ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE SCHOOL OF MEDICINE

DEPARTMENT OF INTERNAL MEDICINE

PREVALENCE OF L ASPARAGINASE ASSOCIATED THROMBOTIC EVENTS AND ASSOCIATED FACTORS IN ADULT PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN TIKUR ANBESSA SPECIALIZED HOSPITAL

PRINCIPAL INVESTIGATOR - DR AMIRA ABRAR (MD, INTERNAL MEDICINE RESIDENT)

ADVISOR- DR FOZIA ABDELA (CONSULTANT INTERNIST, HEMATOLOGIST)

A Thesis to be submitted to Addis Ababa University, College of Health Science, School of Medicine, Department of Internal Medicine in preparation for the partial fulfillment of the requirement for a Specialty certificate in Internal Medicine.

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Acknowledgment

I would like to thank Addis Ababa University Faculty of medicine and department of Internal Medicine for giving me the chance to conduct the study. I also would like to extend my deepest gratitude to my advisor Dr. Fozia Abdela for her support and guidance during every step of the study. I am also thankful for all members of the hematology unit at Tikur Anbessa specialized hospital for their corporation.

List of abbreviations

ALL- Acute lymphoblastic leukemia

AML- Acute myeloid leukemia

AOR- Adjusted odds ratio

aPTT- Activated partial thromboplastin time

ASNase- asparaginase

AT- anti thrombin

AYA- adolescents and young adults

CI- confidence interval

CNS- central nervous system

COR- crude odds ratio

CR – complete remission

CRY- cryoprecipitate

CVC- central venous catheter

CVT- cerebral venous thrombosis

DVT- deep venous thrombosis

FFP- fresh frozen plasma

GRAALL- Group for Research on Adult Acute Lymphoblastic Leukemia

HR-Hazard ratio

IQR – interquartile range

LLN- lower limit of normal

OR- odds ratio

PARKAA-prophylactic antithrombin concentrates in kids with ALL treated with L-asparaginase

PE- pulmonary embolism

Ph – Philadelphia

PT-prothrombin time

SPSS- Statistical package for social sciences

TE –thromboembolism

VTE – venous thromboembolism

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Abstract

Background –L-asparaginase is an important component of acute lymhoblastic leukemia treatment. However, it is associated with increased risk of thrombosis which in turn affects leukemia related outcomes and poses increased risk of mortality and morbidity.

Objective- To assess the prevalence of thrombotic events associated with L-asparaginase treatment and its determinant factors in adult acute lymphoblastic leukemia patients treated in Tikur Anbessa specialized hospital from November 2020- November 2023

Methodology: The study was conducted at Tikur Anbessa specialized hospital. A total of 152 patients who have been treated or are on treatment for acute lymphoblastic leukemia with L-Asparginase containing regimens at Tikur Anbessa Specialized Hospital from November 2020 to November 2023 were included in the study. Data was collected from the patient's medical records (both electronic and paper). Data was entered and analyzed with SPSS version 26, and a chi-square test was used to assess the association of independent variables with the dependent variable. Bi-variate and multivariate logistic regression were used to determine a significant correlation between independent and dependent variables.

Results: A total of 152 patients were included in the study. The median age was 22.5 years (IQR 18, 30.8), and 59.9% of them were male. The pediatric inspired ALL CL10403 regimen was used for treatment in 84.2% of cases, while CALGB 8811 protocol was used in the rest. The prevalence of thrombotic events in acute lymphoblastic leukemia patients treated with an L-asparaginase containing regimen was 11%. All of the events were venous, and cerebral venous thrombosis was the commonest site of thrombosis, accounting for 41.2% of events, followed by lower extremity deep vein thrombosis. The majority of events were symptomatic, and 44.4% occurred during remission induction.

Longer time to achieve complete remission (>4 weeks), adjusted odds ratio AOR 4.8 (95% CI = 1.10, 20.72), and age ≥40 years AOR 10.4(95% CI = 1.47, 75.0) were significantly associated with an increased risk of thrombotic events. Mortality was higher in patients with thrombotic events (47%) when compared to patients who did not develop thrombosis (41.4%) but was not statistically significant (*P*- value = 0.618). Mortality directly attributed to thrombotic events was 23.5% among patients who developed thrombosis.

Conclusion- This study showed that the risk of L-asparginase associated thrombosis in resource limited settings like ours is comparable with previous reports from other parts of the world. Longer time to achieve remission and age above 40 was associated with increased risk of thrombosis.

Key words: acute lymphoblastic leukemia (ALL), L-asparaginase, thrombosis

Contents

Chapter 1 - Introduction

1.1 Title

Prevalence of L-asparaginase associated thrombotic events and associated factors in adult patients with acute lymphoblastic leukemia in Tikur Anbessa Specialized Hospital

1.2 Introduction

Acute lymphoblastic leukemia (ALL) is a clonal expansion of lymphoid progenitor cells. It is classified into B or T –cell ALL based on the type of precursor cells involved. In America incidence of 1.5/100,000 population is reported with peak age in adults 50 years and in children 2-5years. According to population based registry in 2015 there were 1386 new cases of leukemia in men and 1886 new cases in women in Ethiopia. (1) In a study done in Tikur Anbessa in 1982 showed 53.3% of acute leukemia cases were due to ALL. (2) A hospital-based retrospective study of patients evaluated from north-west Ethiopia reported that 7.1% of hematologic malignancies were leukemia, and among this, ALL comprised 23.2%.(3)

L-asparagenase is a chemotherapy drug that is used in the treatment of ALL and B-lymphoblastic lymphoma. It has a proteolytic activity that changes aspargine into aspartic acid and ammonia. Asparagine is a non-essential amino acid that is important for protein synthesis in cells and for normal cell growth. It is synthesized in cells from aspartic acid by the enzyme asparagine synthatase. It can also be obtained through diet. A decreased level of asparaginase will lead to activation of the apoptotic pathway. L-asparaginase works by causing a relative asparagine deficiency. Since lymphocytes can’t synthesize asparagine denovo, they are prone to the asparagine depletion associated with ASP, leading to apoptosis.(4)

There are different L-asparaginase preparations. The native E. coli asparaginase, pegylated (polyethylene glycol) asparaginase, and Erwinia asparagase are the currently used preparations. The first two are derived from E. coli, and the later from E. chrysanthemi. The different preparations are associated with different pharmacokinetics and pharmacodynamics. Pegylated asparaginase has the longest half-life among the three, with a half-life of 5.7 days.The shortest half-life is 0.65 days for Erwinia asparaginase. This affects the dosing schedule in different protocols.(4)

The anti-neoplastic effect of L-asparaginase was first reported in 1953, when a study showed that injection of guinea pig serum led to the regression of implanted lymphoma cells in mice.(5) In 1967 case reports of improvement seen in ALL patients treated with L- asparaginase were reported.(6) It has been incorporated in the treatment of pediatric patients with ALL since 1980 and has shown significant improvement in their survival. Even though studies in children had shown significant improvement in overall and event-free survival with the addition of L-asparaginase studies in older age groups, adults didn't show such results.(7) But adolescents and young adults had significantly better outcomes, including CR and event-free survival, with pediatric-inspired regimens that included ASNase, as seen in the GRAALL 2003 study.(8)

Generally, patients with ALL are at increased risk of thrombosis, like other malignancies. A retrospective study done at the University of Texas MD Anderson Cancer Center, including all acute leukemia patients treated from 1995–2005, showed that the overall prevalence of thrombosis is 10.7%. The incidence was higher in ALL than in AML (17.7% vs. 8.6%).(9) Conversely, a population-based cohort study in California that included acute leukemia patients from 1993 to 1999 showed a higher prevalence of VTE in AML patients. The finding could be because CNS thrombosis was not assessed in this study and is a common site of involvement in ALL.(10)

