Innate immunity in osteosarcoma
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Prognostic factors in pulmonary metastasized high-grade osteosarcoma

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ABSTRACT

INTRODUCTION: Resection of pulmonary metastases has previously been reported to improve outcome in high-grade osteosarcoma (OS) patients. Factors influencing survival in OS patients with pulmonary metastases are important for clinical decision making. METHODS: All 88 OS patients with pulmonary metastases either at diagnosis or during follow-up treated at the Leiden University Medical Center between January 1, 1990 and January 1, 2008 under the age of 40 were included in this study, including 79 cases of conventional, eight cases of telangiectatic and one case of small cell OS. RESULTS: In total, 56 of 88 patients with pulmonary metastases were treated by metastasectomy. Resectability of pulmonary metastases was the main prognostic factor. In patients with primary non-metastatic OS, a longer relapse free interval to pulmonary metastases was significantly associated with better survival (P=0.02). Independent risk factors determining worse survival after metastasectomy in multivariate analysis were male sex (P=0.05), higher number of pulmonary nodules (P=0.03), and non-necrotic metastases (P=0.04). Whether surgery for recurrent pulmonary metastases was performed did not influence survival. Histological subtype of the primary tumor, histological response in the primary tumor after neoadjuvant chemotherapy, occurrence of local relapse, local resection or amputation of the primary tumor and age at diagnosis did not influence outcome. CONCLUSION: This cohort of patients with detailed follow-up data enabled us to identify important risk factors determining survival in OS patients with pulmonary metastases. We demonstrate that after repeated metastasectomies, a subset of patients can be cured.

INTRODUCTION

High-grade osteosarcoma (OS) is a malignant bone tumor mainly affecting adolescents and young adults [71]. Since the introduction of (neo-)adjuvant chemotherapy, long-term overall survival has improved to about 60%, with failure of therapy mainly attributed to chemoresistant metastatic disease. At diagnosis 15–25% of patients present with clinically detectable metastatic disease (synchronous pulmonary metastases) and about 40–50% of patients with primary non-metastatic disease experience relapse, mainly to the lungs (metachronous pulmonary metastases) [4;13;24;141;170;177;243].

Resection of pulmonary metastases with or without second-line chemotherapy has been reported to improve outcome in OS patients with pulmonary metastases and surgery is currently standard treatment in many institutions for patients in whom metastases are deemed resectable. Despite aggressive surgery, however, many patients still relapse. The two largest single-institution studies to date investigating prognostic factors determining survival of OS patients with lung metastases undergoing metastasectomy have had conflicting results. Studies conducted at the Rizzoli Institute concluded that higher numbers of pulmonary nodules, bilateral disease, and incomplete resection were independent prognostic factors for poor survival after metastasectomy [12;14;29]. This was confirmed in a large multi-center study and in several smaller studies [121;228;256;277]. In contrast, several studies concluded that neither number of pulmonary nodules nor other clinical parameters such as disease-free interval, bilateral disease or resection margins significantly affect survival [42;53;97]. In the subgroup of patients with metachronous disease, longer disease-free interval has been associated with a favorable outcome, both in smaller single-center studies and in large multi-center studies [83;121;256;277]. The role of second-line chemotherapy is unclear, with only some authors describing a moderate survival benefit when administered in addition to metastasectomy [23].

In the current study, we sought to determine factors determining outcome in a cohort of patients with extensive follow-up data with high-grade OS metastasized to the lungs, including 79 cases of conventional OS, eight cases of telangiectatic OS and one case of small cell OS.

