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ORIGINAL ARTICLE

Giant cell tumour of the bone treated with denosumab: How has the blood supply and oncological prognosis of the tumour changed?

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KEYWORDS
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Abstract *Background:* Denosumab is gradually applied to refractory or unresectable giant cell tumour of the bone. Whether denosumab can effectively reduce the blood supply of tumour and bring benefit is worthy of study. The aim of the study is to evaluate the related changes after treatment: blood supply, surgical plan downstaging, surgical difficulty and oncological prognosis.
Methods: A self-case—control study was performed from June 2014 to November 2016, and 18 patients were enrolled. Patients received subcutaneous denosumab 120 mg every 4 weeks preoperatively, with additional doses administered on Days 8 and 15 during the first month of therapy. The initial treatment duration was 12 weeks. After 12 weeks treatment, enhanced CT examination was performed for evaluating whether surgical treatment was practicable. The patients received preoperative denosumab treatment for 5 (median 3, range 3—12) months in average. The microvessel density of tumour samples was calculated for evaluating tumour blood supply. The computed tomography (CT) enhancement rate was compared before and after treatment. The related changes of parameters were recorded as the following: clinical benefits, serious side effects, enhancement rate of CT, surgical plans, intraoperative blood loss, operative time, surgical difficulty, histological changes and local recurrence. The patients were followed up every 3 months postoperatively.

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Results: The average CT enhancement rate of lesions was 2.08 and 1.40 before and after treatment ($p = 0.000$), respectively. The unenhanced CT value was significantly increased after treatment ($p = 0.038$). The CT enhancement rate changed more significantly in pelvic or sacral lesions than that in limb lesions ($p = 0.024$). Sixteen cases underwent final surgery, and surgical plan was downstaged. The histological examination showed tumour cells were significantly reduced or even disappeared after treatment. The microvessel density decreased significantly after treatment. The mean postoperative follow-up was 18.8 (10—31) months, and five patients had local recurrence. The high local recurrence rate (4/6) in sacral tumours may be related to the increased difficulty of curettage.
Conclusion: Denosumab treatment can reduce the blood supply of giant cell tumour. The sacral or pelvic lesions changed more significantly than limb lesions. The surgical plan downstaging can also be achieved. The clear margin after denosumab treatment facilitated tumour resection but, increased difficult in curettage surgery, and high recurrence rate of sacral tumour is being concerned.
The Translational Impact of this Article: Denosumab is a new type of humanized monoclonal antibody which showed some effect in the treatment giant cell tumor of bone. Pre-operative treatment with denosamub can reduce intra-operative blood loss and down-stage surgical plan in suitable cases.

Giant cell tumour of the bone (GCTB) is a primary local aggressive bone tumour with which accounted approximately 20% of primary benign bone tumours and 5% of all primary bone tumours [1]. Histologically, GCTB is composed of sheets of neoplastic mononuclear cells interspersed with uniformly distributed large osteoclast-like multinucleated giant cells [2]. The multinucleated giant cells are similar to osteoclasts in morphology, ultrastructure, histochemistry and function of bone resorption [3].

Denosumab is a new type of humanized receptor activator of nuclear factor-κ B ligand (RANKL) monoclonal antibody. It can combined with RANKL specifically and block the RANKL-receptor activator of nuclear factor-κ B (RANK) pathway. Thus, it can interfere with the survival and differentiation of osteoclasts and inhibit osteoclast-mediated bone destruction [4]. Denosumab was gradually applied in the treatment of refractory bone giant cell tumour in recent years [5—7]. These clinical studies showed that the neoadjuvant treatment with denosumab may make the tumour margin clear and reduce tumour size. Therefore, it can transform unresectable lesions into resectable lesions and provide the opportunity of curing patients. The application of denosumab may bring new effective treatment for cases with excessive morbidity because of the complex anatomical location [8,9].

After treatment with denosumab, the changing of lesion reported include cortical and subchondral bone thickening, marginal sclerosis, new bone formation and pathological fracture healing [10,11]. The treatment of denosumab may downstage surgery so that tumour becomes operatable or it may avoid resections. The surgical plan can be changed to less morbid procedures or joint preservation after denosumab treatment [6,10]. Whether the difficulty in intralesional curettage was increased because of tumour ossification with septae formation is noteworthy. If the ossification lesion cannot be completely removed, whether the local recurrence rate may increase but not decrease should be studied and analysed. But when GCTB is large or located in the pelvis and sacrum, intraoperative blood loss and related risk is large. Therefore, we want to evaluate whether denosumab can reduce the tumour blood supply. The microvessel density (MVD) of the tumour samples were calculated for evaluating tumour blood supply. Enhanced computed tomography (CT) examination can reflect the blood supply through the CT value. In theory, if the blood vessels and blood supply decreased, the enhanced CT value should also decrease [12—14]. The aim of the study is to answer the above questions and evaluate the related changes after treatment as following: blood supply, surgical plan downstaging, surgical difficulty and oncological prognosis.

