

Original Research Article

Sequential histological findings and clinical response after carbon ion radiotherapy for unresectable sarcoma



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ABSTRACT

Background and purpose: The efficacy of carbon ion radiotherapy (CIRT) for bone and soft tissue sarcoma has been reported recently. Although histological assessment after CIRT requires skilled interpretation, little information is presently available. In this study, we report sequential histological findings after treatment with CIRT, and evaluate the association between these findings and clinical response.

Material and methods: Seven patients with unresectable sarcoma underwent needle biopsy 12 times at an average of 14.3 months after CIRT and were included in this study.

Results: One patient underwent two biopsies after CIRT for chordoma. Although a few suspected residual chordoma cells were observed at 19 and 30 months after CIRT, the tumor continued to shrink at 75 months. Immunohistochemical analysis of post-CIRT specimens revealed CK AE1/3, EMA, and S100 expression, as in the pre-CIRT specimen. In total, viable tumor cells were found in 9 of 12 specimens; however, only 2 patients showed recurrent masses on radiological examination. The other 5 patients had stable disease.

Conclusions: Viable tumor cells after CIRT did not always cause recurrence. This may be due to observation of dying cells or radiation-induced deformed cells. Histological evaluation after CIRT should be done carefully.

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Introduction

Standard treatment for bone and soft tissue sarcoma includes surgery and chemotherapy. Neoadjuvant chemotherapy is well established, especially in bone sarcoma, for reducing tumor size, which contributes to limb-sparing surgery. As for osteosarcoma, which represents a primary bone malignancy, the 5-year survival rate was 60–70% using a multimodal treatment approach consisting of neoadjuvant systemic chemotherapy followed by local surgical therapy, and then, adjuvant chemotherapy [1]. Neoadjuvant radiotherapy is also utilized, especially for soft tissue sarcoma patients, which contributes to minimized surgical margin and reduction of local recurrence rate [2]. There are several different

methods of neoadjuvant therapy for bone and soft tissue sarcoma to obtain better local tumor control: systemic chemotherapy, radiotherapy, isolated limb perfusion, hyperthermia, or the combination of them [3]. Bone and soft tissue sarcomas may occur in sites throughout the body, and the treatment strategies differ depending on tumor location. For tumors in the extremities, amputation might be an option as curative local management, particularly for tumors that are too large to excise while ensuring preservation of neurovascular bundles. Conventional irradiation is generally less effective for bone and soft tissue malignancies, with the exception of small round-cell sarcomas [4]. In contrast, for tumors located deep in the body, such as in the pelvis or spine, surgical options are severely limited. Obtaining a sufficient surgical margin is critical during sarcoma surgery and wide excision, so resection of normal tissues around the tumor is required to ensure local control. However, due to anatomical difficulties, wide margins are rarely obtained in pelvic and spinal surgeries, resulting

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Table 1
Clinical characteristics of 7 patients who underwent biopsy after CIRT. Abbreviations: Y, years; F, female; C, The National Institute of Radiological Sciences in Chiba; G, Gunma University; CIRT, carbon ion radiotherapy; M, months.

Case	Age (Y)	Gender	Diagnosis	Location	Chemotherapy	CIRT institute	Timing of histologic analysis after CIRT (M)	Histological analysis comments	Viable tumor cells (histology)	Recurrence (radiology) up (M)	Follow-up (M)	Local control period (M)	Clinical outcome
1	76	F	Chordoma	Sacrum		C	19	A few residual chordoma cells	Yes	75	75	75	Alive
2	14	F	Osteosarcoma	Sacrum	Yes	C	6	A few viable cells	Yes	20	20	19	Died of lung metastasis
3	47	F	Osteosarcoma	Ilium	Yes	C	22	No tumor cells	Yes	24	24	24	Alive
4	27	F	Osteosarcoma	Sacrum	Yes	C	3	Fibrosis with viable osteosarcoma cells	Yes	57	57	57	Died of lung metastasis
5	40	F	Extraskeletal myxoid chondrosarcoma	Ilium		C	6	A few viable cells	Yes	36	36	36	Alive
6	76	F	Chordoma	Sacrum		G	27	Same as pre-radiation biopsy	Yes	20	20	20	Alive
7	44	F	Synovial sarcoma	Thoracic spine	Yes	G	3	Recurrent synovial sarcoma	Yes	18	18	3	Alive
Average								46.3	75%	22%	35.7	33.4	

in higher local recurrence rates and lower survival rates [5]. Thus, for tumors located deep in the body for which curative surgical removal is difficult, few treatment options, except for palliative irradiation for local control, are available.

