Research Paper

Pre-treatment serum lactate dehydrogenase and alkaline phosphatase as predictors of metastases in extremity osteosarcoma

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ABSTRACT

Background: The prognosis of patients with metastatic osteosarcoma remains poor. However, the chance of survival can be improved by surgical resection of all metastases. In this study we investigate the value of serum alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in predicting the presence of metastatic disease at time of diagnosis.
Methods: Sixty-one patients with histologically confirmed conventional osteosarcoma of the extremity were included in the study. Only 19.7% of cases presented without evidence of systemic spread of the disease. Pre-treatment serum ALP and LDH were analysed in patients with and without skeletal or pulmonary metastases.
Results: Serum LDH and ALP levels were not significantly different in patients with or without pulmonary metastases ($p=0.88$ and $p=0.47$, respectively). The serum LDH and ALP levels did however differ significantly in patients with or without skeletal metastases ($p < 0.001$ and $p=0.02$, respectively). The optimal breakpoint for serum LDH as a marker of skeletal metastases was 849 IU/L (AUC 0.839; Sensitivity=0.88; Specificity=0.73). LDH > 454 IU/L equated to 100% sensitivity for detected bone metastases (positive diagnostic likelihood ratio (DLR)=1.32). With a cut-off of 76 IU/L a sensitivity of 100% was reached for serum ALP predicting the presence of skeletal metastases (positive DLR=1.1). In a multivariate analysis both LDH ≥ 850 IU/L (odds ratio [OR]=9; 95% confidence interval (CI) 1.8–44.3) and ALP ≥ 280 IU/L (OR=10.3; 95% CI 2.1–50.5) were predictive of skeletal metastases. LDH however lost its significance in a multivariate model which included pre-treatment tumour volume.
Conclusion: In cases of osteosarcoma with LDH > 850 IU/L and/or ALP > 280 IU/L it may be prudent to consider more sensitive staging investigations for detection of skeletal metastases. Further research is required to determine the value and the most sensitive cut-off points of serum ALP and LDH in the prediction of skeletal metastases.

1. Introduction

Osteosarcoma is the most common primary bone cancer in children and adolescents [1]. Surveillance, Epidemiology and End Results (SEER) programme data indicates an annual incidence of 4.4 per million population in patients younger than 25 years of age

[2]. The presence of metastases, at time of presentation, has been shown to be an independently significant risk factor in the prognosis of a patient with osteosarcoma [3]. Pakos et al. analysed the prognostic factors in 2 680 osteosarcoma cases in an international multicentre study and found that metastases at diagnosis increased the risk of mortality by a factor of 2.89 [4]. In developed regions approximately 15% of patients with osteosarcoma present with metastatic disease [5]. In under-developed regions higher rates of metastases have been found at time of diagnosis. This is illustrated in previous studies from South Africa, where evidence of systemic spread was found in 47–66% of patients at time of presentation [6,7].

Implementation of contemporary treatment protocols, incorporating adjuvant chemotherapy, have resulted in an improvement in the prognosis of patients diagnosed with osteosarcoma over the past

decades. The overall 5-year survival rate has improved from less than 20% in the 1960s to approximately 60% [8]. The prognosis, however, remains unsatisfactory in cases with metastases, with an overall 5-year survival rate of less than 30% [8]. Owing to the fact that long-term survival can be improved to over 40%, the European Society of Medical Oncology (ESMO) recommends mandatory excision of all metastatic lesions in patient diagnosed with osteosarcoma [8,9]. It is therefore essential that all patients with metastatic disease are identified timeously. In addition, there is a need for markers which identify patients with a poor prognosis so that more aggressive treatment options can be implemented in an effort to improve their prognosis [9].

In this retrospective review of a cohort of patients with high-grade conventional osteosarcoma of the extremity, we investigate the value of serum alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in predicting the presence of pulmonary and skeletal metastates at time of diagnosis.

2. Methods

A retrospective review was performed of the records of all patients with osteosarcoma who were referred to our tertiary level orthopaedic oncology unit, over the 5 year period from 2010 to 2014. Ethical approval was obtained from the relevant ethics review board prior commencement of the study (UHERB Ref No. 02–012013). All patients with histologically confirmed high-grade conventional osteosarcoma of an extremity were included in the study. Exclusion criteria included involvement of the axial skeleton, soft tissue osteosarcoma, surface lesions and other osteosarcoma subtypes.

