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Sequential Administration of Methotrexate and Asparaginase in Relapsed or Refractory Pediatric Acute Myeloid Leukemia

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Abstract

Background—The efficacy of combination chemotherapy with methotrexate (MTX) and asparaginase is not well known in relapsed and refractory acute leukemia after contemporary therapy.

Procedure—A retrospective study of pediatric patients with relapsed or refractory acute myeloid leukemia (AML) who received MTX and asparaginase as a salvage therapy at St. Jude Children Research Hospital was performed. MTX was given intravenously followed by a dose of asparaginase intramuscularly or intravenously 24 hours later. The chemotherapy cycle was repeated every 7-10 days. Response, survival, and toxicities were evaluated.

Results—Fifteen patients, median age 10.5 years (range, 1.1-18.5 years), were treated. Median number of previous therapeutic regimens was 3 (range, 1-4). Six patients responded to treatment (3 had morphologic complete remission with incomplete blood count recovery, 2 had partial remission, and 1 had stable disease for 16 months), and 4 are still alive. Three of 6 responders had monoblastic leukemia, and also developed tumor lysis syndrome. The 1- and 2-year overall survival rates are 35.6% and 17.8%, respectively. The most common adverse event was transient elevation of transaminases (9 patients). Two patients developed pancreatitis. Episodes of febrile neutropenia were rare (2 patients), and most courses (75 out of 93 total courses) were given in an outpatient setting.

Conclusions—Combination chemotherapy with MTX and asparaginase appears to be an effective salvage therapy and well tolerated in patients with relapsed or refractory childhood AML, even in those heavily pretreated with contemporary frontline or salvage therapy.

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Conflicts of interest
The authors have no competing interests.

Keywords

acute myeloid leukemia; methotrexate; asparaginase; children; relapse

INTRODUCTION

Acute myeloid leukemia (AML) accounts for 17% of all childhood leukemia [1]. Recent risk-adapted therapeutic approaches, including the use of cytarabine (Ara-C), anthracyclines and etoposide, together with improved supportive care, have increased survival rates to 60-70% [2-8]. Nevertheless, up to 40% of patients still suffer from relapse or refractory disease [5,6]. After relapse, the risk of anthracycline-induced cardiomyopathy [7] and the resistance of leukemic cells to chemotherapeutic agents become the major obstacles to treatment [8], and the survival rate is dismal (around 30%) even with the use of novel agents such as deoxyadenosine analogue [3-5] and hematopoietic stem cell transplantation (HSCT) [2,9]. The combination of methotrexate (MTX) and asparaginase has been used in both relapsed acute lymphoblastic leukemia and AML [10,11]. This treatment regimen is based on an in vitro study that showed the synergistic anti-leukemic activity of time sequential combination of the medications [12]. In addition, thymidylate synthase inhibition by MTX [13] and the substantial cytotoxic effect of asparaginase in monoblastic leukemia cells [14] suggested the effectiveness in this particular subtype of AML.

In the present study, we report our recent experience of combination chemotherapy with MTX and asparaginase in children with relapsed or refractory AML after contemporary frontline or salvage chemotherapy.

METHODS

Patients

Patients with relapsed or refractory AML who had previously been treated with frontline therapy, salvage chemotherapy, or HSCT received sequential administration of MTX and asparaginase at St. Jude Children's Research Hospital from 1999 to 2012.

The diagnosis of relapsed or refractory AML was performed by morphology, molecular studies, or immunophenotyping with flow cytometry on marrow or extramedullary specimens.

Treatment

The treatment regimen included an intravenous dose of MTX followed by a dose of asparaginase (intramuscular or intravenous) 24 hours later. *E. coli* L-asparaginase was initially used and replaced by PEG-asparaginase after 2007. *Erwinia* L-asparaginase was used in patients who were allergic to *E. coli* L-asparaginase. The chemotherapy cycle was repeated every 7-10 days. A complete blood count, chemistries including liver, renal, and pancreatic function tests, and physical examinations were evaluated at least weekly. Triple intrathecal therapy (MTX, hydrocortisone, and cytarabine) and prophylactic antibiotics or antifungal agents were given at the treating physician's discretion [15].

