RESEARCH ARTICLE

Comparative Outcome of Thai Pediatric Osteosarcoma Treated with Two Protocols: the Role of High-Dose Methotrexate (HDMTX) in a Single Institute Experience

Worawut Choeyprasert[1], Samart Pakakasama[2], Nongnuch Sirachainan[2], Duantida Songdej[2], Ampaiwan Chuansumrit[2], Usanarat Anurathapan[2], Suradej Hongeng[2]*, Adisak Nartthanarung[3]

Abstract

Background: High-dose methotrexate (HD-MTX) is recognized as an efficient component of therapy against pediatric osteosarcoma in combination with other drugs such as cisplatin (CDP), carboplatin (CBDCA), doxorubicin (ADM), etoposide (VP-16) and ifosfamide (IFO). Objectives: To demonstrate the feasibility and effectiveness of the HD-MTX/CDP/DOX/VP-16/IFO [MTX(+)] protocol comparable to CDP/ADM/CBDCA/IFO [MTX(-)] for treating childhood osteosarcoma at Ramathibodi Hospital (1999-2014). Materials and Methods: A retrospective analysis was conducted of osteosarcoma patients aged less than 18 years treated with two chemotherapeutic regimens between 1999 and 2014. A total of 45 patients received the MTX(-) and 21 the MTX(+) protocol. Results: Overall limb-salvage and amputation rate were 12.9% and 77.7%, respectively. Kaplan-Meier analysis results for 3-year disease free survival (DFS) and overall survival (OS) regardless of treatment regimens were 43.4±6.0% and 53.2±6.1% respectively. The 3-year DFS and OS were improved significantly with the MTX(+) protocol compared to MTX(-) protocol (p=0.010 and p=0.009, log rank test) [69.8±10.5%, 79.8±9.1% for MTX(+) and 31.1±6.9%, 42.2±7.4% for MTX(-) protocol, respectively]. Patients with metastatic osteosarcoma treated with the MTX(+) protocol had statistically significant higher 3-year DFS and OS than those treated with the MTX(-) protocol (66.7±13.6% and 15.0±8.0% for 3-year DFS, p=0.010, 73.3±13.2% and 20±8.9% for 3-year OS, p=0.006, respectively). The independent risk factors for having inferior 3-year DFS and OS were poor histological response (tumor necrosis <90%) and treatment with the MTX(-) protocol. The multivariate analysis identified only the treatment with the MTX(-) protocol as an independent predictor of inferior OS with a hazard ratio (HR) of 3.53 (95% confidence interval of 1.2-10.41, p=0.022). Conclusions: Our study demonstrated the tolerability, feasibility and efficacy of the HDMTX-based regimen improving the survival rate in pediatric osteosarcoma cases, in line with reports from developed countries.

Keywords: Pediatric osteosarcoma - methotrexate - outcome - survival - Thailand

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Introduction

Osteosarcoma is the most common primary bone tumor in children and adults. Approximately 60% of all incidences of osteosarcoma occur in patients aged less than 20 years (Fletcher et al., 2006; Stiller et al., 2006; Mirabello et al., 2009; Mirabello et al., 2009). In Thailand, the annual incidence of pediatric osteosarcoma was approximately 1.9 cases per million children (Wiangnon et al., 2011). After the introduction of neoadjuvant chemotherapy with radical surgery, the disease-free survival rate of patients with non-metastatic osteosarcoma has increased dramatically from below 20%, with surgery only (Coventry and Dahlin, 1957; Dahlin DC, 1978; Marcove et al., 1970; Gaffney et al., 2006), to 60-76% much more recently (Delepine et. al. 1996; Ferrari et al., 2001; Lewis et al., 2007). High-dose methotrexate (HDMTX) with leucovorin rescue is recognized as an efficient component of therapy in childhood osteosarcoma when used in combination with other drugs such as cisplatin (CDDP), carboplatin (CBDCA), doxorubicin (ADR) and ifosfamide (IFO) (Jaffe et al., 1983; Jaffe et al., 1985; Winkler et al., 1990; Rosen et al., 1993; Goorin et al., 2003; Meyers et al., 2008). Since then, several schemes of multiagent adjuvant chemotherapy have been assessed, but recent meta-analysis had still shown no consensus in the addition of HDMTX to treatment of pediatric osteosarcoma. This lack of consensus results from the lack

*Department of Pediatrics, Faculty of Medicine, [1]Chiang Mai University, [2]Ramathibodi Hospital, Mahidol University, [3]Department of Orthopedics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand *For correspondence: suradej.hon@mahidol.ac.th*

of an appropriate randomized- and case-controlled trial comparing the anti-tumor efficacy of HDMTX between treatment groups (Van Dalen et al., 2011).

The purpose of this retrospective study was to assess the therapeutic efficacy in two consecutive eras in order to demonstrate the role of HDMTX-containing chemotherapeutic protocol (MTX(+) protocol) in the treatment of pediatric osteosarcoma at our university hospital. We also aimed to demonstrate the incidence and the epidemiological data, and to identify the prognostic significance influencing the treatment outcome of pediatric osteosarcoma. This approach might provide the legacy of treatment regimen and identify the patient at-risk of refractory disease, as well as who may benefit from other investigational or escalation of treatment.

