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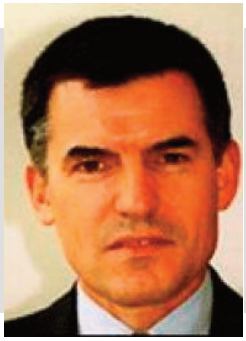
Quality Assurance of Heart Valve and Vascular Allografts for Human Application: Assessment of 30 Years of Activity of the European Homograft Bank (EHB) in Brussels



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ABSTRACT

Introduction and Aims: *Over the last 30 years, the European Homograft Bank (EHB) has been processing, storing, controlling for quality, and distributing heart valves and vascular tissues for some particular indications and patient categories. This article presents an organization, infrastructure, and collaboration network of this TE (Tissue Establishment) with particular accent on the quality of tissues.*

Methods: *Premises and critical equipment is in accordance with GMP and GTP recommendations for cardio-vascular tissue banking. For the selection of donors of heart valves and vessels, very strict exclusion criteria were established regarding the donor age, medical and behavioral history. Additionally, evaluation of morphology and function of allografts has been performed by professionals with sufficient medical and surgical backgrounds. A series of quality tests (donor evaluation for viral infections, bacteriology controls of tissue samples before and after antibiotic decontamination, histology of myocardium, aortic and pulmonary wall, as well as the mitral valve from each processed heart) are part of the quality controls of allografts. Control rate cryopreservation and storage of allograft in extreme low temperatures (below -130°C) is also part of the quality and safety of the allograft.*

Results: *In the past 30 years, 7.805 hearts and 2.517 batches of vascular tissues were recovered and sent to our TE, whereas 7.599 heart valves (Pulmonary, Aortic, Mitral) and 4.199 vascular allografts (ascending and descending thoracic aorta, aortic bifurcation, iliac and femoral arteries, venous grafts) were distributed to the huge implantation network in Europe and beyond. Donation activity increased significantly over time (2176, 2567, and 3062 hearts and 462, 939, and 1116 vascular tissues were donated by our network during the respective first, second, and third decade of activity). Implantation activity also increased significantly (2050, 2550, and 2691 valves and 540, 1397, and 1997 vascular allografts were implanted during the first, second, and third decade, respectively). Only two fatal complications (death of recipients due to immediate postoperative ruptures of the two thoracic aorta-s) were reported during first decade. Excellent results of implanted allografts in adult patients and clear evidence of immune related failure of allografts in newborn and very young children have been demonstrated in some follow-up studies. This issue was the subject of diversity in research projects and prospective clinical trials during last decade. So far, 100 pulmonary and 180 aortic valves were sent for decellularization and implanted for two EU funded clinical trials.*

Conclusion: *Quality and safety of the allografts was attained by quality measures throughout the entire process. The progressive increase and improvement of donation/implantation activity is the result of the high level of availability and dedication of our entire team and excellent relations with cooperating partners. Scientifically confirmed immune based failure of allografts in some patient's cacntegories was a stimulus for further improvement and the development of a more promising "product," a regenerative cell-free valve.*

INTRODUCTION

The pioneering works in the clinical application of the human heart valves (homograft, allograft) were carried on by Gordon Murray in 1956 in Toronto[1]; he implanted the first aortic valve in the descending thoracic aorta for treatment of the diseased aortic valve. He was followed by Donald Ross (in London, UK) and Brian Barratt Boyes (in Auckland, N. Zealand) who in 1962 – almost at the same time, independently of each other – performed the orthotopic implantation of a cadaveric aortic valve. [2-3] The cadaveric valves, before their implantation, were sterilized and stored in an antibiotic cocktail for a few days and up to 6 weeks after being harvested. In 1967, Donald Ross performed another pioneering operation by replacing the diseased aortic valve with the patient's pulmonary valve (*autograft*), and using the cadaveric pulmonary valve (*homograft/allograft*) for the reconstruction of the right ventricular outflow tract (RVOT).[4]

Each year about 2,000 human heart valves (pulmonary, aortic, and occasionally mitral) and some 1,500 vascular allografts (arterial and venous) have been implanted in Europe.[5-7] *The main indications for the use of heart valves* are the congenital valve malformation of newborns and children, young females in reproductive age, athletes and patients with contraindication for use of anticoagulant drugs (this treatment is needed when mechanical valves are implanted in patients), native or prosthetic valve endocarditis with annular abscesses, as well as patients with thromboembolic complication and bleeding risk after valve surgery. *The main indications for the use of vascular allografts* are infection of vascular prosthetic grafts, mycotic aneurysms, critical limb ischemia, hypoplastic left heart syndrome (HLHS) in newborn children, treatment of vascular trauma and the reconstruction of the vascular tree in case of malignant infiltration of great vessels.[5] Gross was the first to use human vascular allografts for transplantation when he corrected the aortic coarctation in 1984 and used the first vascular allograft (homograft).[6]

In the late '80s and early '90s, many cardio-vascular tissue establishments (TE) were created in different European Member States (MS). The aim of these TEs was the donor selection for cardio-vascular tissues, procurement, processing, storage and quality control of the cardio-vascular allografts. Clinical application of the quality-controlled human tissues can guarantee viral and bacterial safety for the patients to be treated and it can assure a long durability of these grafts after implantation. Currently, about 4,000 TE, of which 28 are cardio-vascular, are active in the European Union.[7] The European Homograft Bank (EHB) was established in 1988 as an independent international TE with a non-profit character. It has developed an international cooperation network, which involves many donation and implantation centers in the EU and Switzerland.[8] Since April 1, 2020, it became an integrated service of the University Hospital Saint Luc in Brussels as it merged with the Center for Tissue and Cell Therapies, CTTC.

The European Commission and Council, the National Competent Authorities (NCA), and the Tissue Establishments (TE) in mutual collaboration, have approved and distributed a quality system for organ, tissue, and cell transplantation that assures high quality and safety for both the donor and the patient (the receiver of the human tissue grafts).[9] The security, safety, and efficacy procedures are essential for living donors and recipients alike. Their main goal is to evaluate and document the benefit and harm of transplantation for both the donor and recipient. The level of safety, efficacy, and quality of human tissues and cells for human application as a health product of exceptional nature need to be maintained and permanently improved. Accordingly, implementation of the quality system became mandatory for tissue establishments. It includes, in addition to quality assurance, traceability and vigilance with the reporting of serious adverse events and reactions (SAE/R) nationally (NCA) and internationally (EU), and with the WHO (World Health Organization) funded *Notify Library* which presents a global database for medical products of human origin.[10]

For their optimal and appropriate work, the TEs need the appropriate infrastructure and high quality equipment, which is qualified before its implementation, and needs to be maintained and tested on regular basis. The appropriate management is mandatory, including a responsible person (RP/Director) with the appropriate qualifications and experience. In addition, it needs a qualified and appropriately trained quality responsible person, who collaborates closely with the TE Director. A sufficient number of technical and administrative personnel are necessary, and they need appropriate education and regular trainings in the field.[9]

This paper describes the organization and the results of 30 years of operations in the European Homograft Bank (EHB) in Brussels and its cooperation with donation and transplantation centers

in the EU and beyond, with particular emphasis on the quality assurance of human cardio-vascular tissues.

ORGANIZATION AND STRUCTURE

Collaboration with donation and transplantation centers

The tissue establishment (TE) needs a collaboration network, including donation and implantation partners. A large collaboration network is a necessary condition for obtaining a large donor pool, and subsequent availability of a huge range of allografts (diversity of sizes and conduit lengths) for treatment of an important number of patients for emergency and elective health problems. Figure 1 displays the collaboration network of our TE, including the donation and implanting centers. The responsibilities of each party are clearly determined in the contract of collaboration, with designation of responsible persons from each party. They must exercise care for the fluent exchange of information, notification, and resolution of any difficulties that appear between the cooperating partners.

Infrastructure and critical equipment

For optimal evaluation of the donated material preparation ("manufacturing," "processing"), the TE needs a modern facility with a vertical laminar flow, pressure cascade from compartment to compartment, with control of air quality, temperature, and humidity. The air, before entering the Cleanroom, is filtered by means of the HEPA-filters that are installed on the roof of the processing area. A permanent monitoring of the work environment is mandatory (during the rest and while in activity).[11-13] In 2015, EHB has constructed a new facility adapted for its needs; it is in full compliance with the GMP (Good Manufacturing Practices) and GTP (Good Tissue Practices), (14) and it corresponds fully with the cardio-vascular tissue banking regulation (Figure 2). The Cleanroom facility contains different levels (cascade) of sterile environments (Class A/B/C/D). The quality control regarding the environment for tissue processing is carried on regularly, as specified in the TE SOP.[13-14] (Figure 3)

