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Alkaline phosphatase, lactic dehidrogenase, inflammatory variables and apparent diffusion coefficients from MRI for prediction of chemotherapy response in osteosarcoma. A cross sectional study

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ABSTRACT

Background: This present study aimed to assess if clinical, laboratory and MRI were an accurate benchmark in assessing the effectiveness of neoadjuvant chemotherapy in osteosarcoma patients.
Methods: This was an observational analytic study with a cross-sectional design. We correlated among clinical, laboratory and magnetic resonance imaging (MRI) data before and after neoadjuvant chemotherapy; and percentage of tumor necroses from osteosarcoma patients during the period between January 2017–July 2019.
Results: Of the 58 patients included in this study, 38 were male and 20 were female aged 5 - 67 years (mean: 16-year-old. 37(63.8%) patients underwent neoadjuvant chemotherapy with CAI regimens and 13 (36.2%) with CA regiments. The tumors were classified as stage **IIB** in 43 (74.1%) patients and stage III in 15 (25.9%) patients. Wilcoxon test showed significant differences between alkaline phosphatase (ALP), erythrocyte sedimentation rate (ESR), and neutrophil to lymphocyte ratio (NLR) before and after neoadjuvant chemotherapy in the poor-response group. We found no significant difference between lactic dehydrogenase (**LDH**) and lymphocyte-to-monocyte ratio (LMR) before and after neoadjuvant chemotherapy in the good-response group. MRI revealed decreased tumor volume in patients in the good-response to chemotherapy.
Conclusion: We demonstrated that ALP level was statistically significant in the poor-response group. We also found that LDH value before neoadjuvant chemotherapy had a strong correlation with degree of necrosis and could be used as a predictive indicator. MRI plays an important role in evaluating tumor volumes and preoperative radiological changes to predict histological necrosis.

1. Introduction

Management of osteosarcoma and primary bone malignancies changed after the era of chemotherapy began in the 1970s. With surgery and chemotherapy, the prognosis of osteosarcoma >5 years has dramatically improved, increasing from 10 to 20% (only surgery) to 75–80% [1,2]. In contrast, chemotherapy without surgery causes stable disease and a recurrence of osteosarcoma. Therefore, to achieve a better prognosis, osteosarcoma patients must undergo complete therapy consisting of tumour-free surgery and systemic chemotherapy treatment [1–4].

At this time, neoadjuvant chemotherapy is the gold standard in the management of osteosarcoma patients, planned by limb salvage surgery (LSS). It lasts 6–8 weeks (another study reports 6–18 weeks), depending on the institution and regimen used [2,4,5]. Role of neoadjuvant chemotherapy may kill micro metastases and inhibit local growth of osteosarcoma (causing tumor necrosis), reduce tumor size, and cause death of satellite lesions in the pseudo capsule/reactive zone. Other important advantages of neoadjuvant chemotherapy are the possibility of performing safer LSS, facilitating a resection of tumors en bloc at the time of LSS, and defining prognostic groups based on the observed histologic response to chemotherapy by assessing the percentage of

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necrosis of the osteosarcoma. Until now, histopathology was the gold standard of examination to evaluate the effectiveness of neoadjuvant chemotherapy. However, this procedure requires surgically resected specimens andneeds longer time for evaluation [3].

Laboratory examinations are performed, although not specific, to determine the general condition of patients and to evaluate patient prognosis. In addition to routine blood tests (hemoglobin, leukocytes, platelets and differential blood count, including neutrophils, monocyte, lymphocyteetc.), serum alkaline phosphatase (ALP), lactic dehydrogenase (LDH) [1,2,6,7]. and erythrocyte sedimentation rate (ESR), are required for evaluation of osteosarcoma. ALP and LDH are significant biomarkers in several tumor including osteosarcoma. Both of the biomarkers were considered as effective prognostic factors of osteosarcoma. These tests are routinely evaluated and the results are easy to obtain. It is also universally available and can be monitored easily for better prognostic factors [8].

Magnetic resonance imaging (MRI) is a good modality for evaluating tumor extension into bone marrow, extraosseous, including neurovascular involvement, and skip lesion. MRI also plays a role in determining the location of biopsy and may be used to decide LSS or amputation procedures [1,2].In other words, it is useful for the evaluation ofthe stage of the osteosarcoma before and after neoadjuvant chemotherapy.