Materials and Methods

Patients

As a single-institution retrospective study, we enrolled pediatric patients ≤18 years of age with a diagnosis of osteosarcoma between January 1999 and June 2014, and followed up with treatment until the 30th of June 2014 at Ramathibodi Hospital, the tertiary-care referral and research university hospital in Bangkok, Thailand. Clinical data collected for each patient included clinical characteristics (age at diagnosis, sex, medical background and genetic predisposition to cancer), tumor characteristics (location, size, metastatic status at diagnosis), treatment (preoperative chemotherapy, surgery, postoperative treatment, adverse effects), outcome (remission, relapse, survival) and laboratory investigations (complete blood count (CBC), alkaline phosphatase (ALP)). All patients were assessed for the extension of primary tumor by a magnetic resonance imaging (MRI) scan of involved anatomical sites and the metastatic status at diagnosis by chest roentgenogram, bone scintigraphy and computerized tomography (CT) of the chest.

Chemotherapy Regimens and Administration

The chemotherapeutic protocols for pediatric osteosarcoma are shown in Table 1. Patient characteristics are given in Table 2. After the histological diagnosis of osteosarcoma by tumor biopsy, chemotherapy was administered to all patients according to chemotherapeutic protocol regardless of patients' characteristics, size of primary tumor, osteosarcoma subtypes and metastatic status at diagnosis. For patients diagnosed between 1999 and early 2007, we used 3-4 cycles of CDP/ADM preoperatively and CBDCA/IFO alternately with CDP/ADM postoperatively (MTX(-) protocol) in intervals of 3 - 4 weeks. G-CSF (5 mcg/kg/dose) was administered daily during a period of 8 - 10 days as primary prophylaxis of febrile neutropenia. For patients diagnosed between late 2007 and 2014, we used MTX(+) protocol, consisting mainly of cycles of 2 courses of CDP/ADM and 4 courses of HDMTX preoperatively, and alternating cycles of CDP/ADM, IFO/etoposide (VP-16) and HDMTX postoperatively, as shown in Table 1. Hydration (2,500 ml/m^2/day) and urine alkalinization with intravenous sodium bicarbonate were performed for 24 hours prior to MTX infusion to maintain urine pH 7 - 8. 12gm/m^2 MTX was infused over 4 hours, and then folinic acid rescue was given intravenously at 24 hours from the beginning of MTX infusion, at a dose of 10 mg/m^2 every 6 hours, through a total of 11 doses. The dosage of folinic acid was adjusted according to a plasma MTX concentration at 24, 48 and 72 hours starting from the beginning of MTX infusion by a nomogram for pharmacokinetically guided leucovorin rescue after high-dose methotrexate administration (Bleyer WA. 1981). Plasma MTX concentration was collected every additional 24 hours until the plasma concentrations were <0.1umol/L following MTX infusion. G-CSF was also administered daily for 8 - 10 days as primary prophylaxis of febrile neutropenia in each cycle of CDP/ADR and IFO/VP-16. Complete blood count, blood chemistries, liver function test (LFT) and kidney function were monitored regularly before every cycle of chemotherapy.

After preoperative chemotherapy for 8-10 weeks, all patients were radiologically evaluated for the feasibility of surgical procedure and obtaining patients' informed consent for surgery. The decision of radical surgical procedures, such as wide resection with intraoperative radiotherapy, limb-salvage surgery, rotatoplasty or amputation, was based on orthopedic surgeons' opinion, patients' consent and the volume of primary tumor and neurovascular involvement by MRI before and after

Table 1. Chemotherapeutic Protocols for Pediatric Osteosarcoma

A: MTX(-) Protocol													
									Sx*				
Week	1	4	7	10		16	19	22	25	28	31	34	37
agent	C	C	C	C		J	J	C	C	J	J	C*	C*
	A	A	A	A		I	I	A	A	I	A		

Sx*: evaluation and operation; C: Cisplatin 100 mg/m^2/day, day 1; A: Doxorubicin 37.5 mg/m^2/day, day 1-2y, day 1; I: Ifosfamide 1800 mg/m^2/day, day 1-5; C**: Additional cisplatin (according to clinical adjustment); G-CSF (5 mcg/kg/day) was administered daily after completion of chemotherapy except for HDMTX

B: MTX(+) Protocol													
Week	0	3	4	5	8	9	Sx*	11	14	15	18	19	20
agent	C	M	M	C	M	M		C	M	I	M	M	C
	A			A				A		E			A
Week	23	24	27	28	31	32	35	36					
agent	M	I	M	I	M	I	M	M					
		E		E		E							

Sx*: evaluation and operation C: Cisplatin 100 mg/m^2/day, day 1; A: Doxorubicin 37.5 mg/m^2/day, day 1-2; M: High-dose methotrexate 12000 mg/m^2/day, day 1; I*: Ifosfamide 2800 mg/m^2/day, day 1-5; E: Etoposide 100 mg/m^2/day, day 1-5; G-CSF (5 mcg/kg/day) was administered daily after completion of chemotherapy