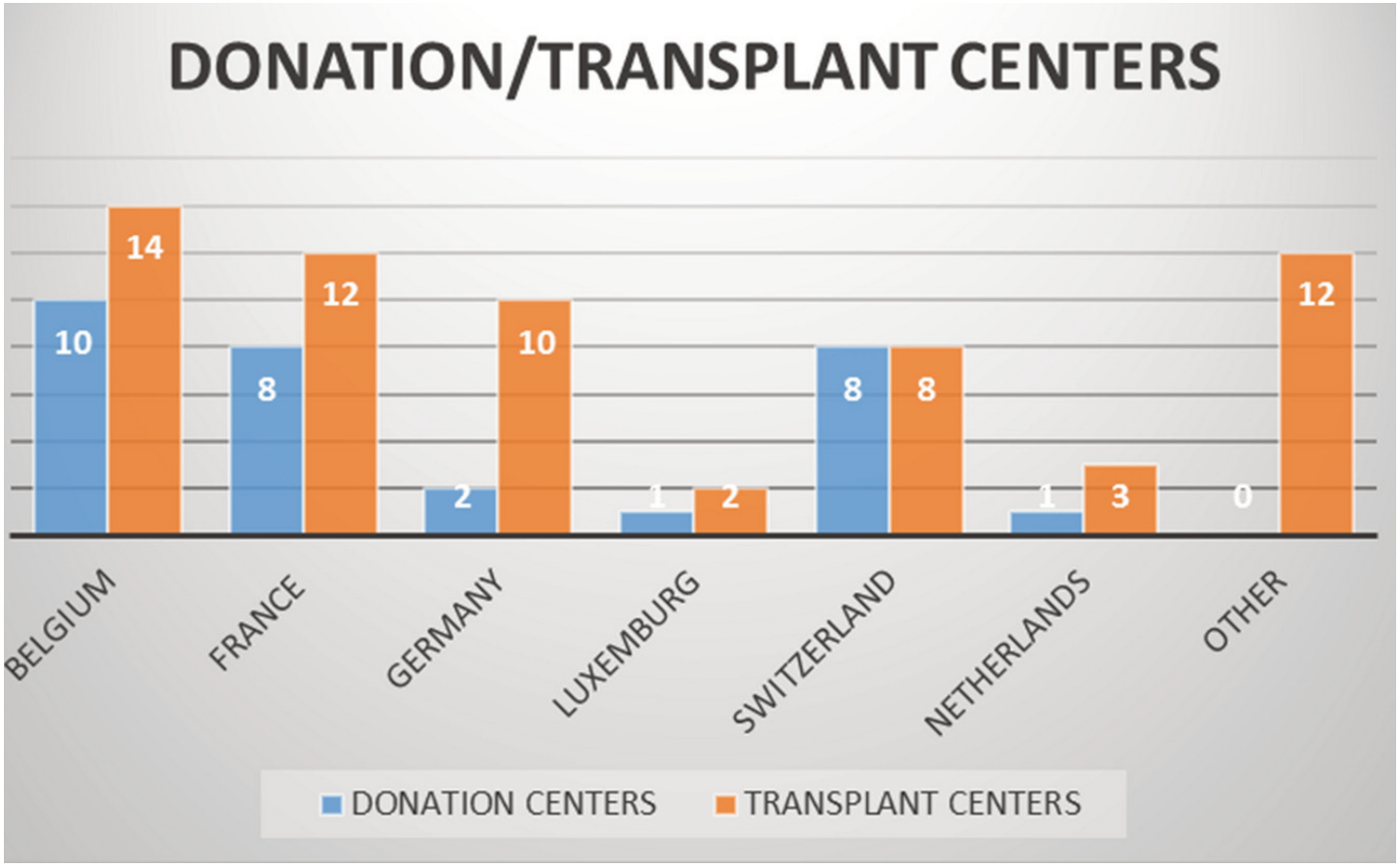

Figure 1. *Donation and implanting centers EHB collaboration network.*

Figure 2. *Cleanroom (GMP/GTP), constructed in accordance with the requirements of the EU Directives, EDQM recommendation, and the Belgian Quality Standards for Tissue Banks (Class A/B/C/D)*

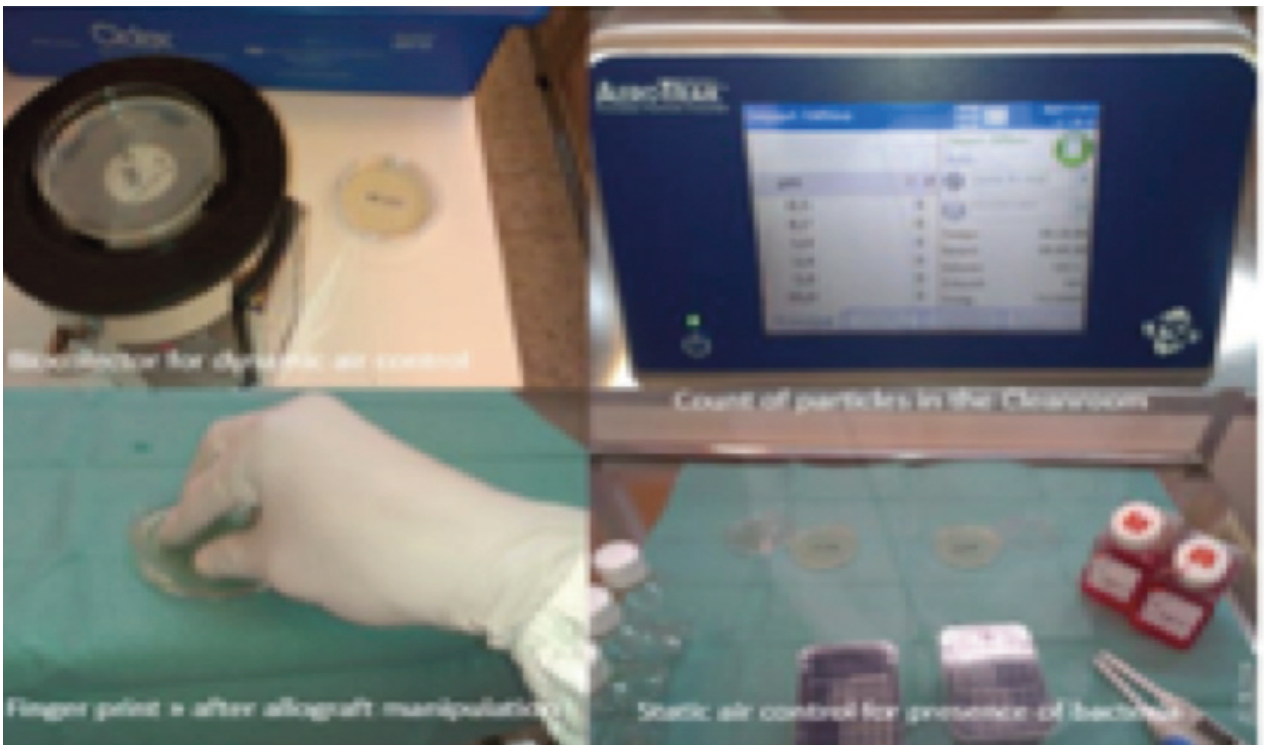

Figure 3. *Monitoring of the Cleanroom (for particles and bacterial contamination (upper images); bacterial control of surgical gloves after tissue manipulation (lower image left) and passive air control (Petri plates, lower image right)*

The TE needs several diverse refrigerators (+4°C, -20°C, -80°C), and a main Cryopreservation and storage infrastructure with a reservoir of liquid nitrogen, cryopreservation devise, and the appropriate number and volume of storage tanks necessary for safe storage of cryopreserved allografts. As to maintain the quality of allografts, the storage conditions have to be constantly monitored in conformity with recommendations and the QMS standard operating procedures (SOP). Supply of the liquid nitrogen must be regular and optimal. The cryopreservation device, *Planer 560/16* (Planer Limited, Middlesex, United Kingdom), a computer guided devise, is of critical importance for our TE (Figure 4). The critical equipment must be validated before its use and regularly maintained. Furthermore, the whole process and equipment need to be covered by the insurance.

Figure 4. *Cryogenic area with storage tanks filled with liquid nitrogen (LN2), containing the stored cardiovascular allografts aimed for clinical application*

DONATION

Donor selection criteria and donor testing

The donor exclusion criteria includes the donor age, clinical and behavioral history (social contact history, contact with toxic substances such as smoking, alcohol, or intravenous drug abuse etc.), travel in some endemic areas (Ebola, Malaria, West Nile virus, Chagas disease, Q-fever, Zika, Dengue, or currently a history of or contact with person in risk of COVID-19, etc.). The complete list of the exclusion criteria for cardiovascular tissue donation is available in Table 1.

For the donor testing for transmissible diseases, blood must be collected either before cardiac arrest or after death (in the last case, the laboratory technique needs to be validated for post - mortem blood testing). In the EU MS, the following tests are obligatory for the clinical application of human tissue grafts: hepatitis B and C, HIV 1&2, HTLV 1&2, Syphilis,

Age	*Morphology of Leaflets & Conduit wall*	*Travel history*	*Malignant Diseases*	*Autoimmune & collagen diseases*	*Risk for viral diseases & Infections*	*Hemodulition risk*	*Other factors Medicament or other treatment*
Valves: *Aortic -Males: >55y Female: >60y Pulmonary Male/Female: >70y*	*Fusion Fibrosis, Atheroma's Calcification Malformation Endocarditis Surgical cut*	***Epidemic/ endemic areas:*** *Zika Ebola WNV Malaria Tuberculosis Q- fever Chagas disease Dengue*	*Solid malignant tumors Blood malignancies* ***Exception :*** *Baseocellular epithelioma or In situ cancer of uterus colon*	*Psoriasis Ulcerous colitis Ankylosing spondylitis Dermatomyositis Polyarteritis nodosa Rheumatoid arthritis Sclerodermia SLE Diabetes Mellitus I Marfan Syndrome*	*AIDS Hepatitis HTLV Syphilis CJD/ vCJD Chagas D. COVID-19 Lyme D. Viral myocarditis Multiple sexual partners Sepsis Septocaemia*	*Blood transfusion of ≥50% of donor's volume last 48h before donation*	*Corticosteroids I.V.drug injection Chemotherapy Other drugs Irradiation Toxic substances: lead, gold,*
Vessels: *Males: >55y Females: >60y*	*Infection, Aneurysm dilation Atheromas Calcification Wall hemathoma Surgical sut*						

Table 1. *Exclusion criteria for cardio-vascular donor of tissues for transplantation consists of AIDS: acquired immune deficiency syndrome; HTLV: human tissue lymphotrophic vitus; vCJD: variant-Creutzfeldt-Jokob Disease; COVID; Coronavirus infectious disease; WNV: West Nile virus; SLE: Systemic Lupus Erythematosus.*

whereas for the living heart donors with dilated cardiomyopathy of unknown etiology, the **Coxsackie viruses** need to be tested as this group of viruses might provoke heart failure. Consequently, all Coxsackie- positive allografts have to be excluded for human application. During the epidemics or pandemics (such as in the case of Q-fever in the Netherlands between 2005 and 2009, endemic of West Nile Virus in Italy and in some other Countries, Ebola in some African Countries, or currently the COVID-19 pandemic) the appropriate clinical and contact history assessment must be established and the best serology tests must be chosen.

Tissue donation, procurement and shipment to the TE

For obtaining the donation of tissues, after confirmation of brain death (donor after brain death, DBD, or donor after cardiac death, DCD), the transplant coordination must verify the donor consent for organ/tissue/cell donation, discussing with the donor (living donor) or family, or consulting the national register, in case of a deceased donor. Only after the verification of the status of consent can the potential donor be considered as suitable for donation. In the EU, the majority of MS have adopted the system *"Opt-out,"* meaning that each citizen is a potential organ, tissue, and cell donor. However, if the person during his life decides not to be a donor, he/she can express his opposition by signing the opposition document at the city hall. Subsequently, this information is registered at the national register. Therefore, when the donor coordination or tissue and cell establishment need this information they have to consult this register. Currently in Belgium, each citizen may decide whether he/she agrees that his/her donated tissues/cells can be used for scientific research as well. The CA has given more possibilities to the citizens for expression of their consent for use of their body parts after death. Some other EU MS

have adopted other systems of donation referred to as the *"opt-in,"* meaning that the citizens need to explicitly express their desire to be a donor. Otherwise, their organs/tissues/cells will not be suitable for transplantation after their death.[15]

Tissue recovery in the donation center is carried on by the qualified persons with sufficient knowledge in the field (medical doctor, cardiac/vascular surgeon or person with a medical/paramedical background who is particularly trained for retrieval of organs and tissues). In case the paramedical personnel is recovering the tissues, his/her work is done under the responsibility of the medical doctor. The TE is obliged to supply the technical instruction (standard operation procedures, SOP) to the procurement teams, as well as the material for packaging and shipment of the donated substances of human origin (SoHO). The donation, procurement, and packaging presents important steps for the TE. Therefore, these instructions need to be updated and improved regularly.[5, 12] The tissue recovery is carried on in the operating theater or in another surgical area with a controlled and monitored environment. Due to the harvesting of cardio-vascular tissues in the morgue, which was a usual practice in the majority of TEs in the 1990s, this resulted in a very high contamination rate of recovered SoHO.[15] The most optimal access to the heart for recovery is a median sternotomy as it allows for very good visualization and access to the entire heart and great vessels (Figure 5).