Patients and methods

Patients

This study was a prospective case self-controlled study of patients with GCTB in our hospital. The ethics committee of our institution had reviewed and approved this study. The inclusive criteria included adults or skeletally mature adolescents (≥12 years of age), histologically confirmed GCTB, active and measurable bone lesions that can be evaluated, Stage 3 tumour (as per Campanacci staging system [2]), unresectable lesion/joint or important structure that cannot be retained. Exclusion criteria included the lesion that underwent arterial embolization and radiotherapy or any other treatment which may affect the blood supply, history of osteonecrosis or osteomyelitis and pregnancy. From June 2014 to November 2016, a total of 18 patients were enrolled (Table 1). Two experienced pathologists in our hospital reviewed the pathologic examinations

Table 1 Profile of patients.

Case	Gender	Age	Primary tumour site	Campanacci staging	Surgical treatment	Preoperative denosumab treatment time (months)	Local recurrence
1	Female	28	Distal radius	3	Resection	6	No
2	Female	24	Sacrum	3	Curettage	9	Yes
3	Female	21	Sacrum	3	Curettage	12	Yes
4	Female	46	Proximal tibia	3	Curettage	3	No
5	Male	26	Distal radius	3	Resection	3	No
6	Male	28	Proximal tibia	3	Curettage	9	No
7	Female	62	Sacrum	3	Curettage	3	Yes
8	Female	29	Ilium	3	Curettage	3	No
9	Male	27	Proximal fibula	3	Curettage	6	No
10	Female	23	Sacrum	3	Curettage	6	Yes
11	Male	48	Sacrum	3	Curettage	3	No
12	Male	25	Proximal tibia	3	Curettage	3	No
13	Male	38	Sacrum	3	Curettage	3	No
14	Male	18	Proximal femur	3	Curettage	3	No
15	Male	26	Distal humerus	3	Curettage	6	No
16	Male	24	Distal ulna	3	Resection	6	Yes
17	Female	29	Sacrum	3	No	3	/
18	Male	41	Ischium	3	No	3	/

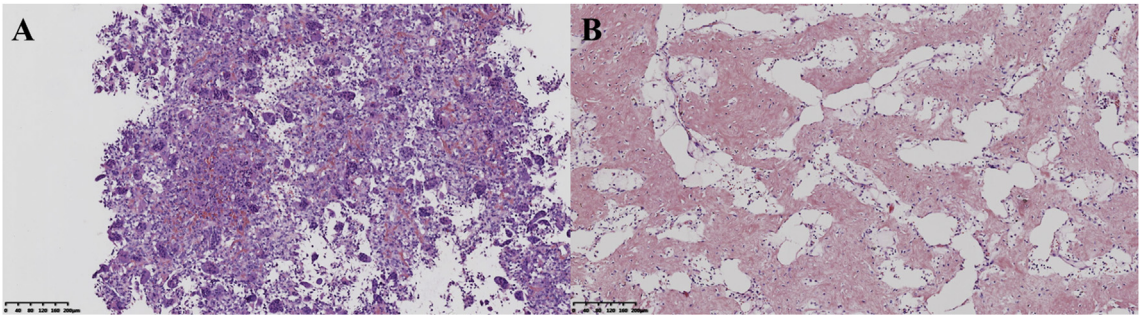

Figure 1 The HE staining of tumour tissue before denosumab treatment showed typical histological features of GCTB; (B) the osteoclast-like giant cells almost disappeared and mononuclears decreased significantly after treatment.
GCTB = giant cell tumour of bone, HE = hematoxylin-eosin after GCTB.

independently. There was no objection to the pathological diagnosis of GCTB (Fig. 1). Eighteen patients received denosumab therapy and underwent enhanced CT examinations. The mean age was 31.3 (18—48) years. There were 9 men and 9 women. Nine lesions were located in pelvis or sacrum (7 sacrum, 1 ilium and 1 ischium), and nine lesions were located in limbs (3 tibia, 2 radius, 1 humerus, 1 femur, 1 ulna and 1 fibula).

Treatment regime and CT evaluation procedures

Enhanced CT examination of tumour was performed before denosumab therapy. Patients received subcutaneous denosumab 120 mg every 4 weeks preoperatively, with additional doses administered on Day 8 and 15 during the first month of therapy only. The initial treatment duration was 12 weeks. After the 12-week treatment, enhanced CT examination was performed again. If the lesion had been resectable/joint or important structure can be retained, denosumab treatment was stopped and the patient received operation. If the above standard was not achieved, the patient continued to receive denosumab treatment. The patients received preoperative denosumab treatment for 5 (median 3, range 3—12) months in average.