After neoadjuvant chemotherapy, MRI may also be used to evaluate response to chemotherapy. By using the diffusion weighted imaging, the response to chemotherapy is represented as changes in cellularity and can be measured with apparent diffusion coefficients (ADC). It is correlated directly with tumour necrosis. Thus, the ADC value is a promising tool to evaluate the response of therapy in osteosarcoma [9]. This present study aimed to assess if clinical, laboratory and MRI were an accurate benchmark in assessing the effectiveness of neoadjuvant chemotherapy in osteosarcoma patients.

2. Methods and patients

This was an observational analytic study with a cross-sectional design. The study was conducted in a tertiary referral hospital, in Jakarta, Indonesia. The study had been approved by the ethical committee of our institution with Approval No. 0055/UN2.F1/ETIK/2019 and registerd in and registerd in University Hospital Medical Information Network UMIN000043289. The work has been reported in line with the STROCSS criteria [10] and had obtained research permission from CMH Research Committee.

Research subjects were selected using the total sampling method from osteosarcoma patients who underwent neoadjuvant chemotherapy during the period between January 2017–July 2019. Fifty-eight osteosarcoma patients with the mean age of 16 years (range was 5–67 years) were included in this study. Informed consent was obtained from all the patients or through their parents (in the pediatric patients) prior to their enrollment in this study.

The inclusion criteria in the study consisted of patients who were diagnosed with osteosarcoma as regards clinicopathological conference (CPC); and who underwent 3 cycles of neoadjuvant chemotherapy. We evaluate all the patients since diagnosis was confirmed to final Huvos results following surgery. The patients with osteosarcoma whose had history of neoadjuvant chemotherapy referred from other hospitals were excluded. They had complete clinical evaluation [pain and musculoskeletal tumor society score (MSTS)], laboratory and radiologic examinations [magnetic resonance imaging (MRI) before and after neoadjuvant chemotherapy)]; and histopathological results before and after surgery (percentage of tumor necroses as regards Huvos grading. Neoadjuvant chemotherapy regimens were adjusted with our institution using cisplatin, adriamycin, and iphosphamide (CAI) for pediatric patients (<18 years old) and cisplatin and adriamycin (CA) for adult patients.

After neoadjuvant chemotherapy was completed, a patient would have a limb salvage surgery or limb ablation. Surgically removed specimens were sent to the musculoskeletal pathologist for evaluation of grading of tumor cell necrosis, using the Huvos classification. Huvos grade I was consistent with less than 50% necroses; grade II with 50–90% necroses; grade III with more than 90–99% necroses; and grade IV with 100% necroses (no viable tumor cells). In our study, we grouped the results of the Huvos grading into two categories: grade III and IV were described as good-response group while grade I and II as poor-response group.

2.1. Statistical analysis

Statistical analysis was performed using Mann-Whitney *U* test for the unpaired group and Wilcoxon test for variables in the paired group. Spearmen's test was used to correlate between the variables. All statistical analyses were performed using SPSS software version 23.0. A p value of<0.05 was considered statistically significant.

3. Results

Of the 58 patients included in this study, 38 were male and 20 were female aged 5–67 years (mean, 16-year-old); 37(63.8%) of the 58 patients underwent neoadjuvant chemotherapy with CAI regimens, while 13 (36.2%) of the 58 patients underwent neoadjuvant chemotherapy with CA. The most common tumor location was the distal femur in 29 (50%) of the 58 patients, followed by the proximal tibia in 16 (27.6%) of the 58 patients, and the proximal humerus radius in 5 (8.6%) of the 58 patients. According to the Enneking surgical staging, the tumors were classified as stage **IIB** in 43 (74.1%) patients and stage III in15 (25.9%) patients.

We documented 55 cases of conventional osteosarcoma, 2 cases of giant cell rich osteosarcoma and a case of telangiectatic osteosarcoma. Based on the Huvos degree of necrosis, 19 (32.8%) patients were categorized as Huvos grade I, 29 (50%) patients as Huvos grade II, 10 (17.2%) patients as Huvos grade III, and no patient with Huvos grade IV. After undergoing neoadjuvant chemotherapy, 4 patients had poor MSTS, 30 patients had fair MSTS, 17 and 7 patients had good and excellent MSTS score, respectively. The patients'characteristics are shown in Table 1.