The efficacy of carbon ion radiotherapy (CIRT) for bone and soft tissue sarcoma has been recently reported. CIRT has higher biological efficacy than X-ray irradiation. It causes cell death by inducing DNA double-strand breaks. The dose distribution exhibits a steep fall-off after the Bragg peak, so precise dose localization can be achieved. Carbon ions, which deposit high linear energy transfer (LET) radiation within the Bragg peak, differ from X-rays and protons, which deposit low LET radiation. Owing to the fundamental physical difference, CIRT could present different therapeutic results compared with conventional irradiation or proton beam therapy [6].

Matsunobu et al. reported that CIRT resulted in a local control rate of 62% in patients with unresectable osteosarcoma, and relatively small tumors were associated with a 5-year overall survival rate of 46% and a 5-year local control rate of 88% [7]. Serizawa et al. reported local control rates at 2 and 5 years for unresectable retroperitoneal sarcomas of 77% and 69%, respectively, without any complications in the gastrointestinal tract [8]. These results are encouraging for patients with unresectable sarcoma who do not have any options for long-term prevention of local tumor progression. Due to these good responses, insurance providers in Japan started covering CIRT for bone and soft tissue sarcoma in 2016. However, because CIRT is a newer treatment, several issues need to be resolved in the near future to optimize its use. For example, which follow-up assessments, such as radiological assessments and histological analysis, should be conducted after CIRT remains unclear. Diagnosis of local recurrence in patients with malignant tumors treated by CIRT remains difficult. Options for evaluating post-radiation recurrence include computed tomography (CT), MRI, and fludeoxyglucose-positron emission tomography (FDG-PET). Yanagawa et al. recommended a combination of FDG-PET and enhanced MRI for detection of local recurrence in patients with sarcomas who undergo CIRT; however, none of the parameters obtained during the assessments performed before and 3 months after CIRT accurately predicted the development of local recurrence [9]. Radiological tumor findings usually do not change immediately after CIRT. Moreover, some cases experience tumor enlargement, despite CIRT being sufficiently effective. More experience with post-CIRT follow-up is required and more accurate tools are needed to determine whether tumors have been killed or remain alive. Rock et al. mentioned the utility of magnetic resonance spectroscopy imaging to detect recurrence after irradiation [10]. Interpretation of histological findings after CIRT also remains unclear and controversial. Only a few studies about post-CIRT histology have been reported. Matsumoto et al. performed spondylectomy after CIRT for chordoma of the mobile spine. These investigators reported 2 patients with histological evidence of viable tumor cells in excised specimens [11]. However, we are uncertain whether these viable cells had the same characteristics as the tumor cells observed before CIRT, and it is important to understand how the viable cells behave if they remain in the body. In this study, we report sequential histological findings after treatment with CIRT obtained through repeat biopsy, and evaluate the association between these findings and clinical response.

Material and methods

Between 2008 and 2015, 20 patients were diagnosed with unresectable sarcoma at the Kanazawa University Hospital, and CIRT was selected as local therapy and performed at the National

