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Apixaban to Prevent Thrombosis in Adult Patients Treated With Asparaginase

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APIXABAN TO PREVENT THROMBOSIS IN ADULT PATIENTS TREATED WITH ASPARAGINASE

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ABSTRACT

The outcomes of pediatric acute lymphoblastic leukemia (ALL) have improved dramatically after intensification of the multi-agent chemotherapy backbone over a period of time starting from 1960's, and asparaginase (ASP) is an important part of this success story. Adoption of such strategy in adult ALL and other high grade lymphoid neoplasms had been difficult due to higher anticipated toxicities including thrombosis. Many thrombosis prevention strategies including antithrombin (AT) replacement, Enoxaparin, unfractionated heparin (UFH) was studied but there is no firmly established evidence-based approach yet. We report the safety and efficacy of thrombosis prophylaxis with apixaban 2.5 mg orally twice daily for up to 3 weeks starting on the first day of receiving ASP. Characteristics of the 20 patients treated with ASP between the years 2017-2020 included a median age of 29.5 years (range 19-63 years), 80% male, 70% whites. Indication for ASP was ALL in 90%. A total of 75% received cryoprecipitate with a median of 10 units (range 0-105 units) in a 4-week period following ASP. The total incidence of thrombosis was 10% and 5% incidence while on anticoagulation. The incidence of major bleeding was 5% while on anticoagulation with apixaban. In conclusion, our study demonstrates the safety of using apixaban for thrombosis prophylaxis and concurrent cryoprecipitate.

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LIST OF ABBREVIATIONS

ALL Acute Lymphoblastic Leukemia

ALT Alanine Aminotransferase

ASP Asparaginase

AST Aspartate Aminotransferase

AT Antithrombin

AYA Adolescent and Young Adults

BMI Body Mass Index

CBC Complete Blood Count

CNS Central Nervous System

COG Children's Oncology Group

CALBG Cancer and Leukemia Group B

DVT Deep Venous Thrombosis

FFP Fresh Frozen Plasma

FISH Fluorescence in Situ Hybridization

Hyper-CVAD Hyper fractionated Cyclophosphamide, Vincristine, Adriamycin and Dexamethasone

ISTH International Society of Thrombosis and Hemostasis

LMWH Low Molecular Weight Heparin

PEG Pegylated asparaginase

PCR Polymerase Chain Reaction

UFH Unfractionated Heparin

VTE Venous Thromboembolism

WBC White Blood Cell

CHAPTER 1: INTRODUCTION

1.1 Acute Lymphoblastic Leukemia

Approximately 6150 children and adults are newly diagnosed with acute lymphoblastic leukemia (ALL) in the USA in 2020 with 1520 estimated deaths[1]. ALL is the most common childhood cancer and the second most common acute leukemia in adults. ALL represents 20% of adult leukemias with a median age of 15 years at the time of diagnosis of ALL; 55% of patients are diagnosed before 20 years of age, 28% are diagnosed at the age of 45 years or older, and 12% after 65 years of age[2]. The outcome of childhood ALL has significantly improved over time even before the advent of many newer agents. One of the cornerstones of such success is the use of L-asparaginase during induction and delayed intensification phases of the treatment. This strategy was adopted later for the treatment of Adolescent and Young Adults (AYA) with ALL resulting in improvement in outcomes [16-39 years]. Based on the analysis of the Surveillance, Epidemiology, End Result (SEER) database, the overall survival of childhood ALL [0-15 years] has improved significantly to around 90%, as compared to 61% in AYA and 10-40% in adults (40-60 years) with ALL with older adults (>60 years) having the worst outcomes[3]. Several factors contributed to improvement in the outcomes of ALL in childhood and AYA including more intense multiagent chemotherapy backbone, aggressive central nervous system (CNS) prophylaxis and incorporation of L-asparaginase.

1.2 Management of ALL

A lymphoblast percentage of 20% or greater is seen in a bone marrow biopsy is diagnostic for ALL and if there is evidence of nodal or extra-nodal disease with <20% blasts in bone marrow, it is referred to as acute lymphoblastic lymphoma. In adults, 75% of the ALLs are B-ALL and the rest are T-ALL. White blood cell (WBC) count at presentation, certain recurrent cytogenetic changes, the ploidy of the chromosomes (hypodiploid Vs hyper diploid karyotype), presence of Philadelphia (Ph) chromosome [t(9;22)] risk stratifies ALL into “standard or good risk” versus “poor risk” ALL[4]. More recently a Ph-like ALL subgroup of B-ALL defined by immunophenotypic expression of CRLF2 by flow cytometry or gene fusion and mutations discovered by Fluorescence in Situ Hybridization (FISH) or gene expression is estimated to have decreased disease free and overall survival in young adults compared to non-Ph-like ALL[5]. Often poor risk changes are more prevalent in AYA and older adults compared to children. Cerebrospinal Fluid (CSF) or CNS involvement is associated with poorer outcomes[6] and proper intrathecal chemotherapy prophylaxis is important to improve outcomes even when leukemic blasts are not detected in CSF. Conventionally the treatment of ALL is divided into a) Induction b) Consolidation c) Delayed intensification, and 4) Maintenance. The presence of minimal residual disease detected by multiparameter flowcytometry or Polymerase Chain Reaction (PCR) based techniques predicts poor outcomes and a higher risk of relapse.

Conventionally, the chemotherapy back bone consists of anthracycline, cyclophosphamide, vincristine, corticosteroid, methotrexate and asparaginase. Tyrosine kinase inhibitors are added to the chemotherapy backbone in Ph positive ALL. Typically, there is higher cumulative dose of chemotherapy drugs including CNS prophylaxis in pediatric ALL regimens and lower in adult ALL regimens. In older ALL (≥60 years) some of the drugs are either significantly dose reduced or avoided all together to minimize the

toxicities. The dose of methotrexate is significantly reduced, anthracyclines and asparaginase completely avoided in older ALL regimens compared to AYA and adult ALL regimens. Modifications make induction regimens “high-intensity”, “moderate-intensity” or “low-intensity”[7].

