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*Original Article*

# A study of the therapeutic effect of allogeneic bone graft repair following bone tumor resection

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**Abstract:** Objective: To compare the therapeutic effects of allogeneic bone graft repair and autologous bone graft repair following bone tumor resection. Methods: A sample of 215 bone tumor patients was studied. These patients were randomly divided into an autologous bone graft repair group (control group; n=107) and an allogeneic bone repair group (treatment group: n=108) according to the source of bone tissue. T lymphocyte subsets, serum alexin, and circulating immune complexes were analyzed. X-ray examinations were performed to estimate the healing of the bones. Bone and joint functions were assessed according to Mankin's standard. Results: There was no statistical difference in C3, C4, and CIC expression between the two groups before and after surgery during the time period tested (P>0.05). There was no statistical difference in the healing rate between the two groups (P>0.05), as indicated by the absence of a significant difference between the mean healing times of the control group (7.25±2.84 months) and the observation group (7.84±2.95 months), (t=1.494, P=0.137). There was no statistical difference between the excellent rate in the two groups as per the Mankin standard (P>0.05). KM survival curves, based on a 5-year follow-up of the two groups of patients, indicated that there was no statistical difference between the 5-year survival rates of the two groups (P=0.513). Conclusion: The results for both grafts were largely consistent and no obvious rejection reaction occurred. Therefore, it is felt that the allogenic bone draft may be suitable for clinical promotion.

**Keywords:** Bone graft, bone tumor, bone defect, heterotopic

## Introduction

Clinical manifestations of bone tumors are mainly ischemia, malnutrition, and cachexia, where local pain and tenderness is extremely evident [1]. Bone tumors are mostly found in adolescents and cause much harm to the quality of life [2]. At present, the main method of treatment is surgery. However, patients usually acquire different degrees of bone defects after the removal of bone tumors, and these defects greatly influence the patient's bone and joint functions, and limits the patient's capability to be active as well [3]. The most common repair method for bone defects is bone graft. Autologous bone graft has natural advantages in repairing bone defects, such as greatly reducing the incidence of immune rejection in patients, and is characterized by rapid healing. However, it cannot be used as the main treatment method due to a lack of bone sources and the possibility for serious damage to the donor site. Autologous bone graft fusion is mainly based on the sacrum where the proportion of cancellous bone is large, which can lead to unsatisfactory intervertebral support and secondary injury [4-6].

Artificial prosthesis transplantation is a method that does not require bone healing to achieve joint remodeling, and its range of motion is close to its own joint. However, it carries many disadvantages such as the risk of post-operative infection and loosening of the prosthesis, which may need to be revised through follow-up surgery. This will inevitably lead to a wide range of bone loss and bone defects [7, 8]. Allogeneic bone graft is an ideal technique to repair bone defects, but the preservation of bone sources poses difficulties, which is a limitation of this type of bone graft [9]. With the advancement of medical technology, the emergence of frozen

allogeneic bone grafting offers a solution to the problem of bone source preservation. The preservation of allogeneic bone through ultra-low freezing technology effectively inhibits the growth of bacteria and prevents bone breakdown, while maintaining the physiological activity of bone [10].

In this study, we used frozen allogeneic bone grafts to repair bone defects following bone tumor resection, and explored the possibility that a frozen allogeneic bone graft may provide clinicians with a more effective evidence-based technique for bone defect repair.

## Materials and methods

### *Method*

We sampled 215 patients with bone tumors who were admitted to our hospital from July 2010 to January 2013. All patients were diagnosed as bone tumor patients via pathological biopsy and imaging. The samples were divided into the autologous bone graft repair group (control group) and allogeneic bone repair group (treatment group) according to the bone sources. The 107 patients in the control group consisted of 58 males and 49 females. Their ages ranged from 18 to 72 years where the average age was 47.58±15.65 years. There 108 patients in the treatment group, included 65 males and 43 females. Their ages ranged from 21 to 77 years where the average age was 46.36±14.87 years. The patients and their families were duly informed and signed informed consent forms were obtained from all patients prior to the trial. The study was approved by the Medical Ethics Committee of the hospital.

