นิพนธ์ต้นฉบับ . Original Article

ภาวะลิ่มเลือดอุดตันหลังการใช้ยาแอสพาราจิเนส ในผู้ป่วยเด็กมะเร็งเม็ดเลือดขาวลิมโฟบลาสต์และมะเร็งต่อมน้ำเหลือง

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Asparaginase with Thrombotic Complications in Pediatric Acute Lymphoblastic Leukemia and Lymphoblastic Lymphoma

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บทคัดย่อ

หลักการและวัตถุประสงค์: ภาวะลิ่มเลือดอุดตันเป็นภาวะแทรกซ้อนรุนแรงที่พบได้ในผู้ป่วยเด็กมะเร็งเม็ดเลือดขาวและมะเร็งต่อมน้ำเหลืองที่ได้รับยาแอสพาราจิเนส วัตถุประสงค์เพื่อหาอุบัติการณ์ ปัจจัยเสี่ยงต่อลิ่มเลือดอุดตัน และผลการรักษาหลังการใช้ยาแอสพาราจิเนส

วิธีการศึกษา: เป็นการศึกษาย้อนหลังเชิงพรรณนาในผู้ป่วยเด็กอายุน้อยกว่า 18 ปีที่เป็นโรคมะเร็งเม็ดเลือดขาวลิมโฟบลาสต์และมะเร็งต่อมน้ำเหลืองในโรงพยาบาลศรีนครินทร์ ตั้งแต่เดือน มกราคม 2540 ถึง ธันวาคม 2550 และได้รับการวินิจฉัยภาวะลิ่มเลือดอุดตัน โดยเก็บและวิเคราะห์ข้อมูลทั่วไปของผู้ป่วย อาการของภาวะลิ่มเลือดอุดตัน ปริมาณยาแอสพาราจิเนสสะสม ยากลุ่มแอนทราไซคลินและสเตียรอยด์ที่ได้รับในระหว่างการรักษาด้วยยาแอสพาราจิเนส ภาพทางรังสีวินิจฉัยภาวะลิ่มเลือดอุดตัน ผลการรักษา

ผลการศึกษา: พบว่า ผู้ป่วยเด็กมะเร็งเม็ดเลือดขาวลิมโฟบลาสต์และมะเร็งต่อมน้ำเหลือง 831 ราย ได้รับการรักษาด้วยยาแอสพาราจิเนส เกิดลิ่มเลือดอุดตัน 16 ราย ค่ามัธยฐานอายุ 12 ปี ค่ามัธยฐานระยะเวลาการเกิดลิ่มเลือดอุดตัน 17 วันหลังจากได้รับยา (ช่วงระหว่าง 1 ถึง 41 วัน) โดยพบลิ่มเลือดอุดตันในตำแหน่งหลอดเลือดดำ (ร้อยละ 62.5) มากกว่าหลอดเลือดแดง (ร้อยละ 37.5) โดยที่ 14 ราย (ร้อยละ 87.5) เกิดลิ่มเลือดในระบบประสาท อาการที่พบบ่อยที่สุด คือ ชัก ผู้ป่วยส่วนใหญ่ได้รับยาแอสพาราจิเนสควบคู่กับยากลุ่มแอนทราไซคลินและสเตียรอยด์ ปัจจัยสัมพันธ์การเกิดลิ่มเลือดอุดตัน คือ ภาวะพร่องโปรตีน S ภาวะขาด antithrombin และ โปรตีน C ผู้ป่วยส่วนใหญ่ได้รับการรักษาด้วยยากลุ่มเฮพารินที่มีน้ำหนักโมเลกุลต่ำ (ร้อยละ 81.1) ผู้ป่วย 8 ราย ภาวะลิ่มเลือดอุดตันสลายหมดหลังการรักษา ผู้ป่วย 4 รายพบลิ่มเลือดอุดตันบางส่วน ผู้ป่วย 2 ราย มีอาการดีขึ้น โดยไม่ได้ติดตามด้วยภาพถ่ายรังสี ผู้ป่วย 10 ราย (จาก 14 ราย) มีลิ่มเลือดอุดตันในระบบประสาท มีผลการรักษาดีมาก (ค่าคะแนน modified Rankin scale 0 หรือ 1) มี 12 รายรอดชีวิต และ 2 ราย เสียชีวิตจากโรคมะเร็งลุกลาม และภาวะสมองขาดเลือด

สรุป: การรักษาโดยใช้ยาแอสพาราจิเนส ในผู้ป่วยเด็กโดยเฉพาะอายุ 10 ปีขึ้นไปที่เป็นโรคมะเร็งเม็ดเลือดขาวลิมโฟบลาสต์และมะเร็งต่อมน้ำเหลืองควรมีการเฝ้าระวังภาวะลิ่มเลือดอุดตัน ปัจจัยเสี่ยงที่พบมากที่สุด คือ ภาวะขาดโปรตีน S ผลการรักษาโรคลิ่มเลือดอุดตัน น่าพึงพอใจ การกลับมาใช้ยาแอสพาราจิเนสอีกครั้งค่อนข้างปลอดภัย อย่างไรก็ตาม ปัจจัยเสี่ยงของลิ่มเลือดอุดตันตามหลังการใช้ยาแอสพาราจิเนส และแนวทางในการป้องกันลิ่มเลือดอุดตันหลังจากการใช้ยานี้ ยังต้องการการศึกษาเพิ่มเติมในอนาคต

คำสำคัญ: ยาแอสพาราจิเนส, ภาวะลิ่มเลือดอุดตัน, ผู้ป่วยเด็ก, โรคมะเร็งเม็ดเลือดขาว

Abstract

Background and Objective: Asparaginase is a standard chemotherapy agent which is successful against childhood acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LL). However, asparaginase increases the risk of Thromboembolic events (TEEs). This retrospective descriptive study aims to describe the characteristics, treatment, and outcomes of children with asparaginase-related thrombosis in Srinagarind Hospital, Thailand.

