Bacterial contamination in postmortem bone donors

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ABSTRACT – **We analyzed factors influencing the contamination rate of allografts and blood samples obtained from postmortem bone donors. 5,710 allografts were harvested, from 550 donors of which 3,164 (55%) were swab culture negative. Blood cultures were positive in 140 donors (26%). The risk of graft contamination increased with each extra team member (Odds Ratio 1.9). It was also higher with organisms of greater virulence in donors with a positive blood culture (OR 3.5).**

The risk of blood contamination increased per hour postmortem (OR 1.1) and the same increase was seen with organisms of high virulence. In donors with multiple trauma, the risk of blood contamination with organisms of high virulence was greater (OR 8.2), but smaller in donors with preceding organ procurement (OR 0.1). To minimize the bacterial load, donors should be obtained in operating rooms, using aseptic techniques with only a few personnel for procurement. The postmortem time should be kept to a minimum. The procurement cultures from donors with multiple trauma should be carefully interpreted. Blood cultures should be taken into account, since these can help to find contamination not detected by swab cultures.

To ensure the safety of bone allografts, tissue banks have adopted procedures to minimize the risks in the transmission of infectious diseases. The medical history of donors is meticulously screened and extensive virological and bacterial testing is done. Moreover, aseptic processing or terminal sterilization methods may give additional safety (Jinno et al. 2000). For limited indications, e.g., reconstruction after resection of bone tumors, minimally processed postmortem allografts are used with mechanical and biological properties which are unaffected. For these grafts, in particular, strict bacterial screening is essential, since the transmission of bacterial microorganisms can lead to severe complications—i.e., an infection in the recipient (Lord et al. 1988, Tomford et al. 1990, Mankin et al. 1996).

In several reports on the bacterial contamination rate of postmortem donors, various factors affecting the risk of contamination have been found (Deijkers et al. 1997, Bettin et al. 1998, Journeaux et al. 1999). However, only a few such donors have been included in these studies, and the results have been partly conflicting. Therefore, we studied factors affecting the risks of graft and blood contamination in a large group of postmortem donors.

Material and methods

All postmortem bone donors met the standards of the EAMST and EATB (1997), which include the absence of clinically manifest infections. We had both heart-beating and non-heart-beating donors and, apart from organ procurement, non-heart-beating heart-valve procurement might precede bone tissue retrieval. Autopsy, when permitted, was always done after the procurement of bone. Bone and soft tissue allografts were harvested only in operating rooms, using standard aseptic techniques. Before the procurement procedure, blood samples for cultures and virological tests were taken from the subclavian vein or artery under aseptic conditions. For heart-beating donors, samples were taken via venous punctures before organ perfusion and, if postmortem heart-valve procure-

ment preceded bone procurement, blood samples were taken after mid-sternal thoracotomy from the inferior vena cava under aseptic conditions.

The blood samples were cultured (Bactec, Becton Dickinson Microbiological systems, Cockeysville, Maryland USA) for aerobic and anaerobic microorganisms for 7 days.

After procurement, every graft was rinsed with sterile saline. In the first 150 donors, antibiotics were added to this solution (Bacitracin 50,000 U/L, Apothekernes Lab, Oslo, and polymyxin-B 500,000 U/L, Pfizer, Connecticut, USA). Then the entire bone surface was carefully swabbed using a 15 cm polyester-tipped applicator (Becton Dickinson), which was placed in transport medium (Port-A-Cul, Becton Dickinson). The swabs were inoculated within 24 h onto blood agar and chocolate plates and cultured under aerobic and anaerobic conditions. The swab sticks themselves were incubated in a brain-heart infusion culture broth (Oxoid Unipath Ltd., Basingstoke, Hampshire, UK), inoculated after 5 days onto blood agar and chocolate plates and cultured for 48 h.