Table 2. Patients' Characteristics According to Treatment Protocol

Patients' characteristics	All (N=70)	MTX (-) protocol (N=45)	MTX (+) protocol (N=21)
Gender, N (%)			
- Male	46 (65.7)	31 (68.9)	13 (61.9)
- Female	24 (34.3)	14 (31.1)	8 (38.1)
Age, median (years), range	11.60 (5.67-15.31)	11.78 (7.90 – 15.31)	10.60(5.67-14.29)
Median follow-up time (months), range	29.65 (3.33-176.30)	38.72 (3.33 – 120.70)	34.47(7.03-84.83)
Median time to treatment (weeks), range	2.14 (0-22.43)	8.80 (0 – 260.71)	2.14(0.14-14.71)
Primary sites, N (%)			
- Knee	44 (62.8)	35 (77.8)	
- Distal femur	25 (35.7)	13 (28.9)	10 (47.6)
- Proximal tibia	19 (27.1)	15 (33.3)	3 (14.3)
- Proximal fibula	4 (5.7)	3 (6.7)	1 (4.8)
- Ankle (Distal tibia)	5 (7.1)	4 (8.9)	1 (4.8)
- Proximal femur	3 (4.3)	1 (2.2)	1 (4.8)
- Proximal humerus	11 (15.7)	7 (15.6)	4 (19)
- Wrist (Distal radius)	1 (1.4)	1 (2.2)	0
- Axial			
- Iliac wing	1 (1.4)	1 (2.2)	0
- Scapula	1 (1.4)	0	1 (4.8)
Metastasis at diagnosis, N (%)	32 (45.7)	20 (55.6)	9 (42.9)
- Distant bony metastasis	5 (7.1)	4 (8.9)	1 (4.8)
- Pulmonary metastasis	32 (45.7)	20 (55.6)	9 (42.9)
- Combined metastasis	5 (7.1)	4 (8.9)	1 (4.8)
Histology, N (%)*			
- Conventional type			
- Osteoblastic	21 (38.9)	11 (36.7)	8 (40)
- Chondroblastic	15 (27.8)	9 (30)	5 (25)
- Fibroblastic	4 (7.4)	1 (3.3)	3 (15)
- Mixed	5 (9.3)	3 (10)	2 (10)
- Variants	9 (16.7)	6 (20)	2 (10)
- No data	16	15	1
Chemotherapy, N (%)	-		
- No chemotherapy	4 (5.7)		
- Neoadjuvant chemotherapy	52 (74.3)	33 (73.3)	19 (76)
- Adjuvant chemotherapy	9 (12.9)	8 (17.8)	1 (4)
- Chemotherapy only	5 (7.1)	4 (8.9)	1 (4)
Surgery, N (%)			
- Amputation	46 (65.7)	34 (75.6)	11 (52.4)
- Rotatoplasty	8 (11.4)	0 (0)	6 (28.6)
- Wide excision with IORT	4 (5.7)	4 (8.9)	0
- Limb-salvage surgery	5 (7.1)	3 (6.7)	2 (9.5)
- Declined operation/Inoperable	7 (10)	4 (8.9)	2 (9.5)
Histological response (Huvos grade), N (%) (Total=63, excluding inoperable/declined surgery)			
- I / II / III / IV (48.6/27/8.1/16.2)	18/10/3/6 (26.7 / 11.1 / 0 / 0)	12 / 5 / 0 / 0 (31.6/26.3/10.5/31.6)	6/5/2/6
- No data	26	24	2
Local recurrence, N (%)	20 (28.6)	18 (40)	2 (9.5)
Detection of lung metastases			
- At initial presentation	32 (45.7)	20 (44.4)	12 (52.4)
- Newly detected during/after chemotherapy	26 (37.1)	20 (44.4)	6 (28.6)

*Percentage was shown excluding missing data; IORT: intra-operative radiotherapy

chemotherapy.

The therapeutic effect was evaluated using clinical (size of primary tumor), radiographic (bone scintigraphy, CT of chest, MRI of primary tumor) and pathological parameters (surgical margin, percentage of tumor necrosis; Huvos score). Progressive disease (PD) was defined by the evidence of subsequently new or worsening metastatic lesions, including pulmonary nodule(s) by CT scan, nuclear uptake lesion(s) by bone scintigraphy or size of primary tumor by physical examination and/or imaging.

Statistical analysis

Survival analysis was done according to the Kaplan-Meier method and was compared by means of the log-rank test based on demographic data, pathological diagnosis and treatment protocol. A value of $p<0.05$ was accepted as statistically significant. Overall survival (OS) rate was determined as the time from the date of diagnosis to death from any cause or by the last follow-up examination. Disease-free survival (DFS) rate was calculated from the date of diagnosis to the first documentation of an event (disease progression, death from any cause or the most recent follow-up examination). The statistically significant parameters in survival analysis were entered into a multivariate analysis by stepwise Cox regression model.