Mann-Whitney test showed no significant difference between the reduction in tumor size and MSTS functional score after neoadjuvant chemotherapy management and histological responses (good-response or poor-response group). Spearman's test revealed no correlation between tumor necrosis after neoadjuvant chemotherapy with a reduction in tumor size and MSTS score (Table 2).

Wilcoxon test showed significant differences between ALP, ESR, and neutrophil to lymphocyte ratio (NLR) before and after neoadjuvant chemotherapy in the poor-response group. In contrast, we found no significant difference between L**DH** and lymphocyte-to-monocyte ratio (LMR) before and after neoadjuvant chemotherapy in the good-response group (Table 3).

In this study, we evaluated the 58 osteosarcoma patients who had had MRI parameters before and after neoadjuvant chemotherapy. However, we had 9 of the 58 patients for ADC value only. From MRI we found a mean value of tumor volume in patients in the good-response group to have decreased in size. In contrast to those, patients in the poor-response group had relatively increased in total tumor volume. No significant statistical differences were found in tumor volumes after chemotherapy in both groups. We found, however, a significant increase in the overall ADC value after chemotherapy (Table 4).

4. Discussions

Preoperative planning can be done more effectively with imaging technology (MRI, CT scan, bone scan, and PET scan), so that 80–85% of osteosarcoma/malignant bone tumor patients in developing countries

Table 1
Characteristics of the osteosarcoma patients.

Variable		n (%)	mean/median
Male		38 (65.5%)	
Female		20 (34.5%)	
Age (years)		16 (5–67)	
Stadium (Enneking)			
IIB	43 (74.1%)		
III	15 (25.9%)		
Tumor Site			
Clavicles	2 (3.4%)		
Proximal radius	5 (8.6%)		
Proximal ulna	1 (1.7%)		
Distal ulna	1 (1.7%)		
Proximal femur	1 (1.7%)		
Distal femur	29 (50.0%)		
Proximal tibia	16 (27.6%)		
Distal tibia	2 (3.4%)		
Iliac wing	1 (1.7%)		
Histopathological type			
Conventional	34 (58.6%)		
Fibroblastic	2 (3.5%)		
Osteoblastic	13 (22.4%)		
Chondroblastic	6 (10.3%)		
Giant-cell rich	2 (3.5%)		
Telangiecstatic	1 (1.7%)		
Duration before admission (months)			5 (1–24)
Decrease of size (L)			−0,054 (−26.8 – 0.3)
Necrosis percentage after neoadjuvant chemotherapy			70 (0–99)
Response to chemotherapy			
Good responder	10 (17.2%)		
Huvos III	10 (17.2%)		
Huvos IV	0		
Poor responder	48 (82.8%)		
Huvos I	19 (32.8%)		
Huvos II	29 (50.0%)		
MSTS Score			12,9 (5–27)
Persentase MSTS(%)			43 (17–90)

Table 2
Effects of two different chemotherapy regimens to the tumor size, MSTS score and histologic response group.

	Decrease in Tumor Size (%)	MSTS Score (%)
CAI (0–18years) (n = 37)	−0.67 ± 4.39 (−26.8 – 0.2)	43.0 ± 18.56 (17–90)
CA (>18years) (n = 21)	−0.042 ± 0.15 (−0.5 – 0.3)	50.0 ± 21.21 (17–87)
p	0.396[a]	0.315[a]
Good responder (n = 10)	0.0 ± 0.15 (−0,3–0,2)	53.3 ± 22.83 (27–87)
Poor responder (n = 48)	−0.06 ± 3.85 (−26,8–0,3)	43 ± 18.79 (17–90)
P	0.097[a]	0.342[a]

[a] Mann-Whitney *U* test.

may undergo LSS [1,10,11]. Unfortunately, although currently imaging technologies in Indonesia are available and neoadjuvant chemotherapy has been administered to the osteosarcoma patients, most of them still report late to the hospital and come with a large tumor, such that amputation is an inevitable surgical procedure of choice because ofthe common poor prognosis. We compiled some factors influencing osteosarcoma patients who underwent amputation in our hospital, including the huge tumor due to massage history, late coming to the hospital due to various reasons, severe contamination of open biopsy from other surgeons, and poor response to chemotherapy characterized by progressivity of the tumors. Of the 132 osteosarcoma patients between 1995 and 2014, only 37 (28%) patients were eligible for LSS [12].