Based on the size of bone lesion, four—eight levels were selected for measurement. The CT value of the same lesion areas were measured before and after treatment in the same CT machine. The injection volume and speed of contrast and CT scan times were the same before and after treatment. The main vascular of the same level was also measured (Fig. 2). The CT enhancement rate was calculated as the ratio of enhanced CT value and unenhanced CT value. With the reference of vascular enhancement rate, the lesion was comparable before and after treatment.

Immunohistochemistry

Before and after treatment, the immunohistochemical staining and MVD calculation of the tumour samples were performed. To identify MVD, immunohistochemical staining

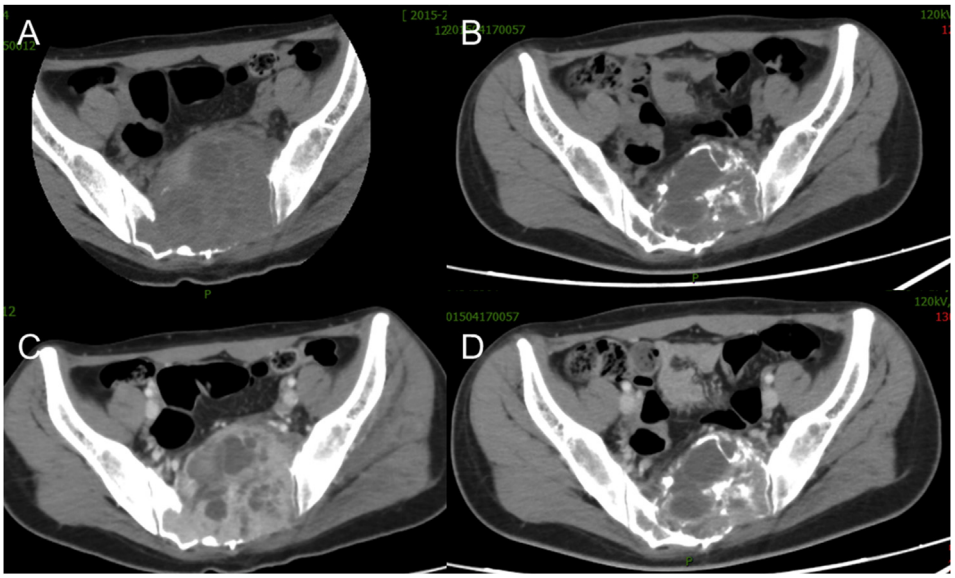

Figure 2 The CT scan showed the significant changing of sacral giant cell tumour before and after treatment in the same lesion. The unenhanced CT (A) and enhanced CT (C) before treatment showed significant enhancement of lesion. The unenhanced CT (B) and enhanced CT (D) after treatment showed the increasing of sclerosis and new bone formation, and also the decreasing of enhancement.
CT = computed tomography.

