1. Introduction

Giant cell tumor of bone (GCTB) is characterized by a large number of osteoclastic giant cells uniformly distributed amongst a background of mononuclear pindle-like stromal cells and rounded monocytes [1]. It is one of the most prevalent musculoskeletal tumors in East and South-East Asia as opposed to Western countries (20% vs 5%) [4,5]. The end of long bone is involved in more than 80% of cases and 75% of them occur in proximal tibia and distal femur [6], and is mainly treated with intralesional curettage [7,8]. As an intermediate tumor with a tendency of local invasion, clinical treatment of GCTB is challenged by a high rate of local recurrence (13–65%) [8,9].

Prognostic factors for local recurrence of GCTB need to be identified in terms of surgical treatments, clinical features, imaging findings, and genetic and molecular aspects [10]. The residual tumor after surgery was considered responsible for local recurrence [9]. Features on tumor border are worthy of clinical investigation. Thankfully, GCTB can be probed in many ways depending on the non-invasive imaging device used or the mode by which it operates [11,12]. Magnetic resonance (MR) imaging is especially valuable for the diagnosis of bone tumors due to its heightened sensitivity to soft tissue disease and multiplanar image acquisition [13]. "Paint brush borders" sign (Figs. 1 and 2) is one of common MR features on the border of GCTB, and can likely be found of common MR features on the border of GCTB, and can likely be found on conventional MR images [10]. However its clinical value was un-}

Retrospective investigation of "paint brush borders" sign in association with local recurrence of giant cell tumor of bone after intralesional curettage

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The pathological basis and the immunohistochemistry (IHC) findings in terms of VEGF, MMP-9, RANKL, and RANK were involved. Also we detected the proliferation of neoplastic GCTB cells in high activities of matrix metalloproteinase (MMP) and vascular endothelial growth factor (VEGF) have been linked to biological aggressiveness of GCTB [14,15]. Kumta et al. demonstrated that elevated levels of VEGF and MMP-9 in GCTB correlated well with local recurrence [8]. In bone tumors, co-overexpression of receptor activator of nuclear factor-κ B (RANK) and RANK ligand (RANKL) was identified as a potential discriminating factor for poor prognosis [17], and the expression of RANKL affected the proliferation of neoplastic GCTB cells in another study [18]. Based on these studies, GCTBs with elevated levels of these proteins might be more prone to recur.

The purpose of this study was to investigate the role of preoperative MR features of "paint brush borders" sign in predicting local recurrence. The pathological basis and the immunohistochemistry (IHC) findings in terms of VEGF, MMP-9, RANKL, and RANK were involved. Also we retrospectively analysed the characteristics of this sign based on a prospectively collected database. At least two years followed up was required.

2. Materials and methods

2.1. Patients

This study was approved by our institutional review board and was carried out in accordance with the relevant guidelines and regulations. Written informed consent was obtained from all patients prior to enrolment in the study. All patients had histopathologically confirmed GCTB located in the proximal tibia or distal femur. MRI scans of all patients were obtained and analysed prior to surgery. Fifty-five patients that underwent intralesional curettage, which is the preferred treatment for GCTB and which was performed consistently by a sub-group of orthopedic specialists in our hospital, were registered and followed up.
in this study from January 2005 to July 2015. Moreover, from March 2013 to July 2016, 36 patients with GCTB around the knee were enrolled for investigation of IHC features, including the protein expressions of VEGF, MMP-9, RANKL, and RANK. Twenty-two of these patients overlapped with the former group; en bloc resection was performed in the other 14 patients, two of whom were enrolled for investigation of the pathologic basis of specific preoperative MRI features. Over 2 years of followed-up results were obtained.

2.2. Imaging procedures

MR examinations were performed on a 1.5-T superconducting whole-body imager (Signa, General Electric Medical System) with dedicated extremity coils.

