High incidence of pathogenic microorganisms in bone allografts explanted in the morgue

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We analyzed the influence of the environment on bacterial contamination in 431 bone allografts from 97 donors (68 multiorgan, grafts taken in the operation theater; 29 morgue donors). From each bone transplant we cultured two aerobic and two anaerobic tissue specimens. The overall contamination rate was 49% (theater 51%, morgue 40%). In grafts explanted in the morgue, we noted more pathogenic microorganisms (60%) than in multiorgan donors, explanted under aseptic surgical conditions (33%). We conclude that the harvesting environment constitutes the major source of pathogenic microorganisms.

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In the last decade deep-frozen large bone allografts have been more extensively transplanted in reconstructive orthopedic surgery (Malinin et al. 1985, Mankin 1987). Femoral head allografts from primary hip arthroplasty and large structural allografts harvested from dead donors are being used currently (Malinin et al. 1985, Delloye and Munting 1989). Contaminated allografts could be responsible for postoperative infections (Tomford et al. 1986). Despite meticulous aseptic explantation techniques, bacterial contamination of the explanted tissue seems to be inevitable (Raahave 1991, Van Veen at al. 1994). Reported contamination rates range from 3% to 66% (Malinin et al. 1985, Carlsen and Jensen 1986, Deijkers et al. 1997). This variation may be due to differences in the quality control or bacteriological screening (Delloye and Munting 1989, von Garrel et al. 1993). We determined whether bone allograft contamination is influenced by different harvesting environments in the morgue or operation theater, using an identical screening protocol.

Material and methods

Since 1980 at the Muenster Tissue Bank, all stages of tissue harvesting, storage and preparation were performed according to the guidelines of the American Association of Tissue Banks (AATB 1979). All donors were identically screened for any sign of malignancy, infection or any other contraindications. There were two types of donor (Tables 1–2). Bone allografts were obtained from multiorgan donors in the operation theater directly after explantation of parenchymatous organs (heart, kidney, liver, pancreas). From other donors, the bone allografts were procured in the morgue within 12 hours of cardiac arrest at a temperature of +22 °C or within 24 hours at +4 °C. Both donor types underwent an autopsy to detect other possible sources of infection.

Between May 1980 and June 1996, 431 bone allografts were procured from 97 donors. The average age of the 29 morgue donors was 70 (45–76) years. The cause of death was cardiac arrest (11), head injury (7), stroke (6) or strangulation (3). Since 1986, bone allografts were also explanted in the operation theater from 68 multiorgan donors. The average age was 29 (16–52) years. The cause of death was polytrauma with a head injury (53), internal hemorrhage or aneurysm (7), stroke (5), other causes (2) and cardiac arrest (1). None of the donors had large skin defects, open wounds in the lungs or the gastrointestinal tract.

Tissue procurement and bacteriological protocol

In both groups, the harvesting procedure was done under aseptic surgical conditions. Standard protocols were used with regard to shaving, scrubbing and wrapping. Skin decontamination was done by applying 10% polyvidone iodine (Dibromol®) for 15 min. The operation field was always covered with steridrapes. Two sets of instruments were used separately on each side. All surgeons used double gloves.

Table 1. Contamination in allografts. Multilocular means that more than one allograft from the same donor was contaminated, systemic means that all allografts from the same donor were contaminated. Number (%)

	Theater donor, 319 allografts	Morgue donor, 112 allografts	Total 431 allografts
Low pathogenic microorganisms	99 (31)	8 (7)	107 (25)
single	8 (3)	0 (0)	8 (2)
mixed	5 (2)	0 (0)	5 (1)
single multilocular [a]	71 (22)	5 (4)	76 (18)
mixed multilocular [a]	15 (5)	3 (3)	18 (4)
High pathogenic microorganisms	65 (20)	37 (33)	102 (24)
single	13 (4)	2 (2)	15 (3)
mixed	5 (2)	3 (3)	8 (2)
single multilocular [a]	18 (6)	19 (17)	37 (9)
mixed multilocular [a]	17 (5)	13 (12)	30 (7)
systemic	12 (4)	0 (0)	12 (3)