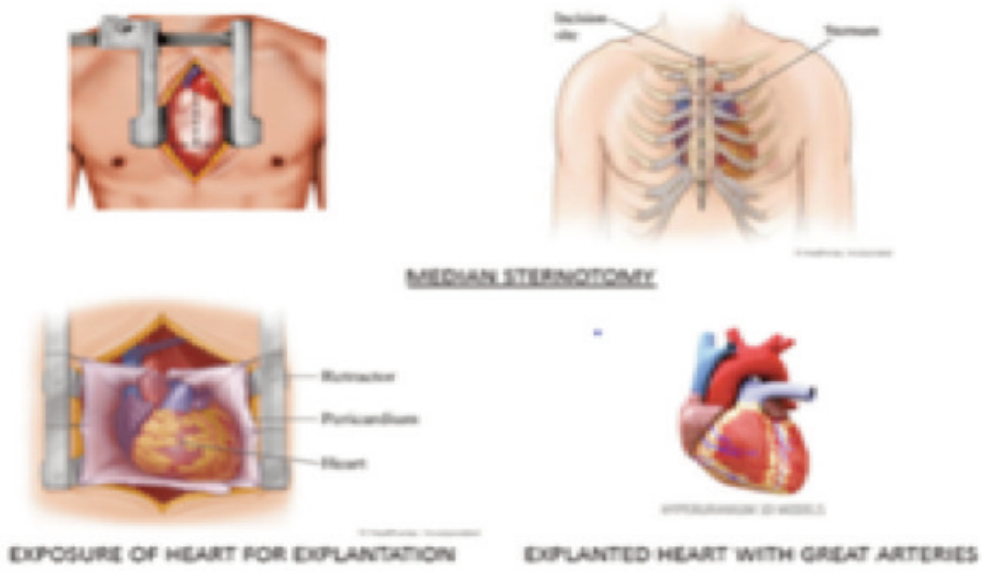

***Figure 5**. Median sternotomy as the best access to the heart and great vessels for harvesting.*

In order to prevent the growth of bacteria, the harvested material has to be rinsed with an isotonic solution in order to remove all blood clots from the tissues. Subsequently, it must be packaged in a sterile bag or pot filled with isotonic solution (saline 0,9%, Ringer, Hartmann, etc.) and placed in a thermo-box of polystyrene, filled with wet ice in order to maintain the cold storage (around +4°C) during shipment to the TE. The shipment has to be completed in the shortest delay; it is best within 24 hours after the recovery or cardiac arrest. Before or during the procedure of recovery, the appropriate volume of blood needs to be collected for the donor serology tests and it needs to join the donated SoHO in the transport box.

Reception and control of the donated SoHO

The person who receives the donated SoHO, must control the conformity of the packaging with instructions sent to the center. If there are multiple donations arriving at the same time, care must be taken for identification, anonymization, and coding of each donor/tissue separately and correctly; this to avoid a mix up of the arriving tissues/documents. The blood samples must be identified, and correctly codified and centrifuged, before being sent to the laboratory of clinical biology for serology testing. All donated material must be carefully handled and

temporarily preserved in a cold solution until the beginning of processing in the Cleanroom. Before being sent to the laboratory, blood samples must be stored in the appropriate temperature conditions. It is preferable to keep a certain volume of plasma or serum in a serotheque for eventual later re-test, if necessary.

TISSUE PROCESSING IN THE TE

As the ischemic time for cardio-vascular tissues is limited for preservation of their quality, the processing needs to start as soon as possible; it is best within 24 hours after cardiac arrest. Dissection and evaluation of morphology and function is carried on in the Cleanroom class A, and background Class B/C, by a qualified person (cardiac surgeon, vascular surgeon, other medical doctor or other trained person). Some activities can be performed by the technical assistants (if they are trained for that), under the responsibility and observation of the TE Director.

The dissection of the heart, separation of aortic and pulmonary valves, and eventually the mitral valve, evaluation of morphology and function, and measurement of the size and length of the vascular conduit are part of the assessment of valves/vessels, before their (provisory) acceptance for clinical application and preservation. The proximal and distal diameter of the valve, as well as the length of the conduit, are measured and notified for implanting surgeons (Figure 6). Also, the morphology of valves or vessels needs to be mentioned via a drawing of the allograft. Even the very small atheroma's or calcifications that might be in the non-important parts of the allograft need to be mentioned for the implanting surgeon.

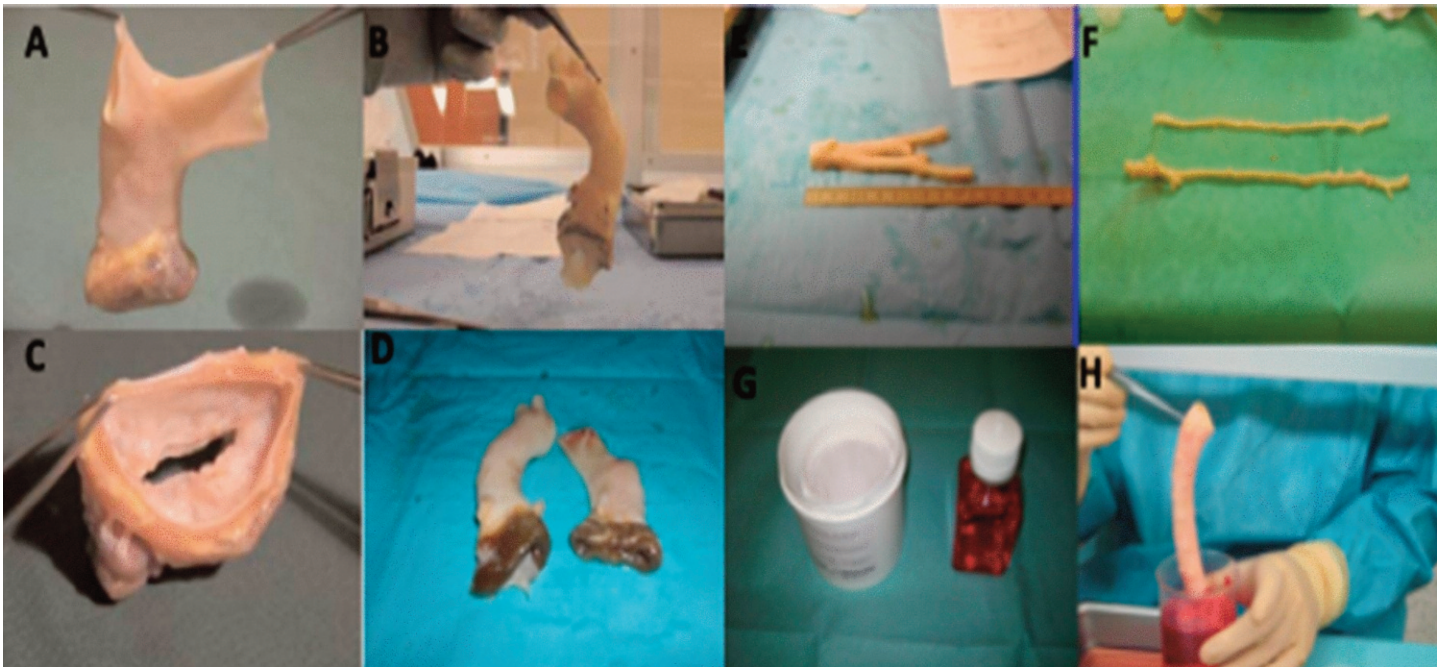

Figure 6. *Heart valves and vascular allografts after dissection and evaluation of morphology and function. A: Pulmonary valve with whole conduit; B: Aortic valve with ascending aorta and part of arch; C: Mitral valve; D: Aortic and pulmonary valve from same donor heart; E: aortic bifurcation with iliac arteries; F: two superficial femoral arteries; G: antibiotic cocktail for decontamination; and H: thoracic aorta after decontamination (ready for cryopreservation).*

After evaluation of the morphology, the allografts are immersed in an antibiotic cocktail for decontamination during 40-48 hours at +4°C. Prior to their immersion in antibiotics, the histology and bacteriology samples are collected for quality testing. Histologically, the microscopic structure (cells, collagen matrix) of the allograft have to be assessed, and the infection and malignancy excluded, whereas the bacteriology tests have to exclude the presence of any contaminant strains in the tissues before their distribution for clinical application. Bacteriology tests are performed before and after incubation in antibiotics. For decontamination purposes, we use an antibiotic cocktail composed of Vancomycin,

Lincomycin and Polymyxin B (low concentration in low temperature), seen in Figure 6.[16-17]

Following decontamination, the allograft is placed in a cryopreservation solution (10% dimethyl sulfoxide in RPMI [Roswell Park Memorial Institute, USA]), and double packaged for cryopreservation. Cryopreservation is carried on in a Planer 560-16 (Planer Limited, Middlesex, United Kingdom) with a computer-controlled rate of freezing (Figure 7). As soon as the cryopreservation is completed (about 2 hours), the allograft is moved to the storage tank in vapors of liquid nitrogen. The shelf life of cryopreserved allografts is 5 years (recommended by the EU Directive 2004/23/EC).[18]