A combination of axial, sagittal and coronal images was obtained using the following sequences: spin-echo T1-weighted (TR range/TE range, 450–600/15–20), fast spin-echo T2-weighted (TR range/TE range, 3500–4000/80–120), fat-suppressed fast spin-echo T2-weighted (TR range/TE range 3500–4000/80–120) was performed on sagittal or coronal plane. Field of view, slice thickness and interslice gap varied depending on diseased region and tumor size. Slice thickness was 5 mm and interslice gap was 0.5 mm. The imaging matrix ranged from 192×256 to 256×256.

2.3. Imaging analysis and classification

‘Paint brush borders’ sign were observed in axial, sagittal and coronal by three senior musculoskeletal radiologists (X. D. [28 years of experience], L.D. [19 years of experience], and H. W. [15 years of experience]). Patients with ‘paint brush borders’ sign were classified into the positive group. However, if a mutual consensus could not be reached, the verdict of the majority was accepted.

2.4. Intralesional procedure

For each case, the intralesional procedure was recorded explicitly. All 56 patients were treated with intralesional curettage conducted by a senior orthopedic surgeon (J. X. [20 years of experience]). The tumor tissue was first removed with a curette after a wide cortical window was created. The remainder of the tumor cavity was eliminated with a high-speed burr. Phenol was applied in the borders of the cavity with cotton-tipped applicators and then neutralized with alcohol in 31 cases; the remaining 25 cases were treated without additional adjuvant. Finally, the tumor cavity was carefully packed with polymethylmethacrylate (PMMA) filling.

2.5. Follow up and recurrence

All patients were reexamined by X-ray or MR annually, regardless of whether or not they were symptomatic. Patients were followed up for at least two years. The extension of the radiolucent zone on radiographs after bone cement filling was a reliable indicator for possible local recurrence. The recurrent tumor represented high signal intensity around PMMA on T2WI. The patients should be reexamined immediately if any relative symptoms (abnormal pain and swelling) occur after surgery.

2.6. Correlation with pathology

Surgical specimens were obtained from two patients treated with en bloc resection. According to the acquired images, formalin-fixed and paraffin-embedded specimens were sectioned and hematoxylin-eosin stained.

2.7. Immunohistochemistry

IHC was performed on formalin-fixed, paraffin-embedded tumor tissue samples, cut into 3-μm-thick sections representative of the tumor. The sections were deparaffinized, rehydrated, and treated using the automated immunostainer BenchMark XT (Ventana Medical Systems SA, Strasbourg, France), following antigen retrieval with citrate buffer (pH 6.0) for 25 min. Subsequently, the sections were incubated with the relevant antibodies for 1 h at 37 °C, followed by addition of the polymeric detection system ultraView Universal DAB Detection Kit reagents (Ventana Medical Systems). Finally, the sections were automatically counter-stained with Gill’s modified haematoxylin and cover-slipped with EUKITT® (ORSAtec GmbH, Bobingen, Germany). The tissues were immunostained according to the manufacturer’s instructions with the following four antibodies: MMP9 (ab88998, polyclonal, 1:1000; Abcam, Cambridge, UK), VEGF (Anti-VEGF Receptor 1 Antibody, Y103, 1:250; Abcam), RANK (Receptor Activator for Nuclear Factor-κ B, 64C1385; Abcam), and RANKL (Receptor Activator for Nuclear Factor-κ B Ligand, 12A668; Abcam). Mouse brain tissue was used as a positive tissue control for the anti-VEGF antibody, and RAW 264.7 cells were used as the positive tissue control for the other antibodies. IHC on adjacent sections in the absence of the primary antibody was performed as a negative control. The sections were analysed with an Olympus light
Fig. 2. A 64-year-old patient with recurrence confirmed after one year follow up. (A-D) Coronal T2WI could not clearly show “paint brush borders” sign (black arrows) compared to T1WI. (E) Coronal fat-suppressed T2WI shows cystic change with massive peritumoral edema (white arrows). (F) Sagittal T2WI after treatment shows a homogeneous high signal intensity (white arrows) indicating a local recurrence in the region of the penetrating irregular margins. (G) Recurrent tumor can be confirmed by surgical specimen.
microscope (Olympus Life Science Solutions, Center Valley, PA, USA) using 10× and 40× objectives. An independent experienced pathologist (F.Y. [21 years of experience]), blinded to the clinical details of the individual patients, evaluated the immunoreactivity of VEGF, RANKL, RANK, and MMP9 using a scoring system. The expressions of VEGF, RANKL, RANK, and MMP9 were semi-quantified using a scoring system. The grade of the immunoreactivity was based on the extent of staining: grade 0 represented no immunoreactivity; grade 1, patchy to diffuse weak immunoreactivity; grade 2, patchy to diffuse moderate immunoreactivity; and grade 3, patchy to diffuse strong or intense immunoreactivity, as previously described [19,20]. However, according to the results of the IHC analysis, there were very few cases of grade 0 and grade 3; thus, grade 0–1 cases were classified as the low-grade group, and grade 2–3 cases were regarded as the high-grade group.