PATIENTS AND METHODS

Definition of cohort

Between January 1990 and January 2008 197 patients under the age of 40 were treated for high-grade OS at the Leiden University Medical Center. Excluded were patients with insufficient follow-up data (n=12) and unresectable primary tumor (n=11). Of the remaining 174 patients, all 88 patients who had pulmonary metastases either at diagnosis or during follow-up were included in this study (Table 3.1). Patients were treated according to one of the consecutive European Osteosarcoma Intergroup (EOI) trials 80861 [243] and 80931 [141] (doxorubicin and cisplatinum, with or without high-dose methotrexate, bleomycin, cyclophosphamide, actinomycin- D, and vincristin) or according to the Euramos-1 trial [1] (doxorubicin, cisplatinum, and high-dose methotrexate with or without interferon-alpha or etoposide and ifosfamide).

Imaging studies

Pulmonary metastases were diagnosed by routine computed tomography (CT) scans of the lungs and additional staging with ^{99m}Tc-bone scintigraphy, and if needed magnetic resonance imaging (MRI). Follow-up included clinical investigations and X-rays of the primary site and lungs. Number and distribution of pulmonary nodules were noted in case of suspected pulmonary metastases.

Histopathology

Primary tumors were histologically classified according to the criteria of the World Health Organization [71]. Resected specimens of primary tumors were evaluated for histological response to neoadjuvant chemotherapy; good response was defined as less than 10% vital tumor tissue. Resected specimens of pulmonary metastases were evaluated for number of resected nodules and viability of resected tumor tissue. In addition, completeness of resection and occurrence of pleural contamination were noted on macroscopic and microscopic examination.

Statistical analysis

Unpaired t-tests, contingency analyses (χ^2 method) and univariate survival analyses (Kaplan–Meier method, log rank test for comparison of survival curves) were performed using GraphPad Prism 5.0. Multivariate survival analyses and survival analyses with continuous input variables were carried out according to the Cox proportional hazards model in SPSS version 16.0. Survival time was calculated from date of diagnosis to date of last follow-up or death (noted as overall survival) or from date of first metastatic event to date of last follow-up or death (noted as overall survival since metastasis). Two-sided *P*-values lower than 0.05 were determined to be significant; *P*-values between 0.05 and 0.10 were defined to be a trend.

RESULTS

Twenty-six of 88 patients had pulmonary metastases at diagnosis (synchronous pulmonary metastases, group A in Table 3.1) and 62 patients developed pulmonary metastases during follow-up (metachronous pulmonary metastases, group B in Table 3.1). The proportion of high-grade OS patients with clinically detectable pulmonary metastases did not change during the study period. About 15% of all high-grade OS patients had pulmonary metastases at diagnosis and 35% developed pulmonary metastases during follow-up. There was a male predominance in our cohort (71.6% males vs. 28.4% females) and there was a trend for males to have a worse overall survival (*P* logrank = 0.08). Most tumors were located in the distal femur (43/88, 48.9%), proximal tibia or fibula (18/88, 20.5%), and proximal humerus (8/88, 9.1%). Less frequently involved were the axial skeleton (5/88, 5.7%) or the other long bones (13/88, 14.8%). One tumor was located in the calcaneus. Histological subtype was conventional OS in 79 cases (including 17 chondroblastic, 2 fibroblastic and 6 unusual histological subtypes), telangiectatic OS in 8 cases and small cell OS in 1 case.

There was no significant difference in overall survival (measured as survival since development of metastases to correct for time dependency of the variable) between patients with synchronous and metachronous pulmonary disease (group A vs. B *P*=0.16). When dividing the 62 patients with

Table 3.1 Overview of patients with pulmonary metastases of high grade osteosarcoma under age 40, treated from 1990 to 2008

	Pulmonary metastases		
	Synchronous (group A)	Metachronous (group B)	Total
No metastasectomy for pulmonary metastases *(group 1)*	1A, 5 (5.7%)	1B, 27 (30.7%)	32 (36.4%)
Metastasectomy for pulmonary metastases *(group 2)*	2A, 21 (23.9%)	2B, 35 (39.8%)	56 (63.6%)
Total	26 (29.5%)	62 (70.5%)	88