Table 3
Effects of neoadjuvant chemotherapy to laboratory changes in good and poor responder groups.

		Good Responder	Poor Responder	P value[mw]
		Median (Min-Max)	Median (Min-Max)	
Before neoadjuvant chemotherapy (n = 58)	**ALP**	183 (74–2708)	229 (51–3354)	0.918
	LDH	638 (266–1365)	404 (60–2225)	**0.046**
	LED	31 (2–117)	40 (2–130)	0.592
	NLR	2.5 (0.75–3.88)	2.26 (0.88–11.63)	0.711
	PLR	9.96 (3.93–28.83)	11.95 (6.93–65.53)	0.209
	LMR	2.5 (2.11–5.00)	2.58 (1.71–4.80)	0.592
Δ (After & Before) neoadjuvant chemotherapy (n = 58)	**ΔALP**	−55(-2565–83)	−28 (-1609–3964)	0.382
	ΔLDH	−280 (-1201–59)	−32 (-1741–1072)	**0.007**
	ΔLED	3 (-29–60)	15 (-75–105)	0.673
	ΔNLR	3.70 (−1.65–28.34)	0.92 (−4.92–16.90)	0.371
	ΔPLR	16.02 (−7.49–98.02)	−0.37 (−60,74–38.77)	**0.028**
	ΔLMR	−0.5 (−1,87-1,38)	−0.35 (−1.73–0.71)	0.929

[mw] Mann-Whitney.

Administration of neoadjuvant chemotherapy in our hospital is normally tailored to the age group, according to the clinical guidelines released by the National Comprehensive Cancer Network and the European Society for Medical Oncology.[13,14]According to the literature, there are several factors that influence the neoadjuvant chemotherapy response: age [15,16], gender [15], staging [1], tumor size, tumor location, histological type, bone morphogenic protein and quantification of (dDNA) P glycoprotein [15], and chemotherapy regimen [1].

In our study, symptoms of pain and swelling were obviously relieved after neoadjuvant chemotherapy. As with previous studies, neoadjuvant chemotherapy reduced tumor volume and improved MSTS functional scores, although it was not statistically significant. Unfortunately, as many as 48 (82.8%) of the 58 patients had histologically poor chemotherapy responses. However, it must be noted that a decrease in volume and improvement of clinical symptoms are not necessarily followed by histologic improvement in chemotherapy response. These findings, therefore, indicate more objective histologic responses are influenced by many factors in osteosarcoma patients. Based on the findings, these may be caused by several factors including patients presenting with a more advanced stage and large tumor size.

Although still debatable, ALP is an important indicator that influences the prognosis of osteosarcoma patients [17] and it is elevated in approximately 40% of cases [18]. Many researchers have used ALP as a prognostic indicator of patients with bone sarcomas and evaluation of the response to chemotherapy [2,7,17]. A study conducted by Meyers et al. [18] reported that the value of bone ALP was higher in patients with osteosarcoma than in benign bone tumor. They also demonstrated a relationship between the decline in the value of bone ALP and improved histological response. Pre-treatment normal ALP value in osteosarcoma patients, according to them, had better 5-year survival rates than the higher one (67% vs 54%) [2]. Increased ALP in osteosarcoma patients who had chemotherapy and surgical procedures showed poor response to the treatment and higher incidence rate of distant metastases [13]. [[,19].

High level of ALP would relate to propensity for malignancy of osteosarcoma and poor clinical outcomes. It is reported that, in most patients with initial elevated ALP, the values decrease to normal levels

Table 4
ADC value and tumor volume from MRI before and after neoadjuvant chemotherapy.