2.8. Statistical analysis

The analysis was carried out using SPSS (IBM SPSS Statistics for Windows, Version 22.0, IBM Corporation, Armonk, US). Percentages have been rounded. Categorical variables were presented as percent and absolute number of patients. The distribution of frequencies of dichotomous attributes in groups was described using Chi-square test or Fisher exact test, if appropriate. All P values were two-sided, and a value less than 0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

A total of sixty-nine patients were enrolled for this study (33 men and 36 women; median age, 29 years; range, 17–64 years). Fifty-five of those patients were treated with curettage, and the positive rate for “paint brush borders” sign is 38.18%. The characteristics of all the patients and patients with “paint brush borders” sign are detailed in Table 1, respectively. IHC were preformed in thirty-six patients, of whom young woman (23 years and 24 years) with GCTB treated with additional adjuvant. While twenty-one showed “paint brush borders” sign, of which sixteen cases developed recurrences (76.19%). Of the remaining thirty-four cases without “paint brush borders” sign, only seven of them had recurrence (recurrence rate 20.59%), \( \chi^2 = 16.496, P < 0.05 \), (Table 2).

### Table 1

| Characteristic               | Value              |
|-----------------------------|--------------------|
| Age, n (%)                  | 35(50.72%)         |
| ≤ 29 years                  | 32(46.38%)         |
| > 29 years                  | 32(46.38%)         |
| Sex, n (%)                  | 36(52.17%)         |
| Location, n (%)             | 32(46.38%)         |
| Proximal tibia              | 37(53.62%)         |
| Distal femur                | 8(12.31%)          |
| “Paint brush borders” sign, n (%) | Yes 30(43.48%)     |
|                             | No 39(56.52%)      |

Table 1

Patient characteristics.

3.2. Prognosis of local recurrence

Of the fifty-five patients with GCTB treated with intralesional curettage, twenty-three patients (41.82%) were diagnosed with recurrent GCTB. While twenty-one showed “paint brush borders” sign, of which sixteen cases developed recurrences (76.19%). Of the remaining thirty-four cases without “paint brush borders” sign, only seven of them had recurrence (recurrence rate 20.59%), \( \chi^2 = 16.496, P < 0.05 \), (Table 2).

### Table 2

| Parameter                           | Local recurrence |
|-------------------------------------|------------------|
| Preoperative MR features            |                  |
| (n = 55)                            |                  |
| “Paint brush borders” sign          |                  |
| Yes                                 | 16               |
| No                                  | 7                |

Table 2

Predicting local recurrence and confounding variable analysis.
3.7. Efficiency of different MRI planes and sequences

The number of patients diagnosed with “paint brush borders” signs on axial, sagittal and coronal planes were 4 (19.05%), 20 (95.24%) and 14 (66.67%), respectively. All “paint brush borders” signs could be detected by T1WI, whereas only 4 cases showed “paint brush borders” sign on T2WI.