[a] related to the most frequent microorganism combination

Table 2. Microorganisms cultured from bone allografts

	Theater donor No. times cultured [a]			Morgue donor No. times cultured [b]		
	Bone	Soft tissue	Total	Bone	Soft tissue	Total
Low pathogenicity	78	103	181	14	18	32
Coagulase-negative staph.	71	95	166	9	15	24
Propionobact.	0	1	1	5	3	8
Corynebact.	4	4	8	0	0	0
Sarcina	2	2	4	0	0	0
Veillonella	1	1	2	0	0	0
High pathogenicity	47	43	90	16	32	48
Staph. aureus	12	18	30	3	1	4
Enterococcus	9	2	11	4	4	8
Peptococcus	10	11	21	4	14	18
E.coli	2	5	7	4	11	15
Candida	7	4	11	0	0	0
Bacteroides	4	2	6	0	0	0
Spores	0	0	0	1	1	2
Acinetobacter	2	0	2	0	0	0
Alcaligenes	0	1	1	0	0	0
Pneumococcus	1	0	1	0	0	0
Pseudomonas	0	0	0	0	1	1

[a] multiple organisms were cultured from 22 allografts
[b] multiple organisms were cultured from 15 allografts

In theater donors, the explantation of the bone allografts was done in an operating room on average 2.5 (0.5–5) hours after circulatory arrest. Allografts were procured in the morgue on average 11 (2–24) hours after circulatory arrest.

Both groups were screened by an identical bacteriological protocol, which was used throughout this study from May 1980 to June 1996 (AATB 1979). From all explantation sites (femur, tibia, fibula), two aerobic and two anaerobic tissue cultures from the bone as well as from soft tissue (muscles, tendons, ligaments) were taken. Anaerobic samples were transported in an airtight box with catalytic oxygen binding (Anaerocult® A, Merck, Darmstadt, Germany). We checked for aerobic and anaerobic bacteria, using dextrose- and Schaedler-broth and Kimmig-(yeasts); blood-, chocolate- and endoagar plates. The incubation period was set at 10 days, in order to confirm or exclude even low-grade contamination. Cultured bacteria and yeasts were isolated, if necessary, and differentiated to an adequate level. If the agar bouillon became turbid, subculturing was done. Intermediate controls were discontinued to minimize the risk of secondary contamination. No techniques to suppress

bacterial growth were used. In accordance with the Leiden Bone Bank, we distinguished low pathogenic microorganisms that are skin-derived (e.g., coagulase-negative staphylococci, *Corynebacterium, Propionibacterium*) from highly pathogenic microorganisms (e.g., streptococci, *Staphylococcus aureus, E. coli*) (Deijkers et al. (1997).

Results

Allograft contamination

The overall contamination rate was 49% (Table 1). In 29 morgue donors, 112 allografts were procured, with an average of 4 (range 1–5) allografts per donor. In this group, 45 allografts (40%) were contaminated. In 68 theater donors, 319 allografts were explanted, with an average of 5 (range 1–8) allografts per donor. In this group, 164 allografts (51%) were contaminated (Table 1). Further differentiation of microorganisms cultured from bone allografts revealed proportionately more highly pathogenic organisms in the morgue donors (60%) than in theater donors (33%) (Table 2). We found no association between contamination rate and the cause of death.

Discussion

Reliability of bacteriological screening methods

The protocol of the study was based on the initial standards for musculoskeletal tissue banking of the American Association of Tissue Banks (AATB 1979). Our overall contamination rate is comparable to other reports (Delloye and Munting 1989, Deijkers et al. 1997). Bacteriological screening of bone allografts is mandatory, since infections can develop due to transmission of bacteria along with the graft (Carlsen and Jensen 1986, EAMST, EATB 1997). Clinical data suggested that the currently used selection criteria may be acceptable, although they could not guarantee the absence of bacterial contamination in transplanted allografts (Van Veen et al. 1994). After transplantation, Tomford et al. (1981) reported a 5% incidence of infection. In musculoskeletal allografts used in reconstructive tumor surgery, the infection rates ranged from 4.7% to 14%, also could be influenced by longer operation times (Makley 1985, Mnaymneh et al. 1985, Mankin et al. 1987).