2.1. Pre-treatment evaluation

Systemic staging involved standard laboratory investigations (including serum ALP and LDH), CT-scan of the patient's chest and abdomen, as well as a Technesium bonescan. The patient's charts were subsequently reviewed and data extracted in order to describe the patient demographics, ALP and LDH levels, tumour volume, as well as the presence of pulmonary or skeletal metastases. Pulmonary metastases was defined as both parenchymal and pleural metastatic lesions, while skeletal metastases included both skip lesions and peripheral bony metastases. A bonescan was not performed on four patients due the fact that their general condition did not permit transport to the centre where this was performed. Serum LDH, reported in International Units per Liter (IU/L) was determined using the Dimension® LDI method (Siemens, Munich, Germany). Serum ALP (IU/L) was determined using Dimension® ALPI method (Siemens, Munich, Germany). Tumour volume was calculated based on MRI (magnetic resonance imaging) images using the formula for an ellipsoidal tumour mass, where volume $= (\pi/6) \times$ length $\times$ width $\times$ height, as previously described [10,11]. Histology was obtained by formal incisional biopsy in all cases and the diagnosis was subsequently confirmed at a combined radiology-histology meeting.

2.2. Statistical analysis

Data were processed and analysed using Stata 13.0 SE (StataCorp, 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP) and R statistical package 3.0.3 (R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). Differences in mean age, LDH and ALP by metastases were tested using the standard two-sample t-test. Receiver operating characteristic (ROC) curves were used to determine the optimal breakpoint for the classification of metastatic cancer based on LDH and/or ALP levels. The criterion of the point on the ROC curve closest to the point (0,1), i.e, upper left corner of the unit square, was used to identify the optimal breakpoints [12,13]. The discriminatory power was evaluated by the area under the ROC curve (AUC). An AUC value of 0.5 indicates no discriminative ability while an AUC exceeding 0.8 suggest good to excellent predictive capability. Sensitivity and specificity based on the optimal identified cut-points were also calculated, along with 95% confidence intervals. Logistic regression analysis was then employed to estimate the strength of association between categorical ALP and LDH versus metastases. A p-value of < 0.05 was considered statistically significant for all tests.

3. Results

Sixty-seven patients were identified with histologically confirmed osteosarcoma involving an extremity. Six patients were excluded from study. One patient passed away prior to completion of systemic staging investigations and five patients were diagnosed with osteosarcoma variants. Sixty-one patients met the inclusion criteria and their clinical characteristics are listed in table 1. The mean patient age was 21 years (standard deviation [SD] 11.9 years) and there was an equal distribution between male and female patients (50.8 vs 49.2%). The incidence of pulmonary and skeletal metastases did not vary significantly according to the age ($p=0.16$ and $p=0.27$, respectively). The majority of patients (98%) where of African descent. The femur (57%) and tibia (31%) were involved in the majority of cases.

Only 19.7% ($n=12$) of patients had no evidence of metastatic disease at time of presentation. Seventy-two percent ($n=44$) had pulmonary metastases. No other visceral metastases, including liver metastases, were detected on the chest and abdominal CT-scans. Twenty eight percent ($n=16$) of patients who had a bone-scan had evidence of skeletal metastases at the time of presentation. The incidence of pulmonary and skeletal metastases did not vary significantly according to patient age ($p=0.10$ and $p=0.14$, respectively). The serum levels of LDH were not significantly different in patients with or without pulmonary metastases ($p=0.88$ and $p=0.47$, respectively) (Table 2). The serum LDH and ALP levels did however differ significantly in patients with or without skeletal metastases ($p < 0.001$ and $p=0.02$, for LDH and ALP, respectively).

Optimal breakpoint analysis of serum LDH as a predictor of pulmonary metastases revealed an area under the receiver operator curve (AUC) of 0.569 (Fig. 1). The optimal breakpoint for serum LDH as a marker of skeletal metastases was 849 IU/L (AUC 0.839; sensitivity$=0.88$; specificity$=0.73$) (Fig. 1). Serum LDH of 454 IU/L equated to 100% sensitivity for detected bone metastases with a positive diagnostic likelihood ratio (DLR) of 1.32 (95% CI 1.1–1.6). The optimal breakpoint analysis of ALP and pulmonary metastases revealed poor correlation (AUC 0.516). The optimal breakpoint for serum ALP as a marker of skeletal metastases was 283 IU/L (AUC 0.771; sensitivity$=0.81$; specificity$=0.76$) (Fig. 2). A serum ALP level of 76 IU/l was 100% sensitive in predicting the presence of skeletal metastases (positive DLR 1.1; 95% CI 1.0–1.2).