1.3 Role of Asparaginase

From the fascinating results of mouse experiments conducted by John Kidd in 1953, it was discovered that transplanted lymphomas regressed following normal guinea pig serum injected intraperitoneally into the mice carrying them[8]. In 1922, Clementi described that serum of guinea pigs and no other mammals is rich in an enzyme L-asparaginase[9]. Inspired by those findings, D. Broome demonstrated in 1963 the evidence showing that L-Asparaginase of guinea pig serum is responsible for its anti-lymphoma effect[10]. Subsequently, many studies have shown significant improvement in event free survival and overall survival in childhood ALL with the addition of ASP to the chemotherapy regimen[11]. Due to frequent toxicities with ASP in adults in the earlier studies in 1970s, its inclusion in the adult ALL regimens had previously fell out of favor.[12] However, due to a significant lag in the outcomes of adult ALL compared to the childhood ALL, the advocacy for inclusion of ASP in the adult regimens has significantly increased in more recent years. ASP hydrolyzes L-asparagine, an endogenous amino acid required for proliferation of ALL cells, which results in cytotoxic effect as asparagine depletion affects RNA, DNA and protein synthesis. This phenomenon is evident in patients receiving ASP with hypoproteinemia, hypofibrinogenemia, as well as low levels of plasminogen, antithrombin, protein C, and protein S[13]. Unlike ALL cells, normal cells are capable of synthesizing asparagine and are not solely reliant on the circulating asparagine, hence are protected from depletion of L-asparagine.

1.4 Pharmacokinetics and toxicities of Asparaginase

ASP has 70-80% of estimated plasma volume of distribution and is minimally excreted in urine. Though ASP has significant anti-leukemia effect, it is associated with significant toxicities, which are more severe in adults than children. Hypersensitivity reactions, and development of anti-ASP antibodies jeopardizing the efficacy of ASP led to development of PEGylated ASP (PEG-ASP), which has lower incidence of such side effects and currently only pegylated formulations of E. coli asparaginase (PEG-ASP and monomethoxy-polyethylene glycol ASP with succinimidyl carbonate linker) are available in the USA due to less side effects and longer half-life requiring lesser injections of the drug with at least equal efficacy. ASP is mainly manufactured from two sources, E. coli and a different gram-negative bacterium Erwinia chrysanthemi. Erwinia ASP was mainly developed to circumvent the frequent hypersensitivity reactions observed with native E. coli ASP. The efficacy and toxicities of ASP depends on the clearance. PEGylation of ASP increase the half-life of ASP and decrease in incidence of development of anti-ASP antibodies. PEG-ASP has lower incidence of hypersensitivity reactions. In patients with anaphylaxis where its use is contraindicated, Erwinia ASP is used. It is less effective in depleting asparagine compared to E. coli ASP. The half-life of various formulations after IM administration were shown to be 0.65, 1.28 and 5.73 days for Erwinia, E. coli and PEG ASP respectively[14]. It is also important to emphasize that the pharmacokinetics also depend on the age as adults have lower ASP clearance compared to children. A study showed IV PEG-ASP at 2500 IU/m^2 maintained therapeutic levels of 0.1 IU/ml or higher for up to 21 days in children, whereas a dose of 2000 IU/m^2 was able to maintain similar level for up to 25 days in adults[15]. Therefore, there is a higher risk of toxicities from ASP in adults compared to children, and the doses used in children cannot be used in adult regimens. In addition to hypersensitivity reactions, ASP has many other

significant side effects including bleeding, thrombosis [including cerebral venous sinus thrombosis (CVST) leading to strokes], neuropathy, nausea, acute pancreatitis, hyperglycemia, hypertriglyceridemia, elevated liver function tests including hyperbilirubinemia, hypofibrinogenemia, acquired deficiency of natural anticoagulant factors like antithrombin, protein C or protein S leading to increased risk of thrombosis.

1.5 Incidence of bleeding and thrombosis after Asparaginase

Asparaginase increases the risk of thrombosis through various mechanisms, some of which include acquired deficiency of natural anticoagulants including antithrombin, protein C, and protein S. ALL by itself is a prothrombotic condition and concurrent use of corticosteroids like prednisone increases risk of thrombosis compared to dexamethasone. The presence of co-existing hereditary thrombophilia like factor V Leiden mutation, prothrombin G20210A mutation may play an additional role. Though thrombosis can either be venous or arterial, ASP often is associated with increased risk of venous thromboembolism (VTE) which adversely affects mortality and morbidity[16]. The incidence of thrombosis differs by age, with higher incidence in adults compared to children[17]. In one study using the Dana-Farber Cancer Institute consortium protocols, the incidence of VTE was 5% in the pediatric age group (0-18 years) versus 34% in adults (19-50 years) with 42% of thrombosis occurring in patients older than 30 years of age[18]. This emphasizes that adults are at much higher risk of thrombosis than children. Majority of the thrombosis occurs during the induction period[16]. Other rare but potentially life-threatening thrombosis like CVST occur in around 2-3% of patients receiving ASP[19] and is often associated intracranial bleeding with hemorrhagic stroke. Thrombocytopenia due to ALL or related to chemotherapy in addition to hypofibrinogenemia and ongoing

anticoagulation increases the risk of bleeding. In one study, 5% of patients experienced grade 3 to 4 bleeding complications during the entire treatment period of which 80% occurred during the induction period[16]. In another study, there was 2%, 9% and 5% incidence of bruising, epistaxis and major bleeding respectively in patients with ASP associated VTE while on anticoagulation[18].

1.6 Prophylaxis for thrombosis

The incidence of thrombosis is the highest during the induction phase of treatment phase. In one study, the rate of VTE recurrence in adults after ASP reintroduction was as high as 47% and thrombosis prophylaxis is important not only during induction but also during the subsequent treatment phases of ALL[18]. For patients treated with ASP, the practice of thrombosis prophylaxis varies from institution to institution due to a lack of strong evidence for optimal strategy. Practice may vary from no anticoagulation, unfractionated heparin (UFH), low molecular weight heparin (LMWH), or antithrombin (AT) concentrates. The safety and efficacy of different prophylaxis strategies vary based on an individual study, and the results may differ particularly in children versus adults. In a randomized study comparing LMWH, AT and UFH for thrombosis prophylaxis in children (age 1-18 years) the incidence of thrombosis was 3.5%, 1.9% and 8% respectively ($p<0.05$) showing advantage of using LMWH in this age group for preventing thrombosis[20]. There is no randomized study in adults to assess the efficacy of using AT replacement but in the GRAALL-2005 trial in adult patients (18-65 years) where AT supplementation was the most commonly used thrombosis prophylaxis, heparin was effective compared to no AT supplementation. In a prior study from our institution comparing patients who received LMWH versus no prophylaxis the incidence of VTE was 0% and 37% respectively, showing potential efficacy of LMWH[21], however, the study cohort was small (n=9). A different study has shown that LMWH prophylaxis

during intensification phase of treatment was not effective in preventing VTE[22]. Direct oral anticoagulants like Rivaroxaban and Apixaban are studied in cancer patients for thrombosis prophylaxis with good efficacy and safety but are not well studied in patients with ALL or those treated with ASP[23,24]. Therefore, International Society of Thrombosis and Hemostasis (ISTH) had suggested either use of AT supplementation with weekly supplementation below 50-60% or LMWH prophylaxis during induction unless the platelet count is <30,000/mm^3 [25].

