Bone bank service in Finland

Experience of bacteriologic, serologic and clinical results of the Turku Bone Bank 1972–1995

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560 bones were harvested by The Turku Bone Bank between 1972–1995. It was started with massive allografts for bone tumor surgery, but today most are femoral heads for hip revision surgery. The increase in harvested bones nearly trebled from 1984–1989 to 1990–1995. Only 1 positive hepatitis C test was found. There were no hepatitis B or HIV positive donors. The incidence of discarding after screening was 24%, with positive bacterial growth (8%, usually *Staphylococcus epidermidis*) as the commonest reason. 2 massive grafts with negative cultures when harvesting were positive after thawing and resulted in deep infection.

369 allografts were transplanted. The infection rate of massive allografts for bone tumor surgery was 5/63 in 1973–1995, and 2/52 in 1985–1995. The infection rate for hip revision surgery was 3.4%. The clinical functional results correspond to those reported in larger international series.

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There has been a bone bank in Turku since 1972, initially as an intrainstitutional organization (Aro et al. 1992). At present, there is a need for and interest in international administration and cooperation for quality and control for safety of human tissues for medical use. We report the donor procurement and screening results of the bone bank service in Turku, and Finland, during the 23-year period 1972–1995.

Legislation and selection of donors

Postmortem tissue retrieval for medical purposes has been legal in Finland since 1957, beginning with cornea transplantation (Statutes of Finland No. 260, 1957), and legal orders for harvesting human tissues for medical use were issued in 1971 and 1985 (Statutes of Finland 1971, 1985).

The main criteria for evaluating donors were the same as those used in large centers in the USA (e.g., Massachusetts General Hospital) or in eastern and central Europe (Central Institute of Traumatology and Orthopaedics, CITO, Moscow; German Central Tissue Bank). The technical model followed by the Turku Bone Bank is a modified CITO-Bank program (Imamaliev 1969, 1970). The history of a potential donor was studied to screen any diseases that might be transmitted by a bone allograft. Contraindications include any infection, positive blood cultures, infected wounds, viral diseases such as hepatitis, HIV, as well as neurologic and rheumatic diseases and malignancies (Table 1). The donors were screened for the presence of serum hepatitis B surface antigen, hepatitis C antibodies and HIV antibodies, using commercial enzyme immunoassay tests. For living femoral head donors, the screening tests are repeated 3 months after donation.

The donors of osteoarticular allografts were young, 18–50 years of age, previously healthy persons dying a sudden cerebral death, usually in a traffic accident. Other diagnoses were shotgun trauma (suicide) and subarachnoidal bleeding. The permission of a next-of-kin or other relatives was always sought. People donating their body or tissue for medical purposes before their death are not included in this material. Femoral heads are retrieved from living donors at the time of a hip replacement, without any donor age-limit.

Bone retrieval

In 1972–1976, bones were retrieved in the autopsy rooms of the Department of Pathological Anatomy, University of Turku, 8–12 hours after death, with the assistance of operating theater nurses. Since 1977, massive skeletal allografts were harvested under sterile operating theater conditions from kidney and multiorgan donors, within 15 min–1 hour after removal of other organs and cardiac arrest. Respiratory assistance time (before retrieval) should not be more than

Table 1. Criteria and laboratory screening tests of cadaver and living donors for bone banking

Relatives
Permission of next-of-kin
Exclusion of donors
Age 18–50 years for long bones; femoral heads, no age limit
Anatomic requirement
Exclusion of transmissible diseases
(medical history, any bacterial/viral disease, HIV, hepatitis, or disease of unknown origin, autoimmune diseases, tuberculosis, malignancy, high-dose corticosteroid treatment, blood transfusion < 6 months before admission)
Abnormal sexual history
Tattoos
History of drug abuse
Artificial respirator time > 72 hrs
CNS diseases
Laboratory tests
Rh-factor
HIV, control at 3–6 months [a]
Hepatitis B
Hepatitis C
Syphilis
Herpes virus simplex 1, 2 and zoster, and cytomegalovirus [b]
HLA antigens
Tissue harvested
Cultures
Bacterial (aerobic and anaerobic)
Fungi

[a] living femoral head donors
[b] important only for organ recipients treated with immunosuppressive medication

72 hours (Tomford et al. 1983). The present technique of retrieval includes working in an operating room with laminar air flow. The first femoral head from a living donor was harvested in 1976. In all, 560 bone specimens, 1–4/donor, have been retrieved (Table 2, Figure). In recent years, the collection of patellar tendons has been added to the bank functions.