Logistic regression analysis confirmed that serum LDH and ALP were significant prognostic factors for skeletal metastases at time of presentation. Univariate analysis of serum LDH > 850 IU/L revealed an odds ratio (OR) of 10.9 (95% CI 2.6–46.1) for the presence of skeletal metastases ($p < 0.01$) and for serum ALP > 280 IU/L the OR was 12.4 (95% CI 2.9–53.0). In a multivariate analysis of serum ALP and LDH both factors remained predictive of skeletal metastases. However, with the addition of pre-treatment tumour volume LDH lost its significance (Table 3).

Table 1
Clinical characteristics and descriptive statistics of cohort.

	n	Percentage	Mean	Range	SD
Age	61		21.3 years	6–56 years	11.9 years
Sex					
Male	31	50.8%	–	–	–
Female	30	49.2%	–	–	–
Site					
Femur	35	57.4%	–	–	–
Tibia	19	31.2%	–	–	–
Humerus	3	4.9%	–	–	–
Fibula	3	4.9%	–	–	–
Ulna	1	1.6%	–	–	–
Pulmonary metastasis					
Yes	44	72.1%	–	–	–
No	17	27.9%	–	–	–
Skeletal metastasis					
Yes	16	26.2%	–	–	–
No	41	67.2%	–	–	–
Unknown	4	6.6%	–	–	–
LDH	61		1156.9 IU/L	269–6135 IU/L	1030.0 IU/L
ALP	61		570.3 IU/L	49–9594 IU/L	1293.4 IU/L
Tumour volume	51		1114.3 cm^3	164–6821 cm^3	1285.8 cm^3

4. Discussion

The presence of metastases at the time of diagnosis is a significant prognostic factor in osteosarcoma [14]. Skeletal metastases at time of presentations appears to carry a worse prognosis than pulmonary metastases [15]. Furthermore, the chance of survival can be improved by surgical resection of all metastases [16]. Systemic staging and the search for the presence of metastases therefore form an integral part of the initial diagnostic work-up of a patient with osteosarcoma. The aim of this study was to determine the value of pre-treatment serum LDH and ALP in predicting the presence of skeletal or pulmonary metastases in patients with conventional high-grade osteosarcoma of the extremities. Neither serum ALP or LDH was found to be of value as prognostic factors for the presence of pulmonary metastases. In terms of skeletal metastases both serum ALP and LDH were found to be significant. Serum LDH, however, lost its statistical significance in a multivariate model that included tumour volume.

Lactate dehydrogenase is known to reflect systemic cancer burden and its prognostic significance has been illustrated in various malignancies, including Ewing's sarcoma [17,18].

A recent meta-analysis of studies evaluating the effect of high LDH levels on overall survival found a pooled hazard ratio of 1.92 (95% CI 1.53–2.40) [19]. The Multi-Institutional Osteosarcoma Study (MIOS) found serum LDH to be the single most predictive factor of adverse outcome [20]. At 6 year follow-up of patients diagnosed with osteosarcoma of the extremities, the event-free survival was 41% for patients with elevated LDH levels compared to 69% for the patients who had normal LDH at diagnosis ($p < 0.001$). A study looking specifically at the prognostic value of LDH in patient with osteosarcoma of the extremities, found that patients who presented without metastases and an increased serum LDH level were also far more likely to develop relapse of disease than those with normal LDH values (60% vs 38%, $p < 0.001$) [21].

Plasma bone-specific ALP has been suggested as a reliable tumour marker for osteosarcoma [22]. Multivariate analysis performed by Mialou et al. identified serum ALP levels in excess of 500 IU/L as an independent risk factor for decreased disease-free and overall survival rates [23]. Furthermore, a reduction in ALP levels following chemotherapy has been shown to correlate with improved response to chemotherapy and survival [24]. On the other hand, some authors have found that serum ALP did not have prognostic value in terms of disease outcome [4].

Although pre-treatment LDH and ALP levels have been shown to serve as a reliable indicator of disease-free survival, its value in predicting the presence of metastases at time of diagnosis remains unclear. In their initial series, Bacci et al. found that the percentage of patients with an elevated serum LDH at the time of diagnosis was significantly higher in patients with metastatic disease than those who had localised disease (64% versus 33%, $p < 0.0001$) [18]. In a larger follow-up series from the Rizzoli institute, involving 1421 patients seen over a 30 year period, it was noted that 18% of patients with localized disease had elevated LDH levels at presentation compared to 36% of patients with metastatic disease ($p < 0.0001$) [25]. Although high LDH levels were able to predict the presence of metastases with a high degree of specificity (0.81), sensitivity was found to be low (0.38). There was however no differentiation made between skeletal and pulmonary metastases in these studies. To the best of our knowledge this is the first report looking specifically at the predictive value of ALP and LDH in detecting skeletal metastases. In our series serum LDH appeared to be predictive for skeletal, but not pulmonary metastases. Serum LDH, with a cut-off value of 849 IU/L, had a sensitivity of 0.88 and specificity of 0.73 as a marker of skeletal metastases.