In addition to the thrombosis risk incurred by ALL, ASNase treatment is also associated with thrombosis and bleeding. The main mechanism is said to be its inhibition of protein synthesis, leading to decreased levels of anticoagulants, mainly antithrombin. This is supported by the decreased AT level in patients who developed thrombosis associated with ASNase treatment in different studies. Additionally, other adult treatment regimens that don't contain ASNase, like hyper-CVAD, didn't report thrombosis as a treatment complication.(3)

Childhood ALL has a good prognosis, with event-free survival and overall survival of 82.5% and 90%, respectively.(7) In adults, the outcome is not as favorable as in the pediatric age group. With the current treatment in adults, the ALL complete remission (CR) rate is 80–85%, with 30–40% leukemia-free survival (LFS) rates.(11)

1.3 Justification of the study

The current treatment regimens given in our setup for all patients incorporate ASNase. There is a lack of data on the prevalence of thrombosis as a complication of the use of L-asparaginase in our setting and also in Africa.

1.4 The significance of the study

ALL patients account for nearly half of the acute leukemia patients admitted for treatment in our setup. These patients are treated with treatment protocols that contain l-asparagenase. The development of thrombosis is significantly associated with increased mortality and morbidity.

There hasn't been any study which looked in to the prevalence of thrombotic events associated with L- Asparginase therapy in ALL patients at TASH. However, personal observations of treating physicians shows that there are a sizable number of events happening each year

This study will address this information gap and give an insight into the burden of these thrombotic events, factors associated with them and their impact on patient outcomes. It also serves as baseline data for future prospective studies regarding the topic. Furthermore, it helps to identify high-risk groups for this complication and to implement a prophylactic measure when applicable.

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Chapter two

2.1 Literature review

Many studies addressed the magnitude of thrombotic events in ALL patients treated with regimens containing L-asparaginase worldwide. Most of these studies are done on children since the disease is more prevalent in the pediatric age group. There is a scarcity of data on prevalence of this complication in Africa and particularly Ethiopia.

The prevalence of thrombosis among ALL patients treated with L- asparaginase is variable. This variability can be due to the study design used and different types of treatment protocols in different setups. The inclusion of asymptomatic thrombosis in some of the studies also contributes to the wide range of numbers reported.

The PARKAA trial reported that out of 60 children, 36.7% had thrombotic event associated with L-asparagiase treatment. Among these, only 5% were symptomatic. Similarly a meta-analysis of 17 studies that included 1752 children showed a prevalence of 5.2 %, with 2.95 being CNS thrombosis. The majority of these thrombotic events happened during induction (4.8%). 2% of the events are during the consolidation and maintenance phases.(12)

Contrary to the data from pediatric studies, a higher prevalence is reported in adult patients. In the Dutch-Belgian HOVON 37 study, which is a multicenter study including 240 adults with ALL treated with ASNase -containing protocols, 10% of patients had symptomatic VTE in the first cycle of induction remission. The median time to develop thrombosis after the start of L-asparaginase was 23 days (ranging from 6 to 46 days). 37% of these thrombotic events involved the CNS, and 46% involved the upper extremity venous circulation.(13)

The Dana Faber Consortium also reported an even higher incidence in adults. Out of 47 adults treated for ALL at DFCI/Brigham and Women's Hospital, 34% developed symptomatic thrombosis, with a median time to thrombosis of 3.5 months. 29% of the thrombosis involved the upper extremity venous system, followed by CVT.(14)

Similarly, in a single institution-based study conducted from 2013–2018 at Ohio State University, 25% of adolescents and young adults with a median age of 23.5 years treated with pegylated asparaginase developed VTE within the first 30 days of starting treatment with

ASNase. The majority of these events occurred during the induction period. Out of the 18 patients, 50% had recurrent thrombosis.(15)

A relatively lower incidence was reported in a retrospective study involving 238 adults with a median age of 29 treated with GIMEMA protocol ALL 0288; 4.2% developed thrombosis. 50% of these events involved the CNS venous system, and 20% involved the CNS arterial system. Other sites involved were pulmonary embolism, portal vein thrombosis, and lower extremity DVT. The median time to thrombosis was 11 days after starting L-ASNase. The relatively lower incidence can be due to a lower total dose of L-asparaginase (6000 iu/m2) for 7 days during induction with a total dose of 42,000 u/m2.(16) Meta analysis of 323 adult patients with ALL had also reported a prevalence of 5.9% during induction therapy. DVT an pulmonary thromboembolism were common site of involvement making up for the 38.9% of thrombotic events.(17) Similarly, a study conducted in Asian patients with ALL and lymphoma that were treated with L-asp also showed an incidence of 2.7% in ALL patients. Like in other studies, the prevalence was higher in adults than children (2.4% vs. 3.6%). The upper extremities were common sites of VTE occurrence. CVT contributed to only 1.7% of cases of thrombosis, unlike the results seen in other studies, which occurred only in the pediatric age group.(14)

Another multicenter prospective study included 1772 ALL patients from the ages of 1–45 years; 7.9% had symptomatic TE events. Adults had an increased risk of PE and CVT, which was more common in the adolescent age group, and in younger patients, the presence of TE was associated with increased mortality.(18)

The GRAALL experience also retrospectively assessed the prevalence of CVT in 708 adult patients treated for ALL and lymphoblastic lymphoma from 2004–2011 with different regimens that contain L- ASNase (eight doses of 6000 u/m2), and 3.1% of them had CVT overall, and 2.8% of ALL patients developed this complication. The median time to thrombosis is 18 days.(19)

As seen in the PARKAA trial, only 5% of children developed symptomatic thrombosis, even though over all, 1/3 of patients developed thrombosis.(15)

High risk groups for thrombosis

A Canadian pediatric study that included 719 patients from 1990–2005 reported a 1.5% prevalence of symptomatic CNS thrombosis. All the events were venous in origin and involved the CNS. 6/7 patients had high-risk ALL (86% of the thrombosis). (20) Similarly, high-risk ALL was identified as a risk factor for ASNase associated thrombosis in the Dana Faber consortium, along with older age at diagnosis and T-cell phenotype.(14) Age above 18 years old was also associated with increase risk in Asian study with OR 1.79. (21) A study from the Mayo Clinic had also shown a similar increased risk in T-cell ALL, but among adult patients, younger patients were at increased risk of thrombosis, unlike other studies.(22)

Other meta-analyses had shown a non-clinically significant increase in the risk of thrombosis with a lower dose of ASNase, a longer duration of use of ASNase, concomitant anthracycline, and predinsolone use. Dexamethasone was associated with a lower risk of ASNase associated thrombosis.(17) The increased risk of thrombosis in lower doses of ASNase is a unique finding in this study.

In the GRAALL 2005 study, the prevalence of thrombotic complications was higher during induction, with a 9.5% incidence, of which 36% involved the CNS.(19) Similar findings were also reported in one retrospective study in the pediatric age group, where all events happened during induction.(20)

Most of the patients included in the studies had indwelling catheters for different indications. In most studies, patients who had thrombotic events had CVC. The majority of patients who developed TE had indwelling catheters.(18).