metachronous lung metastases (group B) into three equal groups of 21 patients each based on the duration of the disease-free interval, longer disease-free interval was associated with better survival, with most deaths occurring in patients in the first tertile (metastasis from day 1 to day 310 since diagnosis, Fig. 3.1) (P=0.02). Other factors associated with poor overall survival for patients with pulmonary metastases were higher numbers of pulmonary nodules as determined by CT scanning (9.3% increase in hazard for each additional pulmonary nodule, P=0.001) and bilateral involvement (P logrank=0.008). Patients with bone or other metastases present at the time of diagnosis of the pulmonary metastases had worse overall survival, mainly because in these cases, resection of metastases (pulmonary and others) was often not performed. Histological subtype, histological response to neo-adjuvant chemotherapy in the primary tumor (<10% viable tumor), location of the primary tumor, age at diagnosis, occurrence of local relapse and year of diagnosis did not affect survival in this cohort of patients with pulmonary metastasis.
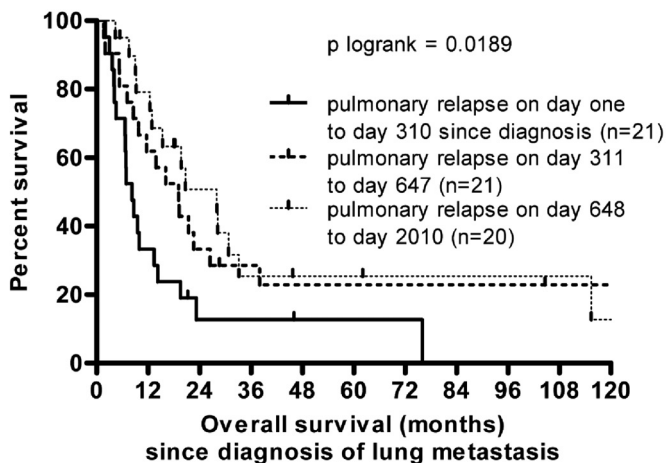

Fig. 3.1 Kaplan–Meier curve of patients with metachronous pulmonary metastases of osteosarcoma (group B), divided into three equal groups based on the duration of the disease-free interval (tertiles), demonstrating worse overall survival for patients with pulmonary relapse from day 1 to day 310 of diagnosis (solid line, P logrank=0.02).

Resection of pulmonary metastases

The majority of patients with pulmonary metastases were treated surgically for these metastases at least once (56/88, 63.6%). Overall survival of these patients (group 2) was significantly better than of patients ineligible for metastasectomy (group 1, Table 3.1 and Fig. 3.2, *P*<0.0001). Irresectability of disease as determined in multidisciplinary meetings including radiologists, pathologists, thoracic and orthopedic surgeons and clinical oncologists was the reason not to perform metastasectomy in most cases (81.3%) (Table 3.2). One patient with radiological evidence of lung metastases who did not undergo metastasectomy is a long-term survivor (duration of follow-up 18 years). In this patient, three pulmonary nodules appeared on CT-scanning 1 year after diagnosis of the primary tumor, ranging in size from 0.5 to 2 cm. The largest nodule had a high density, suggesting calcification. In the following year, lesions progressed in both size and number, after which the lesions stabilized without further treatment. All other patients with clinicoradiological evidence for lung metastases who did not undergo metastasectomy died. In these other cases with unresectable disease, no other curative treatments were available; some patients received palliative chemo- or radiotherapy.

Patients not undergoing metastasectomy for pulmonary metastases (group 2) had more nodules (mean of six vs. three, *P*=0.002), more often had bilateral disease (25% vs. 46%, χ^2 *P*=0.06) and more often poor histological response to neo-adjuvant chemotherapy in the primary tumor (78% vs. 64%, χ^2 *P*=0.05) than patients eligible for surgery (group 1). There was no difference in age.