To increase the stock of femoral heads obtained from living donors, a program involving training of staffs and collection of femoral heads has been organized to include district hospitals within a 60 km radius from Turku.

Retrieval technique

After standard surgical preparation of the skin, a surgical team (1–2 surgeons, 1–2 nurses) removes the bones, using longitudinal, long incisions. The hip joint is opened, the joint capsule and synovia and insertions of ligaments are dissected. Since the 1990s, the proximal humerus has been harvested with its ligamentous capsule—insertion of the rotator cuff tendons. The long bones are cut with a power saw at midshaft, giving sufficient length for an osteoarticular allograft with joint surface. The bones are cleaned mechanically and rinsed with physiological NaCl. The bone marrow is removed by curettage. The skeletal

Table 2. Bones harvested for allograft surgery by Turku Bone Bank Service 1972–1995

	N	%
Long bones [a]	128	23
Hemipelvis	7	1
Patella ligament	10	2
Femoral head	415	74
Total	560	100

[a] Femur, tibia, humerus, radius

defects and the body shape of the donor are reconstructed, using board (floor) stocks, corresponding to the size of the bone harvested.

Microbiological cultures

Sterile swabs are used to obtain samples from the periosteum, cartilage surface and medullary cavity for aerobic and anaerobic bacterial cultures. 6 specimens have been taken for cultures from every bone since 1993. Small pieces are fractured from the surface and the medullary cavity of each massive osteoarticular and femoral head allograft and placed in tubes containing brain-heart infusion broth (BHI, Difco, Detroit, MI, USA) or fastidious anaerobe broth (FAB, Lab M, Bury, U.K.). 2 BHI tubes and 1 FAB tube are inoculated with pieces from each side. The tubes are incubated at 37 °C. After 7 days or earlier if the broth becomes turbid, BHI cultures are subcultured on chocolate agar plates and FAB broths on chocolate and fastidious anaerobe agar plates. The plates are incubated for 2 days at 35 °C, chocolate agar plates aerobically and fastidious anaerobe agar plates in an

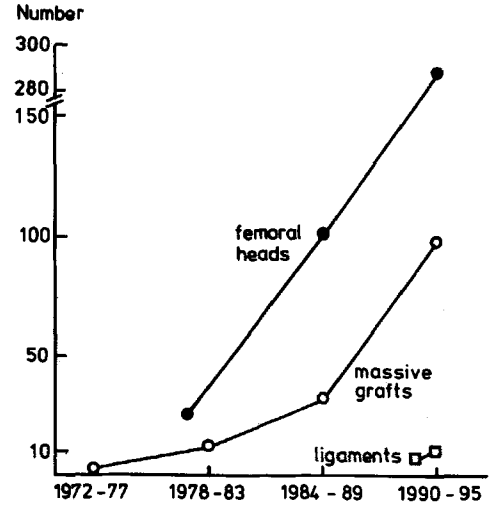

Diagram illustrating the numbers of harvested bone allografts between 1972 and 1995 in the Turku Bone Bank.

anaerobic atmosphere. Bacteria are then identified in the colonies, using normal biochemical and immunological methods. The allografts are approved if all cultures are negative. Cultures are also made for fungal growth. New specimens for bacterial cultures are taken from the grafts before the operation, after thawing.

Storage of allografts, record-keeping, freezers

The bones are deep-frozen at –80 °C, immediately after sampling for bacterial cultures and packaging. Since 1989, the freezing procedure has been carried out in 2 steps: the bones were initially stored at -20 °C for 8–12 hours and the final storage temperature was –80 °C. The freezing procedure was modified because there were reports on the benefits of slow freezing as regards cell packing and intercellular ice crystal formation (Malinin 1985, Schachar and McGann 1986). The freezers have separate shelves for each bone type (femur, tibia, radius, humerus, hemipelvis) and alarms to signal a temperature drop of 10 °C. We have not used cryopreservatives (glycerol, dimethylsulfoxide, DMSO) for cartilage protection, because they seem not to protect against late cartilage degeneration of human allografts.