Response and Toxicity Criteria

Responses to treatment were defined according to international working group response criteria [16]. The National Cancer Institute common terminology criteria for adverse events, version 3.0, were used for evaluation of toxicities.

Statistical Analyses

Overall survival was defined as the time from the date starting MTX/asparaginase to death and was analyzed by the Kaplan-Meier method. Exact Wilcoxon sum rank test was used to compare median of first remission period between responder and non-responder.

RESULTS

Patient demographics

Table 1 lists the characteristics and outcome of 15 patients treated with MTX/asparaginase. FAB M5 was the most common subtype (6 patients), and *MLL* rearrangement accounted for the most common genetic abnormality (7 patients). The median time from diagnosis to the first relapse was 7.3 months (range, 3.4-14.3 months). The median age of the patients was 10.5 years (range, 1.1-18.5 years) at the time of MTX/asparaginase therapy, median time from diagnosis to the initiation of MTX/asparaginase therapy was 9.4 months (range, 2.4-26.3 months), and median number of treatment regimens prior to MTX/asparaginase therapy was 3 (range, 1-4), including 5 patients who had received allogeneic HSCT. Three patients received MTX/asparaginase as the first salvage therapy. One patient (patient 1) had a partial remission after induction and consolidation therapy on the frontline protocol, and MTX/asparaginase was used as a bridging therapy to HSCT, and the second patient (patient 5) had prolonged severe pancytopenia with minimal residual disease after frontline therapy with cytarabine, daunorubicin, and etoposide. The third patient with a relapse (patient7) had previously developed severe axonal degeneration after cladribine/cytarabine. All 15 patients had either relapse or refractory disease after 1 to 4 therapeutic regimens; 3 patients had primary refractory disease, and 7 patients had prior remissions of less than 6 months. The most recent regimens before MTX treatment are listed in Table 1. Before treatment with MTX/asparaginase, 6 patients had isolated bone marrow relapses, 1 patient had combined bone marrow and extramedullary relapses (skin), 1 patient had persistent minimal residual disease, and seven patients had disease that was refractory to immediately prior courses, including 5 with bone marrow disease, 1 with combined bone marrow and extramedullary disease, and 1 with isolated extramedullary disease (skin). The median number of courses of MTX/asparaginase per patient was 2 (range, 1-54). A total of 93 courses were given to the 15 patients, and 75 courses were given in an outpatient setting. Non-escalating MTX doses of 60 to 120 mg/m^2 were given to 14 patients (3 patients with 60 mg/m^2, 5 patients with 100 mg/m^2, and 6 patients with 120 mg/m^2) while escalating doses were given to a patient who received the initial dose at 60 mg/m^2 with increases of 20 mg/m^2 in subsequent cycles until reaching 100 mg/m^2. Six patients were treated with *E. coli* asparaginase (10,000 Units/m^2), 7 PEG-asparaginase (3,000 Units/m^2), and 2 *Erwinia* asparaginase (10,000 Units/m^2).

Responses

Six patients had responses. There was no difference in the duration of response between responders and non-responders (median, 164 days and 290 days, respectively, P=0.37). Three patients had morphologic complete remission with incomplete blood count recovery (CRi), 2 attained partial remission (PR), and one had stable disease, having received a total of 54 MTX/asparaginase courses in 16 months until she died of disease progression. After achieving remission, 4 patients (2 CRi and 2 PR) received HSCT, and 3 of them are alive for 90, 56, and 8 months. One patient (patient 2) who achieved CRi developed pancreatitis after the second course of MTX/asparaginase and was subsequently treated with monthly vincristine, weekly methotrexate, and daily mercaptopurine. Unfortunately, his disease relapsed, and thereafter he received MTX/asparaginase with concomitant octreotide as a prophylaxis for pancreatitis; he remains alive without disease after a total of 8.5 months. Eight of nine patients who did not respond to MTX/asparaginase died of disease and 1 of sepsis after HSCT. The 1-and 2-year overall survival were 35.6% and 17.8%, respectively.