Male sex, higher numbers of pulmonary metastases and viability of resected metastases are independent risk factors after surgical treatment of pulmonary metastases (group 2)

Although patients who were selected for pulmonary metastasectomy (group 2) had improved survival (Fig. 3.2), overall survival was still poor at about 23%. The majority of patients (30/56) underwent thoracotomy just once, but there was no significant survival difference for patients undergoing metastasectomy once or more often (*P*=0.29). This is also reflected in Fig. 3.3, which demonstrates the possibility of achieving complete remission after repeated metastasectomies. Males had surgery for metastases more often than females (70% vs. 48%, χ^2

Table 3.2 Reasons not to undergo surgical removal of pulmonary metastases

| Number of patients (%)	Reason not to undergo pulmonary metastasectomy
26/32 (81.3%)	unresectable disease (including metastases to other sites (n=6) and rapidly progressive disease during neo-adjuvant chemotherapy (n=5))
1/32 (3.1%)	died before metastasectomy could be performed
2/32 (6.3%)	physical or mental condition of the patient did not allow it
1/32 (3.1%)	unknown
2/32 (6.3%)	regression of pulmonary metastasis during (neo-)adjuvant chemotherapy (one patient still alive after 18 years follow up, one patient had unresectable relapse in other organs two years later and died of disease)
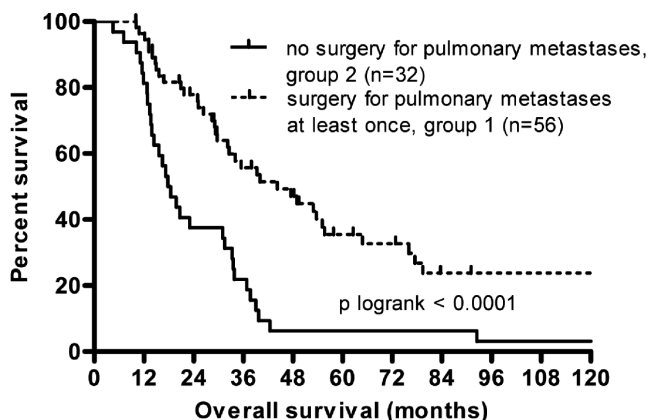
Fig. 3.2 Kaplan–Meier curve comparing overall survival of patients with pulmonary metastases treated surgically (group 1) and nonsurgically (group 2). Patients not treated surgically have a significantly worse overall survival (solid line, P logrank<0.0001).

P=0.05), but had worse overall survival (Fig. 3.4, P=0.04). Almost all of the patients with pleural disruption evident on histological examination or incomplete resection of the metastases in at least one of the metastasectomies died of disease, but this failed to reach significance (P=0.28). Twenty-nine patients (51.8%) had chemotherapeutic treatment before at least one of the metastasectomies. This chemotherapeutic treatment was given to patients either as a part of their primary (neo-) adjuvant treatment or to patients presenting with an initially unresectable pulmonary recurrence during followup. Nine patients had completely necrotic metastases removed at least once (as determined by histological examination) and there was a trend for better survival in these patients (P=0.09). Whether or not additional treatment was given before metastasectomy, did not influence survival, but there was an association between premetastasectomy treatment and subsequent removal of necrotic metastases (χ^2 P=0.04).