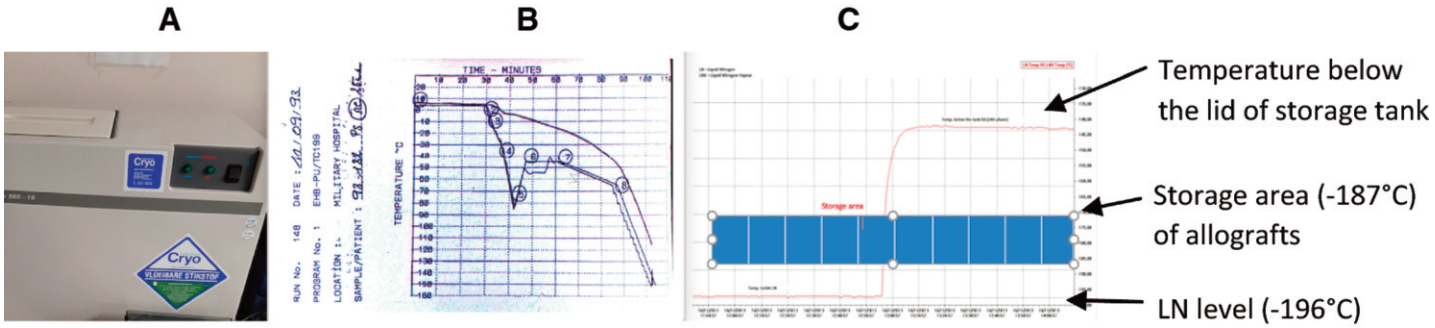

Figure 7. *Cryopreservation and storage of allografts: A: Planner 560-16; B: Cryopreservation curve; C: Storage tanks and storage temperature.*

QUALITY CONTROL AND RELEASE OF ALLOGRAFTS FOR CLINICAL APPLICATION

The Director (and tissue manager) of the TE is legally responsible for the entire process, from donation to the implantation of allografts. For release of an allograft, the following criteria need to be met:

-Donor clinical and behavioral history must be acceptable and cleared.
-Serological tests must show freedom of any viral infection of the donor.
-Morphology and function of the allograft must be within the specification.
-Histology must show normal infrastructure of allograft (extracellular matrix), and no presence of infection and no malignancy (Figure 8).
-At the final testing, the allograft must be free of any contaminant strain.
-Cryopreservation must be acceptable as foreseen with the SOP.
-Storage conditions (temperature, environment) must be within specification.

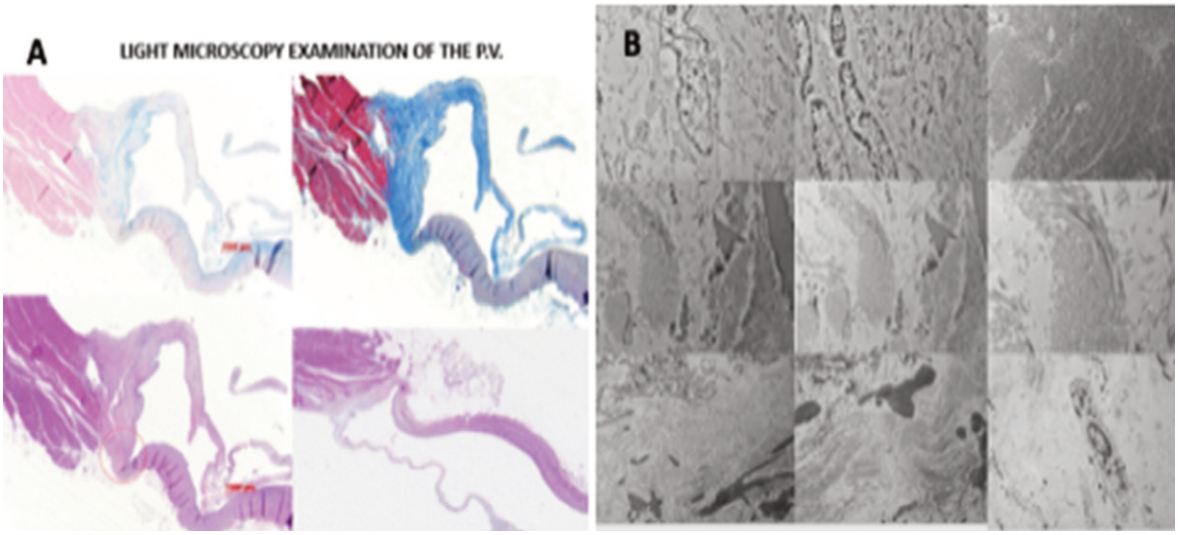

Figure 8. *A: Light microscopic evaluation of the pulmonary valve after thawing showing preserved structure; B: Transmission electron microscopy showing perfectly preserved and intact collagen matrix as well as cellular elements.*

VALIDATION OF THE PROCEDURES WITHIN THE TISSUE ESTABLISHMENT

A diversity of procedures for quality assurance that have been used in the TE were implemented empirically (from their own experience or the experience of other TEs), or relied on the advice of experts in the field (pathologist, clinical biologist, etc.). The initial tissue banking activity followed the organ donation regulation (in Belgium, the law of 1986 regarding the organ transplantation).[18] However, in 2004 the European Commission, in cooperation with experts in the field from the EU MS, proposed and adopted the Tissue Directives: the Directive 2004/23/EC regarding the quality standards on selection, procurement, processing, preservation, storage, and distribution of human tissues aimed for human application. In 2006, two new technical directives were endorsed by the EC and Parliament regarding the technical requirements and notification of serious adverse events and reaction (SAE/R) provoked by human tissues used for human application.[19-21] Accordingly, the tissue banking activity has been separated from the organ transplantation domain. As the organs are considered as *"life-saving"* and the tissues as *"life-improving,"* following the new regulation for tissues, the tissue banking has to respect the principle of *"no risk"* for the patient, in contrast to the organ regulation that has to follow the principle of a *"minimal risk."* Consequently, the donor testing for tissues has now become stricter than that of organs.

Following the new tissue banking regulation, the TEs in Belgium are under the authority of the Federal Agency for Medicaments and Health Products, including donation, processing, storage and distribution of SoHO (FAMHP, *Law of 19 December 2008: "Law on obtaining and use of human body substance with aim of human application and research"*).

Our TE, in accordance with the Belgian and European Tissue Banking regulation, has validated a diversity of procedures (Cleanroom facility, antibiotic cocktail, presence of residual antibiotics or residual cryopreservation solution [DMSO]) in allografts. In the past, some of these procedures have been published in various international scientific journals.[23-25]

RESULTS

Donation

The first donated hearts arrived in our TE in 1989, and the first vascular tissues in 1991.[12] Over the last 30 years, *7.805 hearts* and *2.517 batches of vascular tissues* were recovered and sent to our TE from the donation network in Belgium, France, Luxemburg, Netherlands, Germany, and Switzerland. Donation activity increased significantly over time (2176, 2567, and 3062 hearts and 462, 939, and 1116 vascular tissues) were donated and recovered by our donation network during the respective first, second, and third decade of activity) (Figure 9).

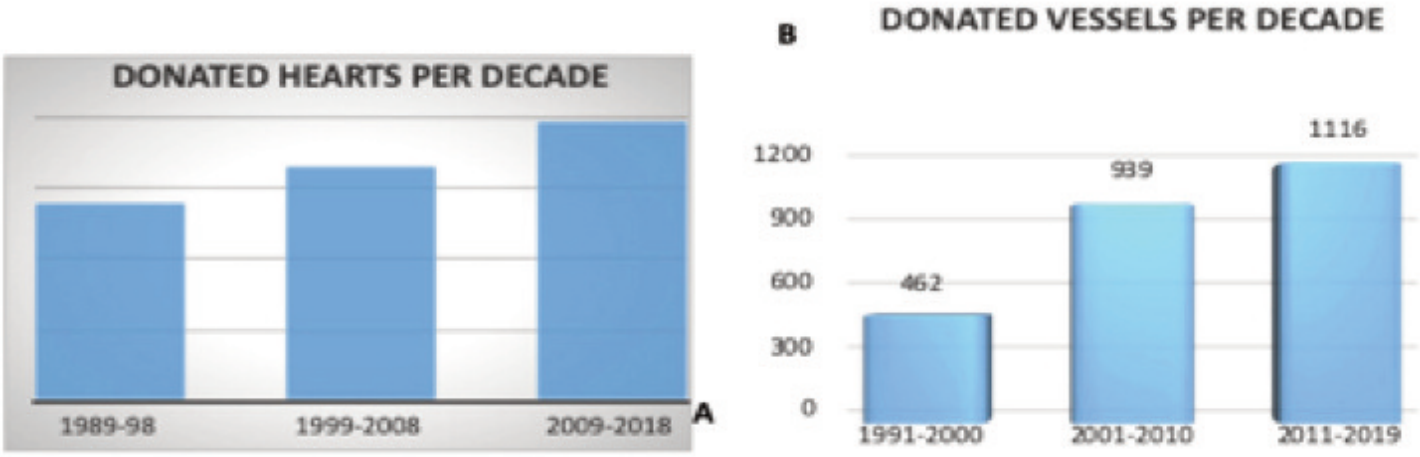

Figure 9. *Donations per decade (A: hearts; B: batches of vessels)*

Results of processing and control

Following the quality control, about 62% of heart valves and about 28% of vascular allografts, are discarded each year for different reasons (Figure 10).

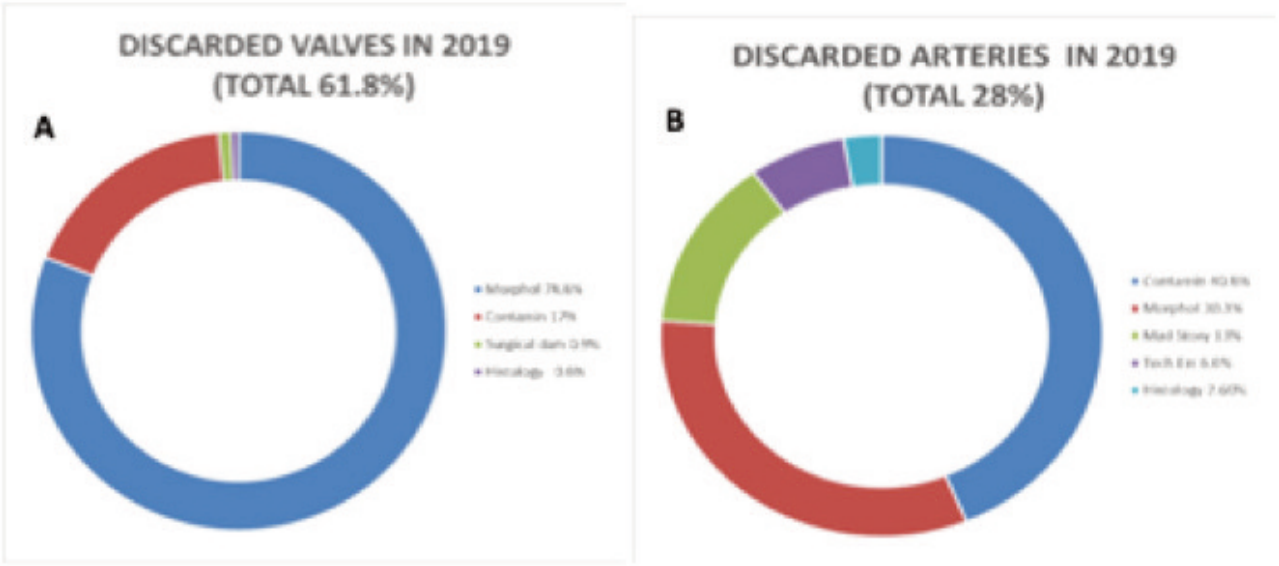

Figure 10. *Discarded allografts and reasons. A: valves, discarded for morphology 79%, and 17% for contamination; B: vessels, discarded for contamination 41%, and 30% for morphology.*

Distribution and implantation

In over 30 years of banking activity in Brussels, the EHB has distributed 7,599 heart valves (Pulmonary, Aortic, Mitral) for implantation, and 4,199 vascular allografts (ascending and descending thoracic aorta, aortic bifurcation, iliac and femoral arteries, venous grafts) to the vast implantation network in Europe and beyond. The donations and the implantation activity increased significantly between the first, second, and third decades (2050, 2550, and 2691 valves and 540, 1397, and 1997 vascular allografts were implanted during first, second, and third decades respectively) in a huge hospital network within the EU and beyond (Figure 11 and Figures 12A&12B).