Some studies have shown an association between the ABO blood group and the risk of thrombosis with asparaginase treatment. In a Dana-Farber Cancer Institute (DFCI) study done in pediatric age groups, the non-O blood group was significantly associated with increased risk (16.35 vs. 10.8%). (23) In another study in Maryland, including 89 patients with a mean age of 38 years, it was found that the non-O blood group was associated with an increased trend towards thrombosis associated with pegylated asparaginase treatment. In the study in Philadelphia, negative ALL was also associated with an increased risk of thrombosis. 30 patients

with Ph-negative ALL developed thrombosis with Pegylated ASNase treatment, while only one patient with Ph-positive ALL had this complication. (24)

Effect of preventive measures

L-asparaginase inhibits the production of AT in the liver. Some studies have addressed the effect of replacing AT on preventing thrombosis risk related to ASNase treatment. A retrospective study in the pediatric age group with a total of 719 patients that compared groups treated with FFP and CRY and those with no prophylactic treatment in two different centers showed that 1.5% of those who did not receive prophylaxis developed CVT during the induction phase of treatment, while none developed this complication in the prophylaxis group. The treatment group received prophylactic FFP when the AT level was below 50% LLN.(20)

The PARKAA study, which was an open-label randomized study performed in children, also reported a lower incidence of both symptomatic and asymptomatic thrombosis in children who were given anti-thrombin infusion with no increased risk of bleeding (28% vs. 37%), suggesting a trend toward the efficacy and safety of AT concentrates in preventing thrombosis associated with ASNase.(25)

Studies done on the adult population also showed similar results regarding the use of FFP. The Dutch-Belgian HOVON 37 study found that FFP administration was associated with a significantly lower incidence of thrombosis, with an OR of 0.28.(13)

In another study conducted in adults with a median age of 32 years, the rate of ASNase - associated thrombosis was lower in those who received AT infusion as prophylaxis compared to those who didn't receive prophylaxis. 4.8% of patients who received AT prophylaxis developed thrombosis, while 12.2% of the patients who didn't receive prophylaxis developed TE.(26)

A study conducted in pediatrics that included 41 consecutive ALL patients showed that the use of enoxaparin was safe and was associated with a decreased incidence of thrombosis. In the study, all patients were provided with daily doses of enoxaparin starting from the first day of ASNase treatment until 1 week after the last dose of ASNase, and none of them developed thrombosis or bleeding during this time. But one patient developed a brain infarction seven days after discontinuation of enoxaparin.(27)

On the other hand, another study in the adult population reported a similar rate of thrombosis despite therapeutic anticoagulation or fibrinogen supplementation.(26)

Laboratory parameters

Treatment with ASP was associated with a decreased level of AT. In one retrospective study, the level of AT dropped from a baseline of 94% to a nadir median level of 47% during treatment with ASNase.(22)

The same study also reported prolongation of PT and aPTT with the use of ASNase. This is also supported by a study conducted in Ghana, where 26 children with ALL and 78 controls were included and coagulation parameters and CBC were assessed. PT and aPTT were significantly higher in those treated with ASNase than controls, but were lower than in all patients who had not started treatment yet. D-dimer, antithrombin level, protein C, and protein S activity levels were also lower in the treatment group compared with controls.(28)

Similarly, plasma antithrombin levels were lower in those patients who developed thrombosis. The median AT level in another study also decreased from a baseline of 120% to 59% at the time of the fourth infusion of ASNase, and almost half of patients also had an AT level less than 60%. In the same study, fibrinogen levels also gradually decreased from the baseline value of 2.9 g/L to median level of 1.1 g/L after 5 doses of ASP.(26)

Effect on treatment outcome

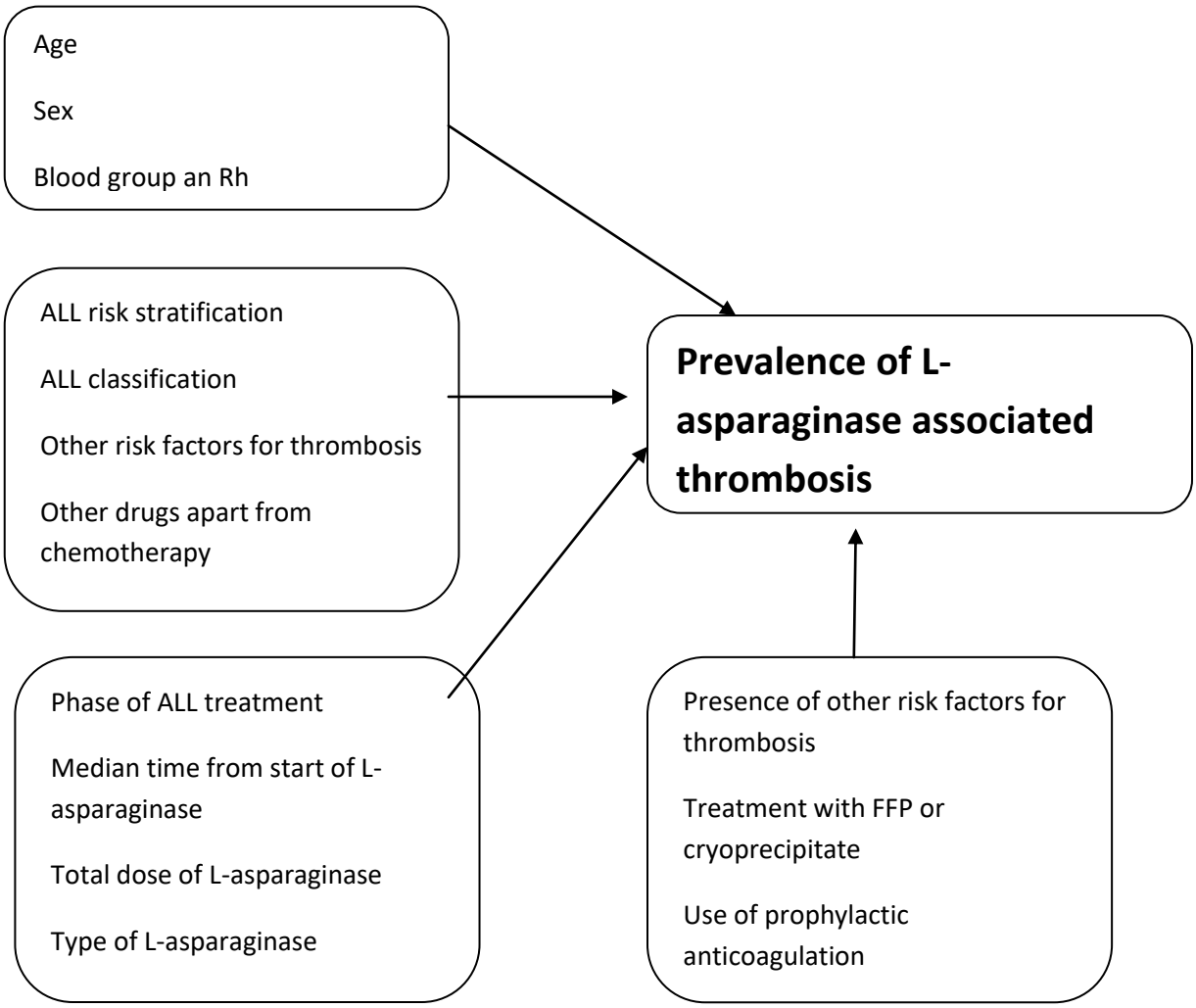
The development of thrombosis is associated with the interruption of planned doses of ASNase or complete discontinuation. This has an effect on the outcome of ALL. In addition, thrombosis is associated with event-related mortality and morbidity.