Before wrapping in sterile 3-fold plastic bags, the bones are immersed in an antibiotic solution for 20 min (cefalexin/cefuroxim natrium or cloxacillin 3 g/ 1–2 L of sterile saline). Each package is labeled and marked to identify the type of bone, date, donor name, and dimensions in cm. Femoral heads are stored in plastic jars or wrapped in plastic bags. Radiographs of massive allografts are taken in two projections before harvesting, to determine the dimensions of the bone as correctly as possible.

Record-keeping is mandatory for medical and legal purposes. It consists of 2 manual records and a third computerized database. The results of the donor laboratory tests and cultures of grafts require a careful control system. In addition, regular random cultures are obtained at 6-month intervals from the freezers, to maintain sterility.

Donors and bones harvested

560 bone allografts have been retrieved in 1972–1995. These include 145 osteoarticular bones and 415 femoral heads (Table 2). The distal femur is the bone most often harvested. Since the early 1970s, the number of retrieved bones has increased slightly. The increases between 1984–1989 and 1990–1995 (Figure 1) were 186% for all bones, 205% for long bones, and 240% for femoral heads. In recent years, 1995–1997, there has been no further increase in long-bone harvesting, but the need for femoral heads is increasing.

Table 3. Primary bacteria cultures of skeletal allografts harvested by Turku Bone Bank Service 1972–1995

		Positive culture	
	N	n	%
Osteoarticular bone	135	12	8
Femoral head	415	35	8
Patellar ligament	10	0	0
Total	560	47	8

Table 4. Distribution of bacteria cultured from harvested bones in the Turku Bone Bank 1972–1995 (n 560)

Staphylococcus epidermidis	19	(51%)
Staphylococcus aureus	3	
Peptococcus	1	
Gram+ rod/Corynebacterium	8	(22%)
Anaerob/aerob. mixed flora	6	
Total	37	

Table 5. Causes of discarding of harvested bone tissue 1972–1995

Positive bacteria culture	47
Technical failures [a]	72
Stored more than 5 years	15
Total	134 / 560 = 24%

[a] Missing laboratory tests, package deficiency

Bacterial growth

47 bacterial cultures taken from 560 (8%) harvested allografts were positive. Osteoarticular, long bones and femoral heads showed similar contamination rates (Table 3). Staphylococcus epidermidis has been cultured most frequently, 19 times (Table 4).

Bacterial cultures taken for freezer and package control in 1994–1996 indicated irregular positive growth in 25–38% of samples. A coagulase negative Staphylococcus was cultured 3 times from samples taken from the freezer. Streptococcus α-hemolyticus, Haemophilus and Pseudomonas were found occasionally.

Serological tests

1 donor with hepatitis C antibodies was identified in 1994. For various reasons (screening tests, technical errors), 134 (24%) bones were discarded (Table 5).

Distribution of bones

The allogenic material of the Turku bone bank service has been distributed without profit to hospitals in the

Table 6. Clinical use of bone allografts and indications at the Turku Bone Bank 1973–1995

	Indication	Grafts implanted
Massive grafts		63
Tumor resection (osteoarticular)	50	
Lengthening	8	
Hip revision (strut, block)	5	
Femoral heads		306 [a]
Hip revision	196	
Fracture	38	
Cavity filling	7	
Spinal fusion	1	
To other hospitals	27	
Total		369 [b]

[a] 1–3 femoral heads/case
[b] An additional 57 bones in freezer

Turku region, Southern Finland (Helsinki), and once to Norway.

Clinical use of the allografts

369 allografts, 63 massive grafts, and 306 femoral heads for 269 objects were transplanted between 1973–1995 (Table 6). 196 femoral heads were used as morselized or as chips in total hip revision for filling a cavity. Massive osteoarticular or segmental intercalary grafts were used in 63 cases, usually after removal of a tumor (Table 6). There has been an increasing demand for all types of allografts and, particularly during the 1990s, the use of femoral heads for total hip revision has increased markedly (Figure). 347/369 allografts were used during 1984–1995.