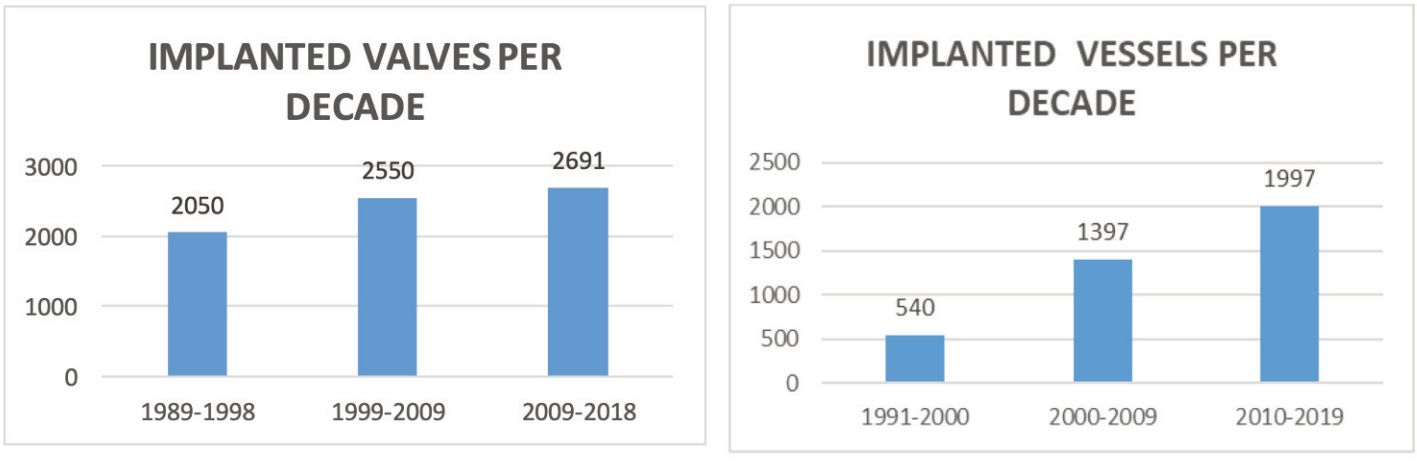

Figure 11. *Implantations (Valves/vessels) per decade*

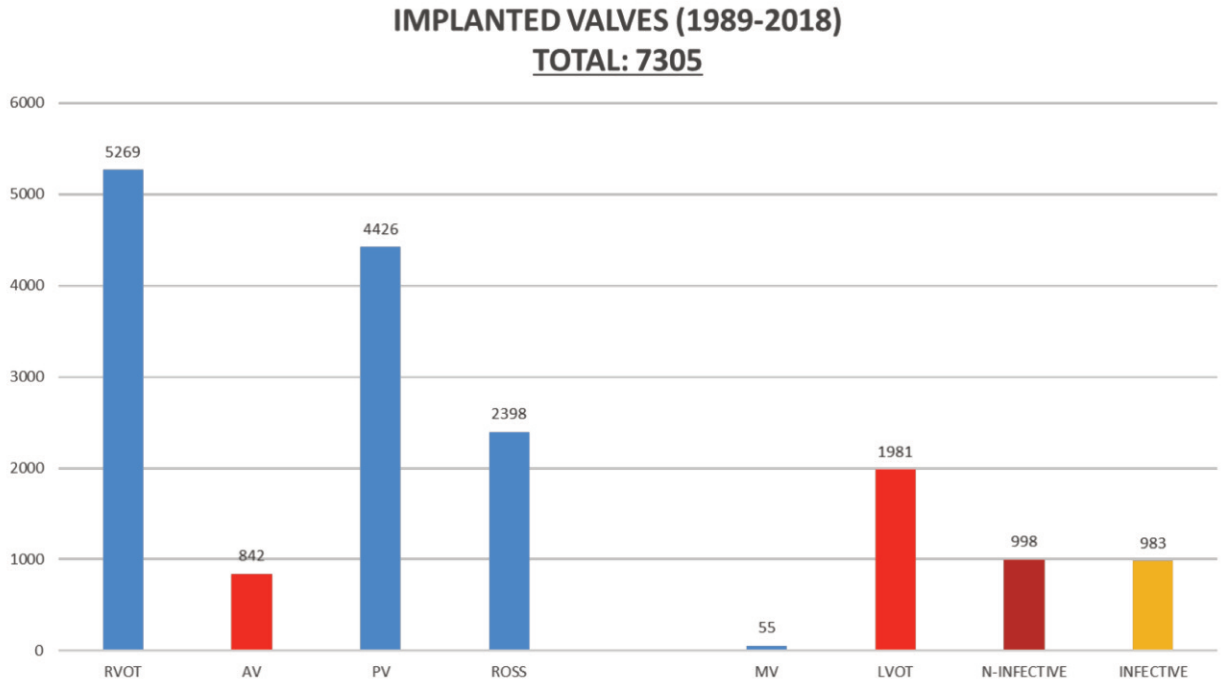

Figure 12A. *Implantation of heart valves (7.305) per position and indication. RVOT: right ventricular outflow tract; LVOT: left ventricular outflow tract; AV: aortic valve; PV: pulmonary valve; Ross: operation following Donald Ross, using pulmonary autograft for replacement of diseased aortic valve and reconstruction of RVOT with allograft/homograft; MV: mitral valve; N-infective: indication for treatment of diseased valve; Infective: treatment of valve endocarditis.*

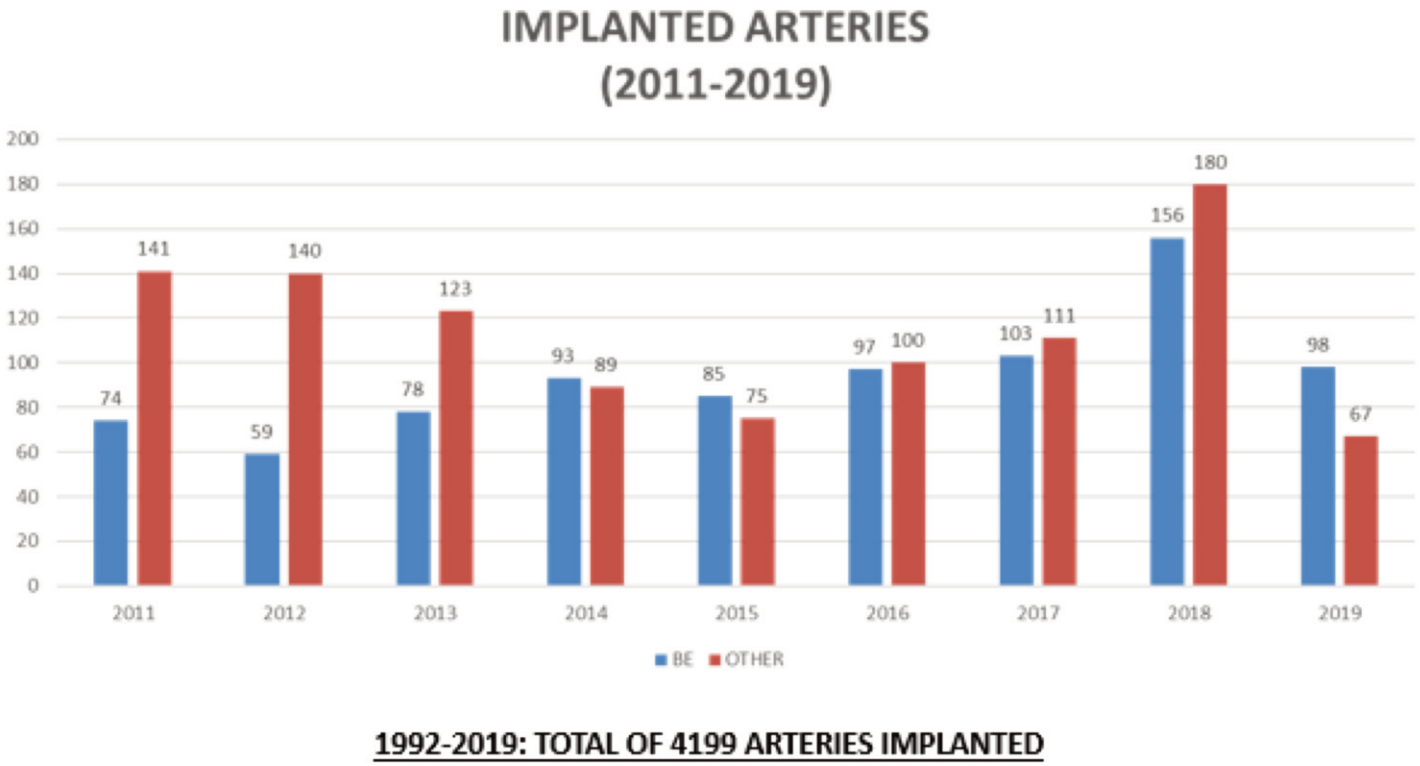

Figure 12B. *Implantations of vascular allografts (Belgium vs. abroad). A total of 4,199 vascular allografts were distributed until December 31, 2019.*

During last 5 years, we have also received some venous grafts, mainly recovered by the abdominal transplant surgeons (liver, pancreas), as from time to time they need an arterial or venous allograft for the reconstruction of the hepatic vascular tree (vascular thrombosis after liver transplantation or for reconstruction of hepatic and pancreatic vascular tree due to malignant infiltration) (Figure 13).