Descriptive statistics were used to define the population. Continuous variables are presented as medians, ranges for data with skewed distribution. Mean and standard variations for data with normal distributions were calculated to describe the samples. The influence

Table 3. Patient Characteristics and Survival Analysis

Characteristics	3-year OS (%)	p-value#	3-year DFS (%)	p-value#
Sex				
Male	51.0±7.5%	0.866	45.2±7.4%	0.868
Female	57.3±10.3%		38.9±10.5%	
Age at diagnosis (by mean age)				
≤11.60 years	59.7± 8.3%	0.209	60.0± 8.3%	0.035**
>11.60 years	46.8±8.7%		27.4±7.7%	
Chemotherapy protocol	.			
MTX(-) protocol	42.2±7.4%	0.010**	31.1±6.9%	0.009**
MTX(+) protocol	79.8± 9.1%		69.8± 10.5%	
Primary tumor				
Axial	0	0.714	0	0.255
Peripheral	53.8±6.1%		44.6±6.1%	
Histology				
Conventional	54.0±7.6%	0.27	48.0±7.6%	0.182
Variants	88.9±10.5%		64.8±16.5%	
Presence of metastases at diagnosis				
Absence	65.1±7.9%	0.058	50.6±8.4%	0.043**
Presence	39.0±8.8%		34.4± 8.4%	
Extent of surgery				
Amputation/Rotatoplasty	54.7±6.9%	0.612	49.2±6.9%	0.888
Limb-salvage surgery	65.1±7.9%		41.7±17.3%	
Histological response*				
Good responders***	100%	0.008**	100%	0.016**
Poor responders	56.1± 9.5%		41± 9.6%	

* Not all data available; **Statistically significance; p=0.05; ***Good responder is defined by tumor necrosis >90%; # Log-rank test

of discrete variables was assessed by the χ^2 test analyses including the Fisher exact test for categorical (nominal) data whenever appropriate, and the Mann-Whitney test or t test for the comparison of the continuous data. All data were analyzed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL), version 18.0.

Results

For the entire cohort, a total of seventy-six patients with pediatric osteosarcoma were identified by reviewing their medical records at the Registry of Division of Pediatric Hematology-Oncology, Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University. Six patients excluded from our study were those who declined treatment after diagnosis and those who were referred out to receive treatment at a regional hospital by Thailand's Universal Health Coverage scheme. Only seventy patients were eligible for our study. Of these patients, the majority were male (65.7%) with a median and mean age at diagnosis of 11.60 years (SD=2.11) and 11.41years (5.67-15.31 years), respectively. 32 patients (45.7%) had initial pulmonary metastasis at the time of diagnosis, while only 2 of them had combined pulmonary and distant bone metastasis. No patient had isolated bone metastasis at diagnosis. Primary tumor site was an extremity in 68 patients (97.1%), the scapula in 1 patient (1.4%) and iliac wing in 1 patient (1.4%). The two most common primary sites were at distal femur (35.7%) and proximal tibia (27.1%). The most frequent presenting symptoms were pain with or without minor trauma (65.7%) and abnormal mass at primary site (64.3%). The median time from initial symptom to diagnosis was 2.45 months (0.5-5.25). After tumor biopsy, chemotherapy could not be given in four patients because of severe osteomyelitis and surgical wound infection. All of them subsequently died with tumor progression locally and metastatically. 7 patients who were declined surgery died, despite intensive chemotherapy. Overall limb-salvage and amputation rates were 12.9% and 77.7%, respectively. Two patients developed acute kidney injury as documented by a rising serum creatinine of more than 1.5 times of the upper normal limit for their ages and by elevated transaminase enzymes, which included total bilirubin after administration of HDMTX. They were treated and fully recovered with leucovorin rescue and force diuresis and also tolerated well a subsequent HDMTX administration. None of the patients had treatment-related toxic death or other serious toxicities, including infectious complications in our study.

According to chemotherapeutic protocols, the presence of good histological response (GHR; percent tumor necrosis of primary tumor >90%) occurred at a significantly higher frequency in patients who received the MTX(+) protocol than those with MTX(-) protocol (42.1% and 0%, respectively, p=0.002). There was no statistically significant difference between groups of treatment protocol in terms of clinical characteristics of patients that included each patient's sex, age at diagnosis, metastatic status at diagnosis, primary sites of tumor, histological subtypes (conventional/variant) and type of surgical procedures (limb-sparing/amputation). All patients who underwent surgery had adequate surgical margin histologically. No correlation was found between age and histological subtypes. Up to 50% of the data regarding primary tumor volume were missing because of poor documentation and missing sections.

Regardless of treatment protocols, the median survival time was 45.0 months (SD=14.20) with a median and mean follow-up time of 29.65 months (SD=40.83) and 43.66 months (3.33-176.30), respectively. The Kaplan-Meier analysis for 3, 5-year DFS and 3, 5-year