In the GRAALL experience, among patients who developed CNS thrombosis, 5% had mortality due to thrombosis, and 20% of thrombosis was associated with permanent sequelae.(15)

In a Canadian study thrombotic events were associated with additional hospital stay including ICU but all patients with thrombosis achieved remission after anticoagulation and continued ALL management.(20)

In an institution-based study that included AYA, 37.5% of patients in whom pegylated asparaginase was discontinued due to thrombosis developed ALL progression. Those patients who completed the planned dose of ASNase had similar progression-free survival and overall survival compared with those who didn’t develop thrombotic complications.(15)

2.2 Conceptual frame work



Chapter 3- Objectives

3.1 General Objective

- To assess the prevalence of thrombotic events associated with L-asparaginase treatment and it's determinants in acute lymphoblastic leukemia patients treated in Tikur Anbessa specialized hospital from November 2020- November 2023

3.2 Specific objective

- To determine the prevalence of thrombotic events associated with L-asparaginase treatment
- To identify factors associated with increased risk of thrombosis in patients who are treated with L-asparaginase
- To identify thrombosis related outcome in patients who had L-asparaginase associated thrombosis

Chapter 4- Methods

4.1Study Design

The study was an institution-based retrospective cross-sectional study done in Tikur Anbessa specialized hospital.

4.2 Study duration

The study was conducted from November 2023 to February 2024. Patients with ALL treated from November 2020 to November 2023 were included in the study.

4.3 Study area

The study was conducted in the Tikur Anbessa specialized hospital, which is found in the capital city of Ethiopia, Addis Ababa. It is the largest specialized hospital in Ethiopia and a referral center for patients from all over the country. The inpatient department has over 700 beds and provides specialty services including internal medicine, gynecology and obstetrics, surgery, and pediatrics. The College of Health Sciences provides training for undergraduate and postgraduate medical students, dentists, nurses, midwives, pharmacists, medical laboratory technologists, and radiology technologists, among others. The Internal Medicine department provides services in different subspecialties in both outpatient and inpatient departments, including emergency and intensive care units. The outpatient department is a medical referral clinic composed of, among other subspecialty clinics, cardiac, pulmonology, neurology, hematology, nephrology, and rheumatology clinics. The hospital serves as the only government setting where treatment for acute leukemia is given. Patients with acute leukemia will have their first phases of treatment as inpatients in hematology wards, then they will be transferred to the Lideta Health Center, which is part of the hospital's hematology and oncology units. They will continue other phases of treatment at the center and will follow as outpatients in the hematology clinic once they reach the maintenance phase.

4.4 Populations

Source population

All adult patients with acute lymphoblastic leukemia treated in Tikur Anbessa specialized hospital during the study period

Study population

All adult patients with acute lymphoblastic leukemia treated in Tikur Anbessa specialized hospital during the study period and fulfill the inclusion criteria

4.5 Sample size and sampling procedures

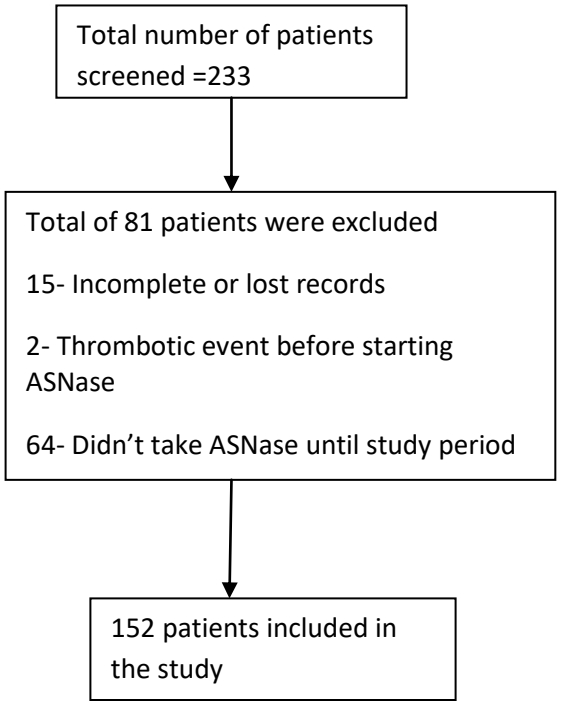
$$n = \frac{\left(Z\alpha_{/2}\right)^2 [p(1-p)]}{(d)^2}$$

Where: n = the sample size ;(Zα/2)2 = at 95% confidence interval Z value (α = 0.05) P= 50%

d = margin of error at 5% (0.05)

P was taken as 10% from a study done in Europe(14). The calculated sample size is 139, adding 10 percent non responder rate, the final sample size is 153. Among screened patient 152 fulfilled the inclusion criteria and all were include in the study. The medical records of patients with a documented diagnosis of ALL were screened from HMIS registries and electronic medical records. 64 patients were excluded because they did not take L-asparaginase until the study period. 2 patients had thrombotic events before starting ASNase, and 15 patients were excluded for incomplete or lost records. Finally, a total of 152 patients were included in the study.

Figure 2 screened patients for the study



4.6 Eligibility criteria

4.6.1 Inclusion factors

- Adult patients 13 years or older according to hospital policy
- Morphological or flow cytometry proven diagnosis of acute lymphoblastic leukemia
- Had taken or started treatment with ALL treatment protocol containing L- asparaginase

4.6.2 Exclusion factors

- Patients who had confirmed thrombosis before taking L- asparaginase
- Patients who had not taken any dose of L-asparaginase until the study period
- Patient with lost medical records

4.7 Study variable

4.7.1 Dependent variable

- L-asparaginase associated thrombosis

4.7.2 Independent variables

- Age
- Sex
- Blood group and Rh
- Risk stratification of ALL
- Immunophenotype of ALL (if flowcytometry was done)
- Type of L- asparaginase used
- Total dose of L- asparaginase taken
- Duration of L-asparaginase treatment
- Presence of other risk factors for thrombosis
- Concomitant treatment with FFP
- Pharmacologic thrombophylaxis

4.8 Operational definition

- Acute lymphoblastic leukemia – Acute leukemia of lymphocyte progenitor origin that is confirmed by morphology or flowcytometry.
- Thrombotic events- any symptomatic or asymptomatic thrombotic event involving the arterial or venous system and confirmed by appropriate imaging modality.
    - For CNS thrombosis confirmation by magnetic resonance imaging (MRI),magnetic resonance venography (MRV) or Compute Tomography scan
    - For pulmonary embolism computed tomography pulmonary angiography
    - For deep venous thrombosis doppler or compressive ultrasound
    - For superficial venous thrombosis ultrasound
    - For myocardial infarction confirmed by electrocardiography or cardiac biomarker
- Complete hematologic remission- Leukemic cells not detectable by light microscopy (<5% blast cells in bone marrow)
- Hematologic relapse- >5% ALL cells in bone marrow/blood
- High risk ALL- patients with any of the following features
    - High WBC count at diagnosis >30,000/microL for B- ALL and >100,000/microL for T-ALL

- BCR/ABL-1 like gene signature
- Progenitor B- cell immunophenotyoes expressed including CD79a,CD19 and cytoplasmic CD22 but not CD10
- Length of time from start of induction therapy to attainment of CR greater than four weeks
- Older age >60 years
- Clonal cytogenetic abnormalities t(4;11),t(9;22),t (1;19)
- MRD- a post remission bone marrow MRD level $\geq 10^{-3}$ using patient specific Ig/TCR gene re arrangements

N.B MRD and cytogenetics are not routinely done in our setup, therefore the other criterias were used to assess risk stratification.