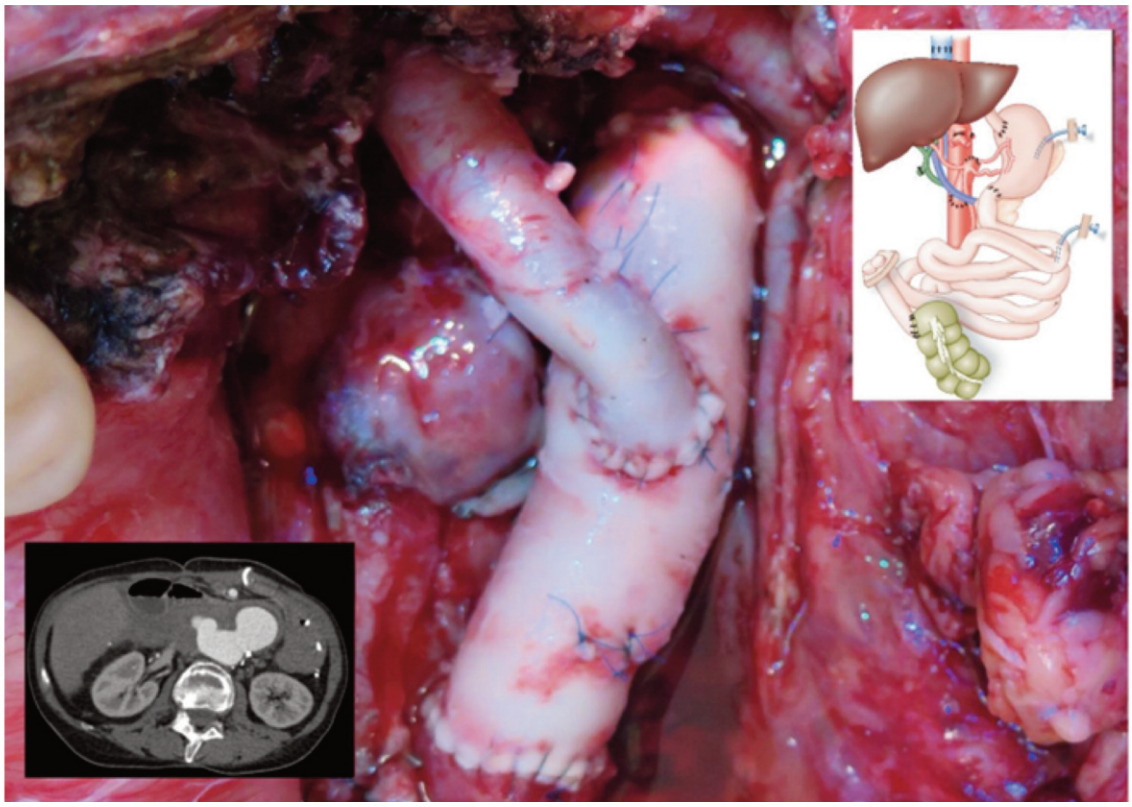

Figure 13. *Composite graft created of two vascular allografts (thoracic aorta and iliac artery) used for aorto-iliac reconstruction after infra-renal aortic resection for malignant infiltration.*

The cardio-vascular allografts are *allocated* on basis of the medical indication and state of emergency, always after the approval of the implanting surgeon, who has the final responsibility for clinical application of allograft. After receiving the necessary information regarding the clinical indication for use of the allograft, the Director of the TE proposes to the implanting surgeon the best available allograft for that case. The implanting surgeon receives from the TE a description of the allograft (drawing) indicating all specification. In the same document, the donor information and all quality test results are indicated in a "release statement," which is signed by the TE Director. If the proposed allograft suites the surgeon, he signs and sends this document for confirmation of the acceptance of the allograft, indicating the date of implantation and the hour of delivery. The TE is in charge of shipment and guarantees its safety until the delivery to the implanting center (while monitoring and maintaining a permanent temperature and avoiding any shock). The implanting surgeon indicates the place of delivery and, eventually, the person who will receive it. The shipment to the implanting centers is carried on either in the Dry Shipper (DS), at a temperature below – 130°C, or in Dry Ice (DI) at -76°C (Figure 14). The allografts shipped in DS can be accepted back if they are not used and the DS is intact (with temperatures that remain below -130°C). However, if the DS was opened, or if there was a temperature deviation, the allograft may no longer be stored in the storage tank with LN, and must be destroyed by incineration. Each year about 0.5 -1% of distributed allografts must be destroyed due to the non-conforming temperatures (DS open by personnel in the OR, or failure of monitoring). Along with the allograft, the implanting surgeons receive all results of testing, as well as the thawing and dilution instructions. The person who carries on this procedure is obligated to read the instructions before thawing.

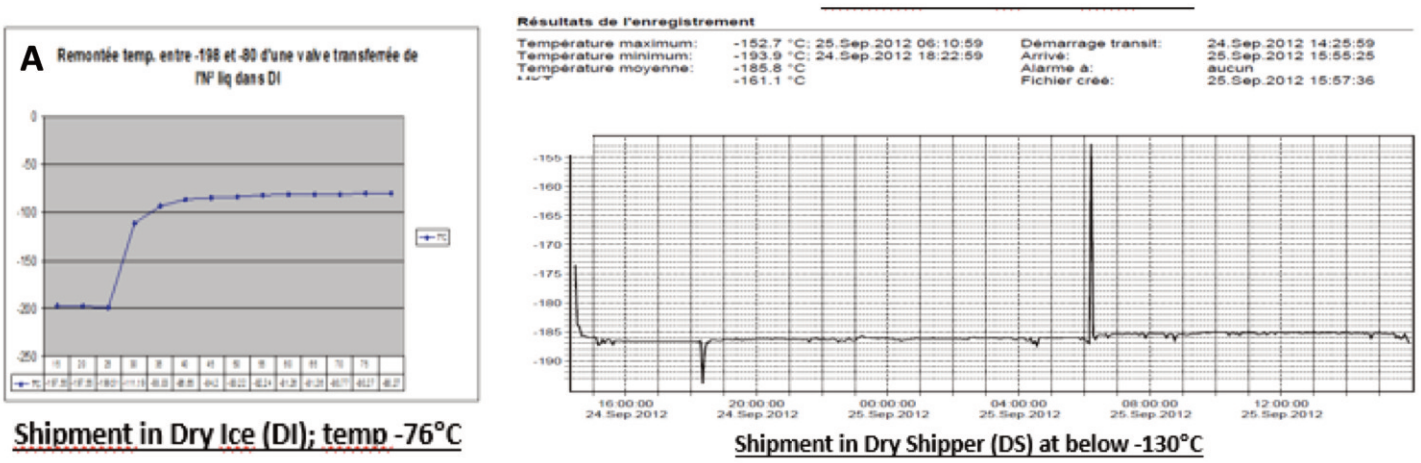

Figure 14. *Monitoring of temperature during shipment of allografts (A: dry ice, temperature -76°C; B: DS, temperature below -130°C).*

In case of shipment in DI, the allograft may not return to the TE for further storage, as the temperature of -76 is above the critical temperature (-123°C) when the micro-crystals might be generated in the allografts matrix and provoke irreparable damages *("fractures" or "cracks")* of collagen. Due to fragilization with ice crystals, the risk of immediate rupture after implantation or premature calcification of the allograft is important. If it is not implanted and not thawed, the cryopreserved allograft may be stored at the implanting center for a short duration (one to a maximum three months) in deep freezer at -80°C.

Together with the allograft, the TE sends the implanting center an accompanying record of the donor/allograft, which contains:

-Release statement including donor information (age, cause of death, explantation date and hour, processing data, specification of allograft, quality test results (bacteriology, histology, serology, and cryopreservation result) and the description of morphology (along with an allograft drawing).

-Thawing and DMSO dilution protocol for the implanting surgeon.

-Notification of any important non-conform situation that surgeon needs to know regarding the allograft.

Traceability of implantation, discharge and follow up information of implanted allografts

After implantation, the surgeon is requested to fill up and send the TE the document *"Traceability sheet,"* which confirms implantation, but also gives his expert opinion about the quality of the allograft. Thus far, 99% of these documents are sent back on the day of surgery or during the week of implantation. Later on, the surgeons are requested to send the second document, *"Discharge form,"* which is received in about 45-50% of all implantations. The information requested by means of this document concerns early performance of the allograft (at the moment of discharge of the patient). This document is not legally requested by the CA.

Regarding the long-term outcome, so far there is no legal obligation for TEs to have this information in their possession. Therefore, the F-U data are not requested as mandatory from the implanting surgeons. However, multiple studies (individual or multi-centric) have been carried on regarding the results of implanted valves and arteries, and show early-, mid-, or long-term outcomes.[26-32] These studies have been carried on in close cooperation between EHB and the implanting institutions (surgical teams).

Financial aspects of the tissue banking

Clinical application of human tissues in EU Countries has been a standard procedure for decades. Use of human heart valves, and/or vascular allografts, is considered as a *"golden standard"* for some procedures. Hence, the Competent Authorities and health care insurances have listed the human cardiac and vascular allografts among the substances that are routinely used for some surgical procedures. Accordingly, they are reimbursed 100% by healthcare insurance. The prizes are fixed and controlled by the CA and follow the national healthcare index.

Long-term outcome of implanted heart valves and vascular allografts

Despite the fact that we are missing important F.U. data of the implanted allografts, many studies have been carried on in cooperation between our TE and implanting centers.[26-32] A study about the reconstruction of RVOT with a pulmonary valve, which we performed with one of our partners and published in 2005, conveyed an important difference in long-term results between new born and very young children (0-5 years) and the adult patients (Figure 15).[26] Similarly, a study carried on with another partner and published in 2019 presented a better outcome for the pulmonary valves in cases with a matching ABO group between the patient and the donor, as compared to the non-matching cases (Figure 16).[32]