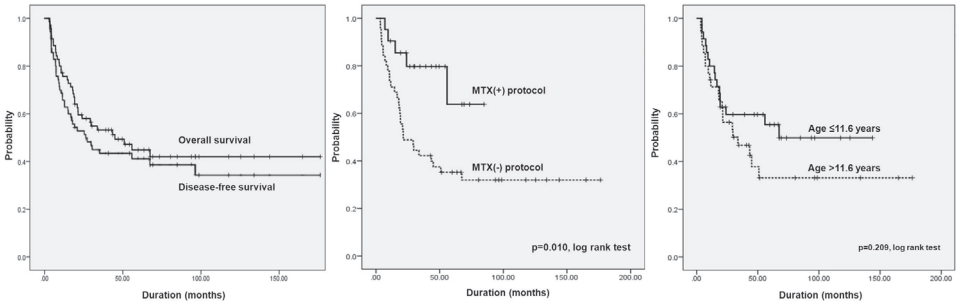

Figure 1. Kaplan-Meier Survival Analysis for Pediatric Osteosarcoma. A) Overall and disease-free survival of pediatric osteosarcoma regardless of chemotherapeutic protocol, B) Kaplan-Meier estimated overall survival according to chemotherapeutic protocol and C) Kaplan-Meier estimated overall survival according to chemotherapeutic protocol according to median age at diagnosis

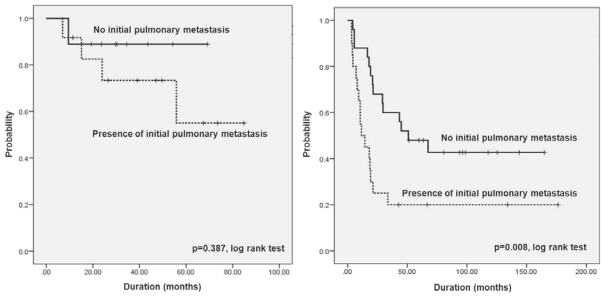

Figure 2. Kaplan-Meier Survival Analysis for Pediatric Osteosarcoma in Presence and Absence of Initial Pulmonary Metastasis. A) Kaplan-Meier analysis for estimated 3-year overall survival for pediatric osteosarcoma treated with MTX (+) protocol were 88.9±10.5% for those without initial pulmonary metastasis and 73.3±13.2% for those with initial pulmonary metastasis (p=0.387) and B) Kaplan-Meier analysis for estimated 3-year overall survival for pediatric osteosarcoma treated with MTX (-) protocol were 60±9.8% for those without initial pulmonary metastasis and 20±8.9% for those with initial pulmonary metastasis (p=0.008)

OS regardless of treatment regimens were 43.4±6.0%, 41.2±6.1% and 53.2±6.1%, 44.8±6.4%, respectively, as shown in Figure 1A. Patients with GHR had a statistically significant higher 3-year DFS and OS than those with poor histological response (PHR) (p=0.008 and p=0.016, log rank test) [100%, 100% for GHR and 56.1±9.5%, 41.0±9.6% for PHR, respectively]. For localized osteosarcoma, the 3- and 5-year OS were 65.1±6.9% and 54.4±8.7%, respectively. The 3-year DFS and OS were higher significantly for MTX(+) protocol as compared to MTX(-) protocol (p=0.010 and p=0.009, log rank test) [69.8±10.5%, 79.8±9.1% for MTX(+) and 31.1±6.9%, 42.2±7.4% for MTX(-) protocol, respectively]. In subgroup analysis, patients with metastatic osteosarcoma treated with MTX(+) protocol also had a statistically significant higher 3-year DFS and OS than those treated with MTX(-) protocol (66.7±13.6%, and 15.0±8.0% for 3-year DFS, p=0.010, 73.3±13.2% and 20±8.9% for 3-year OS, p=0.006, respectively). For patients with localized osteosarcoma who were treated with MTX(+) protocol, the 5-year OS was up to 88.9±10.5%. The presence of initial pulmonary metastasis, with an age at diagnosis ≤11.6 years, had a statistically significant association with lower 3-year DFS (p=0.043 and p=0.035, respectively), but had no significant association with 3-year OS (p=0.058 and p=0.209, respectively). According to the protocol, patients with initial pulmonary metastasis tended to have higher 3 and 5-year OS than those with localized disease for MTX(+) protocol. For MTX(-) protocol, patients with initial pulmonary metastasis had statistically significant higher 3- and 5-year OS than those without metastasis, as shown in Figure 2. However, age at diagnosis, sex, histological subtypes and types of surgery were not found to be statistically significant factors determining DFS and OS.

Two parameters with statistically significant associations with higher 3-year DFS and OS, histological response and treatment protocol, were included in the backward stepwise Cox regression model for a multivariate analysis. The multivariate analysis identified only the treatment with MTX(-) protocol as the only independent predictor of inferior OS rate with a hazard ratio (HR) of 3.53 (95% confidence interval of 1.2-10.41, p=0.022).

Discussion

Osteosarcoma is the most common primary bone tumor in childhood and adolescents. The introduction of neoadjuvant chemotherapy has dramatically improved the outcome in osteosarcoma and has thus minimized the micro-metastasis of the disease. It has also increased the chance of radical resection of primary tumor including feasibility of limb-salvage surgery and has also reduced the risk of local recurrence after limb-salvage surgery as compared to that for amputation (Merkel et al., 1991). The effective chemotherapeutic agents against osteosarcoma are CDDP (Ochs et al., 1978; Baum et al., 1979), CBDCA (Choeyprasert et al., 2014), ADR (Cores et al., 1972; Choeyprasert et al., 2014) and IFO (Harris et al., 1995; Harris et al., 1998) and HDMTX (Jaffe et al., 1983; Rosen G, 1993; Delepine et al., 1996; Goorin et al., 2003; Lewis et al., 2007). Recently, HDMTX seems to be one of the most active agents against osteosarcoma; however, there is still no consensus on a standard chemotherapy approach, including even the HDMTX-based protocol (Jaffe et al., 1985; van Dalen et al., 2011).