Despite very careful screening protocols, the true primary contamination of explanted allografts remains uncertain. All our allografts were evaluated with an identical bacteriological screening protocol, using four tissue samples per graft. Van Veen et al. (1994) cultured total fibular allografts directly after explantation, which is a more precise method. Under aseptic surgical conditions comparable to our study, he noted a contamination rate of 92% (Van Veen et al. 1994). This might imply that our contamination rate would be higher if a more sensitive method had been used. Surprisingly, we found a lower contamination rate in the morgue donors, but the number of explanted grafts per donor and, consequently the bacteriological test numbers were lower in this group. In future, improvements in the bacteriological screening method could include analysis of the filtered "rinsing solution over the allograft surface." Von Garrel et al. (1993) claimed for this method a 95% sensitivity, compared to 10–65% using the method of swab culturing (Van Venn et al. 1994). We think that until this method is validated by other groups, secondary sterilization of every allograft should be considered. Only with this precaution, can surgeons be provided with allografts free of bacterial contamination. At the Muenster Tissue Bank, this subject is part of an ongoing study. It has an important effect, because surgeons nowadays can only use "possibly contaminated allografts" (tested negatively by swab culturing) with some osteoinductive and good biomechanical properties or "secondary sterilized allografts," with some loss of biological and biomechanical properties (Eastlund 1996).

The influence of the harvesting environment

The results of our study of different microorganisms cultured from grafts of theater donors and morgue donors raise some questions. Sometimes, the donor could be the source, due to an unknown infection or bacteremia (Dolan et al. 1970, Deijkers et al. 1997). The cultured microorganisms represent colonizing areas that could not be found in our autopsies. Deijkers et al. (1997) emphasized the need to distinguish between low and highly pathogenic microorganisms. They found highly virulent microorganisms in only 5% with the method of swab culturing. Some studies indicate that these bacteria originate from the gastrointestinal microflora (Minckler et al. 1966, Dolan et al. 1970). In our study, virulent microorganisms were found more often, regardless of donor type. This may be due to the method of procurement rather than infection of the donor, since donors with septicemia, open wounds or other infections do not meet the criteria for donation (AATB 1979, EAMST, EATB 1997).

Despite aseptic explantation techniques, superficial contamination with skin microorganisms is often described (Raahave 1991, Deijkers et al. 1997). The sources are the environment, including the air, con-

taminated staff or instruments. Under operating room conditions, such microorganisms are of low pathogenicity, which was also seen in our theater donor group. The high incidence of pathogenic microorganisms in our morgue donors could be explained by the special environment, a possibility also supported by the studies of McMahon and Lamberson (1989), as well as Minckler et al. (1966).

According to the guidelines of the AATB (1979), all transplants from donors with pathogenic microorganisms or mixed bacterial contamination must be discarded. Allografts with skin-derived organisms, such as *coagulase-negative staphylococci,* could successfully undergo secondary sterilization (Whitby 1984). These allografts do not increase the risk of postoperative infections in alloarthroplasty or tumor surgery (Van Veen et al. 1994). In the discussion on high or low pathogenicity of cultured bacteria, many different features of the reported strains must be taken into consideration. We classified our results according to Deijkers et al. (1997), who termed *Corynebacterium species* and *Bacillus species* as low pathogenic bacteria, despite the fact that *Corynebacterium diphtheriae* and *Bacillus anthracis* are members of these groups. On the other hand, all *Streptococci,* including *hemolytic streptococci,* are regarded as highly pathogenic in his paper. Therefore, we need a more precise definition of the criteria influencing the term 'pathogenicity of bacterial strains', e.g., production of exo- or endotoxins, adhesions or capsules.

We found a higher proportion of highly pathogenic microorganisms in morgue donors, which might be related to the harvesting environment. Consequently, the number of transplantable allografts was low. In multiorgan donors, secondary sterilization of allografts with low pathogenic microorganisms increased the number of transplantable allografts. Because of the low reliability of the present bacteriological screening method and the higher incidence of highly pathogenic microorganisms, we cannot recommend explantation in the morgue. We think, that unless an improvement of the bacteriological screening is achieved, secondary sterilization of every allograft should be considered.

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