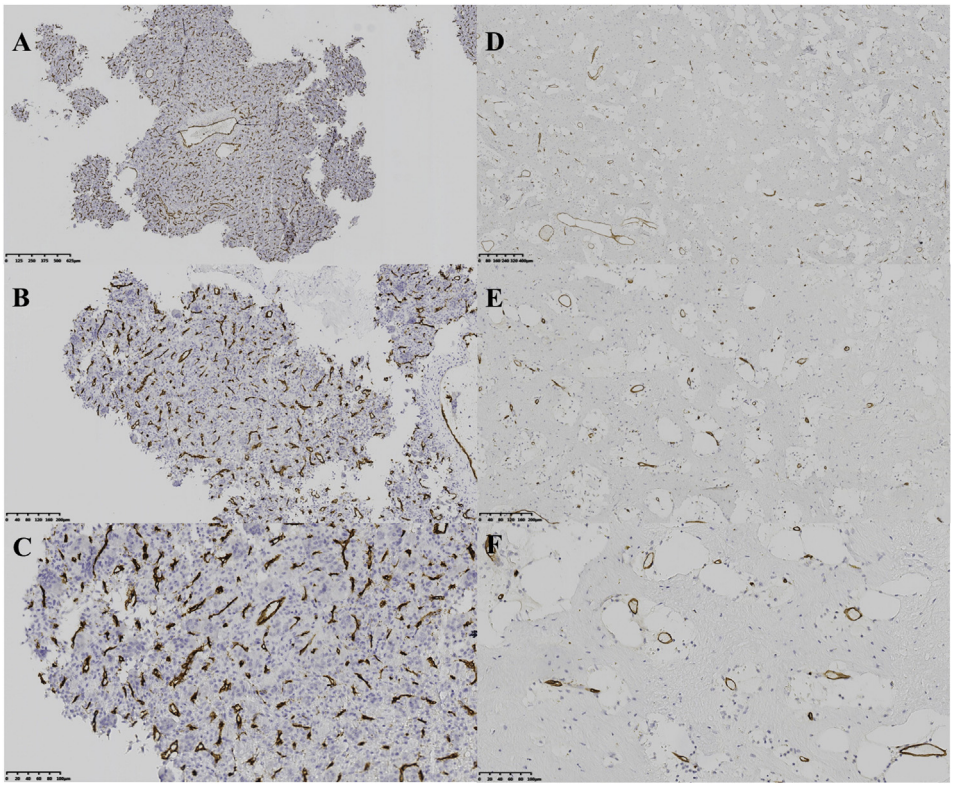

Figure 3 The histopathological changing after treatment. Before treatment (A 40×, B 100× and C 200×): the lesion was full of mononuclears and osteoclast-like giant cells; CD34 antibody showed significant expression of MVD. After treatment (D 40×, E 100× and F 200×): the osteoclast-like giant cells almost disappeared and mononuclears decreased significantly; CD34 antibody showed significantly decreased expression of MVD.
MVD = microvessel density.

was performed using antihuman CD34 antibody. MVD was evaluated by two independent pathologists in a blinded manner, as described previously [15,16]. The slides were scanned at 40× and 100× magnifications (Fig. 3) to identify areas showing conspicuously increased MVD (hot spots). The highest four hot spots were identified for calculation of the stained vessels per sample. MVDs were counted in each hot spot at 200× magnification (0.738 mm^2 field) (Fig. 3). If any

positive staining of vascular endothelial cells was separated by the adjacent vessels, tumour cells or connective tissue were counted as a single vessel. Even if these vessels may be different sections of the adjacent vessels, they should be counted separately. The presence or absence of red blood cells cannot be used to determine blood vessels. At last, the average value of the blood vessel density in three hot spots was calculated. In histology, mononuclear cells are identified as short spindle-shaped and oval cells with single nucleus; osteoclast-like giant cells are identified as multinucleated cells with large size with even hundreds of nuclei (Fig. 1).

Data record and follow-up

The related changes of parameters were recorded before and/or after denosumab treatment as following: clinical benefits, serious side effects, enhancement rate of CT, surgical plans, intraoperative blood loss, operative time; surgical difficulty, histological changes and local recurrence. The patients were followed up every 3 months postoperatively. The clinical examination, X-ray and CT scan of the primary site were performed every 3 months. The bone scan and chest CT scan were performed every 6 months. Postoperative local recurrence was defined as postoperative imaging examinations which showed that the lesion appeared at the site of primary tumour again. The local recurrence time period was defined as the time interval from the operation time to the time at which the lesion appeared again in imaging examinations.

Statistical analysis

The data analysis was performed with SPSS software (version 19.0; IBM Corp., Armonk, NY, USA). Continuous variables were compared by the t-test, and categorical variables were compared by the chi-square test or Fisher's exact test. A p value of ≤ 0.05 was considered statistically significant.

Results

After treatment, all patients had clinical benefits such as pain reduction and increased mobility and function. There were no serious side effects, and all patients tolerated well.

Before treatment, the mean unenhanced CT value and enhanced CT value of the main vascular were 44.0 (38—51) and 136.1 (94—170), respectively. The mean enhancement rate was 3.13 (1.92—4.56). After treatment, the mean unenhanced CT value and enhanced CT value of the main vascular were 43.4 (35—55) and 138.6 (93—158), respectively. The mean enhancement rate was 3.28 (1.90—4.77). There was no significant difference before and after treatment ($p = 0.669$, $t = 0.187$). The CT examinations were comparable before and after treatment.

Before treatment, the mean unenhanced CT value and enhanced CT value of the lesion were 45.7 (33—65) and 92.5 (50—150), respectively. The mean enhancement rate was 2.08 (1.22—4.05). After treatment, the mean unenhanced CT value and enhanced CT value of lesion were 83.9 (32—357) and 105.8 (37—380), respectively. The mean enhancement rate was 1.40 (1.02—2.31). The enhancement rate had significant changing after treatment ($p = 0.000$, $t = 17.664$). The unenhanced CT value had significantly elevated after treatment ($p = 0.038$, $t = 4.650$), and this suggested the increased sclerosis of the lesion and new bone formation (Figs. 4 and 5).

The mean enhancement rate of sacral or pelvic lesion before and after treatment was 2.51 and 1.48, respectively ($p = 0.001$, $F = 18.650$). The mean enhancement rate of limb lesions before and after treatment was 1.66 and 1.25, respectively ($p = 0.042$, $F = 4.909$). The enhancement rate of sacral or pelvic lesions had more significant decrease than that of limb lesions ($p = 0.024$, $F = 6.268$) (Fig. 6).

The average value of MVD was 224.4 (68—324) before treatment and 106.8 (21—197) after treatment ($p = 0.000$, $F = 42.437$). After treatment, the histopathology showed disappearance of osteoclast-like giant cells and significant decrease of mononuclears (Fig. 3). Gross specimens showed that tumour became obviously stiff and firm which were totally distinct from the typical GCTB.

