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Cardiovascular tissue banking in Europe

T.M.M.H. de By[1], R. Parker[1], E.M. Delmo Walter[2], R. Hetzer[1,2]

[1]Foundation of European Tissue Banks, Berlin; [2]Deutsches Herzzentrum Berlin.

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ABSTRACT

Introduction: In the past 50 years, human cardiovascular tissue allografts, also called homografts, have been implanted into patients with different valvular diseases. The use of these allografts and the number of cardiovascular tissue banks and their respective techniques increased. We conducted a survey to establish the quantity of allografts processed, and issued by, European tissue banks. The survey also included the collection of other relevant statistics.
Methods: In 2011, the Foundation of European Tissue Banks collected data from 19 different cardiovascular tissue banks in 11 European countries.
Results: From 2007 to 2010 the data show a decrease in the number of hearts received, from 1700 to 1640 in 18 tissue banks; the average number of hearts received for cardiovascular tissue processing decreased from 113 to 91. The number of heart valves issued for transplantation increased from 1272 in 2007 to 1486 in 2010. The average rate of discard because of microbiological contamination was 20.7 %, while 4.2 % of the grafts were not used because of positive serology. Half of the tissue banks issued arterial grafts, while 3 banks also issued veins and pericardium. An overview of decontamination methods shows considerable methodological differences between 17 cardiovascular tissue banks.
Conclusions: From the experience in Europe, it can be concluded that cardiovascular tissue banks have an established place in the domain of cardiovascular surgery. The statistics show fluctuating data concerning the demand for human cardiovascular allografts and methodological questions. There is room for growth and improvement with respect to validation of decontamination methods.

Keywords: *cardiovascular tissue, tissue donor, tissue bank, homograft, ross operation, discard rate, microbiology, contamination, decontamination, serology, validation.*

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INTRODUCTION

In the early 1960s Ross and Barratt-Boyes introduced the use of human allograft cardiac heart valves, or homografts, into clinical practice (1, 2).
In 2012 the 50th anniversary of the first so-called Ross operation was celebrated.
The Ross operation encompasses implantation of a pulmonary autograft in the aortic position, while an allograft is transplanted in the pulmonary position.
Ever since, there has been a need to store available donor grafts, so that they can be prepared, stored in a tissue bank, and used for implantation, either in elective or in emergency patients. From the end of the sixties and into the eighties tissue banks were founded all over Europe (3).
In the same period studies about the techniques and successes of homograft implantation in larger series of patients were published, followed in the nineties by studies

Corresponding author:
Theo M.M.H. de By
Deutsches Herzzentrum Berlin
Augustenburger Platz 1
13353 Berlin, Germany
e-mail: theodeby@xs4all.nl

which covered more than a decade (4-14). Because, over time, they were the only successful biological heart valve prostheses beside the mechanical ones, the results were very satisfactory.
The advantages were clear: a low rate of thromboembolic events, thus avoiding a lifetime of anticoagulation therapy. In addition, their hemodynamic properties were superior to those of mechanical valves, especially those available in the early 1960s and 1970s.
As time went by, it became clear that the availability and cardiectomy techniques to obtain cardiovascular tissues were a problem as suitable donors were recipients of heart transplants, organ donors whose hearts were not accepted, or donors who were autopsied and their relatives had agreed to their tissues being used (15).
In the last 20 years, the European cardiovascular tissue banks have invested a great deal of finances and effort in improving the safety and quality of their tissue banking methods and facilities. Issues such as donor selection, validation of testing methods, the improvement of sterility systems and clean rooms were addressed.
Regulations based on Directives (16) of the European Union became law in all member states.
The Foundation of European Tissue Banks initiated a survey to obtain an assessment and quantification of the situation in the field of cardiovascular tissue banks, after implementation of the European Directives into national legislation. This study presents the results of that survey.