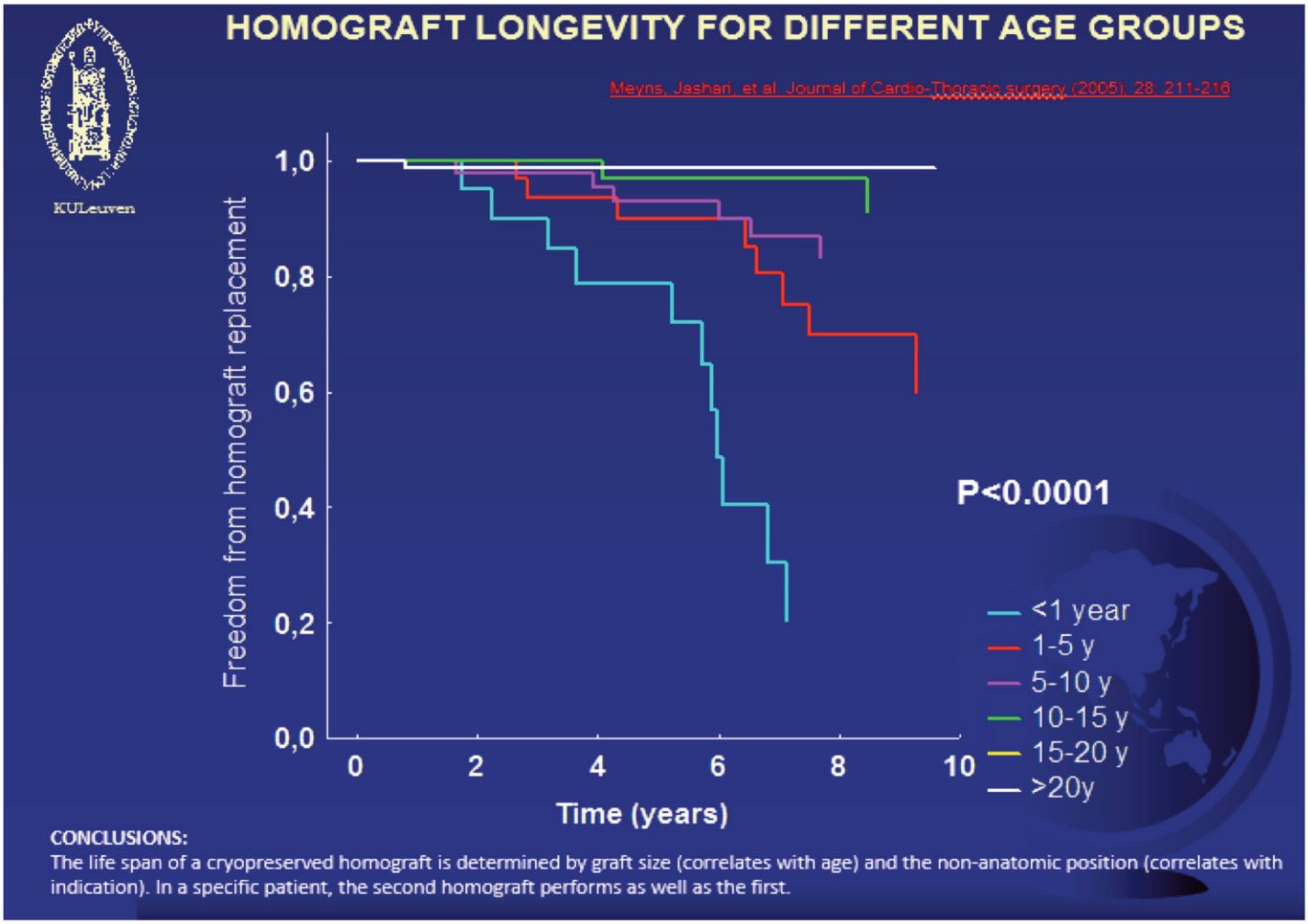

Figure 15. *RVOT reconstruction with pulmonary valve allografts shows excellent outcome if implanted in adult patients, but the valve deteriorates rapidly if it is implanted in newborn or young patients.*[25]

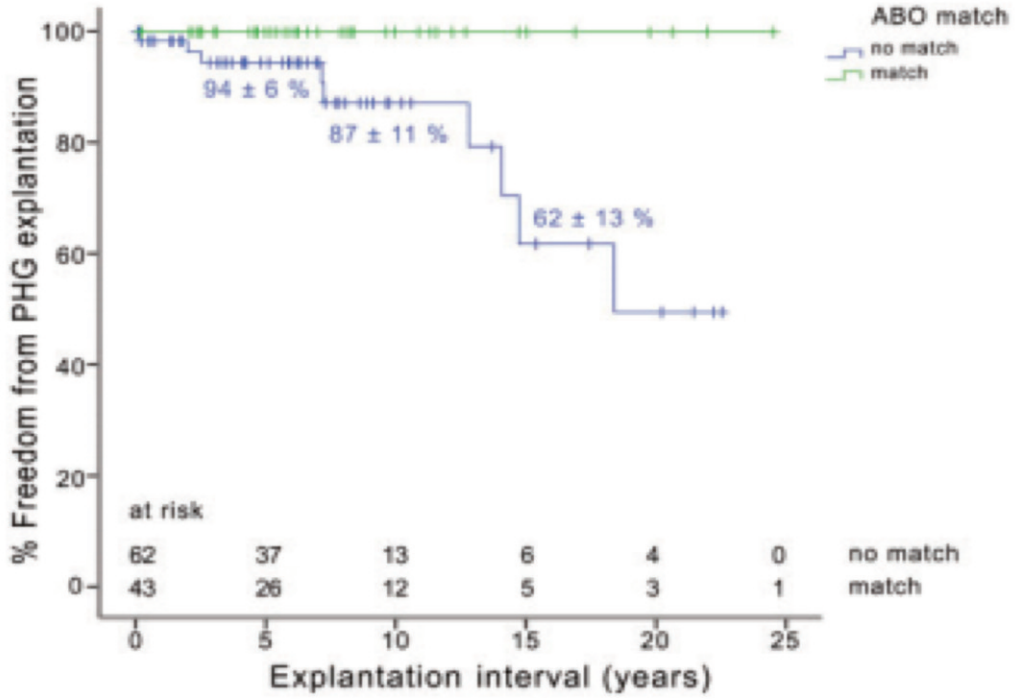

Figure 16. *The long-term outcome of PV in RVOT: excellent result in the ABO matched patients with a faster deterioration the non-matched cases.*

SERIOUS ADVERSE EVENTS AND REACTIONS (SAE/R)

In the last 30 years, some SAE/R appeared and they were detected either in the TE or in the implanting centers. They are listed in the Table 2.

SAE/SAR	VLV	AA	COMMENTS & CONSEQUENCES
Internal pouch taken in the sealing line of external pouch	*1*	*1*	*Important difficulties during opening of allograft at implanting center with contamination risk. Reported to EHB as SAE; no consequences for patient*
Interruption of cryopreservation	*1*		*Cryopreservation interrupted at -100°C. Problem appeared due to low level of LN in the Ranger (feeding of Planner). Allografts discarded*
Allograft fallen on the ground during preparation for shipment	*/*	*1*	*One NPC: allograft discarded and eliminated from the stock; no direct consequences for patient*

Allograft lost in storage tank	*2*	*3*	*Allografts fallen in the liquid nitrogen (behind storage racks); allografts found during disinfection of the storage tank; no direct consequences for patients*
Return to stock: not indicated position in storage tank	*1*	*3*	***4 cases (1 valve, 3 arteries)** "lost" in the storage tank after returning from implanting center. Technician didn't indicate location. In case of emergency, impossible to find allograft (usually can be found, as it is placed in a tank, but takes lot of time and energy)*
Storage tank submerged with LN	*25*	*14*	*Due to a technical defect, the valve of the automatic filling system didn't stop and the tank continued filling until the morning. Despite the fact that histology examination of some valves/arteries of this group didn't show any damaging effect, all 39 allografts were discarded (decision of Board of EHB). Insurance covered 90% of financial loss*
Mix up of distributed valves	*2x*	*/*	***1st case: 2 PV** with approximately same diameters sent to two Belgian Hospitals. Surgeon of one center discovered it and didn't use the received allograft. In emergency 2nd valve was sent and successfully implanted. Surgeon from the other center successfully implanted valve he received (specification almost same as requested valve by him)* ***2nd case:** AV send to the center that ordered the PV and opposite: discovered in one of centers before starting surgery. Both operations re-scheduled and one (aortic) valve eliminated, the other one returned back to stock*
Cracks in allograft	*2*		*Valves were not correctly thawed (EHB thawing protocol was not respected); surgeon called to EHB and was instructed not to use allograft; implanted bioprosthetic valve in both cases*
Death	*/*	*2*	***Descending thoracic aortic allografts**: bad thawing technique (during **first year** of implantations), massive bleeding due to the rupture of allograft*
Acute, non-fatal bleeding	*3*	*5*	*8 cases, re-intervention; either sutured bleeding place or replacement by 2nd allografts/bioprosthesis*
Infection	*2*	*3*	***2 PV**: valve endocarditis; replacement with new allograft; both patients survived infection* ***1 DA**: aorto-intestinal fistula- replacement of allograft with new allograft of thoracic aorta* ***1 IA** infection at the level of distal anastomosis due to liquid collection: evacuation of liquid, antibiotics* ***1 FA** (femoro-femoral cross over): infection and loss of one anastomosis; replacement of allograft with new femoral arterial allograft*

Control of sample from valves after thawing	*3*		***3 PV consecutively implanted in the same center within one month:** 3 consecutive valves tested positive (1x staphylococcus aureus; 1x aspergillus and 1x candida); clinically patients did well, no signs of infection, echocardiography was normal; investigation revealed work around the lab that might have provoked contamination in the lab*
Acute rejection	*/*	*2*	***2FA in implanted in a young patient** for treatment of venous thrombosis after replacement of infected prosthetic graft: severe multiple aneurysms after only one month of implantation; replacement of arteries; immune-histochemical control revealed acute rejection*
Cancer transmission	*/*	*/*	*No reports*
Sero-conversion of patient	*/*	*/*	*No reports*

***Table 2.** SAE/R detected either in the TE or at implanting centers. LN: liquid nitrogen; NPC: non-valved pulmonary conduit; PV: pulmonary valve; DA: descending thoracic aorta; IA: iliac artery, FA: femoral artery.*

CURRENT AND FUTURE DEVELOPMENTS IN THE HUMAN HEART VALVE BANKING

Some studies have shown accelerated deterioration of cryopreserved human heart valves after implantation in young patients and newborn children and point out the immune components as the most important reasons.[26, 29, 32, 33, 34] Hence, some researchers have suggested decellularization and the use of cell-free allografts as a possible solution.[35] Following the preliminary results of implantation of these valves in the animal model by the Hannover Medical School, the EC has approved and financed the European Clinical Trial (**ESPOIR**), involving eight EU centers. This study aimed to explore the implantation of *cell-free pulmonary allograft* in the RVOT of the patients with congenital valve malformation.[36] The short to midterm results of this multi-centric trial exhibited excellent performance of these allografts.[37]

Between 2015 and 2017, another multi-centric trial was carried on and finalized using *cell-free aortic valves* for replacement of diseased aortic valves in children and young patients (**ARISE** Study).[38] Again, the preliminary results of this trial were also promising.[39] Another group (da Costa et al. from Brazil) have also implanted cell-free aortic valves by using another decellularization protocol, and displayed promising early to midterm results.[39]

EHB played an important role in these two studies, as it was the main supplier of the pulmonary valves (ESPOIR) and aortic valves (ARISE) for the patients included in these clinical trials.