	ADC (mm [2]/s)	Tumor Volume (L)		
	Poor Responder	Total	*Good Responder*	*Poor Responder*
N	9	58	10	48
Before chemotherapy	1.43 (1.62–2.32)	247.1 (64–337.1)	299 (93–350.5)	239.1 (64–337.1)
After chemotherapy	1.97 (1.44–2,27)	237.9 (68.5–449.8)	257.2 (48.1–367)	247.9 (68.5–449.8)
P	0.028[a]	0.291[a]	0.139[a]	0,071[a]
Change (95% CI)	−0.52 (-1.05 –1.01)	−160 (-398.66 – 77.13)	34.80 (-1.44 –71.05)	−187.61 (-458.39–83.18)

[a] Wilcoxon test.

after preoperative chemotherapy. Other than the ALP levels at diagnosis, posttreatment ALP values also gain great attention by researchers for their prognostic role[19]. Meanwhile, decreased ALP after chemotherapy and surgery is related to better disease-free survival [1,2,6].

In this study, we demonstrated that ALP level after neoadjuvant chemotherapy was markedly decreased after neoadjuvant chemotherapy, and it was statistically significant in the poor-response group. As an important serum index of osteosarcoma, ALP level indirectly reflects the therapeutic effect of chemotherapy [20]. We assume that the decrease in ALP level might have the positive effect of neoadjuvant chemotherapy. Our finding is supported by Zumarraga et al. [7] who reported no correlation between the percentage of necrosis rates and changes in serum ALP level after neoadjuvant chemotherapy. Unfortunately, no correlation between serum ALP level before neoadjuvant chemotherapy and tumor necroses was demonstrated. Therefore, from the present study, we may not use initial ALP or change of ALP after chemotherapy to predict response of grading necrosis in osteosarcoma. Similar results with our study were reported by Limmahakhun et al. [21] and Adamcová-Krakorova et al. [22] who concluded that the LDH was not relevant for the prognosis of osteosarcoma progression.

The Rizzoli Institute analyzed ALP values after neoadjuvant chemotherapy and surgery in patients with initial high levels of the enzyme but failed to find any significant relationship with relapse [19,23]. However, Han et al. [24] indicated that elevated post-chemotherapyALP correlated with shorter survival and greater incidence of lung metastasis, as well as poor response to chemotherapy. A decrease inALP level during clinical therapy may be a symptom of a positive response to treatment [19].

LDH is reported as the strongest predictor of disease-free survival and an important predictive indicator in osteosarcoma. Ferrary et al. [25] reported that patients with serum LDH less than 460 U/L had better disease-free survival than those with higher LDH level. The baseline serum level of LDH showed an independent prognostic significance for DFS; and patients with normal LDH levels had 55% DFS, whereas those with higher levels had 29% DFS [25]. It was also confirmed by a meta-analysis reported by Fu et al. [26] and Chen et al. [27]concluding that the increase in serum LDH value was significantly correlated with a decrease in disease-free survival and lower survival rates. Meanwhile, other studies reported that LDH was not a prognostic indicator for osteosarcoma [6–8].

What is the correlation between LDH value and degree of necrosis before and after chemotherapy? We demonstrated that LDH value before chemotherapy had a strong correlation with the degree of necrosis and this was statistically significant. LDH value was also decreased after neoadjuvant chemotherapy which was statistically significant. From this study, we concluded that LDH values could be used as a predictive indicator in evaluating responses to neoadjuvant chemotherapy in osteosarcoma. A similar result was reported by Zumarraga et al. [7] concluding that there was a correlation between serum levels of LDH pre- or post-chemotherapy patients and that the percentage of reported tumor necrosis, according to the Huvos grading. In contrast to our study, a study by Limmahakhun et al. [28] and Adamcová-Krakorova et al. [29] concluded that LDH was not relevant for the prognosis of osteosarcoma progression.

NLR and LMR are often used as predictors of malignant prognosis before chemotherapy. Xia et al. [30]and Liu T et al. [31] reported that NLR and LMR might be used as prognostic factors in osteosarcoma cases. Published evidence has shown a significant link between inflammatory markers and poor prognosis in several types of tumors, including leukocytosis, high neutrophil-to-lymphocyte ratio (NLR) and lymphocyte to monocyte ratio (LMR) [31]. Cancer-related inflammation has a role in cancer development and progression [32]. Neutrophils interact with tumor cells by producing cytokines and chemokines, which affect tumor cells' proliferation, angiogenesis, and metastases [32,33]. Tumor-associated macrophages, which arise from blood monocytes, promote tumor progression and metastases [34]. Lymphocytes play a major role in the immune response by mediating the immunologic destruction of various cancers [32,35]. NLR and LMR have been shown to be independent risk factors in various malignant tumors. These factors, when combined, may have stronger prognosis values than any single one [32,36,37].A high value of NLR is related to poor prognosis or decreased survival rate [30,32], while preoperative lower LMR is significantly associated with lower overall survival and disease-free survival (poor prognosis) [31]. Interestingly, A decreased preoperative LMR could be regarded as an independent prognostic factor for both OS and EFS in patients with osteosarcoma. In this present study, we evaluated the correlation between NLR or LMR with degree of necrosis before and after chemotherapy. Contrasted with previous studies, we found that NLR and LMR were not associated with the degree of necrosis after chemotherapy. In other words, NLR and LMR are unable to be an independent prognostic factor for predicting chemotherapeutic response in osteosarcoma.