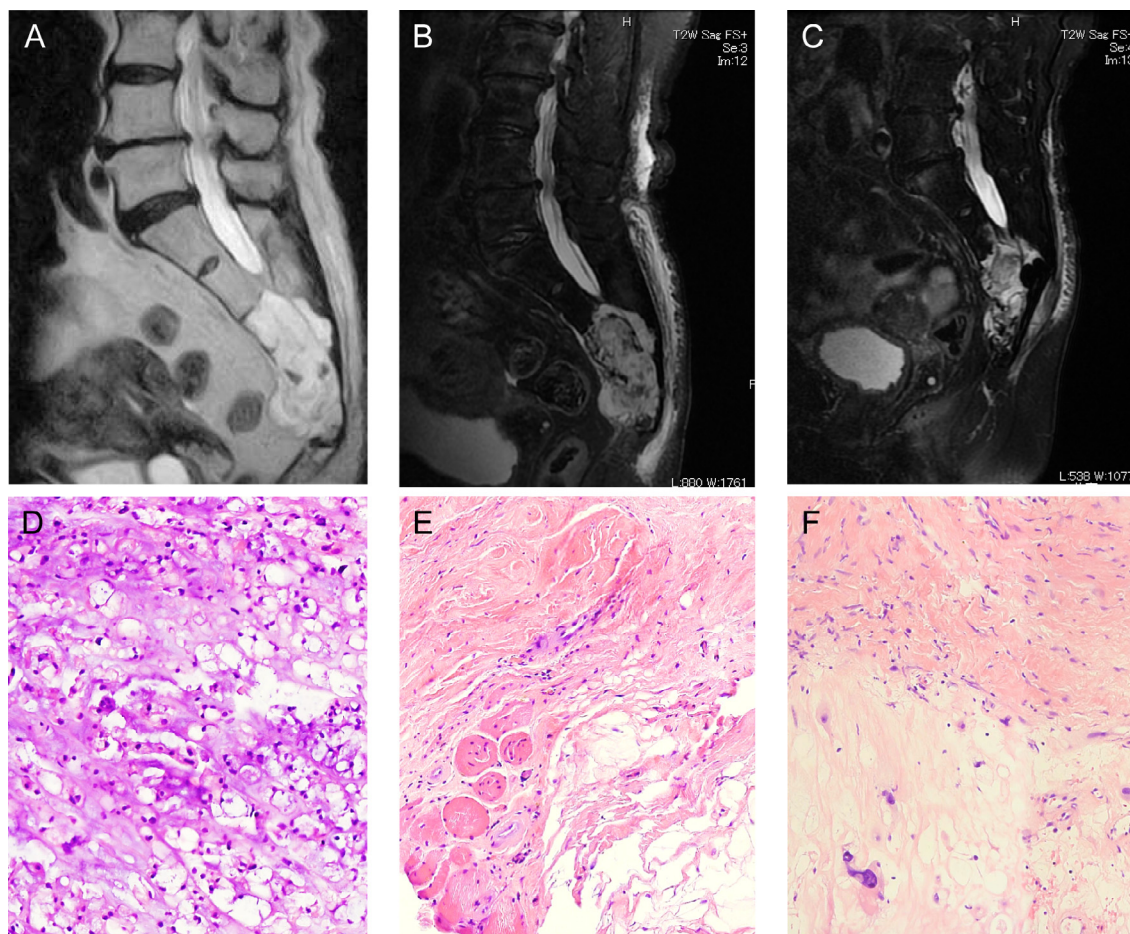


Fig. 1. A 76-year-old female presented with a sacral tumor. Biopsy revealed foamy, vacuolated, physaliferous cells separated by fibrous septa with extensive myxoid stroma. Immunohistochemistry results indicated CK AE1/3, EMA, and S100 positivity. The diagnosis was chordoma, and CIRT was selected to preserve sacral nerves as an alternative to surgical excision. (A) MRI T2-weighted image before CIRT. (B) MRI T2 fat-saturated image 3 months after CIRT. (C) MRI T2 fat-saturated image 75 months after CIRT. (D) Histological section with hematoxylin-eosin staining of needle biopsy specimen before CIRT. (E) Histological section 19 months after CIRT. (F) Histological section 30 months after CIRT. Although a few suspected residual chordoma cells were observed at 19 and 30 months after CIRT, MRI images revealed that the tumor continued to shrink.

Institute of Radiological Sciences in Chiba or Gunma University, Japan. Seven of these patients underwent biopsy after CIRT due to suspected recurrence on radiological findings and were included in this study (Table 1). Diagnoses included 3 osteosarcomas, 2 chordomas, 1 extraskeletal myxoid chondrosarcoma, and 1 synovial sarcoma. Tumor locations included the sacrum ($n = 4$), ilium ($n = 2$), and thoracic spine ($n = 1$). Four patients had previously received standard chemotherapy. Treatment with carbon ion beams was performed once a day at doses ranging from 64.0 to 70.4 Gy for a total of 16 fixed fractions, as described previously [12,13].

The mean duration of follow-up was 35.7 months; 5 patients remain alive, while 2 patients died of lung metastasis. Two patients experienced local recurrences that presented as mildly increasing masses. Needle biopsy was performed 12 times (range, 1–3 times per patient) at an average of 14.3 months (range, 2–30 months) after CIRT. Biopsy specimens were obtained from suspected sites of recurrence, such as those showing gadolinium enhancement on magnetic resonance imaging (MRI). Histological examination was performed by hematoxylin–eosin staining and immunohistochemistry. Histological findings were assessed to determine whether any viable tumor cells could be seen, and immunohistochemistry was performed to confirm similarity to the pre-irradiation conditions in select cases.

Institutional Review Board approval was obtained. Written informed consent was obtained from all patients in this study. Local control was defined as the absence of tumor regrowth based on radiological size.