- Intermediate risk – none of the high risk criteria met and age 30-59 years
- Standard risk ALL- none of the intermediate or high risk criteria are met
- Interruption in treatment of ASNase- patients who Didn't take ASNase on the scheduled date or missed one or more doses

4.9 Data collection procedure and tools

Ethical clearance was obtained from the Institutional Review Board in November 2023, and data collection was started. A structured questionnaire was used to collect the data, and data collection was conducted by the primary investigator. Data was collected from patients' medical records, both paper and electronic, from December 2023 up until January 2024. Data collection included secondary data from the patient's medical records (both paper and electronic).

4.10 Data Quality Assurance

The data was assessed for accuracy and completeness by the primary investigator before proceeding to data analysis. A pre-test was done using 5% of the sample size to clarify any queries and inconsistencies on the checklist before official data collection was started.

4.11 Data analysis

After quality was assessed for accuracy, it was entered into the SPSS version 26 program. The data was re-coded and analyzed. Descriptive statistics were computed for each variable. Depending on the normality of the data mean, the median interquartile ratio and standard deviation were computed and summarized in tables and graphs.

A chi-square test was done to assess the relationship between dependent and independent variables. A bivariate test was done to calculate the crude odds ratio. Those variables that had a significant association based on the Hosmer and Lemshow test were included in the multivariate analysis. The adjusted odds ratio was analyzed, and a *P* value of <0.05 was taken as statistically significant. A 95 percent confidence interval was also calculated for the variables.

4.12 Ethical consideration

Ethical clearance was obtained from the Institutional Review Board (IRB) of Tikur Anbessa Specialized Hospital. A formal letter of permission was received from the hospital's administration before starting the study. The collected data was kept confidential and was used for study purposes only.

4.13 Data dissemination

The final result of the research will be submitted to the Addis Ababa University School of Medicine. The results will be presented during the defense. The copies of the results will be available to the Internal Medicine Department and the hospital's administration. Publication in scientific journals will be attempted.

Chapter 5 –Results

5.1 Socio-demographic characteristics of the study participants

A total of 152 patients were included in this study. The median age of the participants was 22.5 years (IQR 18, 30.8). The majority (47.4%) of the participants were in the age group of 18-29 years, and 59.9% of the study populations were male. The following table explains the sociodemographic and baseline characteristics of the participants.

Table5.5. The socio-demographic and baseline characteristics of the study participants

Variable		Frequency	Percent
Age in years	<18	36	23.7
	18-29	72	47.4
	30-40	21	13.8
	>40	23	15.1
sex	Male	91	59.9
	Female	61	40.1
Blood group and RH	A positive	47	30.9
	B positive	38	25
	AB positive	4	2.6
	O positive	50	32.9
	A negative	3	2.0
	AB negative	1	0.7
	O negative	9	5.9
Blood group category	O blood group	59	38.8
	Non O blood group	93	61.2

5.2 Disease Characteristics of the Study Participants

Out of 152 patients, only 32 (21%) of the participants had flow cytometry done. Among these, 16 (10.5%) were B-ALL and 16 (10.5) were T-ALL. Philadelphia chromosome status was determined in 109 (71.7%) patients, and among tested patients 17 (15.5%) had a positive result.

The median initial WBC count was 27,200/µl. Almost half of the study participants had an initial WBC of less than 30,000. The initial WBC count was more than 100,000 in 28.3% of the participants. When risk stratification was made based on WBC count at presentation according to the criteria mentioned in the operational definition, 25% of B-ALL & 56.2% of T-ALL patients fall in to high risk group.

The commonest blood group was O positive, accounting for 32.9% of participants, followed by A positive (30.9%). On further classification, 38.8% of patents have an O blood type, including both O positive and O negative. 61.2% had a non-O blood group. Details of the disease characteristics of the study participants are summarized in Table 2.

Table 5.6 Acute Lymphoblastic Leukemia Characteristics of the Study Participants

Variable		Frequency	Percent
ALL subtype	B- ALL	16	10.5
	T- ALL	16	10.5
	Not known	120	79
PH chromosome	Positive	17	11.2
	Negative	92	60.5
	Not known	43	28.3
Initial WBC (cells/dl)	<30,000	77	50.7
	30,000-100,000	32	21.1
	>100,000	43	28.3
Initial WBC median(IQR)	27.2(4,109) x 1000/µl		

5.3 Risk factor for thrombosis

Among the study participants, 84.5% of patients were treated with the pediatric inspired ALL CL10403 regimen and the rest with the adult GALGB8811 protocol. Of these, 91(59.9%) of the patients achieved CR as assessed by peripheral morphology and bone marrow aspiration within 4 weeks of remission induction, while 11 (7.2%) required extended remission induction for two more weeks. In 50(32.9%), the time to CR was not assessed because the patients died or went against medical advice during remission induction or thereafter before the assessment of complete remission was done.

Fourty-four percent (n = 67) of the study participants had interruptions in L-asparaginase treatment and, the reason was hepatotoxicity in 41.7%.

Only 2.6% of the participants received FFP transfusions. Similarly, only 5 patients (3.3%) received pharmacologic prophylaxis at one point during the treatment period. None of the participants had previous thrombotic events. Five patients had documented risk factors for thrombosis. These were a recent cesarean section in one patient and, OCP use for abnormal uterine bleeding and immobilization due to ICU admission each in two patients. None of the patients with above risk factors developed thrombosis.

Table 5.7 Risk factor for thrombosis and ALL related treatment

Variable		Frequency	Percent
Documented base line coagulation	Yes	35	33
	No	117	77
PT	median (IQR)	13.4(11.9,15.1)	
INR	mean ± SD	1.21±0.17	
PTT	median (IQR)	31(27.9,35.8)	
Phase of treatment	Remission induction	63	41.4
	Consolidation(EI)	28	18.4
	Interim maintenance/CNS prophylaxis	10	6.6
	Delayed intensification	9	5.9
	maintenance	42	27.6
Duration of CR achieved in weeks	04 weeks	91	59.9
	> 4 weeks	11	7.2
	Not applicable	50	32.9
total dose of L asparaginase (in 1000 IU/m2) Median(IQR)		39(24,108)	
Was L-asparaginase interrupted?	Yes	67	44.1
	No	85	55.9
Reason for interruption (n=67)	Hepatotoxicity	28	41.7
	Thrombotic event	10	14.9
	Medication unavailabilty	17	25.3
	others	14	20.8
Did patient receive pharmacologic thrombophylaxis?	Yes	5	3.3
	No	147	96.7
Did patient received FFP transfusion?	Yes	4	2.6
	No	148	97.4
The regimen for treatment of ALL	Adult CALGB	24	15.8
	pediatric inspired ALL CL10403 regimen	128	84.2

5.4 L Asparaginase associated thrombotic events

In this study, among 152 ALL patients treated with ASNase containing chemotherapy regimens, 17 (11%) developed thrombotic events. All the thrombotic events were venous thrombosis. 41.2% of the thrombotic events occurred during remission induction, followed by 33.3% during consolidation 1 (early intensification).