### *Inclusion and exclusion criteria*

Inclusion criteria: Course of illness lasting for at least half a year, no other inherited diseases, radiotherapy, chemotherapy, autism, memory impairment, or hearing impairment.

Exclusion criteria: The patient was over 18 years old, course of illness was less than half a year, has respiratory system disease, kinship with other patient's, recent blood transfusion therapy, immune function defects, did not cooperate with the examination, did not participate in treatment follow-up, and incomplete clinical information.

### *Allogeneic bone source collection and preparation*

The collection method was in accordance with the standards specified by the AATB (American Association of Tissue Banks) [11]. Allogeneic bone was collected from healthy patients who underwent sudden death, taking care that the bone source was recovered within 8 hours of death. An aseptic operating environment was maintained. The collected bone source was packed in a double sterile bag for transportation at low temperature. Treatment method: The collected bone source surface, including surface tissue, cartilage and, periosteum, was removed, after which, the bone marrow was repeatedly washed with sterile normal saline, and sterilized using γ-rays, at an intensity of 25 kGy. Next it was dried, aseptically, vacuum-packed, and placed in cold storage at -4°C. During storage, the temperature was gradually reduced to -80°C within 12 hours. The selected bone sources were preserved for no more than 6 months. The bones were rewarmed for 30 min at 55°C, before surgery, and soaked in 75% medical alcohol for 30 min. Finally, they were rinsed with saline before use.

### *Surgical methods*

Patients in both groups were treated with brachial plexus or high epidural anesthesia for upper extremity surgery and continuous waist epidural anesthesia for lower extremity surgery. When the tumor site was fully exposed, the tumor and remaining tissue were cleared.

Patients in the observation group were treated with allogeneic bone materials; bone marrow and periosteum were scraped off after which the allogeneic bone was drilled in to the medullary cavity and rinsed. Chemical methods were used to eliminate tumor cells, and the bone graft bed was cleaned. Then the bone was sufficiently compressed. During the operation, air bags were used for hemostasis in order to maintain a clear surgical field, and to expose and free bone tumors. The air bag was loosened to check for nerves damage, and to stop

**Table 1.** Comparison of clinical data of patients

| | Controlgroup (n=107) | Observation group (n=108) | $t/X^2$ | *P* value |
|---|---|---|---|---|
| Sex | | | | |
| Male | 58 (54.21) | 65 (60.18) | | 0.409 |
| Female | 49 (45.79) | 43 (39.82) | | |
| Age (year) | 47.58±15.65 | 46.36±14.87 | 0.586 | 0.559 |
| Hypertension | | | | 0.331 |
| Yes | 39 (34.45) | 47 (43.52) | | |
| No | 68 (63.55) | 61 (56.48) | | |
| Diabetes history | | | | 0.283 |
| Yes | 25 (23.36) | 33 (30.56) | | |
| No | 82 (76.64) | 75 (69.44) | | |
| Smoking history | | | | 0.490 |
| Yes | 60 (56.07) | 66 (61.11) | | |
| No | 47 (43.93) | 42 (38.89) | | |
| Excessive drinking | | | | 0.296 |
| Yes | 15 (14.02) | 10 (9.26) | | |
| No | 92 (85.98) | 98 (90.74) | | |
| Tumor type | | | 3.321 | 0.506 |
| Giant cell tumor of bone | 44 (41.12) | 50 (46.30) | | |
| Osteosarcoma | 30 (28.04) | 35 (32.40) | | |
| Chondrosarcoma | 18 (16.82) | 15 (13.89) | | |
| Metastatic tumor of bone | 10 (9.35) | 6 (5.56) | | |
| Aneurysmal bone cyst | 5 (4.67) | 2 (1.85) | | |

the bleeding in time. The pre-treated allogeneic bone was grafted onto the bone defect for butt joint, ensuring that the bone contact surface and the two ends of the bone defect emerged in a trapezoidal connection. Appropriate internal fixation was selected, in accordance with the criteria of non-weight-bearing activity in the early stage of healing. The soft tissue around the bone defect was restored after fixation. Based on the lesion scraping and lesion status of the patient, allogeneic bone was selected to fill the cavity of the bone defect. Postoperative antibiotics were administered continuously and negative pressure drainage was conducted. If the patient was able to be active after 1 week, passive exercise was allowed.