Methods: Pediatric ALL and LL patients, aged under 18 years old at diagnosis of ALL and LL with definitely diagnosed TEEs, treated between 1997 and 2017, were retrospectively reviewed. Patient demographic data, clinical presentations of TEE, actual dosage of asparaginase, concomitant anthracycline and steroids administration, imaging study results, treatments, and outcomes, were all collected and analyzed.

Results: Of 831 ALL and LL patients, 16 developed asparaginase-related TEE. Median age of patients with TEE were 12 years (range 3-15 years). CNS thrombosis (14 patients). was the most were more venous site more than arterial sites (62.5% versus 37.5%). Seizures were the most common presentation, with a median time of onset of 17 days from the first exposure (range 1–41 days). Most of them received anthracycline and steroids during asparaginase administration. Acquired protein S deficiency was the most common prothrombotic state, followed by acquired antithrombin and acquired protein C deficiency. Most patients were treated with low-molecular weight heparin (81.1%). of 8 patients had complete responses, 4 had partial responses, and 2 had clinical improvement. Ten of 14 patients (71.4%) with CNS TEE had favorable outcomes (modified Rankin scale 0-1). Twelve patients (75%) were alive, two died from advanced cancer, and one died from brain herniation after cerebral infarction.

Conclusions: Caution is necessary concerning asparaginase-related TEE in leukemia and lymphoma patients, particularly in patients aged more than 10 years old. Protein S deficiency was the most risk. Post-TEEs treatment had favorable outcomes. Rechallenge of asparaginase is rather safe. Further larger prospective studies focusing on risk factors and TEEs prophylaxis studies should be conducted to improve outcomes.
EC reference number: HE611459

Keyword: Asparaginase, thromboembolic events leukemia, pediatric patient, leukemia

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Introduction

Thromboembolic events (TEEs) are rare complications of childhood hematologic malignancies but result in morbidity and mortality. Asparaginase is one of the predisposing factors for TEE. Asparaginase is the backbone of childhood acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LL) treatments. Asparaginase is the enzyme that can break down the amino acid L-asparagine to ammonia and glutamic acid. Asparagine is important for cell metabolism but leukemic cells lack the ability to synthesize L-asparagine itself, therefore asparaginase can cause apoptosis of leukemic cells.[1] However, asparaginase can also decrease the synthesis of both procoagulant (fibrinogen, factor II, V, X) and anticoagulant proteins. The predominant effects on the anticoagulant system include the declining levels of antithrombin, protein C, and protein S. Moreover, downregulation of plasminogen and α-2-antiplasmin, an increase in plasminogen activator inhibitor-1 (PAI-1), and increased ADP susceptibility of platelets have been reported.[2] Overall, this results in increasing risks of bleeding and thrombosis.

Asparaginase-associated thrombosis is well reported in western populations. However, there are few reports in asian populations, especially in Thailand. To improve knowledge in the Thai setting, this study has been conducted to describe the incidence of asparaginase-related TEE, the clinical characteristics of patients, and the risk factors, treatments, and outcomes of patients in a tertiary care center in northeastern Thailand.

Methods

Patient selection

The medical records of pediatric ALL and LL patients were retrospectively reviewed. The inclusion criteria were newly diagnosed ALL and LL patients aged under 18 years old at diagnosis of cancers with diagnosis of TEEs, treated at Srinagarind Hospital between 1997-2017. The study protocol was approved by the ethics committee of the Faculty of Medicine, Khon Kaen University (reference number: HE611459) according to the ethics principles of the Declaration of Helsinki (1975) and its revision.

Treatment protocols

Thai Pediatric Oncology Group (ThaiPOG) regimens were used to treat children with ALL and LL. The therapy protocol was optimized using risk stratification (Supplemental Table S1). Patients with LL were given a high-risk ALL treatment regimen. According to the chemotherapy phases, all patients got E. coli L-asparaginase in a variety of total dosages and administration days.

From 1997-2008, pediatric ALL and LL patients were treated according to the ThaiPOG ALL 01-08 for standard risk ALL, ALL 02-08 for high-risk ALL, and ALL 03-08 for very high-risk ALL and LL (Supplemental Figure S1). Asparaginase was used during the induction and delay-intensification phases. All the ALL regimens included 6 doses of 10,000 IU/m^2 asparaginase in the induction phase, along with high-dose prednisolone, vincristine, doxorubicin, and intrathecal methotrexate. For the delay-intensification phase, there were 6 doses of 10,000 IU/m^2 asparaginase in the ALL 01-08 and ALL 02-08, and 12 doses of 10,000 IU/m^2 asparaginase in the ALL 03-08 regimen.

