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ORIGINAL ARTICLE

Prognostic Value of Vascular Invasion in Pediatric Osteosarcomas

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Abstract Metastatic status, histologic response, and quality of surgical resection are prognostic factors for osteosarcomas. Pathology reports sometimes describe peritumoral vascular invasion on surgical specimens after neoadjuvant chemotherapy but their prognostic significance as an independent parameter has never been reported. The aim of this study was to evaluate how the presence of this peritumoral vascular invasion could influence survival. We retrospectively analyzed histology, demographics, and outcomes of pediatric patients treated for osteosarcoma in our institutions between January 2007 and December 2012. A single pathologist analyzed the resection specimens after neoadjuvant chemotherapy. Fifty-one osteosarcomas were diagnosed over a 6-year period; nine had metastatic disease at diagnosis. Surgery was performed after neoadjuvant chemotherapy in all cases. We identified peritumoral vascular invasion in the surgical specimens in 15 cases. Two-year event-free survival (EFS) was 78 % (CI95%[64;93]) for patients without vascular invasion versus 48 % (CI95% [21;75]) in patients with vascular invasion, and 2-year overall survival (OS) was 94 % (CI95%[86;100]) for those without vascular invasion versus 79 % (CI95%[57;100]) for others. Multivariate analysis demonstrated correlation of metastatic status and presence of vascular invasion with survival. The histopathological description of peritumoral vascular invasion in surgical specimens of osteosarcoma after neoadjuvant chemotherapy can be considered a prognostic factor and could indicate modification of the postoperative therapeutic strategy.

Keywords Bone tumor · Vascular invasion · Osteosarcoma · Prognosis

Introduction

High grade osteosarcoma is the most common malignant bone tumor in children and adolescents. Metastatic status at diagnosis, histological response to induction chemotherapy, and quality of surgical resection are the main prognostic factors [1] and determine treatment strategies. Therapies usually comprise neoadjuvant chemotherapy, surgical removal of the primary tumor and metastases, and adjuvant chemotherapy based on the histological response. Histological analysis after induction chemotherapy is essential to stratify risk and select the appropriate postoperative protocol. Pathologists sometimes describe peritumoral vascular invasion on the resected specimen. The prognostic value of these findings has been uncertain, but their strong negative prognostic value for survival has been demonstrated in many tumor types [2]. There is no specific study for osteosarcoma. Subsequent unfavorable evolutions in some patients with osteosarcoma who are deemed "good responders" based on histological analysis suggests that there are still unknown poor prognostic factors.

We retrospectively investigated the impact of the presence of peritumoral vascular invasion after chemotherapy on survival of pediatric patients with osteosarcoma.

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Materials and Methods

We retrospectively reviewed all patients referred to an oncologic orthopedic pediatric center for surgery of a high-grade osteosarcoma between January 1, 2007 and December 31, 2012.

Patients were treated according to the scheme of the national protocol in force during that period, receiving chemotherapy with high dose methotrexate (HDMTX), etoposide, and ifosfamide for 13 weeks followed by surgical removal of the tumor and adjuvant chemotherapy based on the histological response. Good responders continue with the same chemotherapy protocol postperatively, and bad responders received a combination of methotrexate, adriamycin, and cisplatinum [3].

Macroscopic examination was performed by following the national protocol recommendations, cutting entire bone resection specimen in 5 mm thick slices along the long axis with adjacent soft tissue in order to keep the relationship between tumor and surgical margins. The chemotherapy response was evaluated on an entire representative slice. In addition, the interface of the tumor with normal tissue was sampled on all the slices, including the inckedsurgical margins.

Vascular invasion is defined as neoplastic emboli adherent to the walls of veins in the normal soft tissue adjacent to the tumor (Fig. 1). The neoplastic emboli can be viable (Fig. 2) or necrotic (Fig. 3). Review of all pathology reports identified patients with vascular invasion on their resection specimen. Histological response after induction chemotherapy was evaluated using the Huvos-Rosen grading criteria [4].

We analyzed data using XLSTAT software (Addinsoft, *version 2013.6.03*). The risk of error α, in the analysis was set to 5 % (bilateral hypothesis). Patients characteristics were described and compared between groups (with and without vascular invasion) using a Fisher's exact test for qualitative variables. Clinical outcomes such as Event Free survival (EFS) and overall survival (OS) were studied. OS was defined as the time from diagnosis to the date of death (any causes) or censored at date of last follow-up, EFS as the time from diagnosis to the date of recurrence (local or metastatic) or death (any causes) or censored at the date of last follow-up. Survival distributions were estimated by Kaplan-Meier method [5] and compared between groups using the Log-Rank test [6]. Prognostic effect of vascular invasion on EFS was explored using a proportional hazard Cox model [7]. A multivariate analysis was used to adjust vascular invasion effect on parameters known to be prognostic or slightly unbalanced between groups.

