

ORIGINAL ARTICLE

Osteosarcoma with cytokeratin expression: a clinicopathological study of six cases with an emphasis on differential diagnosis from metastatic cancer

K Okada, T Hasegawa, R Yokoyama, Y Beppu, E Itoi

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Aims: To clarify the clinicopathological profile of osteosarcomas showing an intensely positive immunoreaction for cytokeratin.

Methods: Clinicopathological and immunohistochemical features were analysed in 131 patients with non-metastatic, conventional osteosarcoma, treated in Akita University and National Cancer Centre in Tokyo between 1972 and 1999.

Results: Six patients (4.5%; mean age, 32 years; four men, two women) had osteosarcomas showing intense cytokeratin expression. Tumours were located on the long bones of the extremities in five patients and the ilium in one. Osteoid formations were found in biopsied specimens in all cases. Three tumours were classified as osteoblastic osteosarcoma, two as fibroblastic, and one as chondroblastic. In three tumours classified as the osteoblastic subtype, epithelioid features were prominent, and four tumours showed pronounced cellular pleomorphism. In contrast to the expression of cytokeratin, epithelial membrane antigen was negative in all cases. Surgery with a wide excisional margin was performed in six patients. Preoperative and postoperative chemotherapy was given to five of the six patients, but the effects of these agents were negligible. Three of the six patients developed lung metastases, whereas the other three patients have remained well with no evidence of local recurrence or distant metastasis.

Conclusions: Osteosarcoma with intense immunoreaction for cytokeratin was rare. The clinicopathological features were similar to those of patients with conventional osteosarcoma, except for a higher age, chemotherapy resistance, histological epithelioid features, and pleomorphism. This study indicates that osteoid formation and negative expression of epithelial membrane antigen are key features in the differentiation from metastatic carcinoma.

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There have been several reports of osteosarcomas with epithelioid features. In 1975, Scranton *et al* first reported an osteosarcoma showing epithelioid features.¹ After the first report, several authors described epithelioid features in osteosarcomas that were histologically indistinguishable from metastatic carcinoma.²⁻⁶ Some osteosarcomas show an intense and diffuse reaction for cytokeratin on histochemical analysis, although this finding is rare.⁷ Therefore, in cases of epithelioid osteosarcoma with intense and diffuse expression of cytokeratin, the differential diagnosis between osteosarcoma and metastatic carcinoma is a difficult diagnostic problem.

We use immunohistochemistry to examine 131 cases of osteosarcomas treated at Akita University and National Cancer Centre, Japan between 1972 and 1999. Six cases showed an intense and diffuse immunohistochemical reaction for cytokeratin. We describe here a clinicopathological profile of osteosarcomas with intense and diffuse expression of cytokeratin, with special attention to the differential diagnosis between these osteosarcomas and metastatic carcinoma.

"In cases of epithelioid osteosarcoma with intense and diffuse expression of cytokeratin, the differential diagnosis between osteosarcoma and metastatic carcinoma is a difficult diagnostic problem"

MATERIALS AND METHODS

We reviewed more than 200 cases of osteosarcoma involving the extremities or axial bones filed at the Akita University Hospital in Akita and the National Cancer Centre in Tokyo, Japan between 1972 and 1999. Microscopic haematoxylin and eosin

slides of the biopsied specimen and slides of the surgical specimen were reviewed. Patients with osteosarcoma in the jawbones or cranium, and patients who underwent surgery only for metastatic foci were excluded from our study. Similarly, the following conditions were excluded from our study: low grade and high grade surface variants of osteosarcoma, dedifferentiated chondrosarcoma with foci of osteosarcoma, patients with primary metastasis at presentation, and secondary osteosarcomas after radiotherapy. Consequently, 131 patients with non-metastatic, high grade osteosarcoma were investigated in our current study. In all those patients who died, death was the result of tumour progression. All protocols were accepted by the institutional review boards of our institutions. Informed consent was obtained from all patients or their legal guardians, depending on the patient's age.