1.7 Prophylaxis to prevent bleeding complications

Though the incidence of major bleeding is not significantly high in children, ongoing need for anticoagulation prophylaxis, hypofibrinogenemia associated with AT, thrombocytopenia can increase the risk of bleeding. Fibrinogen can be replaced either with fresh frozen plasma (FFP) or cryoprecipitate transfusions. Often further use of ASP is contraindicated if its use leads to major bleeding complications. Due to its importance in the treatment backbone of ALL, if proper precautions are set in place to avoid significant bleeding complications, ASP can be safely used in ALL. Some studies have shown higher incidence of thrombosis when fibrinogen prophylaxis[16] was used, and when to use such prophylaxis (based on fibrinogen level) remains a point of debate. In patients with major bleeding, fibrinogen replacement is recommended. In prophylaxis setting, most institutions use a threshold of fibrinogen level <100 mg/dL, below which cryoprecipitate or FFP is used, whereas some even advocate a level of <50 mg/dL for such use[3]. Due to lack of consistent evidence ISTH recommends against routine FFP prophylaxis as the source of AT replacement but acknowledges lack of good evidence in for using FFP to correct hypofibrinogenemia.

1.8 Study Rationale

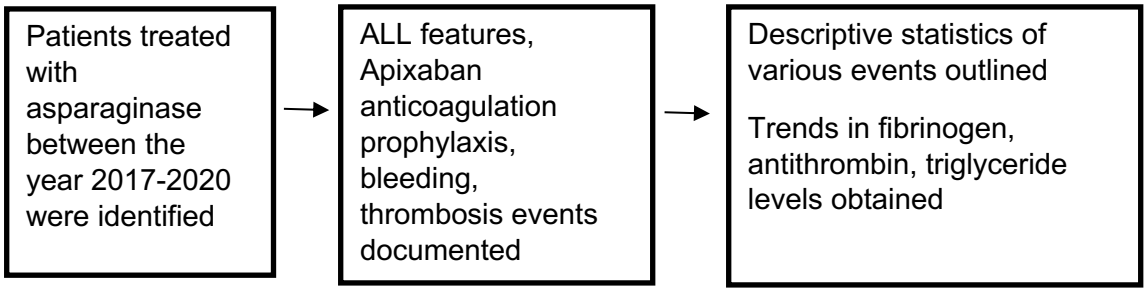
As UFH or LMWH needs adequate levels of AT to be present in the plasma for their efficacy, it is hypothesized that there is resistance to UFH or LMWH anticoagulation if there is acquired AT deficiency due to the use of ASP. Direct oral anticoagulants do not need the presence of AT to be effective, hence offer an advantage over UFH or LMWH. There are no prospective, randomized trials in adults to determine the optimal anticoagulation prophylaxis strategy in patients receiving ASP. Hence, we conducted a study to assess the safety and efficacy of using apixaban for thrombosis prophylaxis in adults receiving ASP.

CHAPTER 2: METHODS

2.1 Study Design

Given a high risk of thrombosis, in 2017, we adopted the use of apixaban for thrombosis prophylaxis and concurrent cryoprecipitate for bleeding prophylaxis based on the severity of hypofibrinogenemia. In this IRB-approved retrospective study, we evaluate the safety and efficacy of thrombosis and bleeding prophylaxis strategies in adults treated with ASP.

Figure 1. Apixaban for thrombosis prophylaxis in patients receiving asparaginase.



2.2 Eligibility Criteria

All adult patients (19 years and above) who received any type of asparaginase, starting from 2017 until 2020 were included in the study. In the absence of any major bleeding complications at the time of treatment of asparaginase, all patients received apixaban prophylaxis and fibrinogen level-directed cryoprecipitate transfusions.

2.3 Treatment Plan

Asparaginase can be given either as an intramuscular or intravenous injection. The formulation of E. coli asparaginase available at our institution is only PEG ASP as the non-pegylated ASP is not available in USA due to the reasons mentioned above. In patients with history of anaphylaxis to ASP or PEG ASP, Erwinia ASP is used for treatment. A dose of PEG ASP 2,500 IU/m^2/dose (no more frequent than every 2 weeks) capped at 3750 IU total dose was used. When Erwinia ASP is used, a dose of 25,000 IU/m^2 three times a week for 6 doses is used. Apixaban is an oral, reversible direct factor Xa inhibitor (direct oral anticoagulant) and does not require AT for antithrombotic activity. It is available in dose strengths of 2.5 mg or 5 mg per tablet. Apixaban has a half-life of around 12 hours while on twice daily dosing and is mainly absorbed in the distal small bowel and is significantly protein bound in circulation. It is mainly metabolized in liver by CYP3A4 and renal excretion accounts for about 27%. Drugs affecting CYP3A4 and or p-gp can have potential drug interaction with Apixaban. In patients younger than 80 years of age with normal renal functions (serum creatinine <1.5 mg/dL), a dose of 2.5 mg orally every 12 hours is effective for thrombosis prophylaxis.

2.3.1 Treatment Schedule

Below is the detailed outline of our institutional adoption of anticoagulation with apixaban and associated supportive care and laboratory monitoring.

Table 1. Institutional policy of apixaban prophylaxis and supportive care

Check Prothrombin time (PT), activated partial thromboplastin time (aPTT), Fibrinogen, AT activity, serum creatinine at baseline
Apixaban prophylaxis with 2.5 mg orally Q 12 hourly started from the day of asparaginase for a total duration of 21 days in the absence of any clinically significant bleeding symptoms or complications
Check fibrinogen twice weekly and transfuse 5-10 of cryoprecipitate if fibrinogen level <100 mg/dL until anticoagulation is used for 3 weeks
If any clinically significant bleeding, transfuse cryoprecipitate to keep fibrinogen >150 mg/dL
Check AT activity levels weekly
Hold apixaban if platelet count is <20,000/mm^3 until platelet count recovers above that value

2.3.2 Supportive care

The institutional practice is to transfuse platelets when platelet count is <10,000/mm^3 or any clinically significant bleeding.

2.4 Duration of study and measurement of effect

A total of 20 patient were identified during the period of 2017-2020 who received asparaginase for the treatment of ALL or other high grade lymphoid neoplasms. From the date of ASP administration, records were reviewed for the parameters outlined below for a period of 4 weeks. Patients' medical records were followed until the last follow up in the electronic health records for any events of thrombosis even when they are unrelated to asparaginase. A fibrinogen level <150 mg/dL is considered low, and <100 mg/dL is severely low. If any bleeding or thrombosis events occurred, records were reviewed to assess if such events were directly related to asparaginase or apixaban. A bone marrow biopsy performed around 21-28 days from the start of induction chemotherapy is used for assessment response to the induction chemotherapy.