The clinical functional outcome mainly in bone tumor patients treated with massive allografts, has been excellent/good in two thirds on the Mankin-Waber scale and fair/poor in one third. The mean individual percentage of the maximum attainable score has been 81 (50–100)% on the Musculoskeletal Tumor Society scale (Aho et al. 1994, 1995). However, late low-degree degeneration of articular cartilage, beginning 3–4 years after transplantation, seems to be common. The osteoarticular grafts of the knee joint, however, showed excellent/good results in four fifths of the patients. The complication seen most often was a fatigue fracture which occurred in one fifth of the patients. Nonunion was not detected.

The infection rate in all patients treated with massive grafts 1973–1995 was 5/63 and 4/52 in 1985–1995 showing a tendency to decrease during the late time period. Hip revision surgery using allografts involved 2 infections (3.4%). In 2 patients treated with massive grafts, cultures taken from the allograft in the operation theater immediately before transplantation, after freezing the bone to be implanted, showed bacterial growth resulting in a deep infection by the same bacteria. In the first case, Pseudomonas aeruginosa was cultured from the distal femoral graft transplanted because of chondrosarcoma in a 28-year-old woman in 1979. The other case, a hemipelvis allograft, contaminated by *Staphylococcus epidermidis* was used in a 13-year-old boy with Ewing's sarcoma in 1995.

Both infections were successfully treated by several debridement operations combined with long-term antibiotic treatment and both allografts were saved. However, the boy died because of metastasis 1.5 years after transplantation.

Discussion

Bone bank and donor tests

The development of tissue banking was slow in Europe, except in the Soviet Union (Imamaliev 1969) after the Second World War. However, one of the largest and oldest multitissue banks in Europe, the German Central Tissue Bank (GCTB) has retrieved more than 50,000 bone grafts in Berlin, consisting of cortical-cancellous strips, struts, long-bone shafts, demineralized bone matrix, etc., since 1956 (von Versen 1992). Finland and the other Nordic Countries have some banking traditions, since patients' bone as autografts were used in the early 1960s at the Invalid Foundation Hospital in Helsinki for scoliosis surgery (Riska 1967). The first distal femoral osteoarticular allografting in humans in Scandinavia was carried out in Stockholm in 1966 (Nilsonne 1969), and allograft operations in dogs and experimental bone banking were started 1968 in Turku (Aho 1973). Since the early 1970s, based on contacts with Russian orthopedics, there were 2 bone banks in Finland for the needs of reconstructive human tumor surgery, temporarily in Helsinki until the early 1980s (Koskinen et al. 1979) and continuously in Turku (Aho 1981, Aro et al. 1992). For the time being, bone banks harvesting femoral heads are operating in many provincial hospitals in Finland (Korkala et al. 1994).

Pathogenic bacteria are often found in donor blood or bones harvested 12–24 h after death. In the study by Malinin et al. (1985), 1 isolate of bacteria was present in 21% of donor blood cultures, whereas 3 or more were found in 2.3%. Deijkers et al. (1997) found microorganisms of low pathogenicity in 50% of bone allografts. However, the correlation between positive blood and positive bone cultures was poor. The reported incidences of positive cultures from bone allografts vary widely, from 5% to 44%. However, microorganisms of high pathogenicity were cultured from only 3% of the grafts (Deijkers et al.). The mi-

croorganisms most often isolated have been coagulase negative staphylococci (70%), Propionibacterium and various Clostridium species (Doppelt et al 1981, Malinin et al. 1985, Hansen et al. 1994), which is in agreement with our findings. A discarding rate of 5% was reported from Hannover with material obtained no later than 6 h after circulatory arrest (Kalbe et al. 1989). Often many organisms constitute a normal skin flora and represent external contamination of blood or bone cultures. The recovery of microorganisms from the blood and tissues of clinically nonseptic cadaver donors can be attributed in most of the cases either to contamination of specimens at the time of their removal or the implantation procedure. The reason for the low contamination rate of massive allografts (8%) of the present survey is probably that most of them were harvested within 3 hours after death (15 min–1 hour after cessation of artificial circulation) in a sterile operating theater. In many services of large national bone banks representing a model for multi-institutional facilities, the contamination risk is higher because the tissues are excised within a 12–24-hour period. In practice, finally, the recovery of pathogenic bacteria even at a single site—1 donor bone—warrants discard of all bones of the donor, because the positive bone culture rate may be as high as 66% (Malinin et al. 1985). The same discard praxis has been followed by us.