From 2009-2017, pediatric ALL and LL patients were treated according to the 2008-revised version of the ThaiPOG regimens. The ALL 1301 was for standard risk ALL, the ALL 1302 was for high risk ALL and LL, and the ALL 1303 was for very high-risk ALL. (Supplemental Figure S2). Asparaginase was used during the induction, consolidation, and delay-intensification phases. Asparaginase dosage in the induction phase was the same as in the former regimens. For the ALL 1301 protocol, two doses of 25,000 IU/m^2 asparaginase were used in the delay-intensification phase. For ALL 1302 and ALL 1303, the protocol contained 4 doses of 25,000 IU/m^2 asparaginase during the consolidation phase and an additional 4 doses of 25,000 IU/m^2 during the delay-intensification phase.

Data collection

All cases of TEEs were included by indicating clinical signs and symptoms and confirmed by imaging studies with a positive thrombus in vessels. The data including basic characteristics of the patients, clinical presentations, actual dosage of asparaginase, concomitant anthracycline and steroids administration, types of steroids, imaging study results, treatments, and outcomes, were all collected.

The time to the onset of TEEs during the induction and consolidation phases was calculated from the start date of asparaginase given, which was on day 4 of the induction, to the first TEE onset. Likewise, the time to the onset of TEEs during delay-intensification

was calculated from the start date of asparaginase, which was on day 8 of the delay-intensification, to the date of the first TEEs onset.

Thrombophilic laboratory results after asparaginase administration were collected, including lupus anticoagulant, protein C, protein S, antithrombin, fibrinogen, factor VIII, factor IX, factor XII levels, and factor V Leiden. All the results were interpreted using age-specific reference ranges.

Outcome criteria

Both of the diseases and the TEE outcomes were reported. The disease outcome was categorized as remission or relapse. The survival status of the patients was also collected. The outcome of TEEs was evaluated by clinical signs and imaging studies. For the imaging, the outcomes of TEEs were classified as follows: complete resolution (CR) if the thrombus was completely resolved, partial resolution (PR) if the thrombus was shrunken in size but not completely resolved, and stable disease (SD) if the thrombus size was unchanged. In particular, patients with CNS TEEs had outcomes reported by using a modified Rankin Score (mRS) with a grade of 0-6.[3] Grading of the mRS is defined as "grade 0" when a patient has no residual symptoms, "grade 1" when a patient has residual symptoms but is able to carry out all usual duties and activities, "grade 2" when a patient has a slight disability but is able to look after his/her own affairs without assistance, "grade 3" when a patient has a moderate disability requiring some help but is able to walk without assistance, "grade 4" when a patient has a moderately severe disability, and is unable to walk and attend to his/her own bodily needs without assistance, "grade 5" when a patient has a severe disability or is bedridden, and "grade 6" when a patient is dead.

Statistical analysis

Descriptive statistics, including mean, median, standard deviation, number, percentage, and range for continuous data, were analyzed by STATA version 10.0.

Results

A total of 831 childhood ALL and LL patients were included. None of patient was excluded. Sixteen patients developed TEEs, with an incidence rate of 1.9%. The majority of patients were female (56.2%), with a median age of 12 years (range 3-15 years). The median time to the onset of TEEs was 17 days (range 1-41 days) after the first dose of asparaginase was given. Most patients (87.5%) developed TEEs during the induction and consolidation phases. Thirteen cases (81.1%) were high-risk ALL, followed by 1 case with standard risk (6.3%), and 1 patient with relapsed ALL (6.3%). Almost all of the patients received asparaginase concomitant with steroids (87.5%) and anthracycline (93.8%). The most common site of TEEs was CNS thrombosis (81.1%), followed by pulmonary embolism (6.3%), deep vein thrombosis of the lower extremities (6.3%), and multiple sites of thrombosis (6.3%). The majority of patients with CNS TEEs presented with seizures, followed by hemiparesis. Nearly half of the patients also experienced other side effects from asparaginase, including hyperglycemia, pancreatitis, and anaphylaxis. None of the patients died from these adverse events. Most of the patients were treated with low-molecular weight heparin (LMWH). Serious bleeding from the anticoagulant therapy was not found (Tables 1 and 2).

Apart from asparaginase, six patients (37.5%) had other predisposing risk factors for TEEs, including infection, indwelling central venous catheter, and progressive ALL/LL. Thrombophilic work-up was performed in 13 patients (81.2%). The most common thrombophilic risk factor found was a low level of protein S (Table 3).

Table 1 Demographic data of TEEs patients

	Number of patients (N=16) (%)
Age	
Median; yr (range)	12 (3-15)
< 10	5 (31.3)
≥ 10	11 (68.7)
Sex	
Male	7 (43.8)
Female	9 (56.2)
Disease	
ALL	13 (81.2)
LL	3 (18.8)
Days of asparaginase	
< 9	8 (50.0)
≥ 9	8 (50.0)
Daily dose of asparaginase	
≤ 6,000 U/m^2	1 (6.3)
≥ 10,000 U/m^2	13 (81.2)
≥ 25,000 U/m^2	2 (12.5)
Total dose of asparaginase	
< 60,000 U/m^2	7 (43.8)
> 60,000 U/m^2	9 (56.2)
Use of anthracyclines	
Yes	14 (87.5)
No	2 (12.5)
Use of steroids	
Yes	15 (93.8)
No	1 (6.2)
Types of steroids	
Prednisolone	9 (56.2)
Dexamethasone	4 (25.0)
Prednisolone and dexamethasone	2 (12.5)