4. Discussion

GCTB margins are often seen as being relatively well-defined or penetrating irregularly. A preoperative MR feature named “paint brush borders” sign on the border of GCTB was quite common on conventional MR [10]. But it was normally overlooked by radiologist and orthopedic surgeons. To illuminate the clinical value of “paint brush borders” sign, its pathologic basis was explored, and found that a number of osteoclastic giant cells were uniformly distributed among monocytes in protrusions. Gross pathologic sections proved that protrusions were invasions of bone around the lesion (Fig. 4). The “paint brush borders” sign was visually matched to the specific gross pathologic sections. This study is a retrospective review of a prospectively collected database. The highlight of this study is that “paint brush borders” sign is firstly investigated and revealed as a risk factor for local recurrence of GCTB after curettage.

Invasions of bone around GCTB could be interpreted as a possible trait of residual tumors responsible for local recurrence. The penetrating irregular margins might decrease the effect of intralesional procedure. We provided reliable evidence to substantiate the viewpoint that residual tumors may be the major culprit in local recurrence and revealed “paint brush borders” sign as a risk factor for local recurrence of GCTB. For clinic treatment, once penetrating irregular margins were detected by MR, surgeons should raise their awareness on GCTBs and pay more attention to eliminate residual tumors in the region of irregular protrusions thoroughly with more aggressive interventions. In this investigation, the lengths of the protrusions varied from 1.5 to 3.6 mm on MR images, as well as being correlated with gross pathologic sections, meant the protrusions were not too difficult to eliminate with surgeons’ raised awareness and meticulous attention. We emphasize that a much closer assessment of the location of the penetrating irregular margins might encourage surgeons intraoperatively to more aggressively curette, drill and thermally treat these specific areas having the most irregular penetrating margins. The analysis of preoperative MR imaging features would be helpful to guide surgical treatment and reduce local relapse.

The MMPs are a family of enzymatic proteins that are often over-expressed in the tumor microenvironment [21], MMP-9 is regarded as a gelatinase B, type IV collagenase and is highly expressed not only during the early stages of osteoclast development, but also in mature
osteoclasts that resorb bone [22]. The main function of MMP-9 in GCTB is to stimulate bone resorption by giant cells [1]. The pathologic basis of the “paint brush borders” sign on preoperative MRI has been identified as invasion of the bone around the lesions. Therefore, the “paint brush borders” sign might indicate a property of osteolytic destruction. Our investigation demonstrated a correlation between the level of MMP-9 expression and the “paint brush borders” sign; this finding may explain the molecular basis of this preoperative MRI feature of the GCTB border.

Several studies [16,23–25] have revealed that co-expression of VEGF and MMP-9 correlates with angiogenesis and invasiveness of human bone tumors. In the present study, IHC staining of MMP-9 correlated with the staining of VEGF and RANKL. The activation of nuclear factor-κ B signalling can also stimulate MMP production in the tumor and surrounding stromal cells [25]. Therefore, the up-regulation of MMP-9 can be triggered by RANKL, which is expressed by spindle-like stromal cells in GCTB. Thus, despite the lack of correlation with the preoperative MRI features, the expression of RANKL is still worthy of further investigation. Denosumab, a fully human monoclonal antibody that inhibits RANKL, has been demonstrated to be safe and effective in the treatment of GCTB [26].

The impact of demographic factors on local recurrence was also significant to clinical management. Siddiqui et al. reported [5] local recurrence of GCTB in proximal tibia (recurrence rate of 60.00%) to be higher than other locations. In this study, recurrence rate of GCTB in proximal tibia was higher than GCTBs in distal femur (53.57% vs 29.63%). However, the result was drawn from statistical analysis with no convincing investigations to explain. Fortunately, our retrospective analysis hit on the answer. “Paint brush borders” signs, a risk factor of local relapse, were found more in proximal tibia compared to distal femur. It indicates the relationship between proximal tibia and local recurrence indirectly via “paint brush borders” sign. Although further research could offer more convincing data, orthopedic surgeons should stress the importance of GCTBs occur in proximal tibia. Moreover, Siddiqui et al.’s report [5] of 60% recurrence rate in proximal tibia could explain why our overall recurrence rate (41.82%) seems extremely high in this day and age.

