Procurement of Cadaveric Bone Allografts: How Long Will It Remain Infection Free?

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ABSTRACT

The common practice by most tissue bankers in harvesting bones from cadaveric donors within 12 hours (or 24 hours if cadavers are refrigerated) were questioned as these guidelines were made by western tissue bankers who did not take into account climate conditions affecting procurement safety. We conducted a study on the contamination rate in 85 cadavers that were about to undergo post-mortem examination in University Malaya Medical Centre. All cadavers were refrigerated within 3 hours from their time of death. Blood cultures and bone samples were taken to identify bacterial colonization in the cadavers. The time interval from death to the time samples were collected ranged between 3.8 to 59.9 hours (average of 15.06 hours). From the data gathered, we found that colonization did not occur in cadavers who had their samples taken less than 12 hours from their time of death. Between 12 to 24 hours from the time of death, contaminations were noted in 9% of cases and even higher after 24 hours. We also noted that in cases where blood samples taken proving bacterial colonization in cadavers have occurred, very few cases of positive bone cultures were noted. Conclusion: Contamination does not occur before 12 hours from the time of death and therefore this period should be accepted as the contamination free period. Blood cultures are not good predictors for contamination of allografts and that bone cultures yields a better option to determine contamination in allograft procurement.

INTRODUCTION

Practices in allograft procurement have been established since the dawn of orthopaedics. Bone grafting as a clinical procedure only became possible with the development of anesthesia in 1846 and antiseptics in 1864. The first documented use of allograft as a procedure is by Nussbaum in 1875[1] when he rotated a fragment of ulna to bridge a 2-inch in this bone[1].

The modern practice of bone grafting was invented by Macewen in the Infirmary of Glasgow in 1880[1]. Macewen described a pioneering clinical application of bone graft in eloquent detail in 1881[2]. Following that Albee, Barth, Lexer and Phemister were responsible for establishing the clinical usefulness of bone grafts in patients who have skeletal disorders and laid the groundwork for the current confidence and popularity associated with the use of bone grafts for enhancing osteogenic potential as well as restoring structural integrity[3].

Since its early use, many clinical applications for allogeneic bone and cartilage have been described[3]. Among other applications are the use tissue in filling benign cystic defects, encourage union of fractures, increase arthrodeses rates and serve as segmental replacements for skeletal deficits, including articular surfaces[2]. Bone grafts have been successfully used to bridge massive defects with satisfactory clinical results[5]. Bone allograft are also used successfully in revision total hip arthroplasty where massive bone deficiency is a common problem particularly in multiply revised hip implants[2].

In Malaysia, tissue banking practice is well recognized with are least 5 servicing tissue banks nationwide registered with the National Tissue Transplant Registry. Infection is the one of the main concerns in any tissue banking practice with rates of infection reported as high as 18% of bones procured[3]. Careful donor selection and stringent microbiological surveillance are essential in bone banking procedures in order to prevent the transmission of micro organism through born allograft[4]. The microbiological screening of the Netherlands Bone Bank Foundation (NBF) consist of blood cultures taken prior to the procurement procedure and of swab cultures taken from the external surface of every graft. Blood cultures however are not required by the joint standards of the European Association of Musculoskeletal Transplantation (EAMST) and the European Association of Tissue Banks[4]. Although it has been accepted that harvesting from cadaveric donors can be done within 12 hours (24 hours if refrigerated below 4° C), not much data have supported this practice locally especially

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considering that cadavers are exposed to the tropical climate and high humidity[5]. With this in mind, evidence of safety should be established as a means to provide guidelines for local tissue bankers to decide the best time to harvest allografts from cadaveric donors. A study was therefore conducted to determine the contamination free period in allografts harvest in cadaveric donors.

MATERIALS AND METHODS

All post mortem cases performed in University of Malaya Medical Center between November 2002 and October 2003 were recruited in the study. All cases were attended by the same technician. Strict selection criteria were adhered to with cases which did not meet selection criteria excluded from this study. Written consent was obtained prior to any procedures.