Table 1 Demographic data of TEEs patients (per)

	Number of patients (N=16) (%)
Phase of chemotherapy	
Induction	12 (75.0)
Consolidation	2 (12.5)
Delayed-intensification	2 (12.5)
Other risk factors	
Infection	4 (25.0)
Central line insertion	1 (6.3)
Progressive disease	1 (6.3)
None	10 (62.4)
Site of thrombosis	
Cerebral sinovenous thrombosis	7 (43.8)
CNS arterial thrombosis	6 (37.3)
Deep vein thrombosis	2 (12.6)
Multiple sites	1 (6.3)
Other side effects of asparaginase	
Hyperglycemia	5 (31.3)
Pancreatitis	1 (6.3)
Anaphylaxis	1 (6.3)
None	9 (56.1)
Treatment modalities	
Low-molecular weight heparin	13 (81.1)
Unfractionated heparin	2 (12.6)
No treatment	1 (6.3)

Table 2 Characteristics of patients with Thromboembolic events (TEEs)

Pt	Age	Sex	Disease	Risk/ Stage	Onset of TEE (days)	Diagnosis TEE (BC)	Phase of chemotherapy	Symptom	Event	Treatment	Rechallenge	Outcome of TEE	Status	Relapse
1	7	F	ALL	VHR	8	2005	Induction	Seizure	CNS arterial thrombosis	No	No	Clinical improve	Alive	No
2	13	F	ALL	HR	12	2008	Intensification	Seizure	CNS arterial thrombosis	LMWH	No	Clinical improve	Alive	Yes
3	6	F	ALL	VHR	21	2010	Consolidation	Eye proptosis	CSVT	LMWH	No	ND	Dead	No
4	12	F	ALL	HR	11	2011	Intensification	Hemiparesis	CSVT	LMWH	ND	CR	Alive	No
5	13	M	ALL	VHR	40	2013	Induction	Dyspnea	PE	LMWH	Yes	PR	Dead	Yes
6	11	M	ALL	HR	20	2014	Consolidation	Hemiparesis	CNS arterial thrombosis	LMWH	No	PD	Dead	No
7	3	M	ALL	Relapse	1	2015	Induction	Reduced consciousness Hemiparesis Reduced consciousness	CNS arterial thrombosis	LMWH	No	CR	Dead	Yes
8	12	F	LL	Stage III	17	2015	Induction	Seizure	CSVT	LMWH	Yes	CR	Alive	No
9	10	F	ALL	HR	20	2016	Induction	Seizure	CSVT	LMWH	Yes	CR	Alive	No
10	15	M	LL	Stage III	25	2016	Induction	Coma	CNS arterial thrombosis	UFH	Yes	PR	Alive	No
11	7	F	ALL	SR	37	2016	Intensification	Seizure	CSVT	LMWH	No	CR	Alive	No
12	13	F	ALL	HR	13	2017	Induction	Coma Leg edema	CNS arterial thrombosis, DVT	LMWH	Yes	PR	Alive	No
13	13	M	LL	Stage II	17	2017	Induction	Seizure	CSVT	LMWH	Yes	PR	Alive	No
14	3	M	ALL	HR	41	2017	Induction	Seizure	CSVT	LMWH	Yes	CR	Alive	No
15	13	M	ALL	HR	8	2017	Induction	Seizure, Hemiparesis	CNS arterial thrombosis	LMWH	Yes	CR	Alive	No
16	12	F	ALL	HR	9	2017	Induction	Leg pain	DVT	UFH	Yes	CR	Alive	No

Table 3 Thrombophilic work-up

Thrombophilic work-up	Number of patients with blood work obtained	Abnormal findings	Number of patients (%)
Antithrombin	13	Low Antithrombin	5 (38.5)
Protein C level	13	Low protein C level	3 (23.0)
Protein S level	13	Low protein S level	11 (84.6)
Thrombin time	1	Prolonged thrombin time	0 (0)
Factor VIII level	1	High factor VIII level	0 (0)
Factor IX level	1	High factor IX level	0 (0)
Fibrinogen level	4	Low fibrinogen level	1 (25)
Factor V Leiden	2	Abnormal factor V Leiden	0 (0)
Lupus anticoagulant	6	Positive for lupus anticoagulant	0 (0)

Thirteen patients (81.2%) had the outcome assessed by imaging studies: eight patients had CR, four patients had PR, and one patient had progressive disease (PD). Two patients had clinical improvement without the follow-up imaging performed. Ten of fourteen patients (71.4%) with CNS TEEs had favorable outcomes (modified Rankin scale 0-1) as shown in Figure 1. Eleven patients (68.8%) were alive, two patients died from advanced cancer, two patients died from infection, and only one patient died from brain herniation after progressive cerebral infarction. Administration of asparaginase was withheld for all patients at the time of TEE occurrence. Eight patients got asparaginase re-challenge with no further TEEs. Only one patient had recurrent DVT after re-administration of asparaginase while having other thrombotic risk factors of central line insertion and immobilization from bedridden status. The remaining 6 patients did not receive asparaginase re-challenge because of persistent seizures (2 patients), death (3 patients), and no additional asparaginase in the protocol after TEEs (1 patient).