For the interpretation of the blood culture results, microorganisms of low virulence, including skin commensals, such as coagulase negative staphylococci and Propionibacterium acnes, were distinguished from microorganisms which are generally considered of higher virulence, including Staphylococcus aureus, Streptococcus species and Escherichia coli (Veen 1994, Deijkers et al. 1997). This resulted in 3 categories of blood cultures: (1) no microorganisms cultured, (2) organisms of low virulence and (3) organisms of high virulence cultured. In the swab culture results, the number of organisms cultured was also taken into account. If the swab culture was positive, the number of micro-organisms was considered high and the broth was not cultured further. If the broth culture alone was positive, the number of microorganisms was considered low. This created 4 categories: (1) no microorganisms cultured, (2) organisms of low virulence cultured from the broth (few), (3) organisms of low virulence cultured from the plate (many) and (4) organisms of high virulence cultured from the broth or the plate.

We studied various factors that might affect the contamination of blood and grafts. These included: a traumatic cause of death, preceding procedure, postmortem time, number of team members, duration of the procurement procedure, type of graft, effect of an antibiotic rinse and sex of the donor. For the effect of a traumatic cause of death, we distinguished between an isolated trauma of the head (n = 37) and multi-trauma (n = 56), defined as multiple traumatic lesions leading to death. The preceding procedure could involve an organ (n = 150) or heart-valve (n = 137) procurement. The postmortem time (mean 8.75 (0.5–24) h) was defined as the time between circulatory arrest and the start of the procurement procedure. The number of team members (range 3–7, mean 4) was the number of procurement and back-table personnel present in the operating room, not including visiting hospital staff. The duration of the procurement procedure (mean 2 (0.75–3.75) h) was defined as the time between the first skin incision and retrieval of the final graft. Other factors were the type of graft, including a group of miscellaneous grafts containing ribs, patellas, scapulas, radii and elbows, and the effect of an antibiotic rinse (n = 150) on contamination of the grafts. We also studied whether the sex of the donor (men = 359, women = 191) affected the incidence of contamination.

Statistics

A multivariate analysis was done, using a logistic regression model (EGRET). A standard model was used to analyze the donors (blood culture). As several grafts were taken from one donor, the correlation between grafts (swab culture) in one donor was accounted for by using a random-effects model for distinguishable data, with the donor number as stratifying factor. The simultaneous effect of the above-stated factors on the probability of contamination was estimated by their respective odds ratios (relative risk). P-values were calculated on the basis of likelihood ratio tests; p-values < 0.05 were considered significant.

Results

Graft contamination

In 10 years, 5,710 grafts were obtained from 550 donors. Microorganisms of low or high virulence were cultured from 2,546 (45%) grafts (Table 1). The commonest isolates of low virulence were

Table 1. Swab culture results according to their category

Category	Number of grafts
1. No microorganisms	3,164 (55%)
2. Organisms of low virulence, broth	1,809 (32%)
3. Organisms of low virulence, plate	592 (10%)
4. Organisms of high virulence, broth or plate	145 (3%)
Total	5,710

coagulase negative staphylococci (n = 2,449). Streptococcus species (n = 65) were the organisms of high virulence most frequently cultured.

The risk of contamination with microorganisms of low or high virulence increased with each extra team member (odds ratio 1.9, $p < 0.001$) (Table 2). The Achilles tendon was contaminated significantly more often than the tibia (odds ratio 1.7, $p < 0.001$) while the fibula was contaminated less frequently (odds ratio 0.6, $p < 0.001$).

The risk of contamination with organisms of high virulence was higher in the humerus (odds ratio 3.3, $p < 0.001$) (Table 3) and the group of miscellaneous grafts (odds ratio 3.8, $p = 0.04$). Blood contamination with microorganisms of high virulence increased the risk of graft contamination with such organisms (odds ratio 3.5, $p < 0.001$), but this risk decreased (odds ratio 0.4, $p = 0.05$) when the blood culture was positive for organisms of low virulence.