The primary surgical plans before treatment in nine patients with limb tumours were as following: two patients cannot receive operation (unclear tumour range); five patients were planned for joint/prosthesis replacement; two patients were planned for amputation. After denosumab treatment, all nine patients were managed with changed surgical plan: 2 patients with unresectable tumours received tumour resection; 5 patients with planned joint/prosthesis replacement received curettage with joint sparing and 2 patients with planned amputation received limb salvage tumour resection. The sacral/pelvic tumours with large bone destruction and very thin/no bone shell received denosumab treatment for the following reasons: decreasing intraoperative blood loss; marginal sclerosis; bone shell formation to avoid collapse of pelvic ring and severe incapability. After treatment, seven sacral/pelvic tumours received curettage, and all patients were avoided from receiving high morbidity procedures. After denosumab treatment, the tumours showed significant sclerosis and intralesional new bone formation. These changes may increase difficulty in intralesional curettage because of tumour ossification with septae formation, especially in sacral tumours. But at the same, the sclerosis tumour with clear margin can facilitate tumour resection because of consolidated tumour, especially in limb tumours with diffuse destruction and unclear margin (Fig. 7).

The mean intraoperative blood loss and operative time in patients with limb tumours were 300 (30—800) ml and 184 (150—240) minutes, respectively. The mean intraoperative blood loss and operative time in patients with sacral/pelvic tumours were 1943 (600—3000) ml and 256 (180—360) minutes, respectively.

The mean postoperative follow-up time was 18.8 (10—31) months. Five patients had local recurrence, and the mean time was 7.8 (5—12) months postoperatively. The recurrent tumours contained four primary sacral tumours and one recurrent limb tumour with multiple soft tissue recurrence. Four sacral cases received operation again, and no recurrence was found again. The limb recurrence was a patient with multiple unclear soft tissue recurrence after

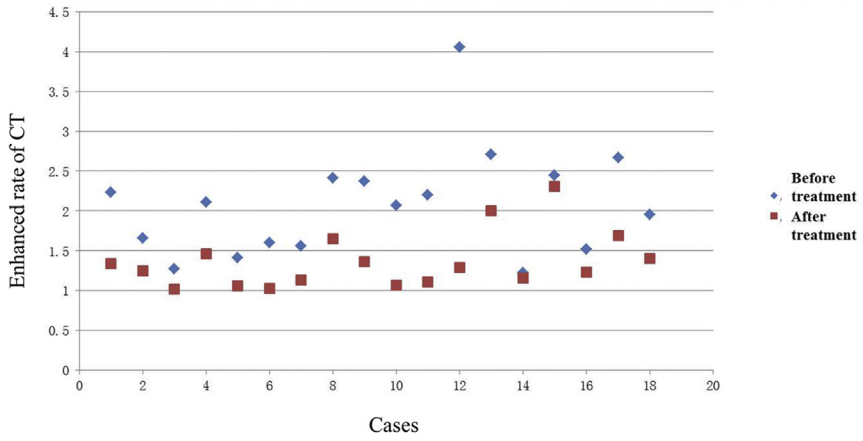

Figure 4 The changing of enhancement rate of lesion before and after treatment (each point corresponds to enhancement rate in each patient).
CT = computed tomography.

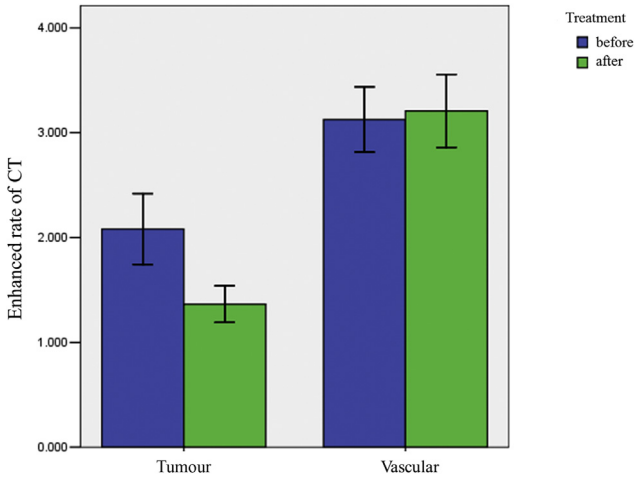

Figure 5 The compartment of changing of enhancement rate of lesion and vascular before and after treatment.
CT = computed tomography.