2.5 Study parameters

The study parameters are described below (Table 2). The following information were obtained at baseline when available and some of them are followed as outlined in table 1 . Fibrinogen level was obtained twice weekly, and cryoprecipitate transfused based on the bleeding symptoms or set limits of fibrinogen level.

Table 2. Study parameters

Age at the time of ALL diagnosis, before initiation of ASP	**At the time of Asparaginase injection**	**Follow up-First 4 weeks and long term**
Gender, ethnicity,		
Splenomegaly by CT or Ultrasound if available		
Thrombosis events	History of thrombosis	Venous thromboembolism (VTE),

		Deep Venous thrombosis (DVT), Cerebral Venous Sinus Thrombosis (CVST); site of thrombosis, treatment of acute VTE, outcome of VTE, duration of anticoagulation for acute VTE, subsequent use of asparaginase after VTE. Arterial or venous thrombosis confirmed by imaging studies like Computerized Tomography (CT), Magnetic resonance Imaging (MRI) or ultrasound (US)
Coagulation parameters	PT, PTT, Fibrinogen, Antithrombin, D-Dimer, platelet count	Lowest weekly fibrinogen, antithrombin level and others including weekly PT, PTT and D-dimer following the treatment were obtained for 3 weeks from ASP when available.
Other laboratory data at baseline	LDH, Uric acid, Triglycerides, White blood cell count, serum creatinine, AST, ALT, Bilirubin; Blood group	Weekly Triglyceride values post treatment were obtained for up to 4 weeks
Other complications		Pancreatitis, Infections. Weekly lipase and amylase if pancreatitis Name of organism if identified and infection reported
History of thrombosis or MI or stroke before treatment		
Body mass index		
Any underlying thrombophilia before treatment with asparaginase, smoking status	Factor V Leiden mutation, Prothrombin gene mutation Antithrombin or Protein C or Protein S deficiency Antiphospholipid antibody syndrome Hyperhomocysteinemia	
	Route of administration, dose, frequency and type	Date and cause of death if any

	of Asparaginase administered	
Risk category of ALL, Karyotype and genetic/molecular studies as available,		Underwent allogeneic hematopoietic stem cell transplantation
Induction regimen used	Consolidation regimen	Date of relapse if any.
	Type and dose of corticosteroids use	
Any underlying bleeding disorders or history documented; history of liver disease		

2.6 Statistical considerations

2.6.1 Study Design: This is a descriptive research study to systematically describe the characteristic features of patients with ALL or similar high grade lymphoid neoplasm who had received asparaginase in the treatment and had received Apixaban for thrombosis prophylaxis. The data is collected based on retrospective review of medical records of the patients who meet the criteria.

2.6.2 Study Population: All patients who received asparaginase as a part of the treatment of their lymphoid neoplasm and also received apixaban for thrombosis prophylaxis fully for the planned duration of 3 weeks or differently based on the clinical circumstances of bleeding or thrombosis during the follow up period of the study.

2.6.3 Sample size: We have identified 20 patients with these requisites who had receive asparaginase from 2017 to 2020 at the University of Nebraska Medical Center and the data from them us used for analysis.

2.6.4 Data analysis: Data will be descriptively summarized using frequencies and percentages.

2.6.5 Safety endpoint: The incidence of bleeding or thrombosis was recorded and when such events occurred, further details were captured.

2.7. Efficacy and safety endpoints

Bleeding events:

Bleeding events was documented using the standard ISTH definition[26] as follows:

Clinically relevant non-major bleeding (CRNMB) in non-surgical VTE studies includes any sign or symptom of hemorrhage (e.g., more bleeding than would be expected for a clinical circumstance, including bleeding found by imaging alone) that does not fit the criteria for the ISTH definition of major bleeding but does meet at least one of the following criteria:

A) requiring medical intervention by a healthcare professional
B) leading to hospitalization or increased level of care
C) prompting a face to face (i.e., not just a telephone or electronic communication) evaluation

ISTH major bleeding in non-surgical patients is defined as having a symptomatic presentation and:

D) Fatal bleeding, and/or
E) Bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular or pericardial, or intramuscular with compartment syndrome, and/or
F) Bleeding causing a fall in hemoglobin level of 20 g/L or more; or leading to transfusion of two or more units of whole blood or red cells.

Any bleeding events which do not fit CRNMB or major bleeding will be described as minor bleeding.

Mortality: The cause of death was described.

Thrombosis events: Thrombosis is diagnosed by imaging studies such as a doppler ultrasound of extremities, neck (to look for DVT or superficial vein thrombosis) or CT angiogram of chest or MRI of the brain or heart or by echocardiogram or coronary or limb angiogram or CT for arterial thrombosis. Description of such thrombosis events was captured.

Transplant status: Describe how many of patients underwent allogeneic hematopoietic stem cell transplantation subsequently.

CHAPTER 3: RESULTS

We identified a total of 20 patients between June 2017-September 2020 who received ASP for treatment of high-grade lymphoid neoplasm of ALL along with apixaban thrombosis prophylaxis. The descriptive analysis of these patient's baseline characteristics and changes in coagulation parameters and other study parameters are outlined below.

3.1 Demographic characteristics

Of the 20 patients, 16 (80%) are males; 4 (20%) are females. The median age of all the patients at original diagnosis is 29.5 years with a range from 13 to 63 years. The median age of all the patients at the time of use of asparaginase is 29.9 years with a range of 19.3 to 63.2 years. A total of 14 patients (70%) were white, and 6 patients (30%) were Latino or Hispanic.

3.2 Baseline risk factors for thrombosis

There was no family history of thrombosis in all the patients. The median body mass index (BMI) of the patients is 30.2 with a range of 19.4 to 40.7. Smoking history is as follows: 4 patients (20%) reported as former smokers; 2 patients (10%) reported as active smokers and 13 patients are non-smokers (65%) and the smoking status is not available (5%) in 1 patient. Corticosteroids are use concurrently in 19 patients (95%). No familial thrombophilia was reported or identified in any of the patients based on family history or when relevant testing (table 2) was performed when performed by the treating physician. Median baseline AT activity is 107% with a range of 79 to 221% (normal range 70-130%).

