diagnosis and flow cytometry based on the exact test.

Table 8- Factors associated with BMA diagnosis in acute leukemia, TikurAnbessaSpeciaizied Hospital, 2018-2019

Flowcytometry final Dx		BMA Diagnosis	
		AML	ALL
	ALL	3	7
	AML	3	7

6. Discussion

This study discusses about the prevalence of acute leukemia and the association between bone marrow aspiration and flowcytometry in one of the biggest specialized hospital, Ethiopia in 2018 and 2019.

6.1 Socio Demographics

The socio-demographics of this study showed majority of the cases were less than 15 years (53.1%). This result is consistent with studies done in other African countries like Zimbabwe and Sudan (29, 31) and integrative literature review done by Marcos A. Ferreira Jr. (42)

The gender distribution of this study showed male predominance (M:F – 2.4:1) similar with other studies done in Zimbabwe, Sudan, Nigeria and previous studies done at the same place (29, 31, 41, 18) and a population based study in 184 countries done by Miranda-Filho A., et al., (21). However, in this study male preference was much higher than the mentioned studies. The possible explanation for this might be male predominance in lymphoblastic malignancies (26) as most of our patients had ALL.

As comparable with a study done in the same hospital a year back (18), in this study more than half of the patients (61.7%) came from outside Addis Ababa. This can be explained by the presence of very few specialized medical centers giving treatment for hematological malignancies.

6.2 Clinical features

The most common clinical presentation documented was symptoms of anemia, fever and bleeding from any site (64.3%, 57.4%, and 42%) and commonest sign were organomegaly and lymphadenopthy (67%, 68% respectively) which is comparable with studies done in Egypt, India and Ethiopia (35, 36, 17).

The finding on complete blood count result was (leukocytosis (59.1%), Anemia (93.9%) and thrombocytopenia (91.9%)) comparable with recent edition of WHO classification of tumors of hematopoietic and lymphoid tissues (26) and a study done by Shamebo M. in the same hospital. (17)

6.3 Outcomes variables

Even if 46 Cases diagnosed as acute leukemia by peripheral morphology only, in this study bone marrow aspiration yield was mainly cellular (40.2%). The possible explanation for this is in this study we only used cases which had acute leukemia on bone marrow examination.

ALL predominance (56.5%) was seen in this study as with a review done by Marcos A. Ferreira Jr. and Tanzania study (42, 30). However a study done in most of African studies including Zimbabwe, Sudan, Nigeria and Ethiopia (29, 31, 41 and 18) showed AML predominance. The absence of ancillary tests could be the possible explanation for this discrepancy.

Similar with other studies done in Zimbabwe, Ethiopia and report of estimates of cancer incidence in Ethiopia (29, 33, 34) this study also showed ALL(69.8%) was more common in pediatric age than AML.

As stated on recent edition of WHO classification of tumors of hematopoietic and lymphoid tissues (26) flow cytometry showed the most common subtype of ALL was B- Cell phenotype (90%) in our study.

6.4 Association studies

In our study age at the time of diagnosis had significant association with bone marrow diagnosis. Similar results were mentioned in studies done in Zimbabwe (29), review done byMarcos A. Ferreira Jr. (42) and population based study done in 184 countries done by Miranda-Filho A., et al,.

This study showed that significant association between fever and bone pain in patients who had diagnosed with ALL. It can be explained by the predominant subtype of ALL which is in case of B-cell ALL bone pain can be the prominent symptoms. (26)

Studies done inEgypt and Ethiopia (35, 17) showed that splenomegaly and lymphadenopathy had association with ALL than AML which was not seen in our study.

The discrepancies between BMA diagnosis andflowcytometryresult were also noticed on a prospective correctional study done at TikurAnbessa Specialized Hospital a year before. (18) Fisher's exact test did not show significant association in our study. This might be explained by the fact that only 20 of the patients included in this study had flow cytometry which is too small to make a generalization.

7. Limitation of the study

- ✓ As this study is retrospective and depends on chart review, it has seen some difficulties in missing data.
- ✓ A lot ofcharts identified to have acute leukemia were missing, therefore, could not be included in the study.
- ✓ Bone marrow biopsy wasavailableforsmallnumberofpatients so it was difficult to study the association between other diagnostic modalities.
- ✓ Since there is no standard reporting method it is difficult to assess the adequacy of the slides and reporting method.
- ✓ Since patients with flow cytometry diagnosis were few, it was difficult to assess the association between BMA and flow cytometry results.

8. Conclusion and Recommendation

Most acute leukemia cases presented with sign and symptoms of bone marrow failure, the commonest subtype being acute lymphoblastic leukemia.

We highly recommend inter-departmental discussion inordertogetadequate bone marrow aspiration andusingICSHguidelinesforthestandardizationofbonemarrowspecimenandreports.

Even though, few patients had flow cytometry diagnosis, there were caseswithdifferent bone marrow aspiration and flow cytometry diagnosis. So, the availability of flow cytometry would decrease this discrepancy.

A prospective multi-centric cohort study would remove the above mentioned limitations.

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Annex 1:Data Collection Form

Patient ID ----------

Patient Demographics

Age _____

Sex _____

Address _____

Clinical Variables

- Presenting features (chief complaint):_____
- Lymphadenopathy (Yes/No/ not available)_____
- Organomegaly (Yes/No / not available)_____
- CBC:
  - WBC:_____
  - HGB:_____
  - HCT:_____
  - Platelet:_____

Outcome Variables

- Peripheral morphology:
  - Smear quality:_____
  - Blast percentage:_____
- Bone marrow aspiration:
  - Yield:_____
  - Blast percentage:_____
  - BMA Dx:_____
  - No of BMAs done for Dx:_____
- Bone marrow biopsy:
  - Adequacy: _____
  - Cellularity: _____
  - Dx: _____

- Flow cytometry
    - Positive CD markers:_____
    - Dx:_____