Blood contamination

Blood cultures were done in 540 donors, of which 400 (74%) were negative, 70 (13%) contaminated with organisms of low virulence and 70 (13%) of high virulence. Propionibacterium species (n = 40) and Streptococcus species (n = 61) were the commonest isolates of low and high virulence, respectively.

The risk of blood contamination was higher in donors with a head trauma (odds ratio 3.3, $p = 0.01$) and multiple trauma (odds ratio 3.4, $p < 0.001$) (Table 4). A similar effect was seen in the risk of contamination with organisms of high virulence in donors who had a head trauma (odds ratio 4.1, $p = 0.01$) (Table 5) and the effect was even more marked in donors with multiple trauma (odds ratio 8.2, $p < 0.001$). The risk of blood contamination

Table 2. Effect of various factors on the relative risk of graft contamination with microorganisms of low or high virulence, estimated by odds ratios

	Logistic regression [a]		
Factors	Odds ratio	95% CI	P-value
Traumatic cause of death			
No (n = 2143)	(1)		
Head trauma (n = 146)	0.7	0.3–1.6	0.4
Multi trauma (n = 257)	1.3	0.8–2.2	0.2
Preceding procedure			
No (n = 1217)	(1)		
Heart valve (n = 656)	1.1	0.8–1.6	0.6
Organ (n = 673)	0.7	0.5–1.1	0.1
Time since death			
Per hour increase	1.0	0.9–1.0	0.2
Team members			
Per person increase	1.9	1.5–2.4	< 0.001
Procurement duration			
Per hour increase	1.1	0.9–1.4	0.4
Type of graft			
Tibia (n = 493)	(1)		
Fascia (n = 34)	0.7	0.4–1.2	0.2
Femur (n = 754)	0.9	0.8–1.1	0.4
Fibula (n = 326)	0.6	0.5–0.8	< 0.001
Achilles tendon (n = 446)	1.7	1.4–2.1	< 0.001
Pelvis (n = 219)	1.2	0.9–1.6	0.1
Humerus (n = 247)	1.1	0.9–1.5	0.3
Miscellaneous (n = 27)	1.0	0.5–1.9	0.9
Blood contamination [b]			
No (n = 1836)	(1)		
Yes, low virulence (n = 324)	0.8	0.5–1.3	0.4
Yes, high virulence (n = 323)	1.1	0.7–1.8	0.7
Antibiotic rinse			
No (n = 1837)	(1)		
Yes (n = 709)	1.0	0.6–1.4	0.9
Sex			
Male (n = 1763)	(1)		
Female (n = 873)	1.1	0.8–1.5	0.6

[a] with distinguishable binominal random effect-account for graft within-donor structure; all estimated effects.
[b] blood cultures could not be done in 10 donors (111 grafts) for technical reasons.

increased with each hour postmortem (odds ratio 1.1, $p < 0.001$) and the same increase was seen with organisms of high virulence (odds ratio 1.1, $p = 0.02$). Preceding heart valve procurement procedures increased the risk of contamination (odds ratio 1.8, $p = 0.01$), while the risk was less in heart-beating donors (odds ratio 0.3, $p = 0.002$). A similar effect of preceding organ procurement was seen in the risk of contamination with organisms of high virulence (odds ratio 0.1, $p < 0.001$). In female donors, the risk of blood contamination decreased (odds ratio 0.6, $p = 0.05$).

Table 3. Effect of various factors on the relative risk of graft contamination with microorganisms of high virulence, estimated by odds ratios