Multivariate logistic regression analysis by Bacci et al. found that only tumour site (femur and humerus), increased alkaline phosphatase (ALP), tumour volume (> 150 ml) and duration of symptoms (less than 2 months) were significant factors in the prediction of the presence of metastases at time of presentation

Table 2
Two-sample *t*-test of serum LDH and ALP as a predictors of the presence of metastasis at time of diagnosis.

	Positive		**Negative**		*p*-Value
	n (%)	95% CI	n (%)	95% CI	
LDH					
Pulmonary metastasis	44 (72.1%)	573.7–1676.7	17 (27.9%)	857.4–1480.9	0.88
Skeletal metastasis	16 (28.1%)	1163.5–2785.8	41 (51.9%)	631.5–892.6	< 0.001
ALP					
Pulmonary metastasis	44(72.1%)	189.3–1101.2	17 (27.9%)	162.2–590.8	0.43
Skeletal metastasis	16 (28.1%)	− 59.8–2398.3	41 (51.9%)	164.3–471.6	0.02
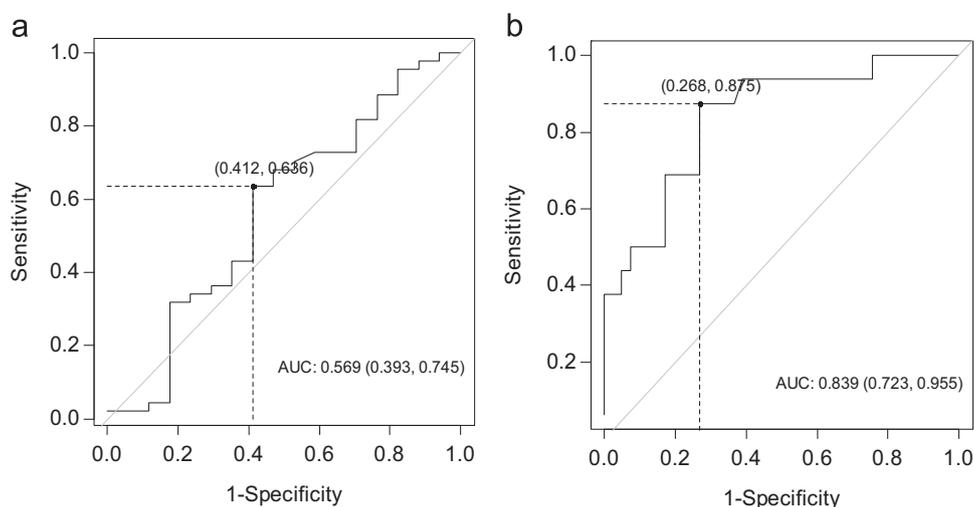
Fig. 1. Receiver operator curve of optimal breakpoint serum LDH as a predictor of metastasis at time of diagnosis. (a) Pulmonary metastases (b) Skeletal metastases.

[26]. Han et al. found an increased incidence of pulmonary metastases in patients with a pre-treatment serum ALP increased more than twice the upper limit of normal (34% vs 12%; $p=0.007$) [27]. Similarly, the Rizolli group found increased ALP levels in patients with metastases at time of diagnosis (91.5% vs 61.3%; $p < 0.001$) [28]. The authors did not distinguish skeletal from pulmonary metastases at time of presentation in relation the ALP. Furthermore, there was an increased relapse rate in patients who presented with localized disease and increased ALP levels (55.1% vs 26.4%; $p < 0.001$). The authors noted however that there was no difference in the site of first metastases related to value of the ALP level. Our findings suggest that normal serum ALP levels may be predictive of the absence of skeletal metastases at the time of diagnosis. With a cut-off of 76 IU/L a sensitivity of 100% was reached. Serum ALP remained a significant predictor in a multivariate model that included the pre-treatment tumour volume, with an odds ratio of 9.8 ($p=0.02$) for skeletal metastases if pre-treatment ALP ≥ 280 IU/L.