In all 131 cases, we obtained formalin fixed, paraffin wax embedded tissues before treatment, including chemotherapy. Representative blocks were cut to a thickness of 4 µm, and were examined by the labelled streptavidin-biotin method with the appropriate use of negative controls throughout, after pretreatment with heat induced epitope unmasking in a 10mM citrate buffer, pH 6.0, in an autoclave at 121°C for 10 minutes. The primary antibodies were applied as follows: cytokeratin (clone AE1/3; 1/100 dilution; Dako, Glostrup, Denmark) and epithelial membrane antigen (EMA; clone E29; 1/100 dilution; Dako). When evaluating the immunostaining for AE1/3 and EMA, only homogeneous staining, along with staining of the cell membranes or cytoplasm, that was of the same intensity and pattern as that in the adjacent normal epithelium was assessed as 2+ positive, and heterogeneous or focal staining was assessed as 1+.



Figure 1 Case 4. (A) Anteroposterior radiograph and (B) lateral radiograph showing a destructive lesion in the metaphysis of the femur with a small amount of fluffy mineralisation and thick periosteal reactions.

In the cases showing 2+ reactivity for cytokeratin, clinical details and follow up information were obtained by reviewing all medical charts, and a histological subtype was determined in each case by biopsy review. Then, the intensity of pleomorphism and the epithelioid features of the tumour cells were evaluated. In surgical specimens after preoperative chemotherapy, the effect of chemotherapy was evaluated by the degree of tumour necrosis as follows: good, $\geq 95\%$; moderate, 90–95%; and poor, $< 90\%$.

RESULTS

Immunohistochemical findings

Six of the 131 tumours (4.5%) showed 2+ immunoreactivity for cytokeratin, and 12 tumours (9%) showed 1+ immunoreactivity. Epithelioid features were extensive in three of the six tumours with 2+ immunoreactivity for cytokeratin. Immunoreactivity for cytokeratin was intensely positive in the epithelioid areas. These three tumours were classified as the osteoblastic subtype according to their predominant matrix production. In two other fibroblastic subtypes and the remaining one chondroblastic subtype, cytokeratin was intensely positive in pleomorphic spindle cells and chondrocytes. EMA was negative in these six cases.

Clinicopathological findings

The six patients ranged in age from 13–66 years (mean, 32). Four patients were male and two were female. Tumours were located on the long tubular bones of the extremities in five patients (upper extremities, two; lower extremities, three), and the ilium in one. The tumours ranged from 4 to 11 cm at the maximal diameter (mean, 6.5). The presenting symptom was pain in all six patients, and the duration before they visited our institutes ranged from one to 11 months (mean, seven). The serum alkaline phosphatase concentration was high in two of the six patients, and within normal limits in the other four patients. Radiological findings were not specific. In four patients whose radiographs were available for review, all four tumours showed osteolytic and destructive lesions in the metaphyses, with various amounts of mineralisation (figs 1 and 2).

Histologically, all six tumours were conventional, high grade osteosarcoma. Definite osteoid formation was identified in the biopsied specimen in all cases. Three of the six tumours were classified as osteoblastic osteosarcoma, two as fibroblastic osteosarcoma, and one as chondroblastic osteosarcoma, depending on the predominant matrix production. In the three tumours classified as the osteoblastic subtype, epithelioid cytological features of the tumour cells—eccentrically