Results

A total of 7 patients were evaluated. Four patients underwent a single biopsy after CIRT. No tumor cells were found in 1 patient, and tumor cells similar to those observed at pre-CIRT biopsy were found in 3 patients. A mass that appeared to be increasing in size on radiologic findings, determined to be a local recurrence, was observed in 1 patient.

One patient underwent two biopsies after CIRT for chordoma, which was positive for cytokeratin (CK) AE1/3, epithelial membrane antigen (EMA), and S100 on immunohistochemistry prior to CIRT. Although a few suspected residual chordoma cells were observed at 19 and 30 months after CIRT, the tumor continued to shrink at 75 months (most recent follow-up) (Fig. 1). Immunohistochemical analysis of post-CIRT specimens revealed CK AE1/3, EMA, and S100 expression, as in the pre-CIRT specimen.

Two patients underwent three biopsies. One patient had a few viable osteosarcoma cells, but no local recurrence was apparent

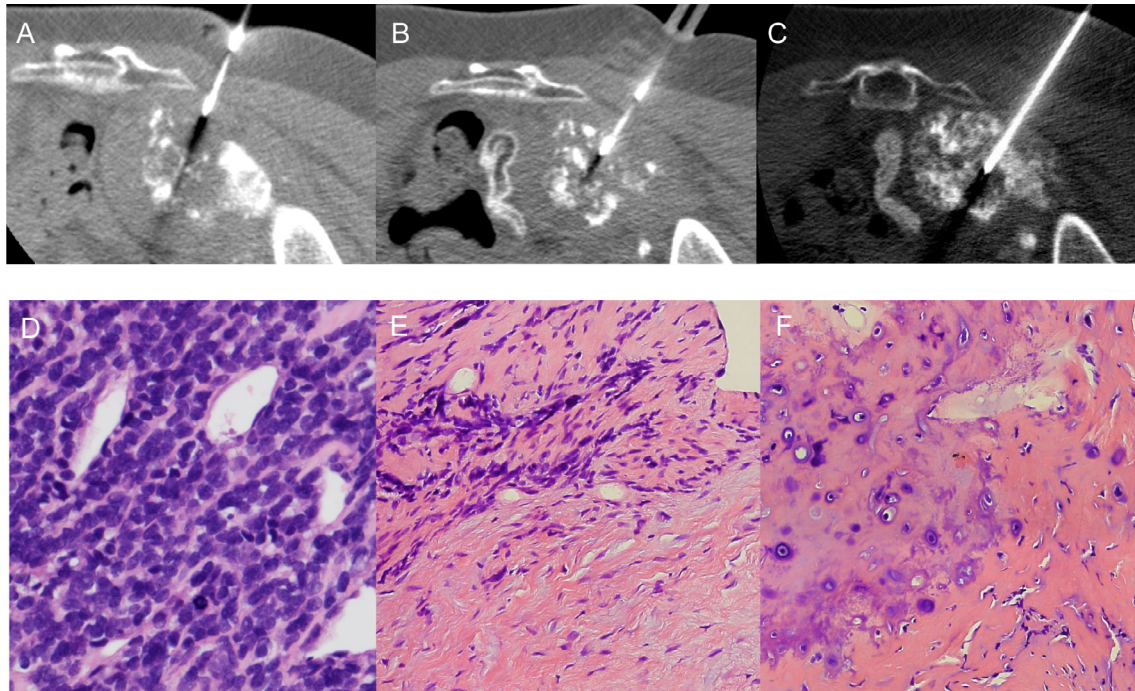


Fig. 2. A 27-year-old female presented with a sacral tumor. Biopsy showed a syncytium of small, round, and uniform malignant cells with abnormal mitoses embedded in a loose, pale pink stroma with an osteoid appearance. A diagnosis of small-cell osteosarcoma was made. Chemotherapy followed by CIRT was performed. Three months after CIRT, fine-needle biopsy (FNB) was performed. Histology revealed fibrosis with viable tumor cells. Twenty months after CIRT, a FNB section shows mainly chondroid tissue with viable cells. CT shows FNB operations for each time point. No signs of tumor regrowth were apparent, but calcification after chemotherapy and CIRT indicated a favorable therapeutic response. CT in FNB operation and histology (A), (D) before chemotherapy; (B), (E) after 3 months; and (C), (F) after 20 months.

on radiologic findings and the patient died of lung metastasis (Fig. 2). The other patient experienced a local recurrence of osteosarcoma following a third biopsy, which showed viable osteosarcoma cells that were not observed on the second biopsy.