Toxicities

Table 2 shows the toxicities from this regimen. Transient elevation of transaminase is the most common side effect (9 of 15 patients); 3 experienced grade III, and 6 had grade II toxicity. One patient also experienced transient grade II hyperbilirubinemia. Two patients had pancreatitis after the second course. Three patients had tumor lysis syndrome, and 1 had capillary leak syndrome. Febrile neutropenia was observed in only 2 patients. No evidence of asparaginase allergy was seen in our cohort including 2 patients who had history of *E. coli* asparaginase allergy and received *Erwinia* asparaginase.

DISCUSSION

In our study, the sequential combination of MTX and asparaginase produced objective responses in 6 of 15 patients with heavily pretreated pediatric relapsed/refractory AML and yielded 1- and 2-year overall survival rates of 35.6 % and 17.8 %, respectively. This therapy was tolerable in the majority of the patients and can be administered in an outpatient setting. Additionally, this regimen is more affordable and available than the novel nucleoside analogues regimen (e.g., clofarabine with or without cytarabine) even in countries with limited resources.

The administration of MTX followed by asparaginase results in a synergistic effect, although the exact mechanisms are unclear [17,18]. On the other hand, the inverse order of administration of asparaginase appears to attenuate the antileukemic properties of MTX [17,19]. From this *in vitro* evidence, it is suggested that a 10-day interval between each cycle of MTX/asparaginase is the most effective when native *E. coli* asparaginase is used [17,20]. However, we have observed responses with the use of PEG-asparaginase, which has at least 2 weeks of therapeutic activity [21,22]. As seen in Dana-Farber ALL protocols, which gives asparaginase prior to MTX administration, the sequence may not be important in vivo [23,24]. *In vitro*, the cytotoxic effect of asparaginase is higher in acute monoblastic leukemia than in other AML subtypes [14], which correlates to the presence of the lowest level of asparagine synthetase in monoblasts [25]. Further, monoblastic leukemia is highly

sensitive to MTX because of the increased polyglutamylation pattern and relatively lower efflux of MTX [13,26]. We have observed 2 patients with CRi (both with extramedullary disease) and 1 with PR among 6 patients with monoblastic leukemia and *MLL* rearrangement, and all 3 responders developed tumor lysis syndrome. However, responses were seen in 3 other patients with non-monoblastic leukemia. Thus, this regimen can be tried in all subtypes of pediatric AML.

Transient mild elevation of transaminase was the most common complication; severe hepatitis and cholestatic jaundice were not seen. Importantly, febrile neutropenia was seen in only 2 cases, and most of the cases were managed as outpatient. There was no incidence of asparaginase allergy in our study; however, pancreatitis was seen in 2 patients. For heavily pre-treated patients, monitoring of pancreatic enzymes is important. Octreotide can be administered together with MTX/asparaginase as a possible prophylaxis for recurrence of pancreatitis [27]. As described above, we observed effective cytoreductions, especially in monoblastic leukemia with *MLL* rearrangement, and elevated uric acid, phosphate, and potassium at the same time (i.e., tumor lysis syndrome). Some of the patients received this regimen in a palliative care setting.

In conclusion, MTX/asparaginase has efficacy in pediatric patients with refractory or relapsed AML after contemporary intensive therapy. The regimen appeared to be well tolerated. This regimen can be a consideration for treatment of relapsed or refractory AML but needs to be prospectively studied.