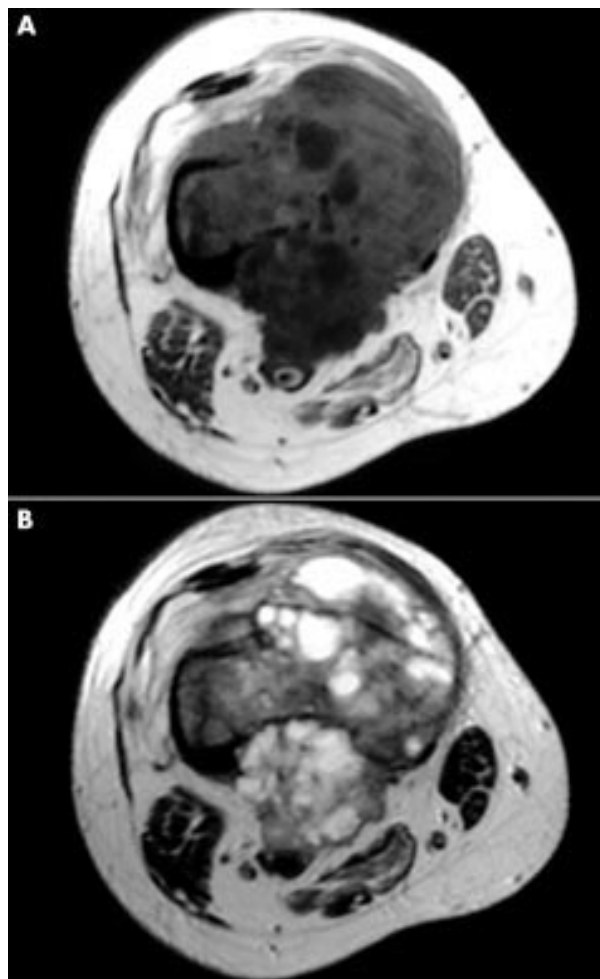


Figure 2 Case 4. Magnetic resonance images of the femoral lesion showing isosignal intensity on (A) T1 weighted images (TR, 519; TE, 10.1) and isosignal to high signal intensity on (B) T2 weighted images (TR, 4000; TE, 10.1).

located vesicular nuclei with prominent nucleoli and abundant pale eosinophilic cytoplasm—were prominent (fig 3). Four of the six tumours (two fibroblastic and two osteoblastic subtypes) showed pronounced pleomorphism (fig 4), and in the other two tumours, cellular pleomorphism was evaluated as moderate. Malignant appearing cartilage was prominent in one case that was diagnosed as chondroblastic osteosarcoma, but neoplastic cartilage was not found in the other cases. Table 1 (table 1) summarises the clinicopathological features of the six cases.

The excisional margins of the initial surgical treatment were wide in all six patients. Chemotherapy was given preoperatively and postoperatively in five of the six patients. In these five patients, three were treated with combination chemotherapy, mainly high dose methotrexate (8–12 g/m² for each course with leucovorin rescue), doxorubicin (60–90 mg/m² for each course), and cisplatin (80–120 mg/m² for each course), with/without ifosfamide (6–10 g/m² for each course). Two other patients were treated with combination chemotherapy of vincristin, doxorubicin, and mitomycin C in varying combinations. Systemic chemotherapy was not administered to one patient who was 66 years old. The effects of chemotherapy in the five patients who underwent systemic preoperative chemotherapy were poor. Tumour necrosis rates in the excisional surgical specimen were less than 90% in the five patients. Follow up information on all six patients was available and covered periods ranging from 24 to 319 months