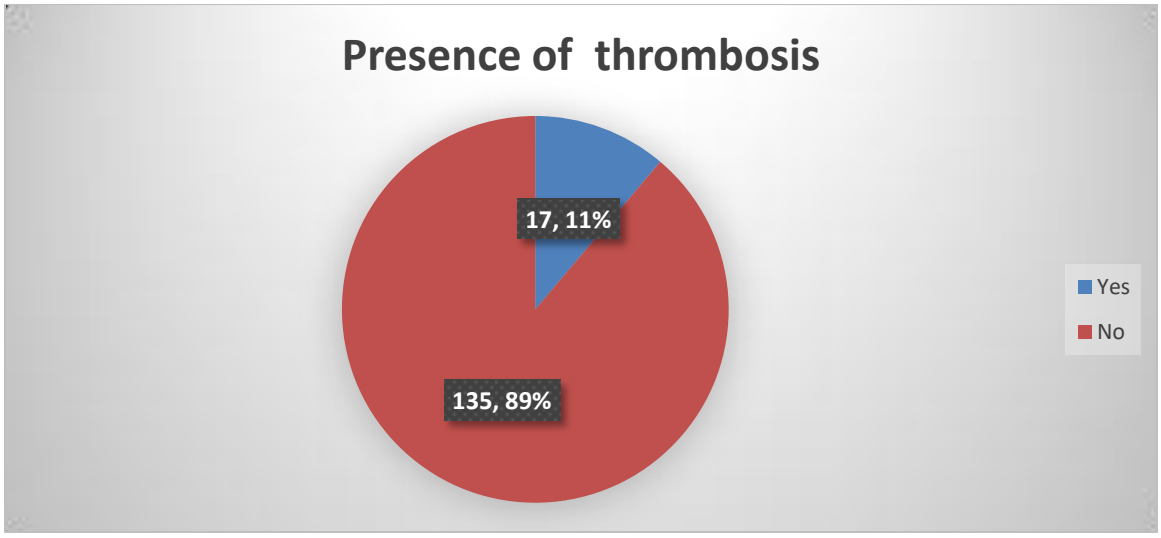


Figure 3. The prevalence of thrombosis among ALL patients.

Cerebral venous thrombosis was the most common site involved, accounting for 41.2% of the thrombotic events. Lower limb DVT was seen in 6 patients (35.3%), one of whom had a concomitant PE. Two patients had an upper limb DVT. One patient had an internal jugular vein thrombosis, and one patient had a cephalic vein thrombosis.

Extremity venous thrombosis and internal jugular vein thrombosis were confirmed by doppler studies. PE was confirmed by CTPA. CVT was confirmed by MRV in 4 out of 7 patients, while it was diagnosed by MRI in two patients & by contrast CT in the rest 1 patient. All of the thrombotic events were symptomatic except one patient who had asymptomatic IJV thrombosis. Among CVT patients, seizure was the commonest manifestation, followed by decreased mentation. One patient presented with a headache and cranial nerve palsy (ptosis). Extremity DVTs presented with swelling and pain. The pulmonary embolism presented with sudden onset respiratory distress and was confirmed by CTPA. Later on, a Doppler study confirmed the presence of DVT in the same patient. All patients had a determination of coagulation at the time of thrombosis before the initiation of anticoagulation. Of these patients, 88.2% had a normal coagulation profile. Details of the thrombotic events are summarized in Table 5.4.

Table 5.4 Characteristics of thrombotic even

Type of thrombosis	Venous	17	100
	arterial	0	0
Site of venous thrombosis	CVT	7	41.2
	Sphlanchnic circulation	0	0
	Lower limb DVT	6	35.3
	Upper limb DVT	2	11.8
	PTE	1	5.9
	others	2	11.8
symptoms	Seizure	5	
	Decreased mentation	3	
	Extremity pain	7	
	Swelling	8	
	Cranial nerve palsy	1	
	Respiratory distress	1	
	Asymptomatic	1	
Time from initiation of L-asp to thrombosis Median (IQR) in days		37(24,60.5)	
Doses of L-asp taken before onset of thrombosis(1000U/m2) median (IQR)		36(33,63)	
Phase of treatment that thrombosis occurred	Remission induction	8	41.2
	Consolidation 1(EI)	6	35.3
	Consolidation 2(Interim maintenance)	1	5.9
	Delayed intensification	2	11.8
	maintenance	1	5.9
Coagulation profile at time of thrombosis	PT median (IQR)	14(12.4,14.9)	
	PTT	30.3 ±5.6	
	INR	1.21± 0.17	

5.5 Treatment Outcome of characteristics of the thrombosis

All patients with thrombosis received anticoagulation. There was overlap between different anticoagulants, and patients were switched from one anticoagulant to the other. Unfractionated heparin was used in 58.8% of cases. Two patients received warfarin. Rivaroxban and enoxaparin were used in was 47.1% and 11.8%, respectively.

Nine of the patients (52.9%) had thrombosis-related complications, including death in four patients. One patient had permanent physical impairment (Ptosis) from thrombosis, and four

required ICU admission and subsequently died. The cause of deaths was increased ICP in 2 patients with CVT, and respiratory failure in the other 2 patients. One of them had PE that was angiography confirmed, and in the second patient, PE was suspected without CTPA confirmation but had a confirmed DVT. The cause of death in the remaining 4 patients with thrombosis was ?intracranial hemorrhage in one patient with history of CVT, sudden cardiac arrest sec ?massive PE in a patient with CVT, and sepsis in the rest two.

Table 5.5 Treatment of L-asparaginase associated thrombosis

Variable		Frequency	Percent
Mechanism of management			
Was anticoagulation administered	Yes	17	100
	No	0	0
Types of anticoagulation (n=17)	Warfarin	2	11.8
	UFH	10	58.8
	Enoxaparin	2	11.8
	Rivaroxaban	8	47.1
Duration of anticoagulation	Median(IQR)	60(4.5,120)	
Presence of complication due to thrombosis(n=17)	Yes	9	52.9
	No	8	47.1
Types of complications (n=10)	Death	4	44.4
	Required ICU admission	4	44.4
	Permanent physical impairment	1	11.1
Was ASNase resumed after thrombotic event? (n=17)	Yes	3	17.6
	No	14	82.4

5.6 The ALL-related outcome of the study participants

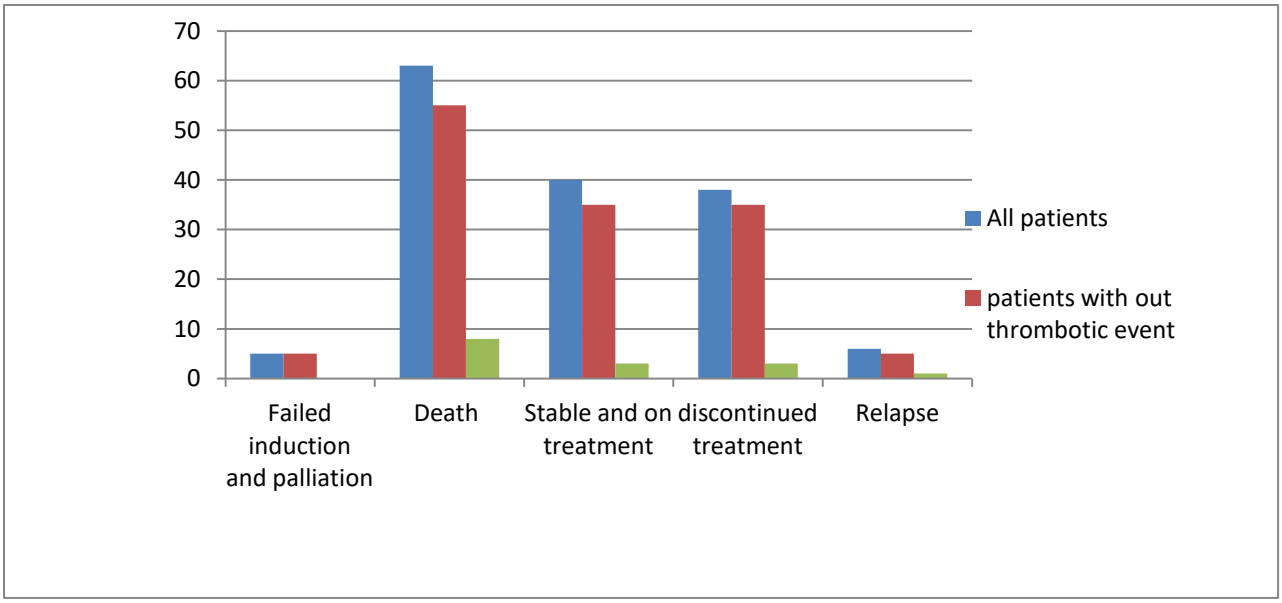
Among patients with thrombosis, 8 (47%) died. Five patients discontinued treatment. One patient had a relapse and was sent abroad for further treatment. Three (17.6%) are stable and on follow – up; currently they are all in the maintenance phase of the treatment. The relation between the outcomes and thrombosis was not statistically significant