In total, viable tumor cells were found in 9 of 12 specimens; however, only 2 patients showed recurrent masses on radiological examination. The other 5 patients had stable disease. No side effects, such as infection or chronic fistula, were observed after biopsy.

Discussion

In 1994, dose-escalation clinical trials using carbon ion beams were initiated at the Heavy Ion Medical Accelerator in Chiba, Japan. A trial for unresectable sarcoma produced favorable local control, except in patients whose lesions were close to the skin and for whom severe skin damage could therefore not be avoided. After improvements in irradiation techniques, skin damage is no longer an issue, and unresectable bone and soft tissue sarcomas are recognized as good candidates for CIRT [14]. A 5-year local control rate of 88% and 5-year survival rate of 86% have been reported for inoperable sacral chordomas, with side effects, such as late sciatic nerve damage, considered acceptable compared to the poor prognosis for patients who do not undergo CIRT [12]. However, optimal follow-up assessments protocols after CIRT have not yet been established. Although histological assessment after CIRT requires skilled interpretation, little information is presently available. Evaluation of the post-CIRT results using histological examinations should be done very cautiously for two reasons. Firstly, the death of tumor cells after CIRT was mainly classified as a mitotic catastrophe; in this case, the histological results could be positive while the tumor is regressing. Secondly, the tumor cells of sarcomas are more radio-resistant than other epithelial tumors; thus, the regression of sarcomas could be slower than other tumors.

Some reports of morphologic alterations of tumor tissues after X-ray irradiation have been published [15,16]. Slmi et al. observed skin metastasis of melanomas in 17 patients given different dose levels of irradiation (40–80 Gy) and found extensive destruction associated with higher doses in excised specimens 10–14 days after irradiation. However, despite the high irradiation dose, viable tumor cells appeared to remain in all cases. Moreover, viable tumor cells were even observed in specimens excised 2 months after irradiation [17].

Histological tumor cell findings after CIRT have revealed decreased cell numbers, nuclear condensation, increased atypical mitotic figures, and cytoplasmic enlargement. Morphologically, radiation-induced cell death is categorized into three classes: (I) apoptosis, (II) autophagic cell death, and (III) necrosis. Oishi et al. observed only autophagic cell death and necrosis, but not apoptosis, in glioma cells after CIRT. These investigators also identified large cells that did not proliferate and were considered to be “senescent cells” or “unclassified dying cells.” However, surviving small cells can regrow and clinically relapse as a tumor recurrence [18]. Jinno-oue et al. also reported that a major population of in vitro glioma cells died after CIRT, and the residual cells could not form a colony [19].

CIRT might have mechanism that would be targeting of cancer stem cells. Cui et al. conducted a study to determine whether carbon ion beams were more effective than X-rays in targeting cancer stem-like cells using human colon cancer cells in vitro and in vivo. A colony assay for cancer stem-like cells showed that carbon ion beams were more biologically effective than X-rays. In xenograft tumors, significantly fewer tumor cells were positive for the putative cancer stem cell markers CD133, ESA, and CD44 following irradiation with 30 Gy carbon ions [20].

In the present cases, viable tumor cells were found in 9 of 12 specimens after CIRT, while only 2 patients showed recurrent masses on radiological examination. The other 5 patients did not

appear to have masses that increased in size. This may be explained by the presence of dying cells or radiation-induced deformed cells observed in biopsy specimens.

Biopsy after CIRT may be associated with complications such as infection or chronic fistula, which were not observed in the present patients but were reported in another study patient. Considering the possibility of biopsy-associated complications and the difficulty in interpretation of histology results, histological evaluation after CIRT should be done carefully; more experience is required to make accurate diagnoses.

Because this is a pilot study, the timing of the biopsy and imaging was not systematically fixed. A prospective study should be conducted with a reasonable schedule for clinical evaluations and data analysis. An imaging study should be conducted every 3 months to demonstrate a recurrence at the original site. A biopsy should be done at least annually or when the size of the tumor increases. A hematoxylin and eosin stain and immunohistochemistry testing should be performed. Tumor-specific antibodies such as CK AE1/3, EMA, and S100 for chordoma need to be stained, and cancer stem cell markers, CD133 and CD44, might be stained to determine the affinity of CIRT for cancer stem cells.

Conflict of interest statement

There is no conflict of interest in this paper.

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