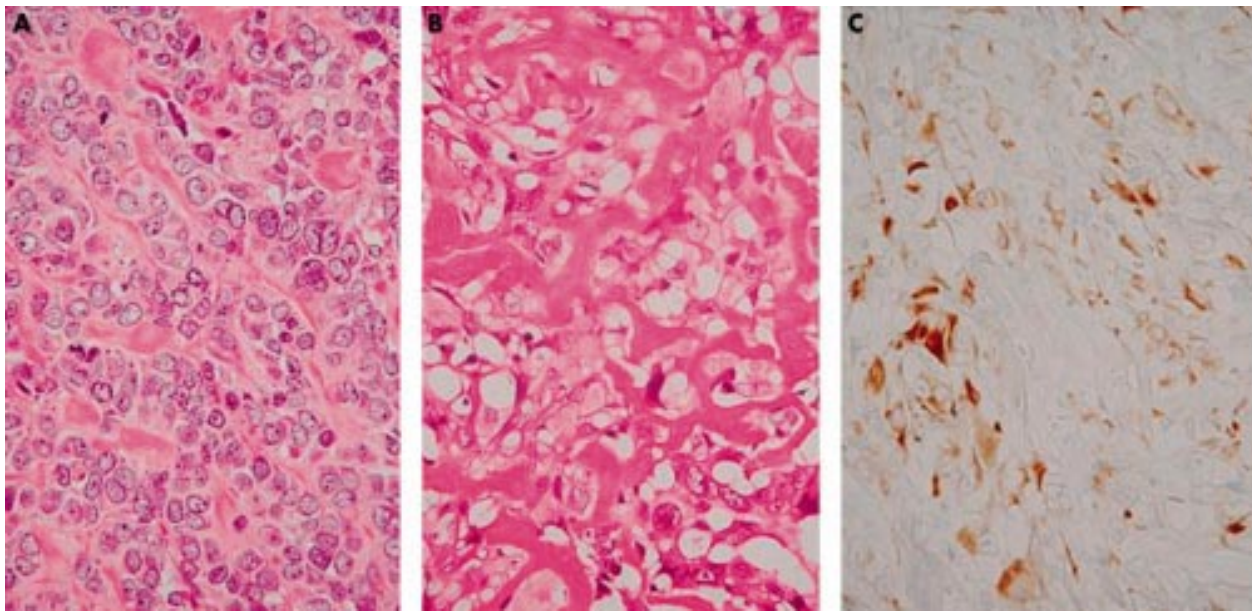


Figure 3 Case 2. (A) Photomicrograph showing the epithelioid cytological features of the tumour cells—eccentrically located vesicular nuclei with prominent nucleoli and abundant pale eosinophilic cytoplasm (haematoxylin and eosin stained; original magnification, $\times 400$). (B) Photomicrograph showing an area of lace-like osteoid formation (haematoxylin and eosin stained; original magnification, $\times 400$). (C) Immunohistochemical stain for cytokeratin showing diffuse and intense positivity of tumour cells (original magnification, $\times 400$).

(mean, 96). None of the patients developed local recurrence. Three (cases 2, 3, and 6) of the six patients developed metastases in the lungs; one died of the expansion of the metastatic foci (case 2) 24 months after surgery, and the other two have remained alive with disease for 38 months, and 53 months, respectively. Three (cases 1, 4, and 5) patients have remained well with no evidence of local recurrence or distant metastasis for 113 months, 30 months, and 319 months, respectively.

DISCUSSION

On immunohistochemical examination of 131 cases of osteosarcoma treated at Akita University Hospital and National Cancer Centre in Japan between 1972 and 1999, six patients

showed a positive immunohistochemical reaction for cytokeratin. The age distribution of these six patients (13–66 years; mean, 32 years) was slightly higher than that of patients with conventional osteosarcomas. Other clinical profiles including sex distribution, site of the lesion, size, and symptoms are similar to those of conventional osteosarcomas.⁶ Similarly, the radiological findings were not specific in most patients, showing an osteolytic and destructive lesion in the metaphyses with varied degrees of mineralisation.

Histologically, three were the osteoblastic subtype, and epithelioid features were also extensive in these three cases. Immunoreactivity for cytokeratin was intensely positive in the epithelioid area. Similarly, cytokeratin expression was seen in osteosarcoma cases with extensive epithelioid cell proliferation