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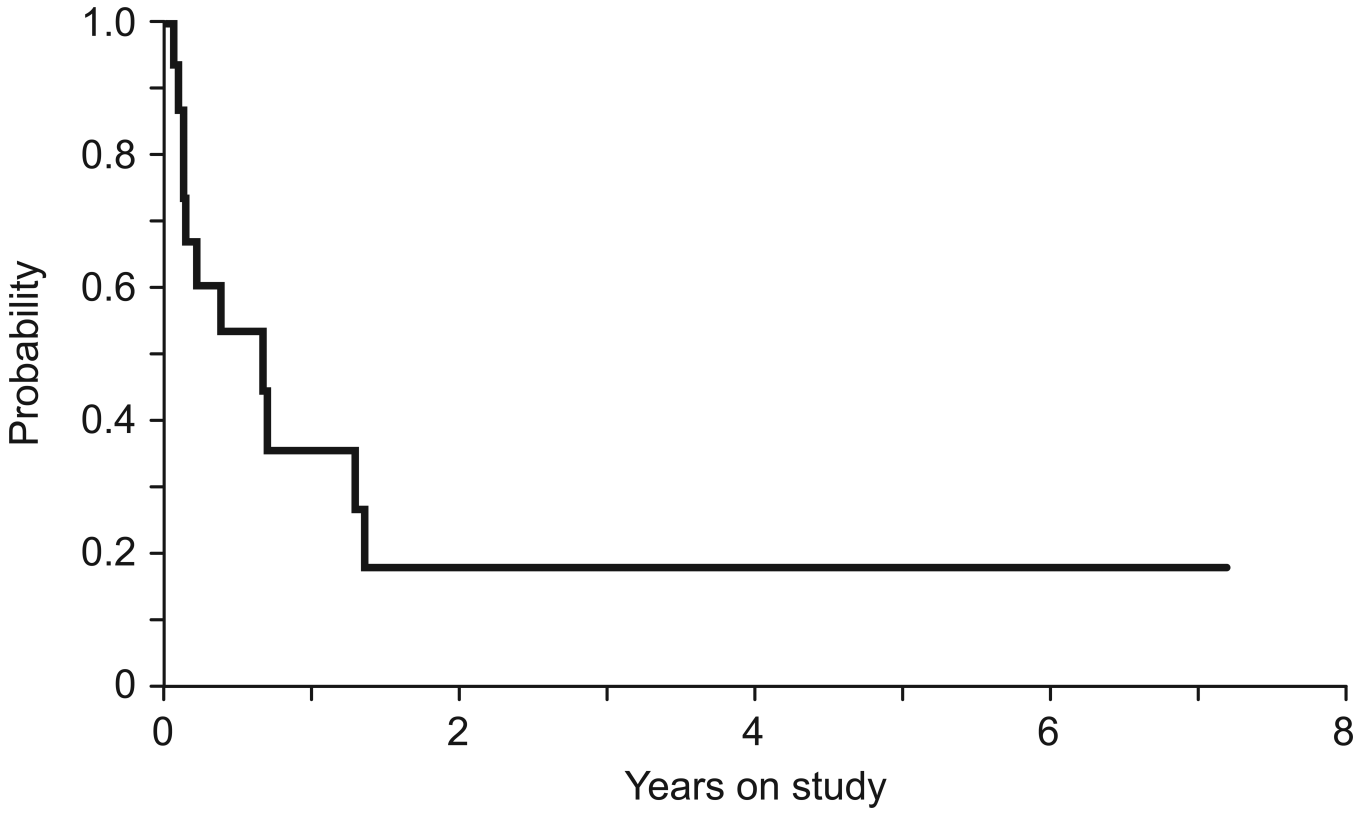

Fig. 1.

The Kaplan–Meier analysis of overall survival in patients with relapsed/refractory acute myeloid leukemia treated with methotrexate and asparaginase.