There are several shortcomings to this study that need to be considered. The small size of this series is an obvious shortcoming. Due to the relative rarity of the disease, this problem is not unique to this series and authors of a recent meta-analysis noted the small sample size of other studies as an obstacle in drawing firm conclusions regarding prognostic implications of LDH in terms of survival. The small number of patients without metastases in this

Table 3
Multivariate analysis of ALP, LDH and tumour volume as a predictors of the presence of skeletal metastasis at time of presentation.

Measure	OR	*p*-Value	95% CI
LDH ≥ 850 IU/L	2.8	0.97	0.4–20.0
ALP ≥ 280 IU/L	9.8	0.02	1.3–70.9
Tumour volume ≥ 1380 cm^3	8.7	0.03	1.1–67.2

series would adversely affect the ROC analysis and therefore the suggested cut-off values. Numerous patients were lost to follow-up in our series and the long-term survival could not be determined. The cohort of patients in our series is also not directly comparable to those in other studies. The incidence of metastases (80%) at time of diagnosis were much higher than the 14% reported by Bacci et al. Furthermore, the mean tumour volume in this series was much higher than in previous reports [29]. These factors suggest that the cancer was either more advanced, both locally and systemically, or more aggressive in nature in our series of cases. The higher incidence of metastases in this series did however enable us to determine more sensitive cut-off points for the proposed prognostic indicators. Our results suggest that the sensitivity of ALP and LDH in predicting the presence of skeletal metastases may be improved by using a cut-off point of 75 IU/L
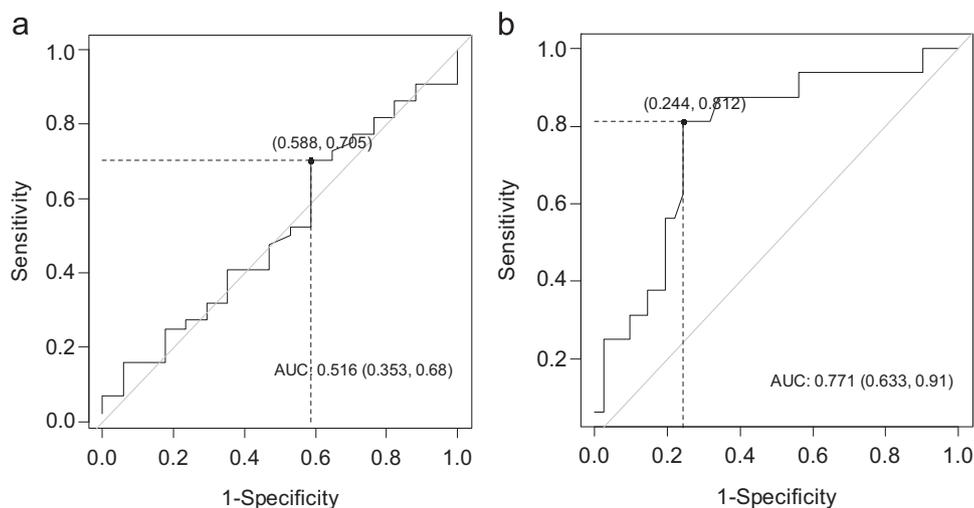
Fig. 2. Receiver operator curve of optimal breakpoint serum ALP as a predictor of metastasis at time of diagnosis. (a) Pulmonary metastases (b) Skeletal metastases.

and 450 IU/L, respectively.

The findings of this study should be borne in mind during the initial staging and follow-up of a patient presenting with osteosarcoma. 18F-fluorodeoxy-D-glucose (FDG) positron emission tomography (PET) has been found to be more sensitive in the detection of skeletal metastases from sarcomas [30]. The sensitivity and specificity of spiral CT (computed tomography) however remains superior to FDG-PET in the detection of lung metastases [31]. In cases of osteosarcoma with LDH > 850IU/L and/or ALP > 280IU/L at presentation it may thus be prudent to consider spiral CT and FDG-PET in the initial systemic staging of the patient. In addition, the follow-up of patients who present with a high ALP and/or LDH should possibly also be more rigorous. ESMO currently recommends the use of X-rays or CT scan in the follow-up of patients [32]. However, thought should be given to the use of spiral chest CT and FDG-PET or technetium bone scintigraphy during the follow-up patients with high LDH and ALP levels in order to detect metastases early.

5. Conclusion

In cases of osteosarcoma with LDH > 850 IU/L and/or ALP > 280 IU/L it may be prudent to consider more sensitive staging investigations for detection of skeletal metastases. Further research is required to determine the value and the most sensitive cut-off points of serum ALP and LDH in the prediction of skeletal metastases. This data may be of value in certain resource constrained clinical environments where special investigations like bone scintigraphy or FDG-PET may not be widely available.

Acknowledgements

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