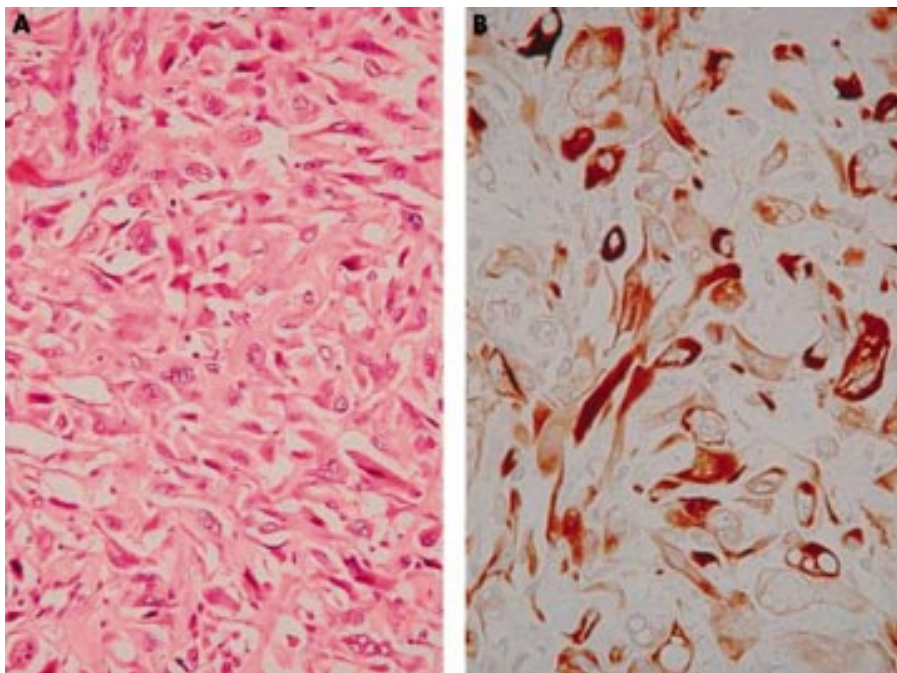


Figure 4 Case 4. (A) Photomicrograph showing proliferation of spindle cells with pronounced pleomorphism and lace-like osteoid formation (haematoxylin and eosin stained; original magnification, $\times 200$). (B) Immunohistochemical stain for cytokeratin showing diffuse and intensely positive pleomorphic spindle shaped tumour cells (original magnification, $\times 400$).

Table 1 Clinicopathological profile of the patients with osteosarcoma with cytokeratin expression

No.	Age/Sex	Site	Symptoms	Size	Histology	Ep	CK	EMA	Pleo	Ch Ef	Surgery	Follow up
1	27 M	Humerus	Pain	8	Os	+	2+	-	2+	Poor	Wide	113 m, NED
2	43 M	Ilium	Pain	12	Os	+	2+	-	+	Poor	Wide	24 m, DOD
3	28 M	Humerus	Pain	4	Fi	-	2+	-	2+	Poor	Wide	38 m, AWD
4	66 F	Femur	Pain	11	Os	+	2+	-	2+		Wide	30 m, NED
5	18 M	Tibia	Pain	6	Fi	-	2+	-	2+	Poor	Wide	319 m, NED
6	13 F	Tibia	Pain	8	Chon	-	2+	-	+	Poor	Wide	53 m, AWD

Size, size (cm); Ep, epithelioid feature; CK, cytokeratin; EMA, epithelial membrane antigen; Pleo, pleomorphism; Ch Ef, chemotherapy effect; OS, osteoblastic; Fi, fibroblastic; Chon, chondroblastic; NED, no evidence of disease; DOD, dead of disease; AWD, alive with disease.

in the literature.²⁻⁵ However, in the other two fibroblastic subtypes and the remaining one chondroblastic subtype an epithelioid element was not seen, and cytokeratin was intensely positive in pleomorphic spindle shaped cells and chondrocytes. Conventional osteosarcoma can show a positive immunoreaction for cytokeratin, even in the fibroblastic or chondroblastic subtypes without an epithelioid appearance.

Intense and diffuse positivity for cytokeratin, mainly seen in epithelial tumours, is rarely seen in osteosarcoma,⁷ although it has also been described in chordomas,⁸ and other soft tissue sarcomas, including synovial sarcomas,⁹ epithelioid sarcomas,¹⁰ malignant rhabdoid tumours,¹¹ and extraskelatal Ewing's sarcomas.¹² In these tumours, chordoma principally occurs in the spine, and is never seen in bones of the extremities. Therefore, it is probably easy to exclude chordoma when a bony lesion of the extremities shows cytokeratin expression. In other soft tissue tumours with immunoreactivity for cytokeratin, location of the lesion is the most important issue in the differential diagnosis.