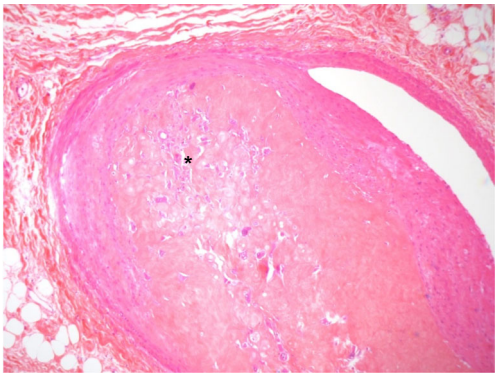

Fig. 2 Magnification × 100, hematoxylin phloxine saffron stain (HPS) * *tumoral cells.*

Results

Between January 1, 2007 and December 31, 2012**,** 51 patients with osteosarcomas were referred to a pediatric center of orthopedic surgery for treatment. The median age was 14 years (range 5 to 20 years); 5 patients aged 18 years or more were nevertheless admitted to the pediatric services. By 31 October 2013, 12 patients died, 11 from tumor progression; one patient had relapsed; one remained under treatment; and 37 were in remission.

In 15 patients, microscopic examination identified one or more foci of vascular invasion located outside the main tumor; five of these had metastases at diagnosis. Histological analysis of Huvos-Rosen grades of resected specimens revealed 2 tumors of Grade I and five of Grade II. Half (8 of 15 patients) were good responders, 6 patients with Grade III tumors and two with Grade IV.

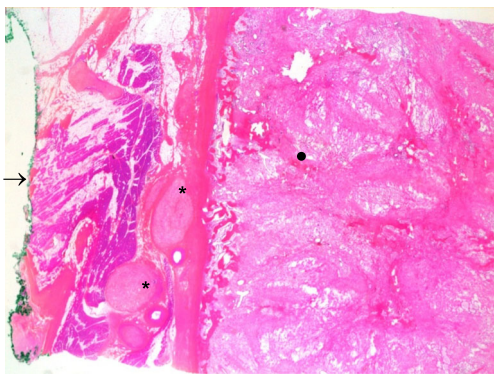

Fig. 1 Low magnification, hematoxylin phloxine saffron stain (HPS) * *tumor emboli,* • *regressive tumor,* → *surgical limit*

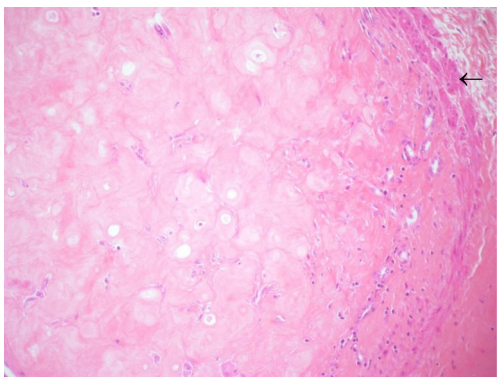

Fig. 3 Magnification × 200, regressive tumor emboli without viable tumor cells ← *venous wall*

No statistical difference was shown between the 2 groups, with ($N = 15$) and without ($N = 36$) vascular invasion, in terms of age, sex, extension at diagnosis, localization, and Huvos-Rosen grade (Table 1), even if some parameters are a bit unbalanced. Indeed, the vascular invasion group has slightly more boys (60 % versus 42 %), more metastasis diagnostic at inclusion (34 % versus 12 %) and less grade 3–4 Huvos-Rosen grade (54 % versus 73 %).

At the time point, in vascular invasion group, 9 patients relapsed: 8 were metastatic, and 1 relapsed locally, and 7 patients died, including 6 deaths after tumoral progression and 1 after infection.

Comparison of survival curves between the 2 groups showed statistically significant difference. Event-free survival at 2 years was 48 % in the group with vascular invasion, CI95% = [21;75] compared to 78 % in the group whitout vascular invasion CI95% = [64;93] (log-rank test: $p = 0.006$) (Fig. 4). Overall survival was 79 % in the group with vascular invasion CI95% = [57;100] and 94 % in the without group CI95% = [86;100] (Fig. 5).

Multivariate analysis demonstrated the emergence of metastatic status and presence of vascular invasion as independent prognostic factors for event-free survival, with hazard ratio (HR) showing a recurrence risk multiplied by 6.91 in case of metastatic disease at diagnosis ($P = 0.001$) and a recurrence risk multiplied by 2.85 when vascular invasion is present ($P = 0.032$).