In the control group, the lesion was completely scraped off, until the incision was fully revealed. A drill was used to open a window on the bone cortex. The bone cortex outside the subperiosteal, subtympanic tumor and the wounded area was resected. The bone window was opened and the tumor cavity exposed. Complete resection was performed, and the medullary cavity was washed repeatedly with normal saline and the wall of the tumor cavity was immersed in 95% ethanol gauze for 15 minutes. Next, normal saline was used to wash the medullary cavity again. Then, depending on the degree of bone defect, corresponding materials (bilateral iliac bones combined with BMP bone graft material) were implanted to fill the bone defect site, tightly combined and pressed, and the gap sutured. Postoperative antibiotics were administered continuously and negative pressure drainage was maintained. If the patient was able to be active after 1 week passive exercise was allowed.

*Observation indicators*

Fasting peripheral venous blood was collected 2 days before surgery and 14 and 28 days after surgery. T lymphocytes, $CD3^+$, $CD4^+$ and $CD8^+$ were detected using flow cytometry, and serum C3, C4, alexin, and circulating immune complex (CIC) were measured via velocity nephelometry. X-ray imaging was used to detect and assess the patient's healing progress. Detection time: Patients were inspected 3, 6, 12 and 24 months after surgery. Evaluation criteria: existence of an external bone callus or bony connection indicated that the patient has healed and the bone joints had clearly resolved. No external callus formation indicated incomplete healing. Healing rate = total healed/total number * 100.

*Evaluation standards*

This study used the evaluation criteria of Mankin et al. [12] as the reference point. The patients were divided into four grades: excellent, good, moderate, and poor. The excellent grade patients showed no postoperative complications or pain, no effect on daily life and normal limb function; Good grade patients

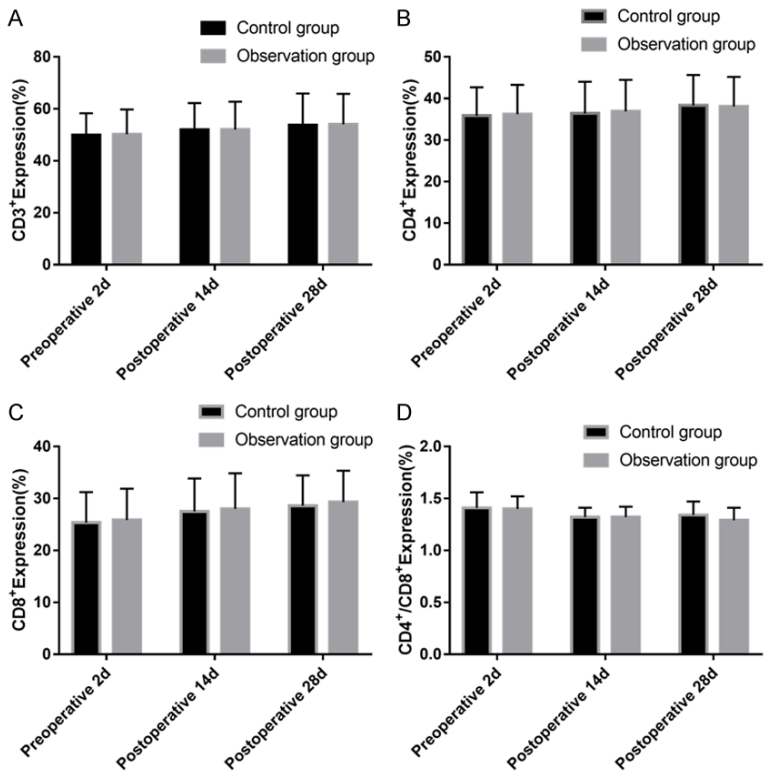


**Figure 1.** A. Comparison of the two groups of patients' preoperative 2 day (d), and postoperative 14 d, and 28 d $CD3^+$ expression. B. Comparison of the two groups of patients' preoperative 2 d and postoperative 14 d, and 28 d $CD4^+$ expression. C. Comparison of the two groups of patients' preoperative 2 d, postoperative 14 d, and 28 d, $CD8^+$ ratio. D. Comparison of the two groups of patients' preoperative 2 d, postoperative 14 d, and 28 d, $CD4^+$/$CD8^+$ ratio.