Histological response to chemotherapy (degree of tumor necrosis after chemotherapy) is one of the most important prognostic factors in patients with osteosarcoma. It usually influences post-operative treatment, including possible changes of chemotherapeutic agents [38,39]. Survival rates are better in good responders to neoadjuvant chemotherapy with high tumor necrosis than in those with poor response. Different degrees of necrosis on histological examination of tumor specimens were first noted by investigators from Memorial Sloan-Kettering Cancer Center and it was observed that significant necrosis was associated with better event-free survival and overall survival [40].

Miwa et al. [38] reported 51 osteosarcoma patients who underwent neoadjuvant chemotherapy showing 36 (70.6%) patients'good response and 15 (29.4%) patients in non-response group. Unfortunately, neoadjuvant chemotherapy in our patients showed 82.8% in poor response and 17.2% in good response. A study reported that, in spite of an aggressive surgical and chemotherapeutic treatment strategy, patients with unresectable primary osteosarcoma (advanced stage in our patients) and those with distant metastases still had a poor prognosis [39].

To define tumor response, the World Health Organization's criteria based on conventional imaging modalities such as CT and MRI have been described [41]. Bajpaiet al. [42] reported tumor volume predicting

histological necrosis was 83% (95% CI = 68–97%), which implies that histological necrosis in 83% of the patients could be determined correctly by baseline tumor volume. In conventional MRI pre- and post-neoadjuvant chemotherapy, tumor volume, pre- and post-chemotherapy average tumor plane and pre-chemotherapy relative-average tumour plane were found to have significant association with histological necrosis. Tumor size is available at baseline and, thus, if size is a criterion to determine prognosis, then risk-adapted therapy can be initiated at baseline; and unnecessary exposure to ineffectual chemotherapy can be avoided, preventing toxicities. Besides the intrinsic biological nature of the tumor, tumor size might also contribute to metastatic disease [13,14,42]. From our study, we concluded that the baseline tumor volume as measured by MRI could not be used to predict the histological response to chemotherapy. However, our data fromosteosarcoma patients in the good-response group showed far more decreased tumor volume after neoadjuvant chemotherapy than in the poor-response group, although a change in volume following chemotherapy did not show a statistic difference (correlation with degree of necrosis). Bajpai et al. [42] mentioned that a decrease in volume was associated with good response in various studies, but that in some patients increased volume was also associated with good response.

By contrast, in our poor-response group, the tumor volume showed more progressive increase during and after neoadjuvant chemotherapy. In this situation we considered and decided on surgery earlier, without waiting for the normal 3 cycles of neoadjuvant chemotherapy; rather we were able to evaluate the regimens or changed to other chemotherapy drugs.

The quantitative evaluation of preoperative radiological changes using diffusion-weighted imaging (DWI) and dynamic magnetic resonance imaging, has been challenged. The operative treatment and neoadjuvant chemotherapy of suspected poor responders might then be intensified earlier, potentially increasing their survival rates and decreasing the risk rates of iatrogenic toxicity [39]. DWI is currently the only imaging method to non-invasively measure the local diffusion characteristics of water molecules in vivo. It is able to reflect the spatial composition and the functional status of water exchange among various tissues in pathophysiological states from the molecular level.

The ADC is used to measure water diffusion and has a decreasing tendency in highly cellular tissue. As the signal of water diffusion is directly associated with the tumour cellularity, necrotic areas in the tumour increase a local diffusion signal. Although the ADC value on DWI may be a promising tool, due to the scanty data currently available, there is no routine practice for DWI to predict the chemotherapeutic response of osteosarcoma [39].