As was the case in the entire cohort of patients with pulmonary metastases, higher numbers of metastases visible on CT scan prior to metastasectomy was associated with worse survival in group 2 patients (who underwent metastasectomy at least once, P=0.04). Similarly, there was a trend for patients with bilateral disease to have worse overall survival (P=0.07). However, 4 of 15 patients with 5 or more nodules on CT scan did survive after resection of these lesions (follow-up since first metastasis 18–38 months). There was a reasonable correlation between number of metastases as estimated by CT scan and the number of metastases found at metastasectomy (r^2 0.41, slope 0.7, P<0.0001), which did not change during the study period. Factors not associated with outcome were histological subtype of the primary tumor, histological response in the primary tumor to neo-adjuvant chemotherapy, occurrence of local relapse, local resection or amputation of the primary tumor and age at diagnosis. In contrast to analysis performed on the entire cohort of 88 patients, disease-free interval was not associated with prognosis in this subset of 56 patients who underwent surgery for pulmonary metastases.We used multivariate
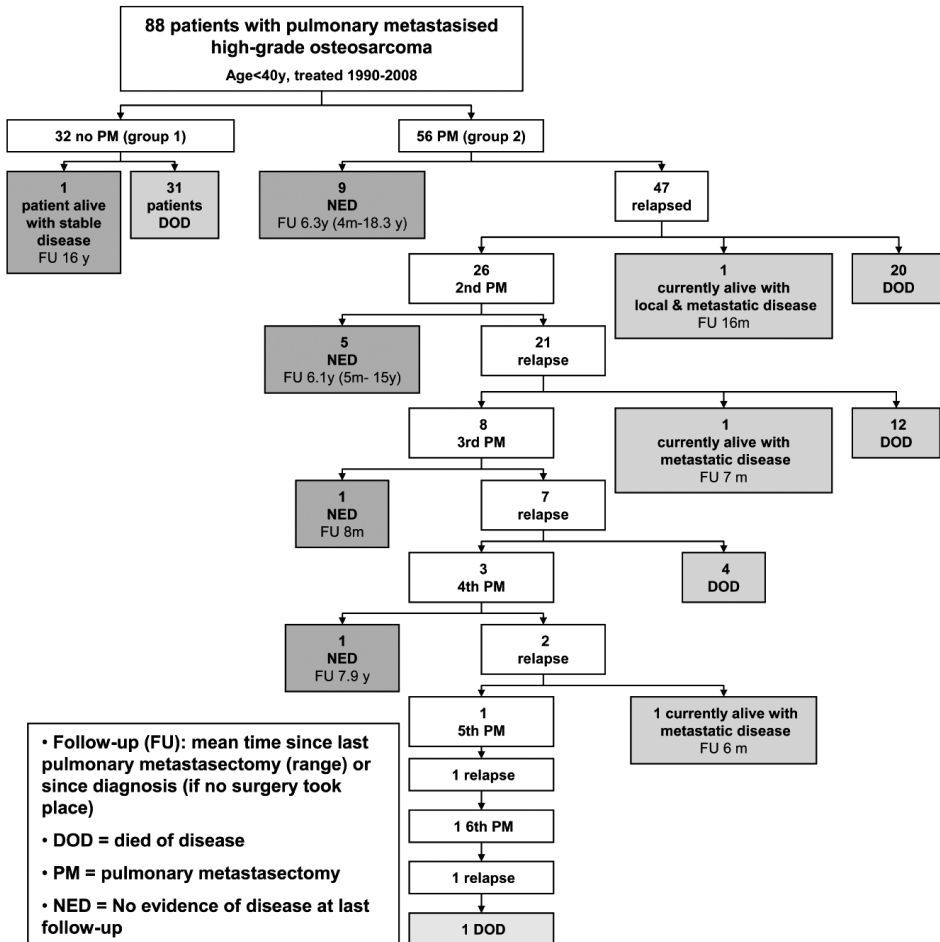
88 patients with pulmonary metastasised high-grade osteosarcoma

Age<40y, treated 1990-2008

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32 no PM (group 1)

56 PM (group 2)

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1 patient alive with stable disease FU 16 y

31 patients DOD

9 NED FU 6.3y (4m-18.3 y)

47 relapsed

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26 2nd PM

1 currently alive with local & metastatic disease FU 16m

20 DOD

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5 NED FU 6.1y (5m- 15y)

21 relapse

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8 3rd PM

1 currently alive with metastatic disease FU 7 m

12 DOD

---

1 NED FU 8m

7 relapse

---

3 4th PM

4 DOD

---

1 NED FU 7.9 y

2 relapse

---

1 5th PM

1 currently alive with metastatic disease FU 6 m

1 relapse

1 6th PM

1 relapse

1 DOD

• **Follow-up (FU): mean time since last pulmonary metastasectomy (range) or since diagnosis (if no surgery took place)**

• **DOD = died of disease**

• **PM = pulmonary metastasectomy**

• **NED = No evidence of disease at last follow-up**

Fig. 3.3 Flow chart of all patients, demonstrating the possibility of achieving complete remission after repeated surgery for pulmonary metastases (including a patient who received surgery for pulmonary metastases four times and is still in complete remission almost 8 years since the last surgery).