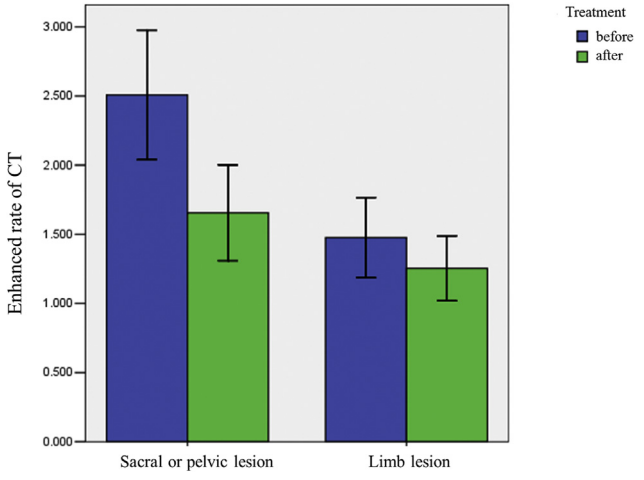

Figure 6 The compartment of enhancement rate of the sacral or pelvic lesion and limb lesion before and after treatment.
CT = computed tomography.

distal ulna resection. After 6 months of denosumab treatment, the clinical symptom was relieved, and nine soft tissue lesions were clearly identified by CT. So, we performed multiple lesions resection (Fig. 7). But multiple extensive soft tissue recurrence was found soon after surgery. The patient received amputation, and the histological result showed no malignant transformation. The multiple lung metastases also showed significant progression after discontinued treatment of denosumab (Fig. 8).

Discussion

With the finding and related research of RANKL and RANK [17,18], the formation and modular mechanism of osteoclast-like multinuclear giant cell in GCTB has become clear. Denosumab specifically combined with RANKL block RANKL-RANK pathway, thereby the bone destruction mediated by osteoclast can be inhibited [4,19]. The aim of the study is to evaluate the related changes after treatment: blood supply, surgical plan downstaging, surgical difficulty and oncological prognosis.

All patients in our study showed clinical benefits such as pain reduction and increased mobility and function. In a Phase 2 clinical trial [10], denosumab was used for 35 cases of recurrent or unresectable treatment of GCTB. After 25 weeks of treatment, 86% of the cases achieved effective response and clinical benefit which included pain relief and functional improvement. The clinical study confirmed the inhibition effect of denosumab on osteoclast formation and activation in GCTB. In 2016, Dubory et al [20] reported eight spinal GCTB with good response to denosumab treatment more than 6 months. The pain and neurological symptoms were relieved.

When the bone destruction and soft tissue mass are large, especially in the sacrum and pelvis, surgical treatment is very difficult. The intraoperative blood loss is usually huge, so the surgical management and the perioperative safety may be seriously interfered. If the blood supply of tumour and blood loss can be reduced, the operation will be more calm and safe. The CT enhancement rate can reflect on blood supply of tumour and even suggest the characteristic and prognosis of some tumours [21,22].