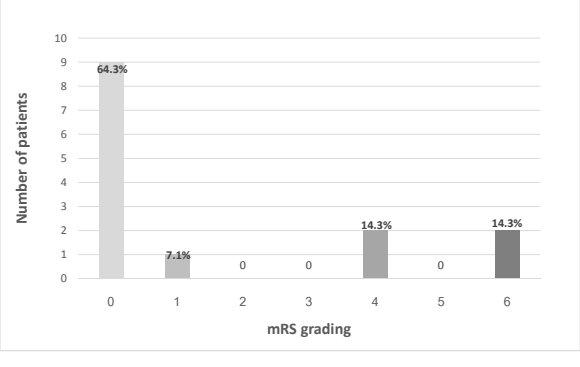


Figure 1 Modified Rankin Scale (mRS) of CNS TEE (N=14)

Treatment protocol for

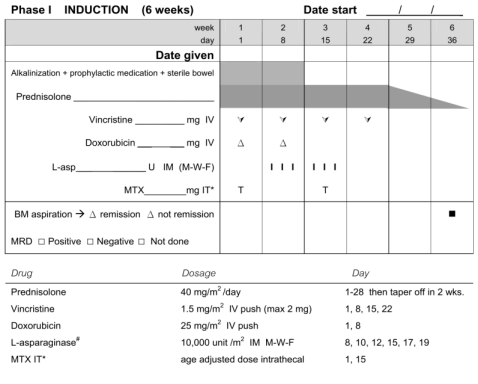
ALL and LL (during 1997-2013)

(A) Induction phase for standard risk (ALL SR 01-08 protocol),

(B) Induction phase for high risk/very high risk (ALL SR 02-08 protocol)

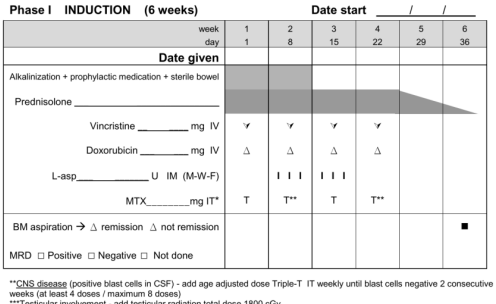
(C) Delay-intensification phase

A



Drug	Dosage	Day
Prednisolone	40 mg/m^2 /day	1-28 then taper off in 2 wks.
Vincristine	1.5 mg/m^2 IV push (max 2 mg)	1, 8, 15, 22
Doxorubicin	25 mg/m^2 IV push	1, 8
L-asparaginase#	10,000 unit /m^2 IM M-W-F	8, 10, 12, 15, 17, 19
MTX IT*	age adjusted dose intrathecal	1, 15

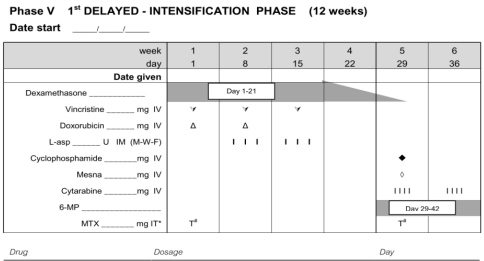
B



**CNS disease (positive blast cells in CSF) - add age adjusted dose Triple-T IT weekly until blast cells negative 2 consecutive weeks (at least 4 doses / maximum 8 doses)
***Testicular involvement - add testicular radiation total dose 1800 cGy.

Drug	Dosage	Day
Prednisolone	40 mg/m^2 /day	1-28 then taper off in 2 wks.
Vincristine	1.5 mg/m^2 IV push (max 2 mg)	1, 8, 15, 22
Doxorubicin	25 mg/m^2 IV push	1, 8, 15, 22
L-asparaginase	10,000 unit /m^2 IM M-W-F	8, 10, 12, 15, 17, 19
MTX IT*	Age-adjusted dose intrathecally	1, 15

C



Drug	Dosage	Day
Dexamethasone	10 mg/m^2 /day PO	1-21 then taper off
Vincristine	1.5 mg/m^2 IV push	1, 8, 15
Doxorubicin	25 mg/m^2 IV push	1,8
L-asparaginase (L-asp)	10,000 unit /m^2 IM M-W-F	8, 11,13, 15, 18, 21
Cyclophosphamide (CTX)	1,000 mg/m^2 IV drip in 1 hr	29
Mesna	500 mg/m^2 IV drip in 15 min at Hr 0, 3 of CTX	29
Cytosine arabinoside (Ara-C)	75 mg/m^2 IV push	29-32, 36-39
6-MP (50mg)	50 mg/m^2 day PO x 14 days	29-42
MTX IT*	age adjusted dose intrathecal	1, 29

*Age-adjusted dose triple drug intrathecal in CNS disease until total 10 doses

Treatment protocol for ALL and LL (during 2014-2017)

(A) Induction phase for standard risk (ThaiPOG ALL-1301)

(B) Induction phase for high risk/very high risk (ThaiPOG ALL-1302/1303)

(C) Consolidation phase for high risk/very high risk (ThaiPOG ALL-1302)