3.3 Baseline risk factors for bleeding

No patients had any family history of bleeding disorders. The median platelet count at the time of initiation of asparaginase is 48,500/mm^3 with a range of 13,000-254,000/mm^3 (normal range 150,000-400,000/mm^3). A history of steatohepatitis (fatty liver disease) was present in 10 patients (50%). Median baseline PT is 12.2 seconds with a range of 10.9 s to 15.7 s (normal range 10.1-14.2 s); median baseline PTT is 26.7 seconds with a range of 21.1 s to 38.3 s (normal range 24-36 s); median baseline fibrinogen activity is 253 mg/dL with a range of 86 to 621 mg/dL (normal range 160-450 mg/dL). The blood group was O in 12 patients (60%), B in 3 patients (15%), A in 3 patients (15%), AB in 1 patient (5%) and unavailable in 1 patient (5%).

3.4 Disease characteristic features

Of the 20 patients, 18 (90%) had acute lymphoblastic leukemia and 2 patients (10%) had nasal NK/T-cell lymphoma. In the 18 patients with ALL, 12 of them had high risk ALL based on WBC at diagnosis (B-ALL: >30,000/mm^3, T-ALL: >100,000/mm^3) and cytogenetics risk stratification. 6 patients had standard risk ALL. Genetic features are outline in table 4. Ten patients (50%) with ALL had precursor B-cell ALL, 2 patients (10%) had Philadelphia chromosome positive precursor B-cell ALL, 5 patients (25%) had precursor-T-cell ALL and 1 patient (5%) had mixed phenotype-ALL.

3.5 Laboratory data at baseline

The laboratory data are outlined in detail in table 3 below. The median WBC count at diagnosis is 11,500/mm^3 with range of 800/mm^3 to 495,300/mm^3 (normal range 4000-11000/mm^3); Median platelet count at diagnosis of 49,500/mm^3 with a range of 7000/mm^3 to 335,000/mm^3 (normal range 150,000-400,000/mm^3). Median lactate dehydrogenase is 539 U/L with a range of 130 U/L to 2594 U/L (normal range 98-192 U/L). Median uric acid is 6.7 mg/dL with a range of 2 mg/dL to 12.2 mg/dL (normal range 3-6.8 mg/dL). Median serum creatinine is 0.73 mg/dL with a range of (normal range 0.44-1.03 mg/dL). Median Aspartate aminotransferase (AST) is 28.5 U/L with a range of 10 U/L to 154 U/L (normal range 15-41 U/L); Median Alanine aminotransferase (ALT) is 37.5 U/L with a range of 9 U/L to 246 U/L (normal range 7-52 U/L); median total bilirubin of 0.6 mg/dL with a range of 0.2 mg/dL to 3.1 mg/dL (normal range 0.3-1 mg/dL).

Table 3. Results of laboratory data at baseline

Test name and units	Mean	SD	Median	Min	Max
WBC (in x1000/mm^3)	62.1	118.8	11.5	0.8	495.3
Platelet count at diagnosis (in x1000/mm^3)	73.9	83.7	49.5	7	335
Lactate Dehydrogenase (U/L)	879	760.3	539	130	2594

Uric acid (mg/dL)	7.3	2.82	6.7	2	12.2
Serum creatinine (mg/dL)	0.79	0.264	0.73	0.38	1.24
AST (U/L)	37.3	31.27	28.5	10	154
ALT (U/L)	50.9	51.23	37.5	9	246
Total bilirubin (mg/dL)	0.83	0.754	0.6	0.2	3.1

3.6 Genetic characteristic features

A description of the karyotype, fluorescence in situ hybridization (FISH) and molecular data is outlined in the table below.

Table 4: Genetic features

	Disease	**Karyotype**	**FISH/Molecular data**
1	Precursor B-ALL	54,XY,+X,+4,+6,+10,+10, +15,+21, +21[2]/46,XY[19]	Positive for trisomy 4 (57.5%); Positive for 3-4 copies of 10 (61.5%); Positive for tetrasomy 21 (65.5%)
2	Precursor B-ALL	46,XY,-7,+9[5]/46,XY[5]	Positive for monosomy 7 (81%); Positive for trisomy 9 (77%)
3	Precursor T-ALL	46,XY[20]	Positive for nullisomy 9p21 (85%)
4	Precursor B-ALL	UKNOWN karyotype	Normal
5	Nasal NK/T cell lymphoma	47,XY,+2,t(3;9)(q21;q34), del(6)(q21q27)[7]/46,XY[1]	
6	Precursor B-ALL	46,XY,add(17)(q25)[2]/46, XY[18]	Normal
7	Precursor T-ALL	48,XY,i(7)(q10),+8,+14[6]/ 46,XY[10]	Positive for 2 copies of 7 centromere with 3 copies of 7q31 (82%); Positive for 3 copies of 7q34 (87%) ; Positive for trisomy 8 (91%); Positive for 3 copies of 14q11.2 (82.5%)
8	Precursor T-ALL	Not available	Positive for rearrangement of the TRB (TCRB ; 7q34) locus (17.5%); Positive for nullisomy 9p21 (22%)

9	Nasal NK/T cell lymphoma	88,XXYY,-2,del(6)(q21q23), del(6)(q15q33),-9,-10,-11,add(15(p12),-16,+mar[17]/46,XY[3]	Positive for 4 copies of 3q27 (47%) Positive for 4 copies of 7q34 (26%) Positive for 4 copies of 8 (49%) Positive for 3 copies of 9 (22%) Positive for 4 copies of 14q11.2 (28%) Positive for 4 copies of 14q32 (44%) Positive for 4 copies of 15q22 with 2 copies of 6q21 and 6q23 (24%) Positive for 4 copies of 17 (51.5%) Positive for 4 copies of 18 (38%)
10	Ph positive Precursor-B-ALL	46~49,X,-Y,t(1;18)(q25;q23),del(2)(p21p25), t(9;22)(q34;q11.2),del(11(q21q23),+21, +der(22)t(9;22),+2~4mar,inc[cp11]	Positive for BCR/ABL1 fusion (99%); Positive for 3 copies of 5q33 (26%); Positive for 3 copies of 21q22 (85.5%); p190 qualitative PCR positive
11	Precursor B-ALL	46,XY,t(5;13)(q33;q14),der(19)t(17;19)(q22;p13.3)[5]/46,XY[15]	Positive for deletion of telomeric TCF3 (E2A; 19p13.3) locus (26%)
12	Precursor B-ALL	46,XX[4]	Positive for monosomies 4 and 17 and loss of ABL1(9q34) (hypopoloidy), gain (but not rearrangement) of IGH, gain of chromosome 10, and for gains of ETV6 (12p13), RUNX1 (21q22), KMT2A (MLL, 11q23.3), and BCR (22q11.2) (doubling).
13	Precursor B-ALL	46,XY[20]	Positive for deletion of the 3' (telomeric) P2RY8 (Xp22.33/Yp11.3) locus (81%); Positive for deletion of the 5' (centromeric) CRLF2 (Xp22.33/Yp11.3) locus (75%)
14	Precursor B-ALL	46,XY[20]	Positive for 4 copies of the IGH (14q32) locus with deletion of 1-2 copies of the 5' (telomeric) IGH locus (12%); Positive for 4 copies of 8 (10%) ; Positive for 4 copies of 11q23 (12%); Positive for 4 copies of 18q21 and the chromosome 18 centromere (12%) Positive for 4 copies of 22q11.2 with 2 copies of 9q34 (11%)