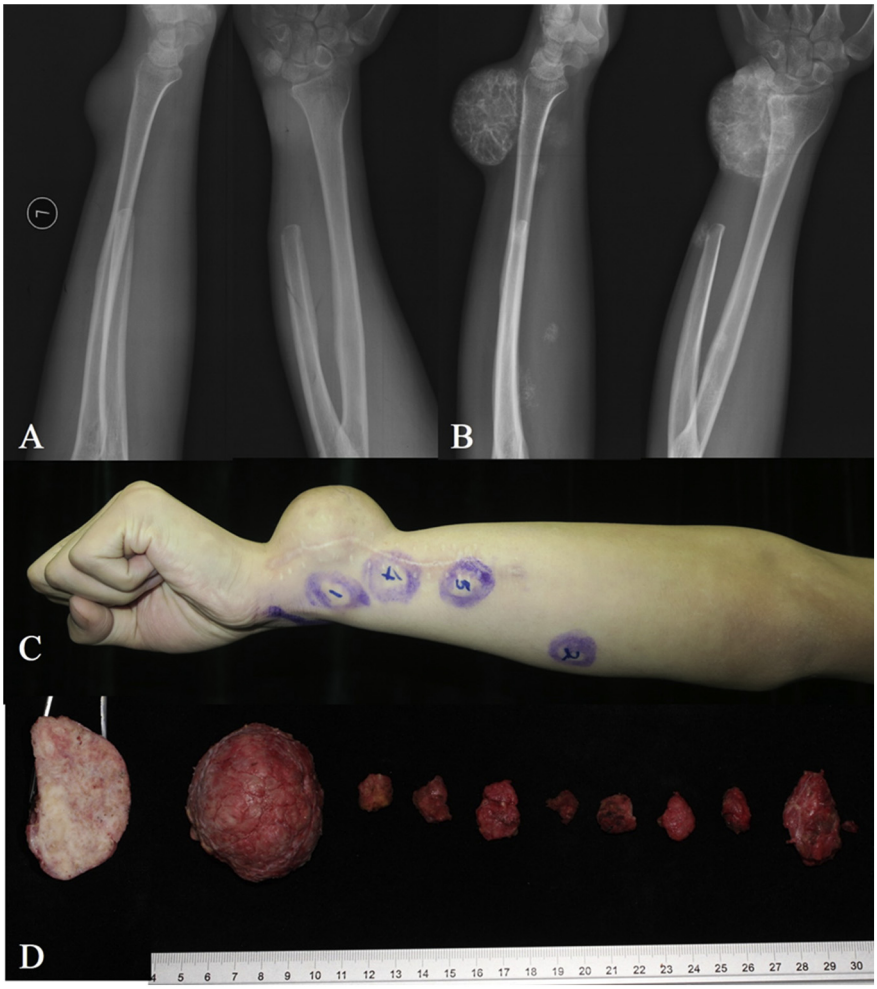

Figure 7 The patient with tumour recurrence of the distal ulna received preoperative denosumab treatment and tumour resection. The recurrence tumours were not clearly shown before treatment (A) and the tumour ranges were clearly showed after treatment (B). The multiple lesions were marked before operation (C), and then all visible lesions were excised (D).

The CT value of the same lesion areas were measured before and after treatment in the same CT machine. The injection volume and speed of contrast and CT scan times were also the same before and after treatment. The vascular enhancement rate before and after treatment was similar, which indicated that the condition of CT examinations was comparable before and after treatment. Therefore, the method of this study is reliable.

Our results showed that the enhancement rate of the tumour was significantly decreased after treatment. This change suggested the decreasing blood supply of tumour. This result was supported by the MVD calculation of tumour. After treatment, the histopathology also showed disappearance of osteoclast-like giant cells and significant decrease of mononuclears. Further analysis showed that the enhancement rate of pelvic and sacral tumours changed more significantly. The mean intraoperative blood loss of sacral/pelvic tumours was 1943 (600—3000) ml. We can obviously feel that the intraoperative bleeding were not as turbulent as previous cases without denosumab treatment. Therefore, preoperative denosumab treatment is more useful in decreasing blood loss in the sacrum/pelvis. Although the CT enhancement rate and MVD calculation of tumour showed decreasing blood supply of tumour, the related mechanism of denosumab-inhibited blood supply is still unclear. We think it may be related with the disappearance of osteoclast-like giant cells and significant decrease of mononuclears. Further study of the mechanism needs to be carried out.

After treatment, the histopathology showed disappearance of osteoclast-like giant cells and significant decrease of mononuclears. Another Phase 2 clinical trial [7] on recurrent or unresectable GCTB also obtained good results of imaging and histology. The osteoclast-like giant cells decreased more than 90% while the tumour stromal cells also decreased. Isabella et al [11] reported significant imaging and histological changes after 6 months of denosumab treatment. The giant cells and expression of RANKL almost completely disappeared; the images after treatment showed that the cortical bone was significantly thickened, and the lesions could be treated by curettage without resection.

A Phase 2 clinical trial [6] which included 222 patients showed that after a median of 19.5 months of treatment,

