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Original Article

Radiological and histopathological examination of apparent lytic lesions in allograft long bones—No cause for concern

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ABSTRACT

Objective: Identify the nature of apparent lytic lesions within human allograft specimens from patients with no known malignancy, using radiological and histopathological analysis
Methods: 123 Post-retrieval radiographs from 23 donors were examined. Sixty-seven radiographs were noted to show apparent lytic lesions. The number, size, character and position of the apparent lesions were recorded.

Results: CT scanning of 9 specimens confirmed the lesions to be of air pockets causing artefact. Histopathological analysis showed no malignant or pathological process.

Conclusions: Apparent lesions were not pathological.

Practice implications: Specimens with similar appearances, in donors with no malignancy, can be safely used in donation.

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1. Introduction

Human bone allografts are crucial in orthopaedic reconstructive procedures including tumour surgery, (most commonly Ewing's sarcoma, osteosarcoma and giant cell tumour), failed arthroplasty and trauma.^{1–3} It is difficult and expensive to identify and screen donors, and allograft is expensive to process, prepare and store. As such this is a rare and valuable resource. Retrieval of such specimens from donors involves

assessment against strict inclusion/exclusion criteria, including screening for previous or active malignancy.^{4–7}

There are several bone and tissue banks in Australia, each required to be licensed by the Therapeutic Goods Administration. PlusLife (Perth Bone and Tissue Bank) procure processes and stores donated bone and tissue, distributing an average of 537 allografts to Western Australia, and 236 allografts across the country each year over the last five years.⁸

PlusLife allograft bone is retrieved from two main sources: 'live' femoral head donation during total hip replacement,

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which is mainly used as milled graft and cadaveric long bone and tendon cadaveric retrieval following or associated with organ donation. These larger retrieved specimens are usually used as allograft in tumour reconstruction and arthroplasty procedures. A proportion of retrieved tissue is stored and used for basic science and clinical research purposes, provided research consent was obtained.⁹

Once a potential donor is identified, a number of strict medical and social inclusion/exclusion criteria need to be met in order for the tissues to be retrieved and to ensure the safety of the recipients. These include screening for blood-borne communicable diseases, previous malignant disease and certain medical conditions, to ensure the safety of recipients.

Following retrieval, tissues are stored for several months at -70°C , awaiting review of medical and social history, serological, histological and microbiological testing; and where appropriate post mortem results. Graft may be stored for up to 5 years from the date of retrieval. Long bone allograft specimens are X-rayed to assess for previously unrecognised bony pathology (neoplastic/traumatic/metabolic), which may deem the specimen unsuitable for transplant. These radiographs and the screening results are assessed by a qualified reviewer. Once the clearance review of all donor information and testing results is performed tissues are thawed and processed.

Processing occurs in a clean room (equivalent to an operating theatre) using sterile equipment and consumables. Extraneous material is removed from the tissue, which is then portioned, and the produced allografts are then washed in warm sterile normal saline. Individual grafts are then measured, weighed, photographed, and sampled. At the completion of processing, allograft is packed, refrozen and stored in a quarantine freezer at -70°C pending microbiological clearance. It is then irradiated at Australian Nuclear Science and Technology Organisation (ANSTO) at a minimum target dose of 25 kGy. The grafts are finally released for distribution and implantation following a final set of reviews of all donor history, retrieval and processing information.

It should be noted that the conditions under which the X-rays are performed are somewhat different from the clinical X-ray. The bones under analysis here have been surgically removed from the donor and stored frozen at -70°C for a short period of time, prior to the x-ray being performed.

PlusLife noted an apparent lytic lesion on a post-retrieval radiograph (Fig. 1) within a retrieved specimen from a cadaveric donor with no known previous or active malignancy. This solitary lesion was contained within the proximal metaphyseal region of a retrieved femur. It was an eccentrically placed medullary lytic lesion, approximately one third of the diameter of the diaphysis and had similar appearances of a myelomatous deposit (Fig. 2). Similar findings were identified by the Donor Tissue Bank of Victoria (DTBV),¹⁰ and for quality control purposes it was decided to reassess the archived radiographs of PlusLife donors, which led to this study.

2. Materials and methods

Following Institutional Review Board and PlusLife Scientific Sub-Committee approval, a protocol was proposed where

Fig. 1 – Plain radiograph of a femoral long bone allograft showing apparent lytic lesions within the diaphysis and metaphysis.

previous X-rays of donated material would be re-assessed for the presence of any similar possible lytic lesions, and where possible, CT examination and histological analysis would be performed to further characterise the observed lytic lesions, and identify a cause.