Regarding serological tests, such as those used for the detection of hepatitis, syphilis or HIV, only 1 positive result indicating hepatitis C has been found in our donors. HIV seroprevalence in Finland is low, 1:10 000 (Kantanen et al. 1996, National Public Health Institution Publications 1997). In western Europe, the reported annual percentage of donors with a positive HIV screening test has been 0.1% (Patijn et al. 1993). In Finland, there has not been a single HIV positive bone donor to date. For HIV testing, we use the new third-generation HIV antibody test (Enzygnost Anti-HIV-PLUS, Behring) with a window period of 3 weeks. All living donors are retested 3 months after donation. Although the window period could be decreased by 1 week using the RNA PCR direct virus detection assay, the assay is not yet practical for large-scale screening and yet a window period of 2 weeks is needed (Busch et al. 1995). Screening donors for hepatitis B and C is more problematic because of their long window periods of about 2 months (Schreiber et al. 1996). Medical history and donor screening are very important to reduce the risk of viral transmission of these diseases (Tomford 1995).

Donors with a history of a blood transfusion within the past 6 months are problematic, because they may not react to HIV antigen enough for a positive test. Hemodilution immediately (48 hours) before testing may lead to a negative result (Patijn et al. 1993). The cut-off date for previous transfusions, about 6 months, is an arbitrary limit. In addition, most donors are now multiorgan donors and often transfused at the time of retrieval of their solid organs. It is therefore difficult to exclude donors transfused within the past 6 months (Tomford et al. 1983).

Risks in the clinical use of allografts

The reported incidence of postoperative bacterial infections of human massive allografts varies between 5% and 25% (Mankin et al. 1987, Delloye et al. 1988, Lord et al. 1988, Alho et al. 1989, Hernigou et al. 1993, Aho et al. 1994, 1995, Loty et al. 1994). We had 2 cases of intraoperative contamination of a massive allograft, and there are more reports on bacterial growth at the time of graft implantation (Ivory and Thomas 1993, Korkala et al. 1994). Usually, a bone graft infection invariably leads to failure (Mankin et al. 1987, Delloye et al. 1988). Our infection rate of 3% after a hip revision using femoral head allografts is in agreement with earlier reports of 3–7% (Oakeshott et al. 1987, Dartée et al. 1988, Allan et al. 1991, Ivory and Thomas 1993, Lawrence et al. 1993, Korkala et al. 1994).

The risk of virus transmission through musculoskeletal allografts is low but 4 infections by HIV and 3 by hepatitis C have been reported until 1995 (Patijn et al. 1993, Tomford 1995). Recently, a case of transmission of human T-cell lymphotropic virus has been reported (Sanzén and Carlsson 1997). In addition, the possibility of developing of Rhesus antibodies after bone allografting should be kept in mind. 3 reports have been published on Rh+ immunization in female patients receiving allografts (Jensen 1987, van Dijk et al. 1988).

Long-term results of massive allografts depend on graft type and size, anatomical site, age of the patient, and the disease to be treated. Large series regarding allograft procedures in extremities show excellent/good results in 31%–71%, fair in 7%–45%, and poor in 4%–24% (Mankin et al. 1987, Mnaymneh et al. 1994, Zatsepin and Burdygin 1994). The results of our series including also a difficult bone, the pelvis, correspond to them. Complications after long bone allografting have led to an enormous international interest in studies on applications of biomaterials.

In Europe, the cooperation of tissue banks started officially in 1991, when the First European Conference on Tissue Banking and Clinical Application was held in Berlin, and the European Association of Musculoskeletal Transplantation was founded in Brussels. Ethical rules, practical processing, policies, etc., to be

observed or "The Common Standards for Musculoskeletal Tissue Banking" (1997) are to be introduced to the European Union officials. International cooperation with the American Association of Tissue Banks (AATB) and the Asian-Pacific Association of Surgical Tissue Banking (APASTB) illustrates the worldwide increase in interest in the medical use of stored tissues.

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