Recently, there were few literatures regarding the epidemiologic data of pediatric osteosarcoma in Thailand (Wiromrat et al., 2012; Choeyprasert et al., 2014). Only one report from the Northern region of Thailand demonstrated the outcome of treatment in pediatric osteosarcoma by the combination of CBDCA and ADR (Choeyprasert et al., 2014). Like the previous literature, males were affected approximately 1.91 times more frequent than females. The age at diagnosis was comparable to previous studies worldwide with a peak age incidence in adolescents (Mirabello et al., 2009; Smith et al., 2010; Wiromrat et al., 2012). Almost all of patients had a primary tumor at extremities, especially around the knee joint (62.8%). Approximately half of the patients had distant metastasis at diagnosis, especially at lungs, which was higher than those of Western countries (Link et al., 1986; Eilber et al., 1987). However, our finding was correlated with a previous study from the Northern region of Thailand (Choeyprasert et al., 2014). For our study, patients with osteosarcoma were managed with a similar chemotherapeutic protocol and with surgical approaches that were aimed at complete resection of primary tumor regardless of metastatic status at diagnosis.

For the entire cohort, the 3- and 5-year OS rate during 1999-2014 for both localized and metastatic osteosarcoma were 53.2±6.1%, 44.8±6.4%, respectively. Our OS rates for patients with localized osteosarcoma were slightly inferior to those with the international data, which show a 5-year OS up to 70% (Bielack et al., 2002; Meyers et al., 2005; Ferrari et al., 2005). In an earlier era, we described a 3-year DFS of 44±9.9% for localized osteosarcoma treated with MTX(-) protocol as compared to 46% from the study of The European Osteosarcoma Intergroup (Lewis et al., 2000), which also treated localized osteosarcoma with CDDP and ADM. The

treatment regimens for pediatric osteosarcoma evolved over time. We adapted the HDMTX-based protocol from the European and American Osteosarcoma Study Group, EURAMOS-1 protocol (Marina et al., 2009), for our institute. Apparently, the MTX(+) protocol statistically improved outcomes for patients with both localized and metastatic osteosarcoma as compared to the MTX(-) protocol. For MTX(+) protocol, patients with localized osteosarcoma had a 3-year OS of 88.9±10.5 %, which was consistent with previous literatures, in which an HDMTX-based regimen was used by such study groups as the Intergroup Study-0133 (Meyers et al., 2005; Meyers et al., 2008; Smeland et al., 2011) and the Scandinavian Sarcoma Group (SSG) XIV protocol (Hegyi et al., 2011). Our study showed a superior outcome compared to those from previous studies from Asia using HDMTX-based regimens (Lin et al., 2011; Han et al., 2012). As regards histological response to chemotherapy, GHR (tumor necrosis ≥90%) induced by preoperative MTX(+) protocol demonstrated a significant correlation with outcomes of pediatric osteosarcoma, which were the same as in previous studies (Hudson et al., 1990; Bramwell et al., 1992; Bramwell et al., 1997; Souhami et al., 1997) We received an unsatisfactory response by the MTX(-) protocol, since no GHR was observed in the MTX(-) subgroup. Types of surgical procedures were not a statistically significant prognostic factor for osteosarcoma in our study, possibly because of the adequacy of surgery in our institute and the efficacy of preoperative chemotherapy making total resection feasible. A younger age at diagnosis was also a good prognostic factor determining DFS, consistent with previous studies (Mankin et al., 2004; Lee et al., 2009; Hagleitner et al., 2011), but not for OS in our study. However, the influence of age on outcome in pediatric osteosarcoma is still inconclusive because of the variability of treatment and metastatic status at diagnosis in each study.

Multivariate analyses showed that the treatment with MTX(-) protocol (HR 3.26, p=0.015) was an independent predictor of OS. The pulmonary metastasis was not the significant prognostic factor in our study; however, patients who had initial metastasis tended to have inferior outcomes, as shown in Figure 2. These results must be interpreted carefully because the small population, especially in MTX(+) arm, might render the subgroup analysis erroneous. The additional limitations were the missing data on the size of primary tumor, type of histology and histological response, especially in early era of treatment in our institute.

In conclusion, this study showed that HDMTX-based regimen has a superior efficacy against pediatric osteosarcoma as compared to other alternatives. Although the high incidence of initial pulmonary metastasis in this study was higher than those of Western countries, treatment outcome was still comparable. The HDMTX-based regimen should be conducted in larger populations in order to determine its efficacy and antitumor activity, especially in those with initial pulmonary metastasis and of an age >11.6 years. Furthermore, the refinement in risk, treatment stratification and dose intensification for pediatric osteosarcoma is a future challenge for researchers to improve outcomes, especially in metastatic patients who may need a more intensive regimen.