(D) Consolidation phase for very high risk (ThaiPOG ALL-1303)

(E) Delay-intensification phase

A

Week / Day	1 / 1	2 / 8	3 / 15	4 / 22	5 / 29	6 / 36
Date due						
Date given						
Medication:						
VCR _____ mg IV	V	V	V	V		
PRED _____ mg po BID						
L-ASP _____ IU IM	A/A	A/A/A/A				
IT-MTX _____ mg	T	T	(T)		T	
Investigation:						
CBC/diff	+	+	+	+	+	Begin Consolidation on Day 36 or when CBC parameter was met (whichever occurs later). Patient with M2 and M3 marrow on day 29 should begin Consolidation therapy irrespective of hematologic values as soon as Day 29 marrow results are known except patient have active infection or life threatening organ dysfunctions.
CSF cell count/ cytospin	+	+	(+)		+	
BUN, Cr, TB,DB, AST, ALT	+				+	
Biopsy and MRD (optional)					+	
ECHOor MUGA and EKG (optional)					+	

Drug	*Route*	*Dosage*	*Days*
Vincristine (VCR)	IV push over 1 min	1.5 mg/m^2/day (Max 2 mg)	Day 1, 8, 15 and 22
PrednisONE (PRED)	PO	30 mg/m^2/dose BID (No max dose)	Day 1-28
L-Asparaginase (L-ASP)	IM	10,000 IU/m2/dose	Day 4,6,8 and 10, 12, 14
Intrathecal Methotrexate (IT MTX)	IT	Age(yrs.) / Dose 1-1.99 / 8 mg 2-2.99 / 10 mg 3-8.99 / 12 mg ≥9 (but < 30 kg) / 12 mg ≥9 / 15 mg	Day 1,8 and 29 *Day 15 only for traumatic tap.

B

Week / Day	1 / 1	2 / 8	3 / 15	4 / 22	5 / 29	6 / 36
Date due						
Date given						
Medication:						
VCR _____ mg IV	V	V	V	V		
PRED _____ mg po BID						
DOX _____ mg IV	D	D	D	D		
L-ASP _____ IU IM	A/A	A/A/A/A				
IT-MTX _____ mg	T	T	(T)	(T)	T	
Investigation:						
CBC/diff	+	+	+	+	+	Begin Aug-Consolidation on Day 36 or when CBC parameter was met (whichever occurs later). Patient with M2 and M3 marrow on day 29 should begin Consolidation therapy irrespective of hematologic values as soon as Day 29 marrow results are known except patient have active infection or life threatening organ dysfunctions.
CSF cell count/ cytospin	+	+	(+)	(+)	+	
BUN, Cr, TB,DB, AST, ALT	+				+	
BM Aspiration					+	
Biopsy and MRD (optional)					+	
ECHOor MUGA and EKG (optional)					+	

Drug	*Route*	*Dosage*	*Days*
Vincristine (VCR)	IV push over 1 min	1.5 mg/m^2/day (Max 2 mg)	Day 1, 8, 15 and 22
PrednisONE (PRED)	PO	30 mg/m^2/dose BID (No max dose)	Day 1-28
Doxorubicin (DOX)	IV push in 15 min	25 mg/m^2/dose	Day 1, 8, 15 and 22
L-Asparaginase (L-ASP)	IM	10,000 IU/m2/dose	Day 4, 6,8 and 10, 12, 14
Intrathecal Methotrexate (IT MTX)	IT	Age(yrs.) / Dose 1-1.99 / 8 mg 2-2.99 / 10 mg 3-8.99 / 12 mg ≥9 (but < 30 kg) / 12 mg ≥9 / 15 mg	Day 1,8 and 29 *Day 15 and 22 only for CNS-3 and traumatic tap.

C

Week / Day	1 / 1	2 / 8	3 / 15	4 / 22	5 / 29	6 / 36	7 / 43	8 / 50	9 / 57
Date due									
Date given									
Medication:									
CPM _____ mg IV	C				C				
ARAC _____ mg IV	Ā Ā Ā Ā	Ā Ā Ā Ā			Ā Ā Ā Ā	Ā Ā Ā Ā			
6-MP _____ mg PO daily									
L-ASP _____ IU IM			A	A			A	A	
VCR _____ mg IV			V	V			V	V	
IT-MTX _____ mg	T	T	T	T					
Investigation:									
CBC/diff	+	+	+	+	+	+	+	+	Begin the Aug Interim Maintenance-I on Day 57 or when CBC parameter was met whichever occurs later.
CSF cell count/ cytospin	+	+	+	+					
BUN, Cr, TB,DB, AST, ALT	+				+			+	

Drug	*Route*	*Dosage*	*Days*
Cyclophosphamide (CPM)	IV over 30-60 min	1000 mg/ m^2/dose	Day 1 and 29
Cytarabine (ARAC)	IV over 15-30 min or SC	75 mg/m^2/dose/day	Day 1-4, 8-11, 29-32, and 36-39
Mercaptopurine (6-MP)	PO	60 mg/ m^2/dose/day	Day 1-14 and 29-42
L-Asparaginase (L-ASP)	IM	25,000 IU/ m^2/dose/day	Day 15,22, 43, 50
VinCRIStine (VCR)	IV push over 1 min	1.5 mg/m^2/day (Max 2 mg)	Day 15, 22, 43, and 50
Intrathecal Methotrexate (IT MTX)	IT	Age(yrs.) / Dose 1-1.99 / 8 mg 2-2.99 / 10 mg 3-8.99 / 12 mg ≥9 (but < 29 kg) / 12 mg ≥9 / 15mg	Day 1, 8, 15, 22