DISCUSSION AND CONCLUSION

Human heart valves and blood vessels were available for many surgeons for almost 60 years with excellent results in some patients and with moderate to poor results in various other categories.[26, 32] Although these valves are considered a *"golden standard"* for the replacement of diseased valves, not every surgeon has the "privilege" of having available allografts supplied by the TE of their own center. Accordingly, many patients and surgeons have to accept less ideal solutions (i.e. a mechanical valve with obligatory anticoagulation for the rest of the patient's life, or xenograft, which has an inferior long-term durability compared to allograft).

In the majority of cases, the TEs function as a *"multi-tissue bank"* and prioritize the other tissues (cornea, skin, bone) that need less complicated procedures for their processing than heart valves, which require a cardiac or vascular surgeon needed in the TE. Usually, these banks have a limited number of heart donations and number of distributed valves

(Figure 17). Among the participants of the study presented in this paper concerning the differences among the TEs, only our TE had more than 300 hearts donated in 2015, with two other "big" banks having received 200 and 150 hearts, respectively. All other TEs received less than 100 hearts.[11]

Our TE is organized as a *heart valve and vascular TE* with an important network of donation and transplantation. This situation gave us the opportunity to have a large diversity of donors, ranging from children to adults, with the possibility of creating of a large allograft pool. Consequently, the availability and diversity of valves make our TE an important asset as compared to other TEs in the EU.[5, 8, 12, 16]

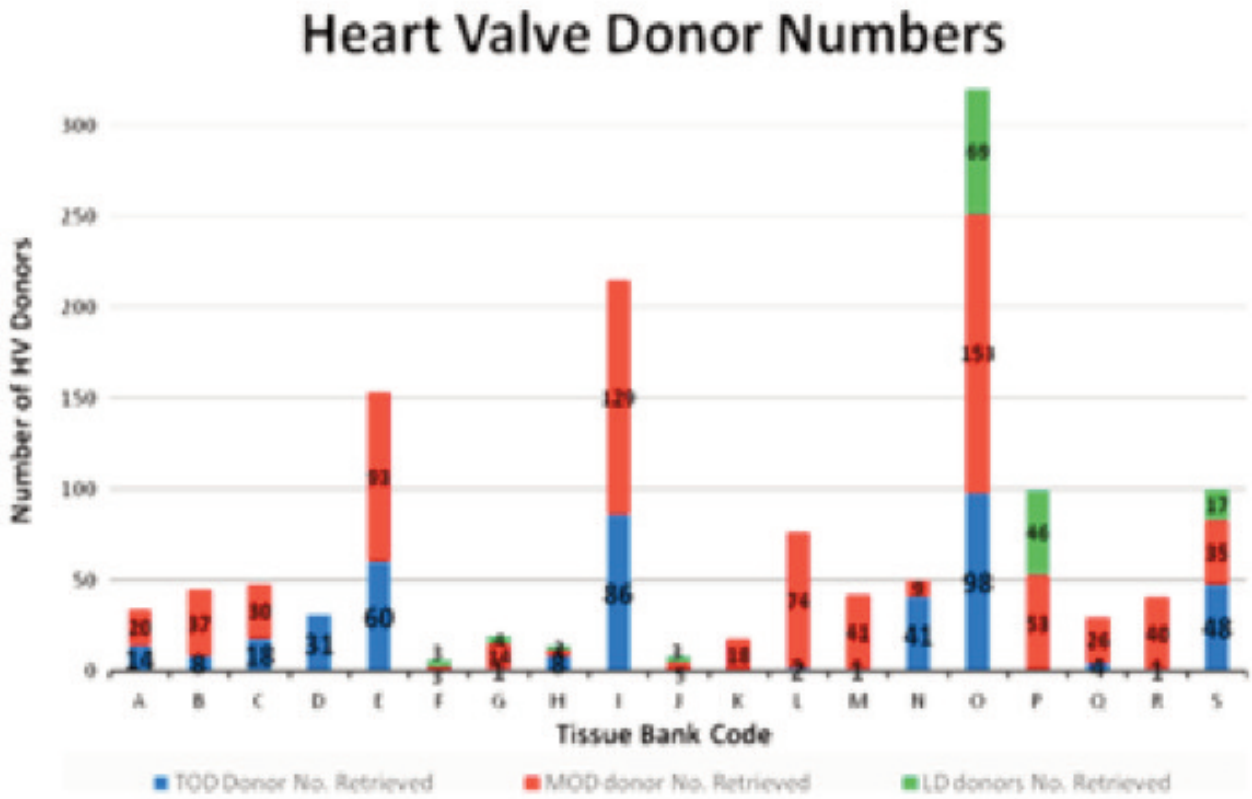

Figure 17. *The number of donated hearts for valves in 19 EU TE. Only 2 TEs had more than 200 hearts in 2015.[11]*

Another important factor of the scarcity of allogenic valves and vessels is the significant number of discarded valves and vessels during processing and quality control (in our TE 62% of evaluated heart valves and 29% of vascular allografts were discarded in 2019). The most important reasons are the morphologic alterations of valves (donors of advanced age). Furthermore, despite very important measures for eradication of infection, it remains a very important challenge for tissue bankers (in our TE, contamination was the reason for discarding respective 17% and 40% of the valves and arteries), (Figure 10). Practically, all TEs were confronted with this issue, either due to a massive contamination during the recovery in the donation centers; due to insufficient decontamination with antibiotics, such as a bad choice and combination of antibiotics; or due to inappropriate work of the laboratory of clinical biology.[41] Accordingly, the European cardio-vascular tissue banking working group has initiated a project for establishing a *regular external quality assessment scheme for cardiovascular TEs at the European level*, with the aim of standardizing the tissue banking procedures and improving the clinical safety of tissue products.

The European Homograft Bank has invested lot of energy in permanent amelioration of the quality, and for working in different ways. *First of all*, we have *improved relations with the donation and procurement centers* (after discussions to reduce repeated mistakes, looking for appropriate solutions, and increasing the vigilance of concerning persons). Avoiding the repeated occurrence of mistakes *was the main goal*. Transparency towards the donation and recovery center (update of the final outcome of donated tissues: discard [and the reason why], accept, implanted or

used for validation purposes or research) has increased the confidence and respect between EHB and its partners. *Secondly,* an important aspect in our work was investing in the *validation of the procedures used in our TE*[17, 23-25], which allowed us to better understand why some of the procedures were performed a specific way after inheriting these procedures from the previous management of the TE. We were also able to demonstrate that techniques and procedures used by our TE can guarantee the safety and security of the patients. *Thirdly*, we have *increased the availability* of highly qualified personnel, including cardiac surgeons (with substantial knowledge of surgical pathology) and biologists that are available 24 hours a day. This has reassured the surgical teams about the quality of our services and determination of our personnel. For understanding the opinion of the surgical teams regarding our services, every two years we send out a *questionnaire about "client satisfaction"* to all implanting surgeons in our network. Interestingly, 80% of them consider our services as *"excellent,"* about 15% *"very good,"* about 4% *"good,"* and only less *than 1% (one surgeon)* considered our services as *"enough"* (personal, unpublished data). *Fourthly*, we have organized a *periodical (biannual) scientific meeting* on the quality issues; we invite our partners (donation/implantation teams) to share their experiences and to discuss ways to improve the quality of our "product" (cryopreserved cardiovascular allograft), as well as listening to their suggestions about the amelioration of our services for them. The tissue quality was the central topic of these meetings. We listened to the remarks of implanting surgeons and we followed their advices and suggestions on how to improve our work. These meetings gave the opportunity to all participants to share their professional experiences with each other and to get to know each other better as well as understanding the difficulties in association with the TE.[42] *Last*, but not least, we have worked together with the implanting surgeons in analyzing the results of our work by initiating and supporting studies about mid to long-term performance of implanted allografts. As a result of important work done by different participants in these studies, several scientific papers have been finalized and published in different international journals (cardiac surgery, vascular surgery, tissue banking etc.).[26-32] As a result of these studies, which were released and utilized by our team, we have learned a lot about the performance of the valves and vascular allografts. Some of these studies also listed the assessment of patients as their objective; these patients were treated for some life-threatening situations (endocarditis with annular abscesses, HLHS in newborn children, critical ischemia of the lower limbs, arterio-digestive fistulas, etc.).[28, 30, 31] As a result of these studies, we understood the differences in performing an implantation of an allograft depending on the patient's category (age related or pathology related). One of these studies provided us with interesting information about the accelerated failure of the pulmonary allograft if it is implanted in the RVOT of children up to 5 years of age.[26] Another study indicated the immune aspects as an important factor for early deterioration of heart valves.[32] Accordingly, some other (fundamental researchers) have shown that the immune issues of allografts, currently considered as the main problem for its accelerated deterioration, might be resolved by removing all living elements of tissue (cells) in order to create the non-immunogenic valves (cell-free scaffolds). Therefore, implantation of an acellular scaffold in the patient might result in a natural re-seeding with the cells of the recipient. Some animal studies have confirmed this supposition.[43-46]

Following the preliminary works of the group from Hanover Medical School and approval of cell-free valves (pulmonary/aortic) by the German Competent Authority, two multi-centric studies were launched by this group and finalized implanting cell-free pulmonary valve in the RVOT ("ESPOIR") and aortic valves in the LVOT (left ventricular outflow tract), and ("ARISE") in patients with congenital malformation of semilunar valves. These two studies were fully financed by the European Commission. There were a number of patients included in these studies: 200 for ESPOIR[36] and 120 for ARISE.[38]

The European Homograft Bank was the main supplier of the valves for both studies, delivering more than 100 pulmonary valves and 180 aortic valves during the projects and beyond. The cell-free valves were implanted in Germany, Austria, Belgium, France, Italy, Switzerland, United Kingdom, Spain, and Moldavia.

As the preliminary results of two Multi-centric trials showed superior results of cell-free allografts compared to the cryopreserved, many papers have been published in this subject in the last 5 years and show evidence of the excellent functioning of these valves.[37, 39, 43-44, 46]

The scientists will have to give an answer in the coming years about whether the cell free allograft recellularizes after being implanted in the patient and whether it can grow together with a very young

patient. These issues are, at this moment, the only the suppositions.

Currently, cell-free valves are authorized (and reimbursed) only in Germany, Austria, and Switzerland due to the very high prices. Consequently, not every patient who might need such a valve will be able to afford the payment and receive it. Hence, all TEs in Europe should create a condition of preparing and distributing such valves for a more reasonable price.

In conclusion, although much improvement has been implemented during the last 3 decades in the selection, preparation, and storage of allogenic cardio-vascular tissues, there is clear evidence of the cell free performing better than the cryopreserved valves. Accordingly, the cardio-vascular tissue community should adapt to the new situation and make both types of valves available.

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