References

Baum ES, Gaynon P, Greenberg L, et al (1979). Phase II study of cis-dichlorodiammineplatinum(II) in childhood osteosarcoma: Children's Cancer Study Group Report. *Cancer Treat Rep*, **63**, 1621-7.

Bielack SS, Kempf-Bielack B, Delling G, et al (2002). Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. *J Clin Oncol*, **20**, 776-90.

Bleyer W (1981). Therapeutic drug monitoring of methotrexate and other antineoplastic drugs. In 'Interpretations in Therapeutic Drug Monitoring', Eds Baer DM, Dita ER. American Society of Clinical Pathology, Chicago pp 169-81.

Bramwell VH, Burgers M, Sneath R, et al (1992). A comparison of two short intensive adjuvant chemotherapy regimens in operable osteosarcoma of limbs in children and young adults: the first study of the European Osteosarcoma Intergroup. *J Clin Oncol*, **10**, 1579-91.

Bramwell VH, Burgers MV, Souhami RL, et al (1997). A randomized comparison of two short intensive chemotherapy regimens in children and young adults with osteosarcoma: results in patients with metastases: a study of the European Osteosarcoma Intergroup. *Sarcoma*, **1**, 155-60.

Choeyprasert W, Natesirinilkul R, Charoenkwan P, et al (2013). Carboplatin and doxorubicin in treatment of pediatric osteosarcoma: a 9-year single institute experience in the Northern Region of Thailand. *Asian Pac J Cancer Prev*, **14**, 1101-6.

Cores EP, Holland JF, Wang JJ, et al (1972). Doxorubicin in disseminated osteosarcoma. *JAMA*, **221**, 1132-8.

Coventry MB, Dahlin DC (1957). Osteogenic sarcoma; a critical analysis of 430 cases. *J Bone Joint Surg Am*, **39**, 57-8.

Dahlin DC (1978). Osteosarcoma of bone and a consideration of prognostic variables. *Cancer Treat Rep*, **62**, 189-92.

Delepine N, Delepine G, Bacci G, et al (1996). Influence of methotrexate dose intensity on outcome of patients with high grade osteogenic osteosarcoma. Analysis of the literature. *Cancer*, **78**, 2127-35.

Eilber F, Giuliano A, Eckardt J, et al (1987). Adjuvant chemotherapy for osteosarcoma: a randomized prospective trial. *J Clin Oncol*, **5**, 21-6.

Ferrari S, Bertoni F, Mercuri M, et al (2001). Predictive factors of disease-free survival for non-metastatic osteosarcoma of the extremity: an analysis of 300 patients treated at the Rizzoli Institute. *Ann Oncol*, **12**, 1145-50.

Ferrari S, Smeland S, Mercuri M, et al (2005). Neoadjuvant chemotherapy with high-dose Ifosfamide, high-dose methotrexate, cisplatin, and doxorubicin for patients with localized osteosarcoma of the extremity: a joint study by the Italian and Scandinavian Sarcoma Groups. *J Clin Oncol*, **23**, 8845-52.

Fletcher CD (2006). The evolving classification of soft tissue tumours: an update based on the new WHO classification. *Histopathology*, **48**, 3-12.

Gaffney R, Unni KK, Sim FH, et al (2006). Follow-up study of long-term survivors of osteosarcoma in the prechemotherapy era. *Hum Pathol*, **37**, 1009-14.

Goorin AM, Schwartzentruber DJ, Devidas M, et al (2003). Presurgical chemotherapy compared with immediate surgery and adjuvant chemotherapy for nonmetastatic osteosarcoma: Pediatric Oncology Group Study POG-8651. *J Clin Oncol*, **21**, 1574-80.

Hagleitner MM, Hoogerbrugge PM, van der Graaf WT, et al (2011). Age as prognostic factor in patients with osteosarcoma. *Bone*, **49**, 1173-7.

Han J, Yong B, Luo C, et al (2012). High serum alkaline phosphatase cooperating with MMP-9 predicts metastasis and poor prognosis in patients with primary osteosarcoma in Southern China. *World J Surgical Oncol*, **10**, 37.

Harris MB, Cantor AB, Goorin AM, et al (1995). Treatment of osteosarcoma with ifosfamide: comparison of response in pediatric patients with recurrent disease versus patients previously untreated: a Pediatric Oncology Group study. *Med Pediatr Oncol*, **24**, 87-92.

Harris MB, Gieser P, Goorin AM, et al (1998). Treatment of metastatic osteosarcoma at diagnosis: a Pediatric Oncology Group Study. *J Clin Oncol*, **16**, 3641-8.

Hegyi M, Semsei AF, Jakab Z, et al (2011). Good prognosis of localized osteosarcoma in young patients treated with limb-salvage surgery and chemotherapy. *Pediatr Blood Cancer*, **57**, 415-22.

Hudson M, Jaffe MR, Jaffe N, et al (1990). Pediatric osteosarcoma: therapeutic strategies, results, and prognostic factors derived from a 10-year experience. J Clin Oncol, 8, 1988-97.

Jaffe N, Prudich J, Knapp J, et al (1983). Treatment of primary osteosarcoma with intra-arterial and intravenous high-dose methotrexate. *J Clin Oncol*, **1**, 428-31.