Discussion

Our results demonstrate that the presence of peritumoral vascular invasion in pathologic specimens of pediatric osteosarcomas is associated with a poor prognosis. This is also true for patients presenting a good response to neoadjuvant chemotherapy who are supposed to reach high survival rates.

So far, the prognostic factors identified in high grade osteosarcoma are presence of metastases at diagnosis [1] axial location of the tumor [1, 8, 9] quality of surgical excision, histologic response rate after neoadjuvant chemotherapy [1, 10–16] and age [17–19] The prognostic value of other factors such as sex [1, 16, 18] or elevation of such serum biomarkers such as alkaline phosphatase or lactate dehydrogenase [16, 20–22] are a matter of debate. More recently, studies have attempted to determine the prognostic value of such tumor molecular markers such as p53, p-glycoprotein, or human epidermal growth factor receptor. Their findings have been controversial [23, 24].

The prognostic significance of peritumoral vascular invasion in osteosarcomas has not been reported in the literature. Multiple case reports of extensive tumor thrombi diagnosed by ultrasound or computed tomography are associated with very pejorative prognosis [25] and some studies have found a pejorative prognostic value of vascular invasion in soft tissue sarcomas [26, 27].

Merimsky and colleagues reported vascular invasion as a poor prognostic factor with regard to tumor aggressiveness, response to chemotherapy, and overall survival in 10 cases of sarcoma (bone and soft tissue) [2]. Vascular invasion is also described as a predictor of the risk of local recurrence and metastatic spread in liposarcoma [28] epithelioid sarcoma [29] and leiomyosarcoma [30]. In an adult series, Carneiro's group proposed a prognostic model for soft tissue sarcomas of the extremities and trunk that was based on 4 specific characteristics of the primary tumors: size, vascular invasion, proportion of necrosis, and tumor growth profile (infiltrative or pushing) and found these characteristics to be significantly

Table 1 Baseline characteristics of study patients

	Group with vascular invasion ($N = 15$)	Control group ($N = 36$)	*P*-value
Age at diagnosis (years)	12	13.5	*0.176*
Sex			
Girls	6 (40 %)	21 (58 %)	-
Boys	9 (60 %)	15 (42 %)	*0.356*
Disease extension at diagnosis			
Localized	10 (67 %)	32 (88 %)	-
Metastatic	5 (33 %)	4 (12 %)	*0.102*
Localization			
Limb	14 (93 %)	35 (97 %)	-
Head and trunk	1 (7 %)	1 (3 %)	*0.506*
Histological response (Huvos-Rosen grade)			
Grade I-II	7 (46 %)	9 (27 %)	*0.206*
Grade III-IV	8 (54 %)	24 (73 %)	-
Non applicable, missing data	0	3	-