showed no postoperative complications or pain, but were unable to perform weight-bearing tasks without physical support, and limb function was limited as well. Moderate grade patients showed no recurrence but needed a brace for daily activities and limb function was severely restricted. Poor grade patients showed recurrent infection and needed further amputation. According to the above method, the "excellent rate" was evaluated as "excellent rate" = excellent + good/total number * 100%. In this study, all patients were followed for 5 years and a survival curve was produced.

## Results

*Statistical analysis*

In this study, SPSS20.0 statistical software package was used. We used the GraphPad Prism 7 software package for graphics. Measurement data were expressed as mean ± standard error (Mean ± SD). The t-test was used for analysis and the enumeration data was expressed as percent rate (%). The chi-square test was used for analysis. Kaplan-Meier was used for survival analysis. The statistical significance level was set at $P<0.05$.

*Comparison of clinical data*

We compared clinical data of the two groups and found no statistical difference in age, gender, hypertension, diabetes history, smoking history, alcoholism, and tumor type between the two groups ($P>0.05$) (**Table 1**). This indicated that the two groups were comparable.

*Peripheral blood T lymphocyte expression*

We conducted flow cytometry counts on $CD3^+$, $CD4^+$, $CD8^+$ and CD4/$CD8^+$ of T lymphocytes in peripheral blood, 2 days before surgery, and 14 and 28 days after surgery. There was no significant difference in the $CD3^+$, $CD4^+$, $CD8^+$ and CD4/$CD8^+$ counts of T lymphocytes in peripheral blood before or after surgery during the time tested ($P>0.05$). As well, there was no significant difference in the $CD3^+$, $CD4^+$, $CD8^+$ and CD4/$CD8^+$ expression in peripheral blood T lymphocytes before and after surgery for the time period tested ($P>0.05$) (**Figure 1**).

*Serum C3, C4 alexin and CIC expression in the two groups of patients*

We evaluated the serum C3, C4 alexin and CIC expression 2 days before surgery and 14 and 28 days after surgery in the two groups. There was no significant difference in the C3, C4 alexin, and CIC expression in the serum between the two groups before or after surgery for the time period tested ($P>0.05$) (**Figure 2**).

*Postoperative healing and joint function assessment in the two groups of patients*

This was evaluated by way of X-ray examination and comparison of healing in the two groups. There was no significant difference in the heal-