	Logistic regression [a]		
Factors	Odds ratio	95% CI	P-value
Traumatic cause of death			
No (n = 112)	(1)		
Head trauma (n = 9)	0.6	0.2–2.0	0.5
Multi trauma (n = 24)	1.3	0.5–3.0	0.6
Preceding procedure			
No (n = 62)	(1)		
Heart valve (n = 41)	1.1	0.6–2.3	0.7
Organ (n = 42)	1.4	0.6–3.0	0.4
Time since death			
Per hour increase	1.0	1.0–1.1	0.5
Team members			
Per person increase	0.9	0.6–1.4	0.7
Procurement duration			
Per hour increase	1.4	0.9–2.2	0.2
Type of graft			
Tibia (n = 21)	(1)		
Fascia (n = 1)	0.8	0.1–6.7	0.8
Femur (n = 41)	1.2	0.7–2.2	0.5
Fibula (n = 20)	1.2	0.6–2.3	0.6
Achilles tendon (n = 24)	1.6	0.8–3.0	0.2
Pelvis (n = 11)	1.6	0.7–3.6	0.3
Humerus (n = 23)	3.3	1.7–6.6	< 0.001
Miscellaneous (n = 4)	3.8	1.0–13.5	0.04
Blood contamination [b]			
No (n = 94)	(1)		
Yes, low virulence (n = 7)	0.4	0.1–1.0	0.05
Yes, high virulence (n = 44)	3.5	1.7–7.2	< 0.001
Antibiotic rinse			
No (n = 106)	(1)		
Yes (n = 39)	1.0	0.4–2.1	0.9
Sex			
Male (n = 83)	(1)		
Female (n = 62)	1.4	0.8–2.5	0.2

[a] with distinguishable binominal random effect-accounts for graft-within-donor structure; all estimated effects.
[b] blood cultures could not be done in 10 donors (111 grafts) for technical reasons.

Table 4. Effect of various factors on the relative risk of blood contamination with microorganisms of low or high virulence, estimated by odds ratios

	Logistic regression [a]		
Factors [b]	Odds ratio	95% CI	P-value
Traumatic cause of death			
No (n = 447)	(1)		
Head trauma (n = 37)	3.3	1.3–8.2	0.01
Multi trauma (n = 56)	3.4	1.8–6.5	< 0.001
Preceding procedure			
No (n = 253)	(1)		
Heart valve (n = 137)	1.8	1.1–2.9	0.01
Organ (n = 150)	0.3	0.2–0.6	0.002
Time since death			
Per hour increase	1.1	1.0–1.1	< 0.001
Sex			
Male (n = 355)	(1)		
Female (n = 185)	0.6	0.4–1.0	0.05

[a] all estimated effects.
[b] blood cultures could not be done in 10 donors for technical reasons.

Table 5. Effect of various factors on the relative risk of blood contamination with microorganisms of high virulence, estimated by odds ratios

	Logistic regression [a]		
Factors [b]	Odds ratio	95% CI	P-value
Traumatic cause of death			
No (n = 447)	(1)		
Head trauma (n = 37)	4.1	1.4–12.0	0.01
Multi trauma (n = 56)	8.2	4.1–16.6	< 0.001
Preceding procedure			
No (n = 253)	(1)		
Heart valve (n = 137)	0.9	0.5–1.6	0.7
Organ (n = 150)	0.1	0.0–0.4	< 0.001
Time since death			
Per hr increase	1.1	1.0–1.1	0.02
Sex			
Male (n = 355)	(1)		
Female (n = 185)	1.0	0.5–1.8	0.9

[a] all estimated effects.
[b] blood cultures could not be done in 10 donors for technical reasons.

Discussion

Our results resemble to those of some other studies (Martinez et al. 1985, Veen 1994, Deijkers et al. 1997, Bettin et al. 1998, Journeaux et al. 1999), although a comparison it is difficult to compare them because they used different procurement procedures and bacterial screening protocols.

Microorganisms frequently found on the skin are known to be dispersed by the operating room personnel and they contaminate the grafts via air (Noble 1975). This phenomenon was shown by the increase in the risk of graft contamination by a factor of 1.9 with each extra team member in the operation room. Moreover, coagulase negative staphylococci were the commonest isolate cultured from grafts in this and other studies reported by by other tissue banks (Martinez et al. 1985, Bettin et al. 1998, Journeaux et al. 1999).