Table I

Patient Characteristics

Pt	Age at Tx (years)	Sex	FAB	Cytogenetics	No of prior regimens	Prior BMT	Response to most recent therapy	Most recent therapy	Site of disease	Doses of MTX/Asp(type)	No. of courses	Response to MTX/Asp	Post-MTX/Asp transplant	Outcome (survival period)	Remission period	Cause of death
1*	3.4	M	M2	normal	1		relapse	ara-C/Asp	marrow	60/10000 (E.coli)	3	CRi	haplo	alive (91 mo)	90 mo	
2	10.5	M	M5	t(10;11)	3	MUD	refractory	clofarabine/ara-C	skin	120/3000 (PEG)	2	CRi		alive (8.5 mo)	8 mo	
3	1.1	F	M5	t(10;11)	3		refractory	clofarabine/ara-C	marrow,skin	120/3000 (PEG)	4	CRi	UCBT	dead (4.5 mo)	2 mo	disease
4	1.8	F	M5	t(10;11)	2		relapse	clofarabine/ara-C	marrow MRD	100/10000 (E.coli)	11	PR	alloCBT	alive (57 mo)	56 mo	
5	4.8	F	M0	normal	1		relapse	ara-C/Dauno/Eto	marrow	120/3000 (PEG)	4	PR	alloBMT	alive (8.8 mo)	8 mo	
6	11.3	F	M1	t(6;9)	3		refractory	hydroxyurea	marrow	100/10000 (Erwinia)	54	stable disease		dead (16 mo)		disease
7	18.5	M	M4	t(16;16)	1		relapse	cladribine/ara-C	marrow	60/10000 (E.coli)	2	NR		dead (8.1 mo)		disease
8	18.3	M	M1	normal	4		refractory	cladribine/ara-C	marrow	60/10000 (E.coli)	2	NR	haplo	dead (16.5 mo)		disease
9	15.8	M	M4	gain 5p	4	MSD	relapse	mitoxantrone/ara-C/GO	marrow	100/2500 (PEG)	1	NR		dead (1.5 mo)		disease
10	1.1	M	M5	t(3;11)	3		refractory	cladribine/ara-C	marrow	100/10000 (E.coli)	1	NR		dead (1.4 mo)		disease
11	2.9	M	M7	t(2;21;3)	3	haplo	refractory	BMT	marrow	100/2500 (PEG)	3	NR	haplo	dead (8.5 mo)		disease
12	1.2	M	M7	t(9;11)	2		refractory	clofarabine/ara-C	marrow	60/10000 (E.coli)	2	NR		dead (1.4 mo)		disease
13	12	M	M5	t(6;11)	3	MSD	relapse	clofarabine/ara-C	marrow,skin	120/3000 (PEG)	1	NR		dead (0.6 mo)		disease
14	16.1	M	M5	t(10;11)	3		relapse	ara-C/idarubicin	marrow	120/10000 (Erwinia)	2	NR	UCBT	dead (2.7 mo)		TRM due to BMT
15	16.7	M	M7	t(3;21)	3	haplo	relapse	BMT	marrow	120/3000 (PEG)	1	NR		dead (1.0 mo)		disease

Abbreviations: ara-C, cytarabine; Asp, asparaginase; BM, bone marrow; BMT, bone marrow transplantation; CR, complete remission; CRi, morphologic complete remission with incomplete blood count recovery; Dauno, daunorubicin; Dx, diagnosis; Eto, etoposide; F, female; FAB, The French–American–British (FAB) classification; GO, gemtuzumab ozogamicin; haplo, haplo identical transplantation; M, male; mo, months; MRD, minimal residual disease; MSD, match sibling donor transplantation; MTX, methotrexate; MUD, match unrelated donor transplantation; NR, no response; PEG, polyethylene glycol- L –asparaginase; PR, partial response; Pt, patient; TRM, treatment related mortality; Tx, treatment with methotrexate/asparaginase; UCBT, umbilical cord blood transplantation.

*Patient who received escalated doses of methotrexate.

Table II

Toxicity of combination therapy with methotrexate/asparaginase

Adverse event	Total events	Grade 1	Grade 2	Grade 3
Gastrointestinal				
Nausea	1		1	
Mucositis	1		1	
Pancreatitis Metabolic/laboratory	2		2	
AST elevation	9		6	3
ALT elevation	8		6	2
Hyperbilirubinemia Infection	1		1	
Febrile neutropenia	2			2
Capillary leak syndrome	1			1
Tumor lysis syndrome	3			3

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase.

No grade 4 or grade 5 adverse events occurred in the study.