Cases that were selected fell into 3 large categories: Brought In Dead (BID), Dead on Arrival (DOA) and Died in Hospital (DIH). By definition, BID is a victim who has been confirmed dead by a medical legal authority (General Practitioner, Police or Fire Department Officer) which was brought to the mortuary requesting for a post mortem. DOA is a victim who has not been confirmed dead by a medical authority (General Practitioner, Police or Fire Department Officer) who was brought in unusual circumstances or with a doubt of clinical death. Resuscitation was attempted but confirmed dead with ECG. DIH are victims who were brought in alive to the emergency department or in collapsed state. Resuscitation attempts were carried out with a recovery (transient cardiac activity) however patient subsequently developed cardiac arrest. Certification of death was done using an ECG.

All cadavers were sent to the mortuary and refrigerated at 4°C. Refrigeration time was documented in the Mortuary Registration. The sampling procedures were performed in the mortuary clean room using aseptic techniques. Bone specimens were taken from the anterior superior iliac spine using a sterilized Biopsy Needle. Blood sampling were performed via direct cardiac puncture following thoracotomy during the post mortem. Following successful specimen collection, all samples sent immediately to the Microbiological Department for cultures.

RESULTS

There were 85 cadavers used in this study. There were 74 male (87%) and 11 female (13%) cadavers. The interval between the time of death and the time samples were collected were between 3.8 to 59.9 hours with an average of hours. Twenty two percent of cases were Malay, 27% were Chinese, 17% were Indian and 34% were classified as others. As most of these cases were work related, the majority of cases were Vietnamese, Myanmar and Bangladeshi. Cadavers were between ages 12 to 66 years (average age of 33.62 years) at the time of their deaths.

The time interval of sample collection from cadavers from their time of death ranged between 3.8 to 59.9 hours with an average time of 15.06 hours (Figure 1). There were 56 cases (65%) brought in dead (BID), 19 cases (22%) died in hospital (DIH) and 11 cases (13%) died on arrival (Figure 2).

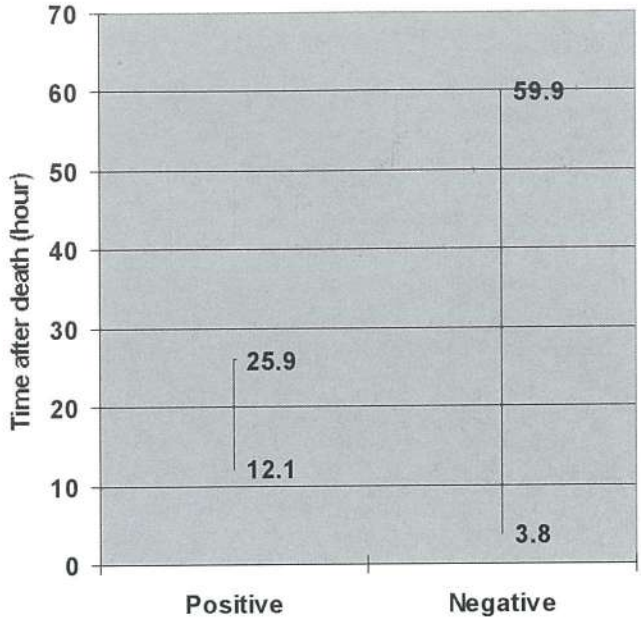

Figure 1. Time-causal relationship of the positive contamination and time of death. Note that positive cultures only occurred in cases where death had occurred after 12.1 hours. Although negative cultures are noted in cases up to 59.9 hours from time of death, we cannot justify the safety period after 12 hours.