15	Ph-like Precursor B-ALL	48,XX,+X,t(1;11)(p34.1;q24),+21c[8]/ 47,XX,+21c[12]	Ph like ALL-CRLF2/IGH rearrangement
16	Mixed phenotype ALL	46, XY[20]	FISH normal at diagnosis; FISH at relapse in 2019 showed Positive for 3 copies of PBX1 (1q23) locus (66%)
17	Ph-positive Precursor B-ALL	46,XY,t(9;22)(q34;q11.2)[7]/46,sl, der(19)t(1;19)(q23;p13)[1]	Positive for variant BCR/ABL1 fusion (80%) Positive for 3 copies of Xp22.33/Yp11.3 (10%); BCR/ABL p210 and p190 positive
18	Precursor T-ALL	46,XX,dic(12;13)(p11.2;q10),r(17), +mar[6]/45,sl,-dic(12;13), +add(13)(p11.2),-mar[12]/46,XX[2]	Positive for deletion of the TP53 (17p13.1) locus (99%)
19	Precursor B-ALL	47,XY,+X,der(9)t(8;9)(q13;q34)[2]/46,XY[18]	Positive for rearrangement of one copy of the CRLF2 (Xp22.33/Yp11.3) locus with 2 intact (non-rearranged) copies of CRLF2 (69%) Positive for rearrangement of IGH (14q32) locus (68.5%) Positive for 3 copies of 8q24 with 2 copies of chromosome 8 centromere (11.5%)
20	Precursor T-ALL	46, XX[20]	Normal

3.7 Treatment details including asparaginase and corticosteroids

The most common induction regimen at original diagnosis is CALGB 10403 in 11 patients (55%). In 1 patient each CALGB 10403 was used as salvage. 19 patients (95%) received PEG and only 1 patient (5%) received Erwinia Asparaginase (during his salvage chemotherapy due to reported history of anaphylaxis with E. coli asparaginase). The dose of Erwinia asparaginase is 25,000 units/m^2 three times a week for a total of 6

doses given as intramuscular injection. PEG was administered as intramuscular injection in 13 patients and intravenous injection in 6 patients. The most commonly used dose of PEG is 2000 U/m^2 capped at a total of 3750 U per dose in 15 patients (75%). Two patients received a dose of PEG of 2500 U/m^2 (10%) and 2 received 1000 U/m^2 (10%). The choice of corticosteroid is prednisone in 13 patients (65%) and Dexamethasone in 6 patients (30%). Prednisone is given at a dose of 60 mg/m^2 orally from days 1 to day 28 of chemotherapy cycle except for one patient who received 100 mg orally daily for 5 days. Dexamethasone was given at dose of 20-40 mg orally daily from day 1-4 and days 11-14 or 3 mg/m2 from day 1-28 of chemotherapy. Leukapheresis along with hydroxyurea was used for cytoreduction before induction chemotherapy in 2 patients (10%), and cyclophosphamide and Hydroxyurea was used in 1 patient (5%).

Table 5 Treatment regimens used

	Induction regimen (% of patients)	**Consolidation (if different)**	**Induction at relapse**
1	COG-AALL 0232 (5%)	Hyper-CVAD	CALGB 10403
2	ECOG 2993 (5%)	CALGB 10403	HD-MTX, Dexamethasone with PEG
3	COG AALL 1231 (5%)		Mini-HCVAD-Inotuzumab
4	CALGB 10403 (45%)		
5	DeVIC (5%) (Dexamethasone, Carboplatin, Ifosfamide, Etoposide)		Nelarabine, Etoposide, Cyclophosphamide

6	Gemcitabine, Oxaliplatin-PEG (5%)		Rituximab-Mini-HCVAD with inotuzumab ozogamicin
7	CALGB 10403-Dasatinib (10%)		FALG-IDA (Fludarabine, Ara-C, G-CSF with Idarubicin) followed by MOAD (Methotrexate, Vincristine, Asparaginase, Dexamethasone)
8	Alliance A041501 (10%)		Hyper-CVAD
9	COG AALL 1131 (5%)		Blinatumomab
10	Hyper-CVAD (5%)		Augmented Hyper-CVAD (with PEG)

3.8 Thrombosis events and anticoagulation prophylaxis

Based on the published literature the incidence of thrombosis in adults treated with asparaginase is in in the range of 30-40%. Two patients (10%) developed VTE within 4 weeks from ASP injection. One patient developed left subclavian catheter associated VTE with thrombophlebitis 18 days after PEG while on apixaban ,and the other patient developed right lower extremity proximal DVT, 20 days after PEG. The later patient received apixaban only for 4 days after which time Apixaban was stopped due to spontaneous splenic rupture (treated with splenic artery coil embolization) due to ALL involvement of the spleen. Hospitalization was further complicated by hemorrhagic shock, traumatic intracranial hemorrhage from fall. In this patient, after appropriate treatment for bleeding, acute VTE was treated with unfractionated heparin followed by enoxaparin for extended anticoagulation. Though in this study, the incidence of

thrombosis was 10% within 4 weeks from ASP *the incidence of anticoagulation failure (thrombosis while on anticoagulation with apixaban) was only 5% in a total of 20 patients.*

3.9 Bleeding events

The reported incidence of clinically significant bleeding while on anticoagulation following asparaginase is around 5-10%. No patients had major or clinically relevant non major bleeding (CRNMB) due to the use of apixaban. One patient (5%) developed major life-threatening bleeding while on apixaban but was related to underlying ALL leading to spontaneous splenic rupture, traumatic intracranial hemorrhage from fall. Cryoprecipitate is used for bleeding prophylaxis. A total of 15 patients (75%) received cryoprecipitate following asparaginase based on their fibrinogen level. The median number of cryoprecipitates used within 4 weeks following ASP is 10 units with a range of 0-105 units. The median number of cryoprecipitates used during weeks 1, 2, 3 and 4 is 5 units (range 0-35 units), 5 units (range 0-40 units), 5 units (range 0-15 units) and 7.5 units (range 0-15 units) respectively. No patients received fresh frozen plasma.

3.10 Other significant events

Two patients developed grade III-IV acute PEG induced pancreatitis (10%) of whom one had severe necrotizing pancreatitis. One patient (5%) developed PEG induced hypertriglyceridemia needing therapeutic apheresis.