Cox regression analysis of risk factors in group 2 patients who underwent metastasectomy at least once, entering only variables with a P-value lower than 0.10 as determined by univariate analysis. This revealed male sex of the patient, higher numbers of pulmonary metastases and viability of resected metastases to be independent risk factors for worse outcome in pulmonary metastasized OS patients treated surgically (Table 3.3).

DISCUSSION

In this cohort of patients with pulmonary metastasized high-grade OS, 26/88 patients had clinically detectable pulmonary metastases at diagnosis (group A, Table 3.1) and 62/88 relapsed
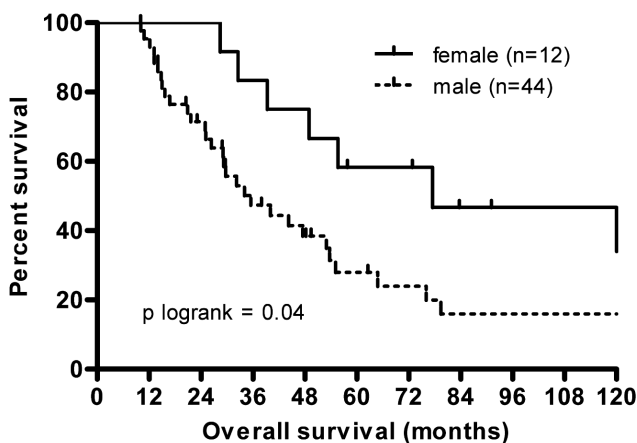
Fig. 3.4 Kaplan–Meier curve of survival after metastasectomy (group 2 patients) for males (dashed line) versus females (solid line). Males had surgery for metastases more often than females (70% vs. 48%, χ^2P=0.05), but had worse overall survival (P logrank=0.04, analysis on subgroup who underwent surgery for pulmonary metastatic disease).

Table 3.3 Multivariate Cox regression analysis to determine independent risk factors for survival in patients surgically treated for pulmonary metastasized high-grade osteosarcoma (group 2 patients)

Risk factor	P-value	Hazard ratio
Female sex	0.05	0.409
Completely necrotic metastases	0.03	0.167
Increasing numbers of metastases	0.04	1.293

with pulmonary metastases (group B). Metastasectomy was almost invariably required for cure, as has been previously described by others [12;97;121]. Since pulmonary metastasectomy is a safe and effective treatment, both in pediatric and adult patients, metastasectomy has become standard of care for patients with pulmonary metastasized OS when these metastases are deemed resectable and there are no other contra-indications for surgery (including metastasis to other sites) [261;269]. In our well-defined cohort of patients with detailed and extensive follow-up data we were able to confirm important risk factors determining survival in OS patients with pulmonary metastases, that is, extent of disease and longer disease-free interval. In addition, we have identified the novel independent risk factors male sex and resection of metastases containing viable tumor cells.

The administration of chemotherapeutic agents before metastasectomy is associated with a subsequent higher chance of resecting necrotic metastases, which in turn is associated with better overall survival. This would suggest a potential beneficial role of second-line chemotherapeutic agents in the treatment of metastasized OS, even when these lesions are deemed resectable. However, since we did not find a direct association between chemotherapeutic treatment and overall survival in the group of patients surgically treated

for pulmonary metastases, the addition of chemotherapy to the surgical treatment of patients with pulmonary metastases remains unproven. In previously published studies it has also been difficult to establish what the value of second-line chemotherapeutic treatment is to the surgical management of metastasized OS [23]. This could either mean that the true benefit is very small, or that the treatments employed are too heterogeneous to draw definite conclusions.