METHODS

In 2011, questionnaires were sent out to 30 cardiovascular tissue banks, 18 of which completed and returned them. One cardiovascular tissue bank had started its activities in early 2011; hence no data could be reported as yet. Three additional questionnaires were received after the statistical analysis was closed, and these data are not included.
The data received were accumulated and statistically stratified. Ranges and means were calculated and tabulated giving insight into the level of activities of these cardiovascular tissue banks. Percentages of detected positive serology were assembled, and a break-down of microbiological contamination as the reason for discarding tissue should yield information on the reasons for tissues being discarded during the process.
Ethical approval was waived given the observational and retrospective design of the study. No data from individual donors and patients were used in this study.

Table 1 - *General statistics.*

	2007	2008	2009	2010
Number of banks providing data	16	17	18	18
Number of countries	8	8	9	10
Number of hearts received	1700	1685	1663	1640
% of grafts issued for grafting	39.3	45	46.8	46.9
Average number of hearts received	113	120	111	91
Range of hearts received	10-312	4-334	9-307	17-262

RESULTS

The statistics in *Table 1* are based on the assumption that every heart received in the cardiovascular tissue banks provided two grafts. Out of 18 tissue banks, 11 had registered the number of donor reports rather than the hearts actually received in the bank. In these 11 tissue banks, 67 % of the donors reported resulted in the receipt of a heart in the bank. *Table 1* shows that from the total of 1640 hearts received by 18 tissue banks in 2010, only 46.9 % provided suitable grafts; hence the discard rate is 53.1 %.

The cardiovascular tissue banks show a considerable difference in their activities: while in 2010 the highest number of grafts received was 262, the smallest bank processed only 17 grafts. When it comes to issuing grafts, similar differences are observed. As shown in *Table 2*, the number of grafts issued ranges from 4 to 243. The statistics in *Table 2* confirm that the demand for pulmonary grafts is about twice as high as the demand for aortic valves: 67 % of all grafts issued were pulmonary valves.

The data provided by the 18 cardiovascular banks show that, in 2010, exporting of tissues to other countries was done by 7 banks, with the proportion varying from 1 % to 72 % of the annual number of processed grafts. *Table 3* provides insight into the information with respect to donors. The average donor age ranges from 40 (in 2007) to 42 in 2010. Fifty-seven percent of the hearts originated from organ donors of whom the heart could not be transplanted, 28 % from non-organ donors (those who become donors after an extended period of cardiac arrest, and are

Table 2 - *Heart valves issued per year.*

	2007	2008	2009	2010
Aortic valves	462	508	514	505
Pulmonary valves	810	953	938	981
Mean number of aortic valves issued	36	34	34	34
Mean number of pulmonary valves issued	62	73	59	61
Range of aortic valves issued	4-95	10-84	5-85	4-79
Range of pulmonary valves issued	16-184	15-226	7-223	17-243

Table 3 - *Donor information.*

	2007	2008	2009	2010
Mean age (yrs)	40	40	41	42
Death to cardiectomy criterion range in hrs.	2-48	2-48	2-48	2-48
Death to cardiectomy in reality, range in hrs	3-18	4-16	4-14	5-18
Death to cardiectomy, average hrs in reality	8	8	7	11
Death to excision criterion range in hours	24-72	24-72	24-72	24-72
Death to excision in bank in reality, range	12-44	13-45	17-42	18-43
Death to excision, average hours in reality	24	24	24	24

thus unsuitable as organ donors) and 15% were retrieved from so called "domino donors". Domino donors are people who undergo a heart transplantation, and whose native heart may still have valves that are transplantable as tissue grafts.

The criteria for the time between cardiac arrest and cardiectomy, as observed by the tissue banks in this study, ranged from 2 to 48 hours. In reality, the average time until cardiectomy was between 8 hours in 2007, and 11 hours in 2010. After receipt in the tissue bank, the valvular grafts are excised from the heart and decontaminated. Also here, the criteria differed greatly between the banks and the time varied from 18 hours to 72 hours, while the average number of hours in practice was 24. *Table 4* shows the reasons for discarding donor tissue. In 2010, 45.3% of the tissue

Table 4 - *Heart valve discards in 2010, average % of all cardiovascular banks.*

Heart valve discards in 2010, average % of all cardiovascular banks		
Not selected because of:		**% of received hearts**
Medical history		32.7
Serology		4.2
Microbiology	Bacteria	10.7
	Multi resistant bacteria	0.4
	Fungi	3.2
	Not specified	0.1
	Suspected	0.35
	Total microbiology	5.9
Morphology		35.8
Technical		7.3
Other or unknown reasons		7.8