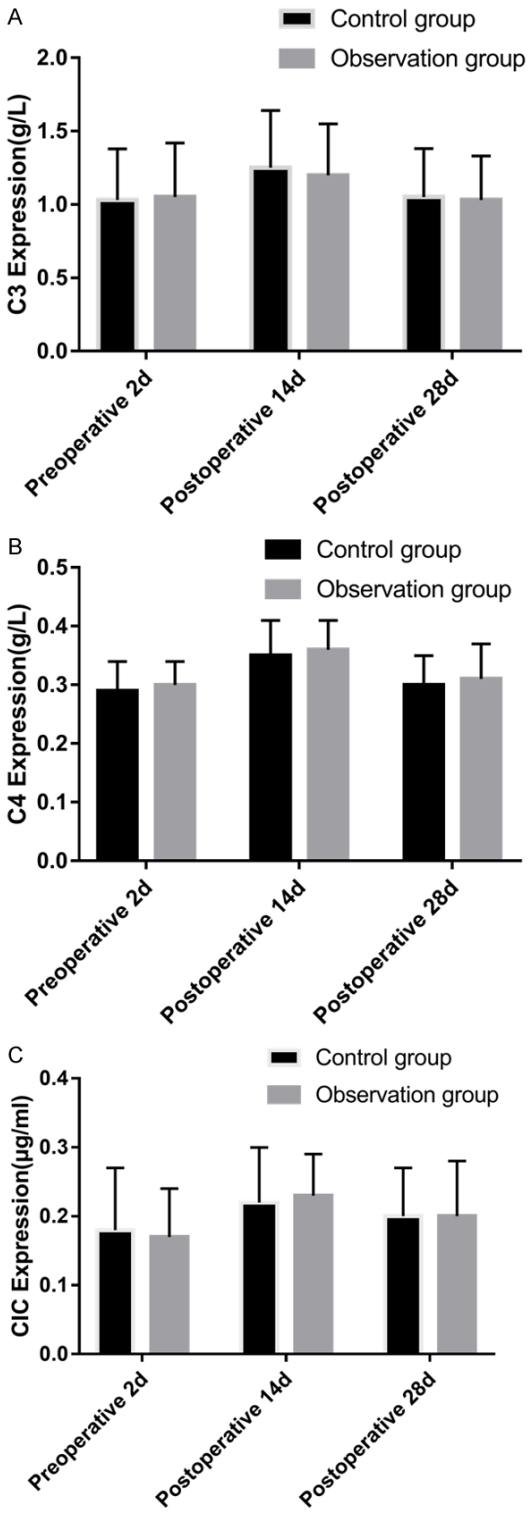


**Figure 2.** A. Comparison of the two groups of patients' preoperative 2 d, postoperative 14 d, and 28 d C3 complement expression. B. Comparison of the two groups of patients' preoperative 2 d, postoperative 14 d, and 28 d C4 complement expression. C. Comparison of the two groups of patients' preoperative 2 d, postoperative 14 d, and 28 d C4 complement expression.

ing rates between the two groups (P>0.05), (**Table 2**). There was no significant difference in the mean healing time between the control group (7.25±2.84 months) and the observation group (7.84±2.95 months) (t=1.494, P=0.137). There was no significant difference in the excellent rate between the two groups, using the evaluation standard (P>0.05) (**Table 3**).

*Patient survival status*

We performed a 5-year follow-up of two groups of patients and produced a K-M survival curve. There was no significant difference in the 5-year survival rate between the two groups (P=0.513) (**Figure 3**).

## Discussion

Bone grafting, by transplanting bone tissue onto a bone defect and strengthening and fixing it, is considered the most effective way to treat bone defects [13]. According to the type of graft, bone grafting can be divided into: artificial bone graft, tumor inactivated bone graft, allogeneic bone graft and autologous bone graft. Artificial bone graft is expensive and cannot meet large-scale bone transplantation needs. Tumor-inactivated bone graft has many limitations including the risk of recurrence after transplantation. Autologous bone graft shows good capability in inducing the healing of bone defects, and therefore provides the most ideal material for bone defect grafting. However, it comes with problems associated with limited bone sources and the risk of secondary injuries [14, 15]. Allogeneic bone graft has the advantage of access to a wide range of bone sources and is able to meet various transplantation requirements. Allogenic bone grafts also have certain bioactivity and are great prospects for clinical application [16]. Therefore, in this study, we compared the healing status of bone defects repaired by autologous bone graft and allogeneic bone graft, following bone tumor resection.

There are several different types of T-lymphocytes. Based on molecular biological characteristics, they can be divided into various sub-populations [17]. $CD3^+$ T cells can be divided into two types, $CD4^+$ and $CD8^+$ T cells, according to differences in the antigens on the surface [18]. $CD4^+$ cells serve as key ce-

**Table 2.** The healing of the two groups of patients

| Group | Complete healing | Unhealed | *P* value |
|---|---|---|---|
| Control group (n=107) | 99 (92.52) | 8 (7.48) | 0.482 |
| Observation group (n=108) | 96 (88.89) | 12 (11.11) | Observation group (n=108) |