In this study, “paint brush borders” signs were mostly detected on the sagittal plane, and only 4 cases (19.05%) showed the sign on axial plane, while coronal MR had a moderate efficiency. On the other hand, all the patients with “paint brush borders” sign could be diagnosed on T1WI. As stark contrast between the high signal intensity of the bone marrow and the low signal intensity of tumor tissue, T1WI images showed a better effect on manifesting irregular protrusions than other sequences (Fig. 2). Collectively, we propose sagittal T1WI is crucial for preoperative MR examination of GCTB. It could provide useful information about tumor border which play a key role to direct surgical approach.

The application of phenol is controversial in reducing local relapse [8]. By correlation analysis, the impact of the application of phenol on the prognosis was excluded. Moreover, the long span of our database probably interfered with the investigation. In this study, the rates of local recurrence in the first and last 5 years were 45.83% and 38.71%, respectively, which were not significantly different.

There are some notable limitations in this study. The first limitation of this study was its retrospective character. Secondly, even though our single-center study was standardized by enrolling GCTB around the knee, the number of patients with GCTB was comparatively small. More patients and multi-institutional studies are necessary for a confirmation of the results with additional statistical power. Thirdly, the inadequate MRI reliability makes it difficult to use it in clinic in current stage.

In conclusion, penetrating irregular margins on preoperative MR correlate well with invasions of the bone around GCTB, and MMP-9 might play a key role in formation of “paint brush borders” sign. To guide surgical treatment, we propose sagittal T1WI is crucial of preoperative MR examination to diagnose the “paint brush borders” sign, coupled with coronal T1WI, MR imaging can provide useful information of tumor border to predict local recurrence of GCTB.

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Table 3

|                        | VEGF | MMP-9 | RANK | RANKL |
|------------------------|------|-------|------|-------|
| "Paint brush borders" sign | 0.31 | 0.03 | 0.708 | 1     |
| Cystic change          | 0.732 | 0.039 | 0.467 | 1     |
| Peritumoural edema     | 1    | 0.471 | 1    |       |
| Adjacent soft tissue invasion | 0.694 | 0.682 | 0.388 | 1     |
| VEGF                   | ×    | ×     | ×     | ×     |
| MMP-9                  | 0.017 | ×     | ×     | ×     |
| RANK                   | 1    | 0.475 | ×     | ×     |
| RANKL                  | 0.516 | 0.041 | 0.481 | ×     |

* Significant statistical difference (p < 0.05).

Table 4

Correlation of "paint brush borders" sign with demographic factors.

| Parameter                        | "Paint brush borders" sign |
|----------------------------------|-----------------------------|
| Location                         | Yes(n = 21) | No(n = 34) |
|                                  | χ² = 5.723, p < 0.05       |
| GCTBs in proximal tibia          | 15                    | 13            |
| GCTBs in distal femur            | 6                     | 21            |
| Gender                           | χ² = 0.029, p > 0.05     |
| Male                             | 10                    | 17            |
| Female                           | 11                    | 17            |
| Age                              | χ² = 0.147, p > 0.05     |
| Young patients(≤ 29 years)       | 10                    | 18            |
| Elderly patients(> 29 years)     | 11                    | 16            |

* Significant statistical difference (p < 0.05).

Conflicts of interest

The authors declare they have not conflicts of interest.

Institutional Review Board approval was obtained. And no animal subjects were involved in this study.

Author Contributions

Guarantor of integrity of entire study: Xiaoyi Ding
Study concepts: Xiaoyi Ding; Yifeng He
Study design: Xiaoyi Ding; Yifeng He
Literature research: Yifeng He; Juiming Lin
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