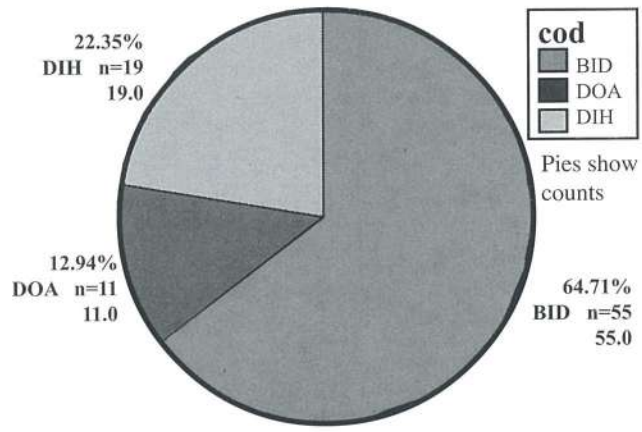

Figure 2. The distribution of cases following the different category of death (COD).

Of the blood cultures collected, seventy (85%) cases were positive while the other 13 (15%) were negative (Table 1). In contrast, only 4 (5%) of the bone cultures were positive while 81 others (95%) were negative. Of the 72 cases with positive blood cultures, only 4 (6%) had positive bone cultures.

Table 1. Distribution of the number of cases at different time intervals (from time of death)

Time after death	Total number of cases (cadavers)	Positive Bone culture	Percentage (%)
0-11.9	39	0	0
12.0 – 23.9	35	2	6
24.0 – 35.9	8	2	25
36.0 – 47.9	1	0	0
48.0 – 59.9	2	0	0

There were 81 cases that are negative against 4 cases positive for contamination. In comparing two distinct groups (samples collected before 12 hours and after 12 hours), there are no contaminated cases in the first group whereas 4 cases were seen in the later. Mean comparison analysis showed significant statistical differences between the two (p value < 0.01). There were no statistical significant differences between the cases examined at 36 hours and above with cases with positive cultures (p value > 0.05). There were strong correlation between time of death and positive bone cultures (p value < 005).

Majority of the blood cultures had multiple organisms isolated with 19 (22%) of cases having isolated single organisms (Table 2). Commonest organisms isolated were Streptococcus viridans. In contrast, all positive bone cultures grew single organisms (Table 3). No significant correlation were noted between positive blood and bone cultures (Table 4).

Table 2: The distribution of colonized organism in blood cultures with the number of cases found positive

Organism isolated	No. of specimens
Streptococcus viridans	24
Klebsiella pneumoniae	21
Acinetobacter baumanii	16
Group A streptococcus	13
Escherichia coli	11
Group B streptococcus	10
Citrobacter sp.	10
Enterococcus sp.	9
Pseudomonas aeruginosa	8
Enterobacter sp.	8
Bacillus sp.	8
Coagulase neg. staphylococcus	7
Proteus sp.	6
Staphylococcus aureus	3
Aeromonas sp.	3
Group D streptococcus	2
Group G streptococcus	2
Clostridium botulinum	1
Clostridium bifermentes	1
Clostridium sordellii	1
Diphteroids	1
Flavobacterium sp.	1
Eubacterium lentum	1
Serratia marcescens	1
Porphyromonas asaccharolytica	1
Brevundimonas vesicularis	1
Alcaligenes sp.	1

Table 3. Organism isolated from bone cultures and the time sample collections were performed

Time after death (hours)	Organism isolated
25.9	Klebsiella pneumoniae
22.2	Group A streptococcus
15.2	Clostridium sordellii
12.1	Staphylococcus aureus

Table 4. Correlations of positive bone cultures and blood cultures. Note that there is no correlation between the two categories with p value > 0.05

		Blood	Bone
Blood	Pearson Correlation	1	.094
	Sig. (2-tailed)		.390
	N	85	85
Bone	Pearson Correlation	.094	1
	Sig. (2-tailed)	.390	
	N	85	85

DISCUSSION

Careful donor selection and stringent microbiological surveillance are essential in bone banking procedures in order to prevent the transmission of micro organism through bone allograft [1]. To ensure safety to the recipient, through evaluation of a cadaveric organ donor is important in order to prevent clinical infection and malignancy. The Malaysian Institute for Nuclear Technology (MINT) Research recommends that the age of donor is preferably between 15 to 65 years old[10]. Delmonico et al (2000) suggested that a cadaveric donor should be less than 80 years of age owing to concerns of poor bone quality[6].