D

Week / Day	1 / 1	2 / 8	3 / 15	4 / 22	*5 / *29	6 / 36	7 / 43	8 / 50	9 / 57
Date due									
Date given									
Medication:									
CPM _____ mg IV	C				CCCCC				
ARAC _____ mg IV	Ā Ā Ā Ā	Ā Ā Ā Ā							
6-MP _____ mg PO daily									
ETOP _____ mg IV					EEEEE				
L-ASP _____ IU IM			A	A			A	A	
VCR _____ mg IV			V	V			V	V	
IT-MTX _____ mg	T	T	T	T					
Investigation:									
CBC/diff	+	+	+	+	+		+	+	Begin the Aug Interim Maintenance-I on Day 57 or when CBC parameter was met whichever occurs later.
CSF cell count/ cytospin	+	+	+	+					
BUN, Cr, TB,DB, AST, ALT	+				+			+	

Drug	*Route*	*Dosage*	*Days*
Cyclophosphamide (CPM)	IV over 30-60 min	1000 mg/ m^2/dose	Day 1
Cytarabine (ARAC)	IV over 15-30 min or SC	75 mg/m^2/dose/day	Day 1-4, 8-11
Mercaptopurine (6-MP)	PO	60 mg/ m^2/dose/day	Day 1-14
Cyclophosphamide (CPM)	IV over 15-30 min	440 mg/m2/dose	Day 29-33
Etoposide (ETOP)	IV over 60-120 min	100 mg/m2/dose	Day 29-33
L-Asparaginase (L-ASP)	IM	25,000 IU/ m^2/dose/day	Day 15,22, 43, 50
VinCRIStine (VCR)	IV push over 1 min	1.5 mg/m^2/day (Max 2 mg)	Day 15, 22, 43, and 50
Intrathecal Methotrexate (IT MTX)	IT	Age(yrs.) / Dose 1-1.99 / 8 mg 2-2.99 / 10 mg 3-8.99 / 12 mg ≥9 (but < 29 kg) / 12 mg ≥9 / 15mg	Day 1, 8, 15, 22

E

Week / Day	1 / 1	2 / 8	3 / 15	4 / 22	*5 / *29	6 / 36	7 / 43	8 / 50	9 / 57
Date due									
Date given									
Medication:									
VCR _____ mg IV	V	V	V				V	V	
DOXO _____ mg IV	D	D	D						
DEX _____ mg po BID									
L-ASP _____ IU IM		A	A		*CCCCC		A	A	
CPM _____ mg IV					*EEEEE				
ETOP _____ mg IV					*T				
IT-MTX _____ mg	T					T			
Investigation:									
CBC/diff	+	+	+	+	+	+	+	+	Begin IM-II on Day 57 or when CBC parameter was met whichever occurs later.
CSF cell count/ cytospin	+				+	+			
BUN, Cr, TB,DB, AST, ALT	+				+				
ECHOor MUGA and EKG (optional)	+								

Drug	*Route*	*Dosage*	*Days*
Dexametasone (DEX)	PO	5 mg/ m^2/dose BID	Days 1-7, 15-21
Vincristine (VCR)	IV push	1.5 mg/m^2/day (Max 2 mg)	Day 1, 8, 15, 43, 50
Doxorubicin (DOXO)	IV over 15 min	25 mg/m^2/dose	Day 1, 8, 15
L-Asparaginase (L-Asp)	IM	25,000 IU/ m^2/dose/day	Day 8 , 15, 43, 50
Cyclophosphamide (CPM)	IV over 30-60 min	440 mg/m2/dose	Day 29-33
Etoposide (ETOP)	IV over 60-120 min	100 mg/m2/dose	Day 29-33
Intrathecal Methotrexate (IT MTX)	IT	Age(yrs.) / Dose 1-1.99 / 8 mg 2-2.99 / 10 mg 3-8.99 / 12 mg ≥9 (but < 30 kg) / 12 mg ≥9 / 15mg	Day 1, 29, 36

Note:

- *Begin Day 29 of DI only when ANC≥750/µL and Plt ≥75,000/µL

Abbreviations : L-asp, L-asparaginase; CTX, cyclophosphamide; ARA-C, Cytarabine; 6-MP, 6-mercaptopurine; MTX, methotrexate; IT, intrathecal; IM, intramuscular

Discussion

Asparaginase-related TEEs are serious side effects that cause morbidity and mortality in pediatric ALL and LL patients. Our study demonstrated the clinical characteristics, treatments and outcomes of patients with asparaginase-related TEEs. The incidence rate of TEEs in patients treated with asparaginase was 1.9%, which was comparable to the earlier reported incidence of 1.5-6.2%.[4,14,15] The incidence rate of CNS TEEs is higher than non-CNS TEEs (2.9% versus 2.3%).[4] Similar to our study, the majority of TEEs were CNS thrombosis and the venous site was more common than the arterial site.