Table 5.6 ALL related outcome f patients

ALL related outcome of the patient			Thrombotic event				*P* value
	Total		Yes		No		
	n	%	n		%		
Failed induction and palliation	5	3.3	0	0	5	3.7	0.42

Death during RI or afterwards	63	41.4	8	47	55	40.7	0.618
Still on treatment and stable	40	26.3	3	17.6	37	27.4	
Discontinued treatment	38	25	5	29.4	33	24.4	
Relapse	6	3.9	1	5.9	5	3.7	0.664

Figure 4 The ALL-related outcomes of the study participants



5.7 The chi-square relation between thrombotic events and independent variables

The chi-square test revealed that study participant age (*P*-value = 0.131), blood group (*P*-value = 0.057), and duration to achieve CR (*P*-value = 0.004) had a statistically significant correlation with thrombotic events, as shown in the table below. In addition, there was a statistically significant association for the ALL subtype in those who had flow cytometry and use of pharmacologic prophylaxis.

On the other hand, Philadelphia chromosome (*P*-value = 0.341), sex (*P* value = 0.253), use of FFP (*P* value = 0.472), initial WBC count (*P* value = 0.935), phase of treatment (*P* value = 0.742), and total dose of L-asparaginase (*P* value = 0.298) did not show significant correlation.

Table 8.7 The chi-square relation between the thrombotic events and independent variables

Variable		Thrombosis event		Chi-square value	*P*-value
		yes	No		
Age in years	<18	2	34	5.62	**0.131**
	18-29	6	66		
	30-40	4	24		
	>40	5	11		
Sex	Male	8	83	1.307	0.253
	Female	9	52		
ALL sub type	B-ALL	4	12	2.13	**0.14**
	T-ALL	1	15		
PH Chromosome	Positive	3	14	.906	0.341
	Negative	9	83		
Blood group	O	3	56	3.612	**0.057**
	Non-O	14	79		
Duration to achieve CR	4 weeks	11	80	8.26	**0.004**
	>4 weeks	5	6		
Regimen	CALGB	5	19	2.671	**0.102**
	Pediatric inspired regimen	12	115		
Pharmacologic prophylaxis	Yes	3	132	4.322	**0.038**
	No	2	15		
Interruption in L-asparaginase	Yes	80	6	3.530	**0.060**
	No	55	11		

5.8 The determinant factors of thrombotic event

A bi-variate regression analysis was done for the independent variables, and if a statistically significant association was seen based on the Hosmer-Lemeshow test, they were included in the multivariate regression analysis. The type of regimen was not included in the multivariate analysis, despite having statistically significant association in the bivariate logistic regression. This is because it overlaps with age, since patients older than 40 were treated with an adult CALGB regimen.

As described in Table 8, study participants' age, blood group, time to achieve CR, and use of prophylactic anticoagulation were significantly associated with thrombotic events by the bivariate logistic regression. In addition, the type of regimen also has a significant association. ALL subtype was determined only in 21% of the participants for this reason; it was not included

in the multivariate regression analysis, despite a significant association in the bi-variate regression analysis.

The multi-variate regression analysis showed that age above 40 years had 10.4-fold increased thrombotic event compared to age <18 years (AOR = 10.4, 95% CI =1.47, 75.0). Similarly, patients whose time to achieve complete remission was more than 4 weeks had a 4.8 fold increase in thrombotic events when compared to those who achieved CR within 4 weeks (AOR = 4.8, 95%CI = 1.10, 20.72).

Table 5.8 The bivariate and multivariate regression analysis for association between independent variable and thrombotic events among ALL patient.

Variable		Thrombosis event		*P*-value	COR(95%CI)	*P*-value	AOR (95%CI)
		yes	No				
Age in years	<18	2	34	1		1	
	18-29	5	66	0.606	1.5(0.30, 8.07)	0.671	1.5(0.25, 8.48)
	30-40	4	24	0.130	4.0(0.66, 24.06)	0.501	2.1(0.24, 19.1)
	>40	6	11	0.080	4.7(0.83, 26.81)	0.019	**10.4(1.47, 75.0)**
Blood group	O	3	56	1		1	
	Non -O	14	79	0.070	3.31(0.91, 12.05)	0.126	3.2(0.72, 14.01)
Time to achieve CR	4 weeks	11	80	1		1	
	>4weeks	5	6	0.009	6.06(1.58,23.23)	0.037	**4.8(1.10, 20.72)**
Pharmacologic therapy	Yes	2	3	1		1	
	No	15	132	0.063	0.17(0.03,1.10)	0.176	0.21(0.02,2.04)

Chapter- 6 Discussion

L-asparaginase is an important component of treatment regimens for acute lymphoblastic leukemia. However, it is associated with a significant risk of thrombotic events. In this study, we analyzed the prevalence of thrombosis in ALL patients treated from November 2020 to November 2023 with ASNase containing regimens in Tikur Anbessa specialized hospital.

The prevalence of thrombosis in our study was 11.2%. This is comparable to a multicenter study done in Europe that reported a prevalence of 10%. The prevalence is lower than the Dana Faber Consortium study, which showed 34%.(14) This could be due to differences in the characteristics of patients. In our study, 23.7% of patients were <18 years old. There was also no indwelling catheter use in our study that might affect the thrombotic events.(18)

The prevalence is also lower than in a study from Ohio University, where the reported rate was 23.5% among adolescents and young adults treated with pegylated asparaginase. (15) In addition to the aforementioned reason of the absence of an indwelling catheter, in our study, patients were treated with E.coli L-asparaginase, which might contribute to the difference in incidence. The prevalence is higher than some of the studies done on the adult population.

Differences in treatment protocol, genetic variation, use of antithrombin infusions and the presence of other risk factors for thrombosis could contribute to the difference in prevalence.