Table 5 - *Different decontamination methods in 17 European cardiovascular tissue banks.*

Valve bank	**Antibiotics**	**Duration**			**mg/L**	**Medium**
		Antibiotics: concentration	**of culture**	**Temperature**		
Barcelona, BST	Cefoxitin	240 µg/mL	24hrs	5°C (2-8°C)	240	
	Vancomycin	50 µg/mL			50	
	Polymyxin B	120 µg/mL			120	
	Clindamycin (Lyncomicin)	100 µg/mL			100	
	Amphotericin B	5 µg/mL			5	
Barcelona, TSF	Penicillin	50 U/ml	24 hrs	5° C (+ /- 3°C)	50 U	
	Vancomycin	50 µgr/ml			50	
	Streptomycin	50 µgr/ml			50	
	Amphotericin B in medium 500ml RPMI	10 µgr/ml w/o L-glutamine			10	RPMI

Bad Oeynhausen	Mefoxitin	0.024 % (m/V)	18-24 hrs	6° C	240	
	Lincocin	0.012 % (m/V)			120	
	Colistin	0.0099 % (m/V)			99	
	Vancomycin	0.005 % (m/V)			50	
Berlin	Amikacin	1.2 mg/2 ml Syringe	18-24 hrs	5° C (+/- 3° C)		
	Metrodinazol	1.2 mg/2 ml Syringe				
	Flucytosin	3.0 mg/2 ml Syringe				
	Vancomycin	1.2 mg/2 ml Syringe				
	Ciprofloxacin	1.2 mg/2 ml Syringe				
Bristol	Amphotericin	0.05 mg/ml	21-24 hrs	22° C	50	
	Ciprofloxacin	0.20 mg/ml			200	
	Vancomycin	0.05 mg/ml			50	
	Gentamicin in Hanks' BSS	4.00 mg/ml			4000	HANKS
Brussels	Lincocin	120 µg/ml	48 hrs	4° C	120	
	Vancocin	50 µg/ml			50	
	Polymixine B in medium 199	124 µg/ml			124	M199
Cracow	Gentamicin	100 mg/ml	24 hrs	4° C	100	RPMI
	Vancomycin	50 mg/ml			50	
	Clindamycin	120 mg/ml			120	
	Colistin	100 mg/ml			100	
	Ampicilin + Sulbactam	200 mg/ml			200	
	Amphotericin B	25 mg/ml			25	
London	Cefuroxime	250 ug/ml	24 hours	37° C	250	
	Gentamicin	80 ug/ml			80	
	Ciprofloxacin	200 ug/ml			200	
	Vancomycin	500 ug/ml			500	
	Colistin	1000 IU/ml			1000 UI	
	Amphotericin	100 ug/ml			100	
Linz	Amphotericin B	125 µg/ml	24 +/- 2 hrs	+ 4° C	125	RPMI
	Gentamicin	600 µg/ml			600	
	Metronidazol	600 µg/ml			600	
	Ciprofloxacin	150 µg/ml			150	
	Vancomycin	600 µg/ml			600	
Lund	Amphotericin	250 ug/ml	24 hours	5°C (+/- 3° C)	250	
	Ketokonazol	100 ug/ml			100	