In diffusion-weighted MRI, water diffusion is used as a surrogate marker to distinguish highly cellular regions of tumor (with restricted diffusion) from acellular and necrotic regions (with free diffusion). This helps to detect treatment response, which manifests as a change in cellularity within the tumor over time. Changes in the degree of restricted diffusion, for example, by an alteration in cell membrane integrity or permeability to water, are reflected in changes in the diffusion-weighted signal, and can be quantified by changes in ADC. Therefore, in diffusion-weighted images we would expect different degrees of signal change in necrotic and viable tumor tissue. In viable tumors, we did not observe free diffusion of water with corresponding low ADC values, while necrotic areas had free diffusion of water with corresponding high ADC values [4]. The kinetic curve assessment in dynamic contrast-enhanced MRI depicts the permeability **characteristics** of the tumor, which might alter with neoadjuvant chemotherapy. Thus, it was hypothesized that a change in these kinetic curves from higher permeability to lower permeability characteristics would correlate with response to chemotherapy [42].

In the present study, a change in ADC values did not significantly correlate with the degree of necrosis; instead, ADC value remained increased in the poor-response group. 9 of the 58 osteosarcoma patients who were reviewed in this study had varying degrees of necrosis less than 90%. This result is similar with Bajpai's study [42] and Oka's study [43]. Other studies from a meta-analysis had not identified a significant association between ADC values and tumor necroses, meaning that the predictive value of ADC remained undetermined [39]. However, in previous studies, ADC values and their change after chemotherapy had correlated well with histological necrosis in osteosarcoma [44]. Furthermore, a similar correlation had been detected in other cancers, such as breast metastases, liver [45] and hepatocellular carcinomas [46]. One possible reason for the wide range of measured ADC values in tumor tissue could be the susceptibility of echo planer imaging sequences to artefacts, like distortion and low signal-to-noise ratios. One possible explanation for our study findings is supported by Bajpai's study which showed that our patients presented us with relatively large tumors.

5. Conclusions

There were no significant changes in tumor size and MSTS scores after neoadjuvant chemotherapy. We demonstrated that ALP level after neoadjuvant chemotherapy was markedly decreased, and was statistically significant in the poor-response group. However, there was no correlation between serum ALP level before neoadjuvant chemotherapy and tumor necrosis. We also demonstrated that LDH value before neoadjuvant chemotherapy had a strong correlation with degree of necrosis, and was statistically significant. LDH value was also decreased after neoadjuvant chemotherapy which was statistically significant too. We concluded, therefore, that LDH value could be used as a predictive indicator to evaluate patients' responses to neoadjuvant chemotherapy in osteosarcoma.

A decreased preoperative LMR could be regarded as an independent prognostic factor for both OS and EFS in patients with osteosarcoma. However, we found that NLR and LMR were not associated with any degree of necrosis after chemotherapy. In other words, NLR and LMR cannot be independent prognostic factors for predicting chemotherapeutic response in osteosarcoma.

In our study, osteosarcoma patients who underwent neoadjuvant chemotherapy showed 82.8% poor response and 17.2% good response. One possible reason for that could be that our patients presented to us with relatively large tumors.

MRI plays an important role in evaluating tumor volumes and preoperative radiological changes, using diffusion-weighted imaging (DWI) and water diffusion to predict histological necrosis. We concluded that the osteosarcoma patients in the good-response group showed far more decreased tumor volume after neoadjuvant chemotherapy than patients in the poor-response group; although the change in volume following chemotherapy did not show a statistic difference (correlation with degree of necrosis). A change in ADC values did not significantly correlate with the degree of necrosis; rather, ADC values remained increased in the poor-response group.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Declaration of competing interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2021.102228.

Ethical approval

The study had been approved by the ethical committee of our institution with Approval No. 0055/UN2.F1/ETIK/2019; and had obtained research permission from CMH Research Committee.

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The authors declare that sponsors had no such involvement.

Author contribution

AFK contributed to performed data collection, analysis and interpretation, manuscript drafting, revising, and approval for publishing; IA contributed to performed data collection, analysis and interpretation, manuscript drafting, revising, and approval for publishing; TS performed data collection, analysis and interpretation, manuscript drafting, revising, and approval for publishing.

Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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