Another source of skin commensals is the donor's skin, as shown by the increase in the risk of contamination of Achilles tendons, an area colonized with these organisms. Its harvesting involves repeated manipulation of the skin. In contrast, the fibula had a low risk of contamination, because its retrieval is relatively easy and involves no manipulation of the skin.

We found a higher risk of graft contamination with organisms of greater potential virulence which tend to spread hematogenously in humeri. The source of this contamination may be the use of indwelling catheters in patients' arms.

We were unable to confirm the higher risk of contamination with organisms of greater virulence in the pelvis reported by Journeaux et al. (1999). This may be related to retrieval in the morgue.We were unable to confirm this. Others have reported a higher incidence of organisms cultured from grafts harvested in mortuaries (Bettin et al. 1998).

The events preceding the death of donors clearly affect contamination with organisms of high virulence, as shown by the greater risk of blood contamination with these organisms in donors with a traumatic cause of death. The limited sensitivity of the swab culture technique, also noted by Veen, was confirmed in these donors as no similar risk was found with graft contamination (Veen et al. 1994). The presumed hematogenous contamination is difficult to detect by swabbing the external surface of the graft, since the microorganisms are mainly located in the marrow of the graft, but other studies have suggested that blood cultures may be of help in this matter (Vehmeyer et al. 1999).

Blood contamination was also significantly affected by preceding heart valve and organ procurement. The increased risk of contamination with organisms, presumably of low virulence, in donors with preceding heart valve procurement may be due to the method by which these cultures were obtained. The blood samples were taken after thoracotomy and preparation of the inferior vena cava during which the operating field could have become contaminated with skin commensals.

In donors of organs, the risk of contamination with, organisms of presumably high virulence was lower. This may be because antibiotics are routinely given to organ donors, although older studies in the 1970s showed that they do not affect postmortem blood cultures (Koneman et al. 1971, Koneman and Davis 1974). A better explanation is probably that the circulatory system of these donors was intact, which enabled microorganisms to be cleared.

The risk of blood contamination increased by 10% per hour in the time between circulatory arrest and the start of bone procurement. This increased risk was mainly attributed to organisms of high virulence. Others have reported that the incidence of positive cultures from postmortem specimens showed no increase with time after the postmortem (Koneman and Minckler 1970, Nehring et al. 1971). It seems advisable, however, that the time after the postmortem should be reduced since a positive blood culture with organisms of high virulence also increases the risk of graft contamination by these organisms.

Female donors ran a lower risk of blood contamination with organisms of low or high virulence, but this effect was not seen with organisms of high virulence alone. This is probably because men disperse more skin flora than women (Noble 1975, Tammelin et al. 2000).

The categorization of microorganisms in the present study has also been used by other tissue banks, but it has been criticized (Bettin et al. 1998). Some microorganisms regarded as of low virulence, like coagulase negative staphylococcus, can cause infections in patients, especially in the presence of foreign bodies (Hope et al. 1989, Bettin et al. 1998, Ringberg et al. 1998). To avoid confusion about the terminology of microorganisms cultured from postmortem donors we recommend that skin commensals be distinguished from nonskin commensals instead of describing organisms as of low or high virulence. This permits identification of possible sources of contamination.

The method of culture used in our study is useful for semi-quantitative assessment of the bacterial load of a graft, although the value of an additional broth culture has been disputed (Morris et al. 1995, Silletti et al. 1997).

On the basis of the present study, the following conclusions can be drawn. To minimize the bacterial load of retrieved allografts, donors should be procured in operating rooms using aseptic techniques with the number of procurement and back-

table personnel kept to a minimum. In addition, the postmortem time should be kept to a minimum.

The procurement culture results using donors with multiple trauma causing their death should be carefully interpreted. The isolation of non-skin commensals may indicate a hematogenous spread of these organisms. When such a spread is suspected, tissues obtained from that donor should be processed by an acceptable method for the destruction of isolated organisms.

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