The highest number of cases consists of males contributing 87% of the study population while only 13% were females. Non Malaysians made the majority of our subjects (34%) followed by Chinese (27%), Malays (22%) and Indians (17%). This figure was due to the fact that the majority of death were due to industrial accidents and the main labour force in this country consists of foreigners. The number of Malay subjects would have been higher if not for religious and social stigma regarding the management of human remains.

Apart from careful donor selection, stringent microbiological surveillance is crucial in preventing infection through allografts. The microbiological screening of the Netherlands Bone Bank Foundation (NBF) consist of blood culture taken prior to the procurement procedure and of swab cultures taken from the external surface of every graft[4]. Blood cultures however are not required by the joint standards of the European Association of Musculoskeletal Transplantation (EAMST) and the European Association of Tissue Banks (EATB)[4]. Most tissue banks rely on swab cultures taken from the external surface of every graft. The Bone Bank of University of Malaya Medical Center

microbiological screening consists of swab culture and do not include blood culture.

In our study, we compared the result of bone cultures and blood cultures to determine the significance of blood culture as a microbiological procedure. Blood cultures were taken through a cardiac puncture while bone samples were taken from the marrow of the anterior superior iliac spine under aseptic technique. These were similar to the study by Vehmeyer et al (1999)[7].

In a similar study, Vehmeyer et al (1999) reported that 30 out of 95 blood samples (32%) taken from cadavers within 24 hours were positive and of that 22% of the bone cultures were positive. In comparison to our study, we found that as high as 85% of cases were recorded to have positive bacterial blood culture. However, of this number only 6% of the bone cultures were positive.

Therefore we can conclude that positive blood culture does not reflect bacterial colonization of bone allografts. Performing a blood culture is thus unnecessary in microbiological screening for a potential donor.

It is worth noting that 3 of 4 (75%) of the positive bone cultures had the same organism isolated from the blood cultures. Martinez et al (1985) found that identical organisms were isolated in 73% of cases when both blood and bone marrow cultures were positive[8]. However Vehmeyer et al (1999) found that only 1 out of 4 (25%) with positive bone and bone marrow cultures yielded identical isolate[7].

In our study, we found that positive bone cultures were noted as early as 12.1 hours after death. Six percent of bone culture taken between 12 to 24 hours after death and 25% between 24 to 36 hours after death were positive. None of the bone cultures taken in the first 12 hours after death were positive. The delay in refrigeration of these positive bone cultures were between 2 to 3 hours. Our analysis showed no correlation noted between the duration of delay in refrigeration and positive blood cultures. Therefore the safe period for the harvesting of bone allograft should be 12 hours if there is a delay in refrigeration of up to 3 hours. Kumta et al (1997) stated that it is possible to extend the safe period up to 96 hours following death. In their study rats were immediately refrigerated at 4°C after being sacrificed, as a result there were no delays in refrigeration[9].

As stated by Martinez et al, delay in refrigeration affects colonization of bone allograft and therefore shortened the safe period for harvesting them[8]. This is of particular importance in tropical climates where humidity remains above 90% all year round with average room temperature of 28°C. However, due to the small number of cases of positive bone cultures, significant correlation between delay in refrigeration and safe period cannot be established.

CONCLUSION

Proper donor selection and stringent microbiological surveillance are crucial in preventing allograft related infection. Blood cultures taken prior to procurement do not signify bone allograft colonization. Blood culture is therefore unnecessary in microbiological surveillance of bone allograft.

Time interval between death and procurement contributes to bone allograft colonization. The safe period to harvest bone allograft without the risk of contamination should be less than 12 hours after death and delay of refrigeration accepted up to 3 hours.

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