3.11 Trends in liver functions, fibrinogen, antithrombin and triglycerides

One patient had known Gilbert's syndrome and mild baseline hyperbilirubinemia. One patient had significant elevation of AST/ALT (2051/2226 U/L) at diagnosis which was

attributed to extramedullary involvement of ALL with improved to normal by the time ASP was administered. One patient had grade II (5%) and three patients (15%) had grade III-IV ASP induced elevation in liver enzymes and bilirubin (20%). Below is the trend in various laboratory data with baseline being the day of injection of asparaginase. The figures 2 to 4 illustrate the trends in the median values of fibrinogen, AT and triglycerides respectively. Due to the retrospective nature of the study, the below laboratory data was not available for all the patients at weekly time points for 4 weeks. The data below is an analysis of the available data only.

Table 6: Trends in LFT, fibrinogen, AT and triglyceride (TG) levels

Test name and normal range)	Mean	Median	Min	Max
TG-baseline (<150 mg/dL)	213.7	171.5	67	594
TG - week 1	339	190	93	1692
TG- week 2	543	378	64	2860
TG – week 3	850	257	39	4190
TG- week 4	311	258	40	813
Amylase- baseline (20-70 U/L)	27.5	32	12	41
Amylase – week 1	33	31.5	10	55
Amylase – week 2	47.3	43	10	125
Amylase – week 3	35.2	32	13	71
Amylase – week 4	37.4	24.5	14	129
Lipase – baseline (15-60 U/L)	17.5	14	6	49
Lipase – week 1	25.3	22	16	63
Lipase – week 2	54	38.5	3	209
Lipase – week 3	278	68.5	5	2959
Lipase – week 4	85.6	33	3	402
AST (U/L)-Baseline (15-41 U/L)	37.3	28.5	10	154

AST - week 1	21.7	20.5	11	42
AST - week 2	71.9	30.5	11	276
AST - week 3	121.8	44	15	1299
AST – week 4	64.1	64.5	15	152
ALT (U/L)- Baseline (7-52 U/L)	50.9	37.5	9	246
ALT – week 1	55.2	56	20	139
ALT – week 2	163.5	71	18	948
ALT – week 3	190.5	83	19	1044
ALT – week 4	140.1	118	37	313
Total bilirubin (mg/dL)- Baseline (0.3-1 mg/dL)	0.83	0.6	0.2	3.1
Bilirubin - week 1	1.44	1.4	0.6	2.6
Bilirubin - week 2	2.23	1.3	0.6	12.1
Bilirubin – week 3	2.3	1.3	0.4	10.5
Bilirubin – week 4	1.67	0.9	0.4	9.8
Fibrinogen – baseline (160-450 mg/dL)	302.2	253	86	621
Fibrinogen – week 1	132.5	112	70	344
Fibrinogen – week 2	123.4	116	65	210
Fibrinogen – week 3	174.3	169.5	68	359
Fibrinogen – week 4	240.3	217	147	424
Antithrombin- baseline (70-130%)	114	107	79	221
Antithrombin – week 1	69	65	43	102
Antithrombin – week 2	70.1	66	22	118
Antithrombin – week 3	90.4	83.5	39	146
Antithrombin – week 4	93.1	85.5	39	150