Applied tests: *Fisher test for qualitative variables.* $\alpha = 0.05$

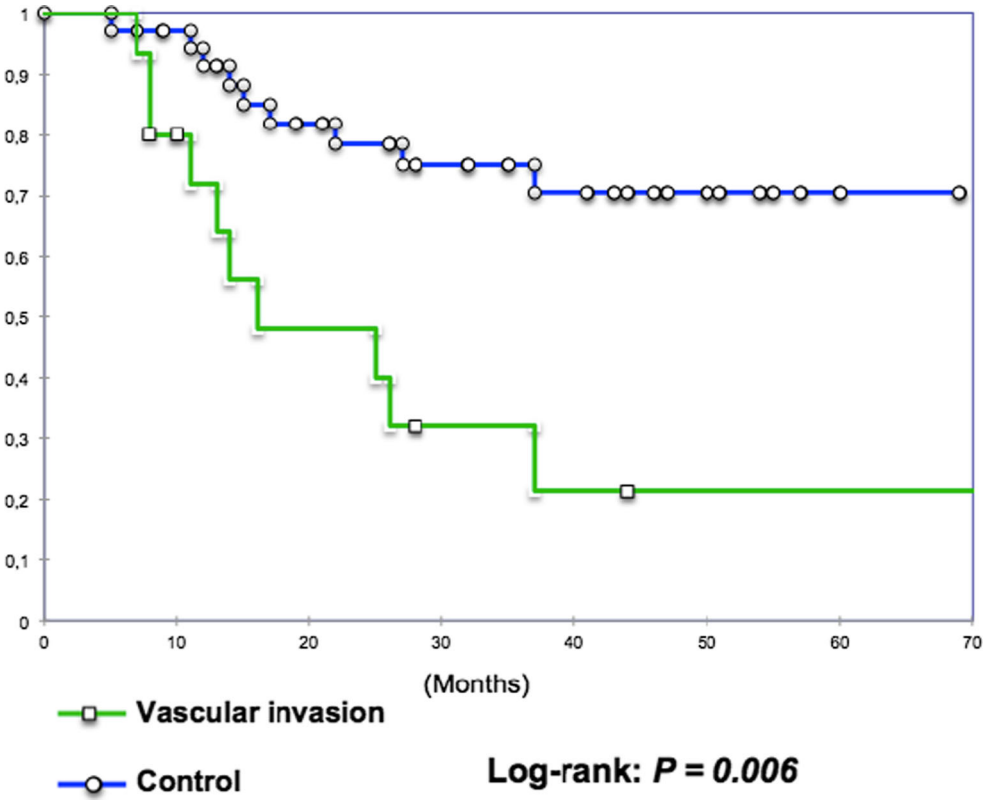

Fig. 4 Event-free survival compared using Kaplan-Meier method

associated with metastasis-free survival [31]. Moreover, those researchers demonstrated that the presence of vascular invasion allows to identify a high and a low risk group, with a powerful prognostic value.

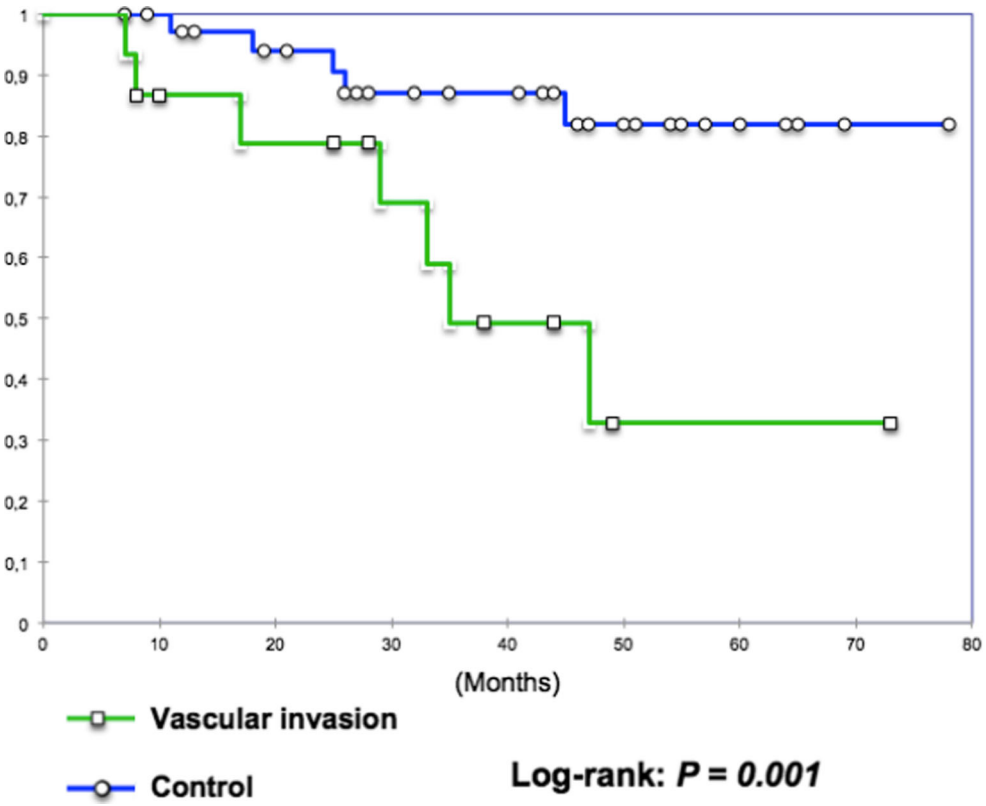

Fig. 5 Overall survival compared using Kaplan-Meier method

Our study is limited by the small sample size and it's retrospective character. Probably because of the small sample, poor histologic response to neoadjuvant chemotherapy, a poor prognostic factor validated in multiple studies, did not appear significant. For the same reason, we also did not include tumor location in the multivariate analysis; tumors from only one patient in each group had an axial location. On the other hand, it would have been interesting to have data on the size of the tumors to test a possible link between size and presence of vascular invasion. However, the absence of a standardized method to account for the size of the tumors due to the heterogeneity of the reports of the imaging examinations utilized for diagnosis prevented such investigation.

Despite significant progress during the last 40 years in therapeutic strategies for patients with osteosarcoma, high risk osteosarcomas (either poor responders or metastatic) still have bad outcomes. Efforts to identify patients with poor prognostic factors either at diagnosis or during treatment remain necessary. The identification by pathologists of peritumoral vascular invasion in osteosarcoma specimens after neoadjuvant chemotherapy can be considered a prognostic factor and could allow modification of the therapeutic strategies following surgery for osteosarcoma. The presence of these vascular invasions should be prospectively investigated.

Compliance with Ethical Standards

Conflict of Interest Statement None declared.

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