2.1. Radiology

The post-retrieval radiographs of 123 long bone specimens from 23 donors contained within the PlusLife archive were re-examined by a Biomedical Scientist, an Orthopaedic Surgeon and a Radiologist. Assessment of bony lucencies was performed both quantitatively and qualitatively. Quantitative analysis included determination of the number of lucencies. Qualitative analysis included the presence, shape (ovoid or linear) and distribution (epiphyseal, metaphyseal and shaft) of the lucencies.

The donor files pertaining to these specimens were re-examined to confirm that none of the donors had a medical reason for the lesions, and no abnormal results had been identified on screening.

Nine long bone specimens containing apparent lytic lesions from 6 donors, where consent to biomedical research had been obtained, were identified. These tissues had not met the criteria for transplant (identified post retrieval) and were being held for potential use in education/validation/investigation

Fig. 2 – Plain radiograph of femur displaying metastatic myelomatous deposit. Case courtesy of Dr Frank Gaillard, Radiopaedia.org, rID: 12559.

related to the current PlusLife products; the intended function or purpose of current products, e.g. staff training, validation of production and transport procedures and investigation related to developing changes in our processes.

These nine specimens (all transected femora) were selected to undergo computed tomography scanning to further characterise any lucencies, which were then evaluated for number, pattern, location and size. Density of the lesions measured on CT was used to determine if the lucencies corresponded to gas or soft tissue (e.g., metastases). A multi-detector scanner Toshiba Aquilion™ ONE 320 was used to acquire images with a 1.5 mm slice thickness. Images were analysed with DICOM viewer Osirix® v.4.0.

2.2. Histopathology

Three of the specimens had been cleared for histological analysis. The frozen specimens were thawed and sectioned through lesional areas and were localised using measurements obtained from CT. Where multiple lesions were identified on CT, only the largest lesion was targeted. Sections were fixed in 10% neutral buffer formalin for 48 h and subsequently decalcified in EDTA solution for a further 24 h, before routine tissue processing and paraffin embedding. Four micron sections were cut from the paraffin blocks and stained with haematoxylin and eosin (H&E) for microscopic examination.

3. Results

3.1. Radiology

One hundred twenty-three radiographs were analysed: 40 femora, 2 distal femora, 1 proximal femur, 42 tibiae, 2 proximal tibiae, 15 fibulae, 1 distal fibula, 1 proximal fibula, 2 hemipelvises, 16 iliac crest and 1 proximal humerus. All

Table 1 – Number of specimens with lucent lesions and relation with transection.

	Transection	No transection	Total
LUCENT LESIONS	27	40	67
NO LUCENCIES	43	13	56
TOTAL	70	53	123

radiographs were taken before processing, 70 were transected specimens and 53 were complete bones. It is noted in Table 1 that 38.5% of transected specimens had lucencies while 75.5% of the non-transected specimens presented this lesions, showing that transected bones had fewer lesions than non-transected ones. Of the 27 transected specimens with lucencies, only 7 (26%) had lucencies adjacent to the transection.

Among the different types of bones, femora showed more lucencies than the other specimens, with 78% presenting lucencies, followed by 59% of the tibiae (Table 2). A smaller percentage of fibulae and iliac crest were affected, both 12%. One of the two hemi pelvises and the humeri did not show lucencies.

Six specimens had additional post-processing radiographs. Two had lucencies that disappeared post-processing. The other four specimens had no lucencies pre and post-processing.

CT scanning of the 9 femoral specimens confirmed the lucencies corresponded to gas with no other cause identified. In 5 of 9 femorae, the predominant pattern was ovoid, with all specimens presenting both linear and ovoid patterns. While the ovoid pattern was seen in both shafts and proximal bone (metaphysis and epiphysis), the linear pattern was present only in the shafts (Table 3). Ovoid lesions were numerous, with more than 30 in all specimens, with sizes ranging from 1 mm to 20 mm. The majority of ovoid lucencies measured up to 5 mm and were seen in the proximal aspect of the femorae (Table 4). All specimens demonstrated vascular channels filled with gas. It was noted that the ovoid lesions communicated with the linear lucencies. Small enostosis were observed in 5 (55%) specimens as incidental findings.

3.2. Histopathology

Macroscopic examination of cross-sections cut in lesional areas revealed an intact cortex of preserved thickness. The medullary cavity, however, demonstrated soft marrow with small, circumscribed empty defects, ranging from 2 to 5 mm in

Table 2 – Plain radiograph distribution of lucencies per specimen.