	Colistin	200 ug/ml			200	
	Vancomycin	500 ug/ml			500	
	Gentamicin	500 ug/ml			500	
Milano	Polimyxine B sulphate	100 µg/ml in RPMI1640 medium	24 hours	4° C	100	
	Vancomycin	50 µg/ml in RPMI1640 medium			50	
	Cefoxitin or Cefotaxime	240 µg/ml in RPMI1640 medium			240	
	Lincomycin	120 µg/ml in RPMI1640 medium			120	
Oxford	Amikacin	1g/L	18-24 hrs	20 - 30° C	1000	M199
	Cefuroxime	500 mg/L			500	
	Vancomycin	1g/L			1000	
	Timentin	3.2g/L			3200	
	Polymixin B	10,000,000 iu/L				
	Nystatin	1440,000iu/L				
Paris	Vancomycin	500 mg/L	18/24 h	4°C	500	
	Gentamicin	320 mg/L			320	
	Clindamycin In RPMI medium	600 mg/L			600	RPMI
Prague	Amikacin	0.1 mg/ml	24 hrs	20 - 30° C	100	
	Ampicilin + Sulbactam	0.2 + 0.1 mg/ml			200 + 100	
	Cefoperazon	0.2 mg/ml			200	
	Fluconazol	0.1 mg/ml			100	
	Amphotericin B 0.1 for NHBD in medium 199	0.1			100	
Rotterdam	Amikacin (as sulphate)	0.6 mg/mL	5-6 hours	37°C	600	
	Vancomycin	0.6 mg/mL			600	
	Ciprofloxacin (as lactate)	0.15 mg/mL			150	
	Metronidazole	0.6 mg/mL			600	
	Flucytosine	1.5 mg/mL			1500	
Treviso	Vancomycin	100 mg/ml of RPMI 1640 medium	72 hrs	+ 4° C	100	RPMI
	Polimyxine	100 mg/ml (1.000.000 IU/ml) of RPMI 1640 medium			100	
	Ceftazidima	240 mg/ml of RPMI 1640 medium			240	
	Lincomycin	120 mg/ml of RPMI 1640 medium			120	

Warsaw	Tazocin (Piperacillin/ Tazobactam)	0.5 mg/ml	24 hrs	20° C (+/- 2° C)	500	
	Gentamicin	0.05 mg/ml			50	
	Nystatin	2 500 j./ml				
	Vancomycin	0.5 mg/ml			50	

Barcelona, BST = Banco de Sang y Tejidos; Barcelona, TSF = Transplant Services Foundation; RPMI = Roswell Park Memorial Institute; HANKS' BSS = Hanks Balanced Salt Solution; M199 = Medium 199; NHBD = Non Heart Beating Donors.

grafts had to be discarded. In many cases there was more than one reason for not accepting the heart, or its tissue grafts, for transplantation.

In 32.7 % of the cases the reason for discard was that there were contraindications for transplantation of the tissue in the donor's medical history. During processing 35.8 % of the cardiovascular tissue was found to be unsuitable because of its morphology. In 17.65 % and 4.2 % of the cases, respectively, microbiology or serology test results were a reason not to accept the grafts for transplantation.

Technical and unknown reasons were responsible for 7.3 % and 7.8 %, respectively, of the discards. *Table 5* gives an overview of decontamination methods in 17 cardiovascular tissue banks. Substantial differences can be observed in the number of hours during which the tissue banks culture the tissue to detect and/or eliminate micro-organisms; the range is 5-72 hrs. Also, the temperature under which incubation takes place shows a large variety: from 4o C to 37o C. The banks use 25 different antibiotics in many different concentrations.

In *Table 6* a breakdown of other tissues provided by the banks in this study shows that pericardium, arteries and veins are processed alongside valvular allografts.

DISCUSSION

The level of activity in cardiovascular tissue banks is determined by the numbers of donors. This study shows that the range of

Table 6 - *Other tissues issued.*

Other tissues issued		**2007**	**2008**	**2009**	**2010**
Pericardium	banks	3	3	3	3
	in % of all banks	19 %	18 %	17 %	17 %
	tissues	39	50	54	81
Arteries	banks	7	7	7	9
	In % of all banks	44 %	41 %	39 %	50 %
	tissues	307	305	423	481
Veins	banks	3	3	3	4
	in % of all banks	19 %	19 %	17 %	22 %
	tissues	245	229	314	286