**Table 3.** Scores of patients in two groups

| Group | Excellent | Good | Secondary | Difference | *P* value |
|---|---|---|---|---|---|
| Control group (n=107) | 39 (36.45) | 45 (42.06) | 15 (14.02) | 8 (7.48) | 0.211 |
| Observation group (n=108) | 27 (25.00) | 49 (45.37) | 20 (18.52) | 12 (11.11) | |

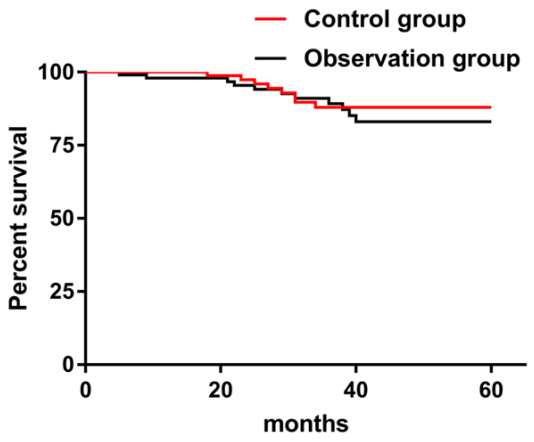


**Figure 3.** There was no difference in survival rate between the two groups after K-M survival curve.

lls for T cell guidance and induction, and can regulate humoral and cellular immunity. For example, they produce antibodies by coordinating the differentiation of B cells. $CD8^+$ cells serve as important cells for inhibiting and killing T cells and, and play an inhibitory role against antibody production and secretion. Studies have shown that overexpression of $CD8^+$ cells is conducive to the continued growth of cells [19-21]. Under normal conditions, the ratio of $CD4^+$ to $CD8^+$ cells is in the range of 1.4 to 2.5. A ratio above 2.5 indicates an active cellular immune function which is prone to autoimmune reactions. A ratio lower than 1.4, indicates an immunosuppressed state. In this study, we found that there was no significant difference between $CD3^+$, $CD4^+$ and $CD8^+$ expression between the two bone graft types before or after surgery during the tested time period. $CD4^+/CD8^+$ ratio in the two groups remained stable at about 1.4. This indicated that postoperative cellular immune response was activated in both groups.

We also detected serum C3, C4 and CIC in both groups. C3 can be specifically lysed with protein segment-receptor during an inflammatory reaction or B cell-mediated immune reaction [22]. C4 play an important regulatory role in complementing activation and precipitating prevention of the immune complex [23]. CIC is a product of the combination of the body's own antibodies and antigens, where differences in their expression play an important role in determining the prognosis of many diseases [24]. Our results showed that there were no significant differences in C3, C4 and CIC before and after surgery for the two different bone grafts during the time period tested. This further confirms the feasibility of both approaches in repairing bone defects after bone tumor resection. We presume the reason that the experimental group did not display immune rejection was due to pretreatment of allogeneic bone sources prior to the experiment. Thorough removal of periosteum, tissue, and cartilage from the femoral surface was performed, and repeated washes were utilized to remove bone marrow, resulting in a loss of protein activity, which reduces immune activity. Significant sterilization was also achieved through irradiation with gamma rays (25 kGy).

Finally, we found no significant differences in the postoperative healing status, average healing time, and joint function assessment, between the two groups of patients. There was no difference in the survival rates of the two groups of patients 5 years after surgery, which may indicate that the effects of treatment and prognosis were basically the same for both graft methods.

However, this study had certain limitations. Firstly, the sample size was insufficient to determine experimental bias. This issue needs to be further assessed. Moreover, the immune mechanism was not researched in depth. Therefore, further studies to elucidate the immune mechanism involved may be needed to supplement

our findings, as also to provide an evidence backed basis for clinicians to decide on effective treatment methods.

In summary, the effect of allogeneic bone grafting and autologous bone grafting as treatments for repairing bone defects was basically the same, and there was no obvious rejection reaction. Therefore, allogeneic bone grafting may be suitable for clinical promotion.

## Disclosure of conflict of interest

None.

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