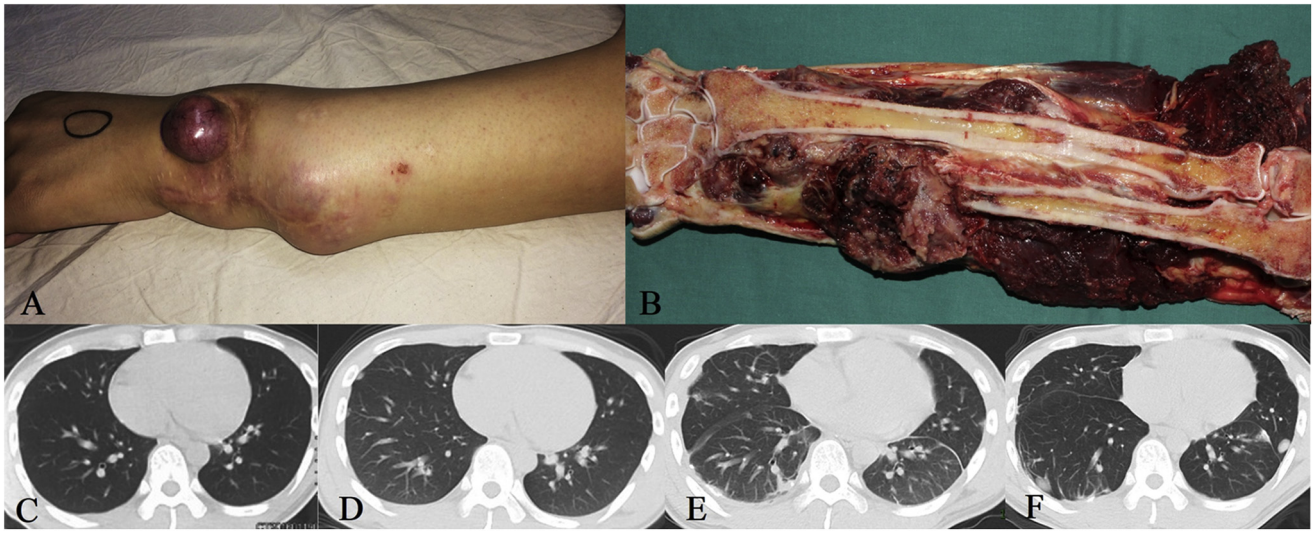

Figure 8 Tumour relapsed and progressed after discontinued treatment of denosumab. Multiple extensive soft tissue recurrence was found after operation (A), and amputation was performed (B). The lung metastases showed significant progression after discontinued treatment of denosumab: chest CT before operation (C), 1 month postoperative (D), 6 months postoperative (E) and 9 months postoperative (F).
CT = computed tomography.

96% of the original plan for joint replacement and 86% of the original plan for joint arthrodesis changed to operation which can retain the joint. Denosumab treatment may also downstage surgical plan in pelvic tumour [9]. After denosumab treatment, the patients in our study were managed with downstaging surgery: two patients with unresectable tumours received tumour resection; five patients with planned joint/prosthesis replacement received curettage with joint-sparing and two patients with planned amputation received limb salvage tumour resection. In limb tumours, surgical treatment was downstaged, and local recurrence rate was low. The clear tumour range facilitated resection, and the thickened cortical bone facilitated curettage. The sacral/pelvic tumours received curettage, and high morbidity procedure was avoided. Although small blood loss and operative time were showed, we found that such curettage actually reduced the tumour range in original surgical plan. This kind of downstaging surgery did not remove sclerosis of the lesion, which may lay a hidden danger for postoperative recurrence.

The significant elevated CT value of tumour suggested the increasing sclerosis of the lesion. These changes may facilitate tumour resection because of clear margin but increase difficulty in intralesional curettage because of tumour ossification with septae formation, especially in sacral tumours. Although the new bone shell formations facilitate tumour curettage, this sclerotic bone which contains stromal cells of GCTB may be the source of tumour recurrence after discontinuation of denosumab. A recent study [23] examined the viability and osteoclastogenic capabilities of neoplastic stromal cells of GCTB. It showed that the stromal cells are quiescent during denosumab treatment, but the neoplastic cells remain proliferative once the microenvironment is free of denosumab.

Sometimes, it was difficult to distinguish tumour ossification form the normal bone. Separating the sclerosis lesion from the nerve or vascular was also a difficult and challenging work. The previous reports showed the recurrence rate of sacral GCTB after intralesional curettage as about 20—40% [24—27], but the local recurrence rate in our study was 66.7% (4/6). The high recurrence rate of sacral tumours after denosumab treatment should be related with these surgical difficulties. A prospective study of GCTB [14] showed the local recurrence rate was 17% with preoperative denosumab treatment. The authors considered that the thickened cortical bone and osseous tumour matrix increased the difficulty of determining tumour range [14]. In sacral tumours, it is impossible to achieve such extended curettage as we performed in limb tumours, so we found more recurrences in sacral tumours and little recurrences in limb tumours. The difficulty of resection was decreased after medication, because tumour border became clearer and we can obtain the originally planned surgical margin.

After analysis of the only one limb recurrence in our study, we found that the recurrence was very rapid and severe after discontinuation. The patient had to receive amputation. Although the uncontrolled local recurrence and rapid progression of lung metastasis were found, the histological result still showed no malignant transformation. As nine cases of malignant transformation of GCT after denosumab therapy have been reported [6,10,28—30], we should be alert to the safety of denosumab. But the new bone formation after denosumab therapy should not be misinterpreted as malignant transformation.

There were some limitations in our study. First, it was a retrospective study. Second, we reported a relatively small sample size series because the incidence of GCTB is relatively low and only cases eligible for entry were selected for inclusion in this study. Third, we tended to include Stage 3 or more aggressive tumours which may lead to selection bias. This bias may be related to the high recurrence rate of sacral tumours. It may be because of selection bias as we tend to use denosumab in higher grade or more aggressive tumours with more difficult intralesional

curettage. The role of intraoperative navigation to increase accuracy can be investigated in future studies.

Our study showed significant effects of reducing blood supply of tumour and intraoperative blood loss. The surgical plan downstaging can also be achieved. The clear margin after denosumab treatment facilitated tumour resection. But the increased difficult in curettage surgery and high recurrence rate of sacral tumour are being concerned. Surgical plan designed based of the tumour range before treatment may be useful in decreasing local recurrence. The best preoperative treatment duration and the concern about withdrawal rebound phenomenon are still need to be solved.

Conflict of interest

None.

List of abbreviations

MVD	micro vessel density
GCTB	giant cell tumour of bone
RANK	receptor activator of nuclear factor-κ B
RANKL	receptor activator of nuclear factor-κ B ligand

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