CVT was the commonest site of involvement with thrombosis in our study, which is a similar finding with other studies. (12)(16)(18) However, upper extremity DVT was the most common site of involvement in some of the studies, but in our study, the prevalence of upper extremity DVT was lower (11.1%). (14)(13)

Thrombotic events were more frequent during remission induction in this study (44.4%), but the association was not statistically significant (*P* value = 0.74). This has also been reported in other studies. A higher number of thrombotic events occurred during remission induction in the GRAALL 2005 study. All thrombotic events were during remission induction in a retrospective study in pediatric age. Other studies have also shown similar findings. (12)(13)(15)(19)(20)

In this study, older age, specifically age ≥ 40 years, was associated with a statistically significant risk of ASNase associated thrombosis, with an AOR 10.4 (95% CI =1.47, 75.0). This finding is in accordance with the finding from different studies. The Dana Faber Consortium identified that older age was associated with an increased risk of L-asparaginase associated thrombosis. (14) On the other hand, a study from the Mayo Clinic reported that among adult patients, younger age was associated with an increased risk of thrombosis. (22)

High risk ALL has been shown to be associated with an increased risk of thrombotic events in previous studies.(14) In a Canadian study conducted in the pediatrics age group, six out of seven patients that developed thrombosis (86%) had a high risk ALL. Similarly, in the Dana Faber Consortium, high risk ALL was associated with an increased risk of thrombosis. (14)(20)

In this study among variables used to assess for high risk ALL patients who didn't achieve complete remission after initial RI were at increased risk of thrombosis with AOR 4.8(1.10, 20.72).

The absence of BCR/ABL mutation was associated with a lower risk in the chi-square test but was not significant (*P* = 0.34). Similarly, a high WBC count at presentation was not significantly associated with an increased risk of thrombosis in our study (*P* value = 0.935).

Some studies have shown that patients with a non-O blood group were at a higher risk of L-asparaginase associated thrombosis, when compared to patients with an O blood group. In a study done in pediatric age group, 16.35% of patients with a non-O blood group developed thrombosis, compared to 10.8% of patients with an O blood group. (23)(24) In our study, on the bi-variate analysis, the non-O blood group was associated with an increased risk of thrombosis with a COR of 3.31 (0.91, 12.05). But the association was not statistically significant in the multivariate analysis (*P* = 0.13).

Preventive measures, including the use of prophylactic anticoagulation, showed a decreased risk of thrombosis in one pediatric study. (27) Similarly, in our study, the use of unfractionated heparin showed a decreased risk of thrombosis with a COR of 0.17 (0.03, 1.10), but the result was not statistically significant on the multivariate analysis. This could be explained by the very low number of patients who were given prophylactic anticoagulants (5 out of 152).

Even though a decreased risk of thrombosis was reported with the use of FFP, in our study the association was not statistically significant (*P* value = 0.472). (13)(20)(25) This could also be because of the use of AT infusions in addition to the FFP in these studies. In addition, only four of the patients in our study received FFP.

In this study, the mortality rate of patients who developed thrombotic complications was slightly higher than ALL patients with no thrombosis (47% vs. 41.4%) but was not statistically significant (0.618). The thrombosis related mortality was also higher (23.5%) in our study, compared with the other studies. (15) This could be explained by that of overall higher mortality and the difference in the availability of treatments and the treatment setup. Four out of the seventeen patients also required ICU admission as a complication of the thrombotic events. However, there was no statistically significant association of thrombosis with death, relapse or failed induction.

Strengths of the study

The study is the first in our setup to assess the thrombotic complications associated with L-asparaginase. It gives an insight into the burden of the condition and the factors contributing to this complication.

Limitations of the study

Since the study is retrospective it is difficult to assess detailed risk factors and laboratory parameters, including the coagulation profile. Due to missed medical records, not all patients treated during the study period were included in the study. For some variables, for instance, the use of FFP and prophylactic anticoagulation, the number of patients who received such treatment is very small, and that may affect the correlation. ALL subtype and Philadelphia chromosome were not assessed for all patients, and that might affect the outcome. The study is an institution based study done in one center; hence, the result may not reflect the general population.

Recommendations

The result of this study reflects that a significant number of patients develop thrombosis related to ASNase treatment. We recommend further prospective studies to look deep into the contributing factors and comparative studies, including preventive measures.

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Annex

Addis Ababa University College of Health Science

School of Medicine Department of internal medicine

Questionnaire

Title of the study - Prevalence of L asparaginase associated thrombotic events and associated factors in adult patients with acute lymphoblastic leukemia in Tikur Anbessa Specialized Hospital

1. Socio demographic data

1.1 Age

1.2 Sex

2. Baseline patient characteristics

2.1 ALL subtype

A) B ALL

B) T ALL

C) Not known

2.2 Philadelphia chromosome

A) Positive

B) Negative

C) Not known

2.3 Blood group and Rh

2.4 What was the initial WBC count at time of presentation?

A) <30k

B) 30-100k

C) >100k

3. **Risk factors for thrombosis**

3.1 did the patient have previous history of thrombosis

3.1.1 If yes site of thrombosis

3.2 Does the patient have any of the following risk factor for thrombosis?

A) Recent surgery

B) Immobilization

C) Indwelling central venous catheter

D) Current OCP use

E) Family History of thrombosis

3.3 Was there determination of coagulation profile before the start of L- asparaginase? If yes document the value

4. Treatment for ALL

4.1 Which phase of treatment did the patient complete or was taking?

A) Remission induction

B) Consolidation

C) Interim maintenance

D) Delayed intensification

E) Maintenance

4.2 If CR was achieved, how long did it take to achieve it?

A) 04 weeks

B) >04 weeks

c) not applicabe

4.3 How much was the total dose of L asparaginase patient took?

4.4 Was L-asaraginase interrupted during treatment protocol?

4.3.1 If yes what was the reason for discontinuation?

A) Thrombotic Event

B) Hepatotoxicity

C) Pancreatitis

D) Other (specify)

4.5 What is the type of L asparagines the patient was taking?

4.6 Did the patients take any pharmacologic prophylactic anticoagulation?

4.7 Did the patient receive FFP transfusion?

4.8 What regimen was used for treatment of ALL?

A) CALGB

B) Pediatric inspired CL10403 regimen

5. Treatment related complication

5.1 Did the patient have venous thrombotic events during treatment?

5.2 If the answer to question number 5.1 is Yes Where is the Site of thrombosis?

A) CVT

B) PE

C) Splanchnic Vein Circulation thrombosis

D) Lowe limb DVT

E) Upper limb DVT

F) Others (Specify

5.3 Did the Patient have arterial thrombotic Event?

5.4 If the answer to question number 5.3 is yes where is the site of thrombosis?

A) Cerebral arteries (Stroke)

B) Peripheral extremity arteries

C) MI

D) Others (Specify)

5.5 How was thrombosis confirmed?

5.6 Time from initiation of L asparaginase and onset of thrombosis?

5.7 How many doses of L asparaginase taken before onset of thrombosis?

5.8 Was thrombotic event

A) Asymptomatic, incidental finding

B) Symptomatic?

If symptomatic- Mentions symptoms

5.9 Was there documentation of coagulation profile result at time of thrombosis? If yes document the values

6. Outcome of treatment

If the answer to question number 5.1 was yes (for questions 6.1-6.4)

6.1 If patient had thrombosis how was thrombosis managed?

A) Anticoagulation administered

B) Not administered

6.1.2What anticoagulation did the patient receive?

6.1.3 How long the patients receive anticoagulation?

6.2 Any complication due to thrombosis?

A) Yes

B) No

6.3 If the answer to question 6.2 is yes what was the complication?

A) Death

B) Required ICU admission

C) Permanent Physical impairment

D) Prolonged the hospital stay

E) If others mention

6.4 Was L-Asparginase resumed after discontinuation following the thrombotic event?

A) Yes

B) Never resumed

6.5 Final ALL related outcome of the patient?

A) Refractory (Failed Induction) & discharged for palliative intent

B) Death (during RI or afterwards)

C) Still on treatment & stable

D) Went against during treatment

D) Lost from follow up before completing treatment

E) Lost from ff after completing therapy including long-term maintenance

F) Relapse