	With lucencies	No lucencies	Total
Femur	36	7	43
Tibia	26	18	44
Fibula	2	15	17
Hemipelvis	1	1	2
Iliac crest	2	14	16
Humerus	0	1	1
Total	67	57	123

Table 3 – CT Characteristics of lucent lesions per specimen.

	Pattern			Location		
	Ovoid	Linear	Ovoid + Linear	Shaft	Metaphysis	Shaft + Metaphysis
Femur	19	4	13	31	1	4
Tibia	8	17	1	25	1	0
Fibula	2	0	0	2	0	0
Hemipelvis	1	0	0	–	–	–
Iliac crest	2	0	0	–	–	–
Humerus	0	0	0	–	–	–
TOTAL	32	21	14	58	2	4

maximum extent, suggestive of entrapped gas (Fig. 3–5). There were no solid lesions identified.

Microscopic examination of the medullary cavity show poorly preserved marrow fat and haematopoietic elements, which in all cases, were of a severity which precluded complete histological assessment. However, within the limits of the exam, there were no obvious features to suggest either suppurative or granulomatous inflammation or malignancy.

4. Discussion

Due to their superior biological, materialistic and structural properties, human bone allografts have been used in reconstructive surgery for over 100 years.¹ Over this time their use has been increased markedly with the largest increases in the last decade. Bone allograft has a wide range of uses, from milled cancellous bone for impaction grafting of small bone

defects, to partial long bone allografts for limb salvage orthopaedic oncology reconstructions.

Allograft bone is a valuable resource and its retrieval and processing is complex, time consuming and expensive. Every effort must be made to ensure that the graft is of the highest quality and will not have a deleterious effect on the recipient.

Myeloma and other malignancies causing multiple small lytic bony lesions are relatively rare with only 26 850 new cases in the US in 2015, accounting for 1.6% of all new cancer cases. It is most common in patients over the age of 70.^{11,12} Therefore, it is very unlikely that more than half of our retrieved specimens, included in this study, actually had a pathological process accounting for the lesions. However, it is extremely important to ensure that implanted allograft does not cause the recipient harm, and as such this is an important and relevant study to undertake.

In 2007, the Victorian Bone Bank (Donor Tissue Bank of Victoria, DTBV) reported similar findings of apparent lytic

Figs. 3–5 – Clinical photographs of anatomical specimens—sections through apparent lytic lesions in retrieved long bone allografts.

Table 4 – Number of ovoid lesions per specimen.

	0–5	6–10	>10
Femur	24	6	2
Tibia	8	1	0
Fibula	2	0	0
Hemipelvis	1	0	0
Iliac Crest	2	0	0
Humerus	–	–	–
TOTAL	36	3	6

lesions, within the medullary cavity of long bone allografts.¹⁰ They described similar uncertainty as to the suitability of the specimen for use in donation due to the potential for possible disease communication and voiced their concerns about a potentially unnecessary and expensive waste of a precious and rare commodity.

The DTBV undertook CT scans of a selected number of their long bone specimens to further investigate and characterise the lesions in an attempt to exclude any pathological process. They concluded that the lesions were not pathological in nature, but that they were air pockets, which had occurred as a result of the retrieval process, or even early decomposition of the bone.

We have shown in radiological and histological assessment that the apparent lytic lesions seen on plain radiograph assessment of retrieved specimens are air pockets either resulting from the retrieval process, freezing artefact or early decomposition of the specimen, and do not represent structural or pathological reason for the specimen to be discarded.

Retrieval occurs up to 24 h of post-mortem, and cadavers are stored in refrigerated conditions prior to retrieval and the specimens undergo microbiological testing, making decomposition a very unlikely source of the gas pockets. Similarly, it was less likely for specimens that had been sectioned to contain apparent lesions, which is unusual as it would be more likely for the sectioned bone to contain air pockets. This finding contradicted that of the DTBV.

They hypothesised that as the linear air pockets were of similar shape to blood vessels and pre-existing trabeculae. We concur that the lesions occur as a result of the retrieval and freezing processes. In addition to the DTBV paper, we have

histopathological evidence that these apparent lesions do not represent a pathological process.

5. Conclusion

Radiological and histopathological examination of apparent lytic lesions in retrieved long bone allograft specimens has confirmed that these lesions are air pockets and do not represent a significant pathological process that would render the specimens redundant.

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