Figure 2: Trend in Fibrinogen

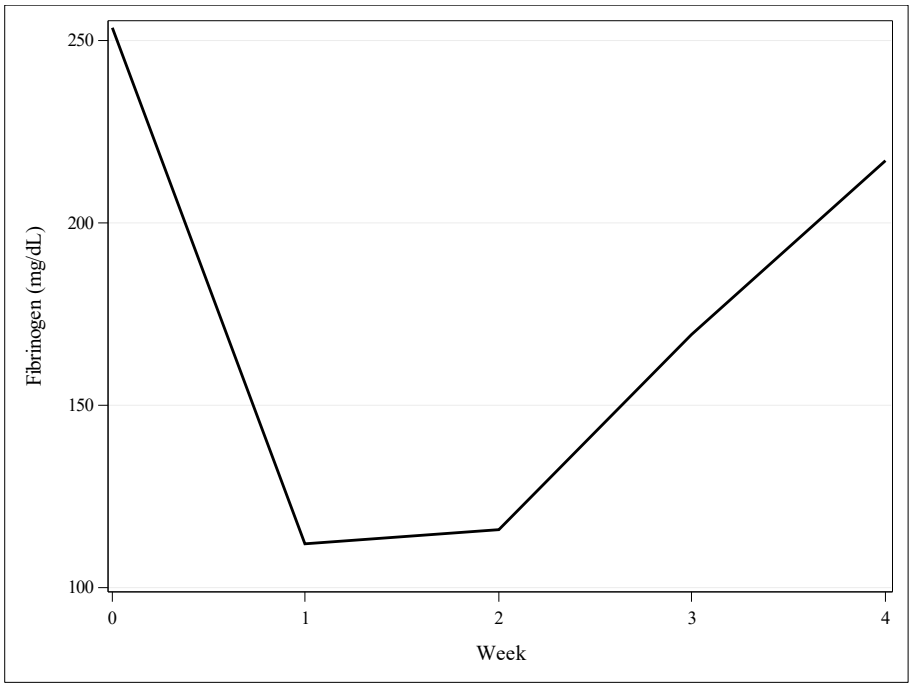


Figure 3: Trend in Antithrombin activity

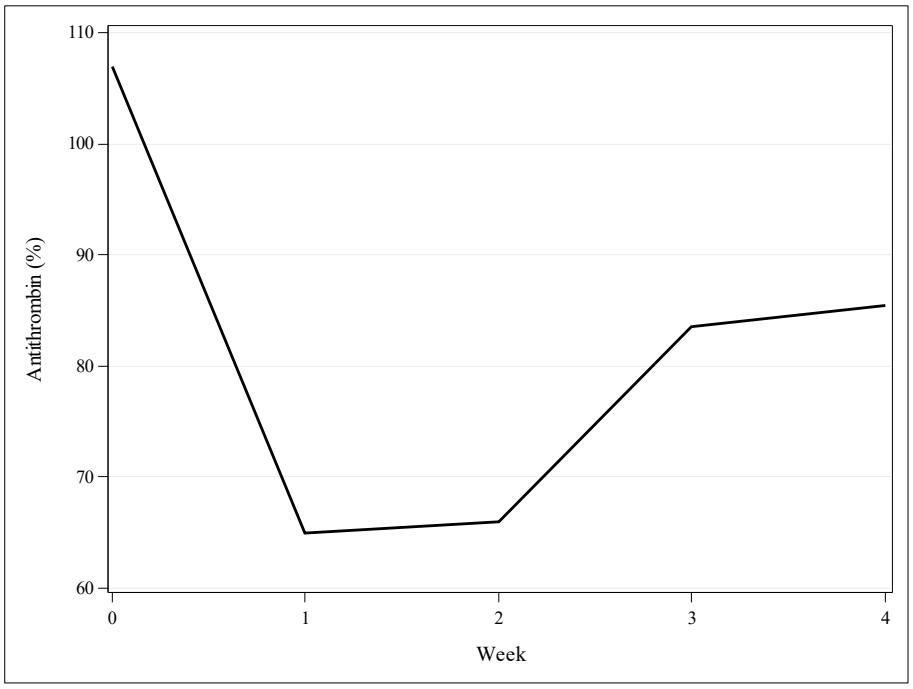
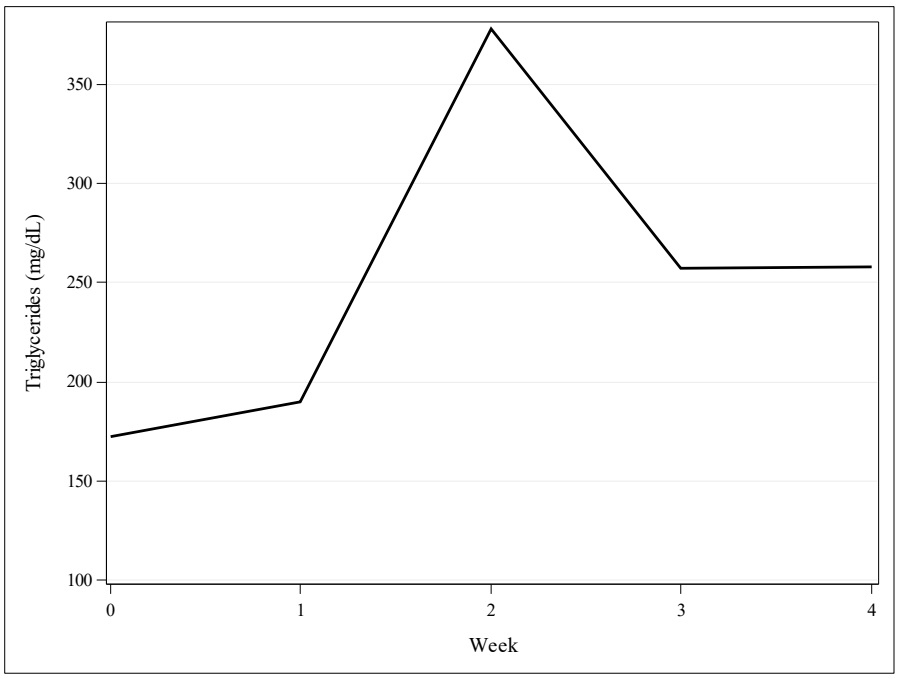


Figure 4: Trend in Triglycerides



3.12 Mortality results and hematopoietic stem cell transplantation

Four patients died (20%), and the cause of death is relapsed/refractory disease in 3 patients (75%) along with severe life-threatening infections (75%). In one patient the cause of death is severe infection alone. First patient had suffered Streptococcus mitis, Pseudomonas aeruginosa pneumonia/bacteremia with septic shock. Second had angio invasive pulmonary mucormycotic needing total right pneumonectomy and subsequently died (Primary cause of death is infection). Third had invasive fungal sinusitis with Fusarium blood stream infection with CVST. Nine patients underwent an allogeneic hematopoietic cell transplantation (45%). Out of the four patients who died only one (25%) had undergone a transplant.

CHAPTER 4: DISCUSSIONS

The management of ALL has evolved significantly over the past half century. Though drugs like methotrexate and corticosteroids had a positive impact on short term outcomes, the greater success was witnessed after the use of effective multi-agent chemotherapy, and CNS prophylaxis. Asparaginase played a significant part of this success story, but is associated with substantial risk of toxicities, particularly thrombosis and bleeding complications. Many studies have shown a very high incidence of thrombosis in adults after the use of ASP[18]. Unlike the higher incidence of thrombosis of 30-40% seen in other studies, our study has shown a total incidence of 10% in patients treated with ASP followed by apixaban prophylaxis and 5% of the patients developed thrombosis when apixaban was held due to major bleeding. One of the reasons for not using anticoagulation prophylaxis in spite of the awareness of thrombosis associated with ASP is the risk of bleeding due to hypofibrinogenemia. To balance the risk, often FFP or Cryoprecipitate are used for fibrinogen replacement therapy. A growing body of evidence points towards concerns of higher risk of thrombosis with the use of fibrinogen concentrates[16]. Our institutional practice of using apixaban concurrently with fibrinogen replacement therapy appears to be safe unlike the recent report of data from the adults with ALL treated with ASP in the GRAAL-2005 study where most patients received AT supplementation (87%) along with UFH or LMWH for thrombosis prophylaxis. This difference is likely due to the choice of anticoagulant and differences in its mechanism of action as an anticoagulant. It is important to emphasize that the only patient with true anticoagulation failure (thrombosis while on apixaban prophylaxis) did not receive any fibrinogen concentrates during his induction therapy preceding the occurrence of

thrombosis. The patient who experienced thrombosis and received cryoprecipitate infusions, did not receive any anticoagulation for 16 days due to the occurrence of preceding major bleeding. Apixaban being an oral anticoagulant with no need for additional monitoring, offers a great advantage over other modes of anticoagulation coagulation prophylaxis like UFH, LMWH or AT replacement therapy which are given parenterally and need monitoring. AT replacement therapy also adds a significant cost burden to the overall treatment cost with no clear advantage in improving overall survival.

The trends in laboratory values highlight the fact that the anticipated acquired AT deficiency from ASP use peaks during week 1 and 2 after administration and returns to normal range by week 3. Similar pattern of hypofibrinogenemia was seen in weeks 1 and 2 returning to normal range by week 3. This pattern emphasizes that the risk of thrombosis and bleeding are likely the highest during the first 2 weeks post ASP.

This study has certain limitations including a smaller number of patients, and a possibility of missing data in a retrospective study. Most of the patients in our study received PEG and only one received Erwinia asparaginase. The efficacy of apixaban for anticoagulation prophylaxis is unlikely to be confined to a specific formulation of ASP but as most patients received PEG, its efficacy with various available formations cannot be fully established.

In conclusion, our study provides initial evidence that Apixaban anticoagulation prophylaxis following the use of ASP can reduce the risk of thrombosis significantly without increasing the risk of bleeding. The use of concurrent cryoprecipitate transfusion does not appear to increase the risk of thrombosis among patients receiving apixaban prophylaxis.

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