As was found in other studies, large metastatic tumor burden (defined as five or more pulmonary nodules or bilateral involvement), has prognostic relevance. It is now well known that spiral CT scanning (1mm slices) is more sensitive than conventional CT, allowing the detection of significantly larger number of nodules and also smaller nodules <5mm in diameter. Even these small pulmonary nodules should be regarded as probable pulmonary metastases when other risk factors for pulmonary nodules, such as smoking history or prior granulomatous disease, are absent. The proportion of patients diagnosed with pulmonary metastases in this study did not change during the study period. Previously, Kayton et al. [120] reported that CT scanning of the chest underestimates the number of metastatic lesions in OS. In our cohort there was a reasonable correlation between number of metastases as predicted by CT scan and number of metastases as determined at resection. However, we feel that the presence of high numbers of pulmonary nodules should not guide decisions regarding resection if the nodules are resectable; a third of patients with high tumor burden, that is, five or more nodules onCT scan prior to surgery, survive after resection of the lesions with a median duration of follow-up of 25 months. Similarly, patients experiencing relapse after first metastasectomy can still benefit from repeated metastasectomies if these lesions are resectable and if there is no risk of respiratory compromise.

The reason for the relationship with gender and outcome in our cohort is unclear. There was a male predominance in our cohort (71.6% males vs. 28.4% females). Males had their metastases significantly more often surgically resected than females. However, men had lower overall survival even after resection of metastases. A recently published review of OS incidence and survival rates from 1973 to 2004 in the United States noted a worse overall survival for males over all age groups, concordant with previous smaller studies [177;205;227]. In another study, a strong correlation between male sex and poor histological response to pre-operative chemotherapy was found, although this did not result in worse overall survival [24]. OS cells express sex steroid receptors and it has been observed that 2-methoxyestradiol (2-ME), a naturally occurring metabolite of 17beta-estradiol (E2), induces cell death in OS cells [57;155]. It remains unclear whether direct effects of sex steroids on neoplastic cells play a role in the observed better outcome for females, or if other mechanisms are underlying this observation.

Patients with OS which has a good histological response of the primary tumor to neo-adjuvant chemotherapy have better overall survival [98]. In our cohort of patients with OS and metastatic disease (group A and group B), there was no association between response to chemotherapy and survival. Similarly, the previously reported association of chondroblastic tumors with good histological response to chemotherapy and better overall survival, was not present in this cohort of patients with OS and lung metastasis. However, when analyzing all patients diagnosed with OS in our center, including those that did not develop pulmonary metastases the association between poor histological response and poor survival was

also present in our cohort. This suggests that poor histological response to chemotherapy probably determines poor survival through risk of developing clinically detectable, pulmonary metastases. However, once these metastases have developed, the histological response in the primary tumor was no longer relevant in our series.

Analysis of patients with resectable non-metastatic OS treated in three consecutive EOI trials demonstrated the association between early recurrence and poor survival [83]. In the current study, this association was also present in the subgroup of patients presenting with metachronous pulmonary metastases, but this association disappeared when patients eligible for surgical treatment of the metastases were selected. This indicates that patients presenting with relapse roughly within the first year after diagnosis have a higher chance of presenting with unresectable pulmonary involvement, but treatment of resectable pulmonary disease results in a similar outcome in this group.

In conclusion, this well-defined cohort of OS patients with pulmonary metastases treated within a single institution allowed us to establish that higher number of pulmonary nodules, resection of vital metastases and male sex were associated with poor overall survival. In the present study we confirm extent of disease, that is, number of pulmonary nodules, to be an important independent risk factor determining survival in OS patients with pulmonary metastases. In addition we demonstrate that female sex and resection of necrotic metastases are associated with better survival after pulmonary metastasectomy. Importantly, we demonstrate that even after repeated metastasectomies, cure can be achieved in a subset of patients.

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Prognostic factors in metastasized osteosarcoma