"Although a poor chemotherapy effect may indicate that an alternative method of treating this type of osteosarcoma is needed, two of the five patients who received chemotherapy and one patient who did not receive chemotherapy remained continuously disease free"

Thus, the differential diagnosis between metastatic carcinoma to the bones and cytokeratin positive osteosarcoma with extensive epithelioid cell proliferation is the most difficult. History of cancer and the following systemic survey for any lesion is mandatory in the differential, although these do not provide specific information. Careful attention should be paid because osteoid or bone formation in cases of melanoma¹³ and metastatic carcinoma⁶ have been reported in the literature. However, the most important histological findings to make a diagnosis of osteosarcoma are still osteoid formation and malignant appearing cartilage.⁶ In our current series, all six cases showed osteoid formation, but only one case contained malignant appearing cartilage. Furthermore, none of the patients had a history of cancer treatment and there was no lesion found on systemic survey. Thus, the diagnosis of cytokeratin positive osteosarcoma was made.

In 2001, we reported several osteoblastic osteosarcomas showed rosette-like features.¹⁴ These osteosarcomas with rosette-like features also frequently had epithelioid histological features, and were frequently positive for EMA, but negative for cytokeratin. In contrast, the current six cases had intense and diffuse immunohistochemical positivity for cytokeratin only. Interestingly, osteosarcoma with rosette-like features had a worse prognosis than that of classic osteoblastic osteosarcoma, suggesting a correlation between EMA expression and poor prognosis. However, cytokeratin expression in osteosarcomas was not correlated with poor prognosis. Among the groups of epithelial markers, EMA and cytokeratin expression in osteosarcoma may show a different correlation with clinical behaviour. In our current study, none of the

Take home messages

- Osteosarcoma with intense immunoreaction for cytokeratin was rare and was seen in only 4.5% of patients with osteosarcoma
- The clinicopathological features were similar to those of patients with conventional osteosarcoma, except for a higher age, chemotherapy resistance, histological epithelioid features, and pleomorphism
- In the differential diagnosis between cytokeratin positive osteosarcoma and metastatic carcinoma, clinical history of cancer, existence of other cancers in the visceral region, osteoid formation on histological slides, and negativity for epithelial membrane antigen provide useful information

patients showed coexpression of EMA and cytokeratin. However, we have previously reported two cases of osteosarcoma in the thoracic and second lumbar vertebrae with immunoreactivity for both cytokeratin and EMA.^{3,4} Thus, it should be borne in mind that negative results for EMA in cytokeratin positive osteosarcomas are not always consistent findings.

Three of the six cases showed good clinical results, but the other three developed lung metastases. Characteristically, the effect of chemotherapy on the five patients who received preoperative chemotherapy was negligible. Although a poor chemotherapy effect may indicate that an alternative method of treating this type of osteosarcoma is needed, two of the five patients who received chemotherapy and one patient who did not receive chemotherapy remained continuously disease free. Because of their rarity, further studies should be undertaken to evaluate the relation between the effect of chemotherapy and clinical outcome of this subtype of osteosarcoma.

In conclusion, in the differential diagnosis between cytokeratin positive osteosarcoma and metastatic carcinoma, clinical history of cancer, existence of other cancers in the visceral region, osteoid formation on histological slides, and negativity for EMA provide useful information.

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ECHO

Detection of *Staphylococcus aureus* by 16S rRNA directed in situ hybridisation in a patient with a brain abscess caused by small colony variants

F Kipp, W Ziebuhr, K Becker, V Krimmer, N Höß, G Peters and C von Eiff



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A 45 year old man was admitted to hospital with a right sided facial paralysis and three month history of seizures. Computed tomography showed a left temporal mass including both intracerebral and extracerebral structures. Ten years earlier the patient had undergone a neurosurgical intervention in the same anatomical region to treat a subarachnoid haemorrhage. In tissue samples and pus obtained during neurosurgery, *Staphylococcus aureus* was detected by a 16S rRNA-directed in situ hybridisation technique. Following long term cultivation, small colony variants (SCV) of methicillin resistant *S aureus* were identified. The patient was treated successfully with a combination of vancomycin and rifampin followed by prolonged treatment with teicoplanin, with no sign of infection on follow up nine months after discharge. This is the first report in which *S aureus* SCV have been identified as causative organisms in a patient with brain abscess and in which in situ hybridisation has been used to detect *S aureus* in a clinical specimen containing SCV. Antimicrobial agents such as rifampin which have intracellular activity should be included in treatment of infections caused by *S aureus* SCV.

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