donor hearts received in 18 banks varied from 1640 in 2010 to 1700 in 2007.
As the number of hearts received represents only 67 % of the number of donors referred, it may be worthwhile to analyze the reasons why the hearts of 33 % of the reported donors were eventually not allocated to the tissue bank. By eliminating factors preventing the donation from materializing, banks would be able to increase their activity.
On the other hand the statistics document that in 2010 45.3 % were not suitable for transplantation and had to be discarded. Better donor screening beforehand, and a more effective process from cardiectomy to excision and for decontamination in the bank are three factors which could decrease this high number of discards.
This study shows in statistics what cardiovascular tissue bankers have known for a long time, that the demand for pulmonary valves is about twice as high as the demand for aortic valves: 66 % of all grafts are pulmonary valves.
Although this study does not extend to the use of grafts, the literature shows that for many centers the pulmonary valve is the allograft of choice in congenital as well as in acquired cardiac diseases (11).
The activity of the banks varies from processing less than 20 to 262 donor hearts in 2010. One has to wonder about the routine capabilities of personnel as well as about the optimal use of the investment and costs of maintenance of a class A laboratory.
The donor age *(Table 3)* has gradually increased from an average of 40 in 2007 to 42 years in 2010. As the average age in the European population increases, the donor age increases accordingly. Some cardiovascular tissue banks receive hearts from organ donors only. The reason is twofold:

1) some authorities forbid the use of non-organ donors;
2) to set up a cardiectomy team on a 24/365 basis requires additional organizational constraints and investments which some banks wish to avoid.

Most cardiovascular tissue banks strive to increase the volume of available tissue. The dependency on the receipt of organ donor and domino donor hearts brings them into a vulnerable position. The need for additional cardiovascular grafts could be compensated by an effort to set up a non-organ donor program.
The discard because of morphology can hardly be avoided. However, the differences in decontamination methods, use of antibiotics and their concentrations, as well as temperature should be a subject to cause concern in the cardiovascular tissue banks participating in this study.
In 2010, a conference of these tissue bankers and their microbiologists was organized by the Foundation of European Tissue Banks. Substructuring and validation methods were exchanged, and some arguments were proven to be right. At that conference, and from the questionnaire in this study, no adverse events were reported by any of the participating tissue banks.
While most of the cardiovascular tissue banks in this study concentrate on the processing and distribution of the "classic" homograft heart valves, nine banks showed activities with respect to processing tissues such as arteries, veins and pericardium. Table 6 clearly shows an increase in the distribution of arterial grafts. Correspondence with different tissue bank representatives revealed that the demand for arterial grafts is growing throughout Europe. While veins are used in access surgery (shunts), pericardium serves as patching material to bridge larger gaps of deficient tissue during cardiothoracic operations.
The numbers of these tissues issued over the period 2007-2010 also show a considerable increase.

CONCLUSION

For the first time since the start of the clinical use of human allogeneic heart valves, data from a number of European cardiovascular tissue banks could be accumulated.
Statistics with respect to numbers, discard and use of cardiovascular tissue provide insight into the magnitude of their activities as well as into some of the parameters they use.
First of all, looking at the number of tissue grafts issued for transplantation, one can conclude that the demand for tissues has not decreased during the period of 4 years encompassed in this study. Apparently the demand increased by 16.8 %, from 1272 to 1486, over a 4-yearperiod.
The results show that cardiovascular tissue bank activities have remained relatively stable over the years, though the number of donors has somewhat decreased (3.5 %). While the demand for pulmonary grafts still increased from 810 to 981 (21.1 %), only 505 aortic grafts were issued in 2010. What happens with all the aortic grafts which are not issued is a logistical as well as an ethical question.
In order to cope with the persistently high demand for pulmonary grafts and arteries, those cardiovascular tissue banks which do not retrieve hearts from non-organ donors should seriously consider initiating such a donor program.
Although not clinically proven, studies show that stem cell techniques may eventually contribute to the quality and availability of human heart valves, yet none of the cardiovascular tissue banks indicated that they are in any way involved in stem cell research.
The differences in accepted time lapses from death to cardiectomy, and from cardiectomy until excision of the valves and further processing find their origin in viewpoints with respect to quality and safety. A consensus between the tissue banks contributing to this study should be based on data with respect to the potential loss of tissue quality starting at cardiac arrest and measured over time.
As there are very large methodological differences with respect to microbiology testing, incubation and decontamination of cardiovascular tissue between the 17 contributing tissue banks, there is a necessity to validate procedures and room for improvement (17-19). This survey shows an increased demand for other tissues, which may be worth further exploration.
After all, where alternatives seem to fail or are absent, it is the task of tissue banks to satisfy the clinical demand for tissue grafts.

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