Jaffe N, Robertson R, Ayala A, et al (1985). Comparison of intra-arterial cis-diamminedichloroplatinum II with high-dose methotrexate and citrovorum factor rescue in the treatment of primary osteosarcoma. *J Clin Oncol*, **3**, 1101-4.

Lee JA, Kim MS, Kim DH, et al (2009). Risk stratification based on the clinical factors at diagnosis is closely related to the survival of localized osteosarcoma. *Pediatr Blood Cancer*, **52**, 340-5.

Lewis IJ, Nooij MA, Whelan J, et al (2007). Improvement in histologic response but not survival in osteosarcoma patients treated with intensified chemotherapy: a randomized phase III trial of the European Osteosarcoma Intergroup. *J Natl Cancer Inst*, **99**, 112-28.

Lewis IJ, Weeden S, Machin D, et al (2000). Received dose and dose-intensity of chemotherapy and outcome in nonmetastatic extremity osteosarcoma. European Osteosarcoma Intergroup. *J Clin Oncol*, **18**, 4028-37.

Lin F, Wang Q, Yu W, Tang L, et al (2011). Clinical analysis of Chinese limb osteosarcoma patients treated by two combinations of methotrexate, cisplatin, doxorubicin and ifosfamide. *Asia Pac J Clin Oncol*, **7**, 270-5.

Link MP, Goorin AM, Miser AW, et al (1986). The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. *N Engl J Med*, **314**, 1600-6.

Mankin HJ, Hornicek FJ, Rosenberg AE, et al (2004). Survival data for 648 patients with osteosarcoma treated at one institution. *Clin Orthop Relat Res*, **429**, 286-91.

Marcove RC, Mike V, Hajek JV, et al (1970). Osteogenic sarcoma under the age of twenty-one. A review of one hundred and forty-five operative cases. *J Bone Joint Surg Am*, **52**, 411-23.

Marina N, Bielack S, Whelan J, et al (2009). International collaboration is feasible in trials for rare conditions: the EURAMOS experience. *Cancer Treat Res*, **152**, 339-53.

Merkel KD, Gebhardt M, Springfield DS (2008). Rotationplasty as a reconstructive operation after tumor resection. *Clin Orthop Relat Res*, **270**, 231-6.

Meyers PA, Schwartz CL, Krailo M, et al (2005). Osteosarcoma: a randomized, prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose methotrexate. *J Clin Oncol*, **23**, 2004-11.

Meyers PA, Schwartz CL, Krailo MD, et al (2008). Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival--a report from the Children's Oncology Group. *J Clin Oncol*, **26**, 633-8.

Mirabello L, Troisi RJ, Savage SA (2009). International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons. *Int J Cancer*, **125**, 229-34.

Mirabello L, Troisi RJ, Savage SA (2009). Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. *Cancer*, **115**, 1531-43.

Ochs JJ, Freeman AI, Douglass HO Jr, et al (1978). cis-Dichlorodiammineplatinum (II) in advanced osteogenic sarcoma. *Cancer Treat Rep*, **62**, 239-45.

Rosen G (1993). An opinion supporting the role of high-dose methotrexate in the treatment of osteosarcoma. *Cancer Treat Res*, **62**, 49-54.

Smeland S, Bruland OS, Hjorth L, et al (2011). Results of the Scandinavian Sarcoma Group XIV protocol for classical osteosarcoma: 63 patients with a minimum follow-up of 4 years. *Acta orthopaedica*, **82**, 211-6.

Smith MA, Seibel NL, Altekruse SF, et al (2010). Outcomes for children and adolescents with cancer: challenges for the twenty-first century. *J Clin Oncol*, **28**, 2625-34.

Souhami RL, Craft AW, Van der Eijken JW, et al (1997). Randomised trial of two regimens of chemotherapy in operable osteosarcoma: a study of the European Osteosarcoma Intergroup. *Lancet*, **350**, 911-7.

Stiller CA, Bielack SS, Jundt G, et al (2006). Bone tumours in European children and adolescents, 1978-1997. Report from the Automated Childhood Cancer Information System project. *Eur J Cancer*, **42**, 2124-35.

van Dalen EC, van As JW, de Camargo B (2011). Methotrexate for high-grade osteosarcoma in children and young adults. *Cochrane Database Syst Rev*, **5**, 6325.

Wiangnon S, Veerakul G, Nuchprayoon I, et al (2011). Childhood cancer incidence and survival 2003-2005, Thailand: study from the Thai Pediatric Oncology Group. *Asian Pac J Cancer Prev*, **12**, 2215-20.

Winkler K, Bielack S, Delling G, et al (1990). Effect of intraarterial versus intravenous cisplatin in addition to systemic doxorubicin, high-dose methotrexate, and ifosfamide on histologic tumor response in osteosarcoma (study COSS-86). *Cancer*, **66**, 1703-10.

Wiromrat P, Jetsrisuparb A, Komvilaisak P, et al (2012). Incidence and survival rates among pediatric osteogenic sarcoma cases in Khon Kaen, Thailand, 1985-2010. *Asian Pac J Cancer Prev*, **13**, 4281-4.