Seventy-five percent of the patients were older than 10 years of age. The majority of patients (93.8%) had high-risk disease and received the high-risk chemotherapy protocol, which consisted of asparaginase concomitantly given with steroids and doxorubicin. Corticosteroids have been reported to increase the risk of thrombosis by increasing the levels of factor VIII, factor IX, and von Willebrand's factor (VWF), while also inhibiting fibrinolysis by secreting PAI-1.[5–8] Doxorubicin, which is a cytotoxic agent in the anthracycline group, has also been found to augment the risks of TEEs by inducing vascular toxicity, and enhancing platelet adhesion and aggregation.[9-10] Our study emphasized the evidence that intensive chemotherapy treatment, and particularly the concurrent use of asparaginase with steroids and doxorubicin, increased the risks of TEEs in ALL/LL patients.

The effects of asparaginase dosage and duration were not concluded in our studies, because most patients were treated following the ThaiPOG protocol, which uses high-doses and short-durations of asparaginase. According to Caruso et al., patients who received a lower dose (≤ 6,000 mg/m^2) and longer duration (> 9 days) of asparaginase had higher risks of TEEs than those who received a higher dosage and shorter duration.[4] Therefore, the effect of dosage and duration of asparaginase on TEEs might require further study.

The central venous catheter insertion has been established as an important risk factor for thrombosis in several studies.[8,9] In our institute, central venous catheters were not routinely used for chemotherapy infusion if the peripheral veins were accessible. There was only one patient with an indwelling central venous catheter when TEE occurred. Therefore, the association between central venous catheters and asparaginase-related TEEs might not be adequately demonstrated in this study.

Our study also reported the thrombophilic laboratory investigation of asparaginase-related TEE cases. The most frequent abnormality was a decreased free protein S level, followed by decreased antithrombin and protein C levels. These findings were similar to the previous studies.[16,17] Thromboembolism prophylaxis strategies including the utilization of fresh frozen plasma (FFP), antithrombin concentrate (AT), and heparin have been widely published.[7,18-20] FFP supplementation had no effect on the primary prevention of TEE in pediatric ALL patients. The PARKAA study demonstrated the efficacy of AT infusion to prevent thrombotic events. However, the results were underpowered.[19] The THROMBOTECT trial has been conducted, focusing on the efficacy of unfractionated heparin (UFH), LMWH, and AT for TEE prophylaxis during the induction period of ALL. The trial demonstrated that LMWH or AT concentrate seemed to effectively decrease the incidence of TEEs when compared to UFH.[20] However, the increased incidence of late relapse in patients who received AT concentration was reported. In our study, the primary TEE prophylaxis was not used in the patients receiving asparaginase in a normal setting. Nonetheless, seven patients with low levels of antithrombin, protein C, or protein S, received FFP prophylaxis for the subsequent doses of asparaginase. Despite FFP prophylaxis given, one out of seven patients still developed recurrent DVT. However, this patient also had multiple other risk factors for TEEs, including central venous catheter insertion and immobilization. Earlier research suggested that L-asparagine in plasma-derived products may decrease the efficacy of asparaginase and affect leukemia and lymphoma survival outcomes.[7,20] In our cohort, no patient had relapsed disease within at least a 3-year follow-up period. The cut-off levels of protein C, protein S, and AT should be further studied to optimize the replacement therapy in order to prevent TEEs.

Seven of 16 patients (43%) developed other complications from asparaginase. Five patients who had hyperglycemia were older than 10 years. Among these patients, one received insulin therapy because of diabetic ketoacidosis. Similar to the study in Hispanic children, an older age and a high body mass index increased the risk of hyperglycemia.[21] Despite having these complications, no patient died from anaphylaxis, hyperglycemia, or pancreatitis.

The majority of TEE patients had favorable outcomes after treatment, confirmed by the resolution of thrombosis from imaging studies, notably in patients who were diagnosed in recent years. This could result from the introduction of the Thai National Chemotherapy Protocol in 2014, which focuses on optimal chemotherapy and supportive care strategies to improve patient outcomes. Our study also displayed the functional outcomes of CNS TEEs, in which the majority of patients (71.4%) had no serious neurological consequences. In this series, only one patient died from progressive arterial infarction.

Even though asparaginase-related TEEs occurred, omitting asparaginase may affect the survival outcomes of ALL. Re-exposure to asparaginase following TEEs is a challenging topic. In the UKALL 2003 study, no recurrent TEE was observed in the patients with asparaginase-related venous thrombosis after re-challenging and concurrent with heparin prophylaxis.[8] Likewise, our cohort found only one patient with multiple pre-existing thrombotic risks who had recurrent TEE.

This report is the first to collect patients with asparaginase-related TEE over a 10-year period in our institute. Regarding the retrospective study design, some data might not be sufficient to generate conclusive results. With this limitation, prospective studies about TEEs and preventive strategies should be conducted in the future to improve the outcomes of pediatric leukemia and lymphoma patients.

Conclusions

Caution is necessary concerning asparaginase-related TEE in leukemia and lymphoma patients, particularly in patients aged more than 10 years old. Protein S deficiency was the most common abnormal thrombophilic laboratory investigation. Post-TEEs treatment had favorable outcomes. Rechallenge of asparaginase is rather safe. Further larger prospective studies focusing on risk factors and TEEs prophylaxis studies should be conducted to improve outcomes.

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