INTERNATIONAL BENCHMARKING IN CARDIO-THORACIC SURGERY

Quality Improvement by Comparison of Outcome Data.

Theo M.M.H. de By

"International Benchmarking in Cardio-Thoracic Surgery"

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"Internationale Benchmarking in Cardio-Thoracale Chirurgie"

Kwaliteitsverbetering door middel van het vergelijken van resultaten

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Thesis

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For my family

"As it is not one swallow or a fine day that makes a spring,
so it is not one day or a short time that makes a man blessed and happy."

-Aristotle.

Table of contents

CHAPTER 1

Introduction, aims and outline of the thesis

Observed differences in practices, outcomes and quality.

The common denominator in the chapters of this thesis is the observation of the application of different methods in cardio-thoracic surgery, multicenter registries, tissue banking, quality improvement by comparison of clinical outcomes data, and (international) benchmarking. All studies and publications are aimed at analysing these differences, and to compare outcomes. Consequently, the outcomes are valued in terms of differences in quality. Obviously, quality is what everyone, specifically medical professionals and patients righteously strive for and desire. However, considering the definition of quality in scientific management literature, one is confronted with a large range of interpretations about what quality encompasses. In industry the term Zero Defects was introduced in the 1960's and focuses on eliminating all defects in industrial production. (1) Already hard to apply in industry, where incoming basic materials must comply with standardised quality characteristics, the principle is hardly applicable in health care or more specifically in cardio-thoracic surgery where medical history, congenital conditions, morbidity, age and related factors are a predominant pre-existent risk with respect to a default outcome.

Another definition, that is congruent to application in health care is the belief that products should be designed to reflect customers' desires and tastes. (2) Although the primary desire to be healed may be assumed to be any patient's rightful mindset before entering a hospital, other considerations play a role as well. (3) Management literature as well as studies in medicine, specifically if they originate from the United States, include value for money in their definitions (4). In Europe such considerations are scarce when it comes to assess what quality in health care should encompass. "To meet justified customer expectations and demands", derived form an EU Parliament study is perhaps most consistent with the purpose of the EuroSCORE. (5,6) However, specifically because the terminology "desired" and "consistent professional knowledge" is included, we consider "quality of care is the degree to which health services for individuals and populations increase the likelihood of desired health outcomes and are consistent with current professional knowledge" as the most suitable. (7)

Current challenges

Instruments and platforms to benchmark by comparing objective outcomes, have been developed during the past 15-20 years. The aforementioned instruments consist of databases and registries, the reach of which has tremendously grown as a result of the possibilities offered by the Internet and by the innovative statistical and imaging software development. Platforms are offered by professional societies, peer-organisations in focus-meetings, through workshops, by organising joint projects and the like.

Studies concerning the observed differences in outcomes, whether it be in the use of allografts, in mechanical circulatory support or in cardio-thoracic surgery in general,

have demonstrated that the use of benchmarking instruments and platforms provides benefits (8,9,10). When checking local outcomes against risk-assessed data of peer organisations strengths and weaknesses can be identified. The search for best practice is called benchmarking. (11) In doing so, those who are responsible to oversee the level of performance, should not only analyse weaknesses in the core activity but consider the entire structure, process and outcome of the sequence of treatment. (12)

The next step will then be to focus on the observed performance gap with similar units, the result of which should be the initiation and implementation of an improvement project. At the closure of such an improvement project the new outcomes are to be compared with those from the pre-improvement period to determine whether the desired improvements have been realised. Once consolidated in renewed procedures or in adapted structures, the plan-do-check-act circle leads to a new check to reveal the next performance gap and area for improvement. (14) Over time the number of process-constraints will be reduced to an absolute minimum, though there will always be room for improvement. (15)

For those for whom this method is new it will be hard to comply with this consistent and continuous approach of their own processes. However, once results become tangible, the culture of continuous quality improvement has been realised.

The platforms where professionals meet should ideally offer an atmosphere in which differences in methods, insights and outcomes can be openly communicated with peers. Thus, the objectivity of the organization is of great importance for the success of registries and the resulting improvement initiatives. This is preferably carried out by an association of professionals from the field who practice principles of good governance. So, when organised in a setting of objectivity, trust among participants will gradually grow. Examples from practice show that growing trust, using anonymous data at first, leads to the 'uncovering of the veil', thus adding to the power of the benchmarking process.

Taking all of this into account, registry reports show that patient morbidity and survival of therapies have improved over time. Registries are constructed with overall results and defined outcomes and do not make a difference between the different professionals or circumstances involved. For instance, registries don't measure is the positive influence of the resilience of theatre nurses. (16) Neither do they register improvements as a result of technological innovation. When it comes to cardio-thoracic surgery in general, the introduction of new diagnostic possibilities such as magnetic resonance imaging (MRI), innovation in professional areas such as perfusion and anaestesiology have had, and will have, positive effects on the process of care. More specifically in mechanical circulatory support, the changes that were instigated by technological renewal have led to the new era in advanced heart failure therapy. (17). Additionally, the application of statistical methods have enabled scientists to derive and validate risk-scores. (18) The challenge is not only in the adoption of new therapeutic insights, but also in setting up the structure

from diagnosis and patient selection to surgical therapy, and from out-patient care to long-term follow-up. Technological renewal combined with checking and benchmarking outcomes amplify the cycle of continuous improvement. This should ultimately lead to a situation in which professionals in health care reach a situation in which the likelihood of desired health outcomes has increased for individuals as well as for populations and in are in consistency with current professional knowledge.

Aims and outline of this thesis

As we investigated applied methodologies and outcomes in several areas of cardio-thoracic surgery in Europe, differences have become evident. The aim of this thesis is therefore to assess these differences, the methods that are used, to define areas for improvement, as well as approaches toward harmonisation and improvement of outcomes. Harmonisation and improvement lead to best practices and improved quality. As the objective assessment of quality takes place by comparison with external sources, the chapters in this thesis are all characterised by this adagium. Formal scientific comparison between organisations, measuring quantitative results, lead to insights in the causes of quality differences in critical operational data.

The studies, presented in this thesis demonstrate that diversity of methods and systems is large; particularly in Europe. In order to provide the desired insights in the diversity of European practices, we aim to demonstrate where there is room for improvement in order to pave the way for future quality advancement projects.

Starting with the hypothesis that benchmarking leads to quality improvement, a selection of literature is presented in **Chapter 2**. By executing a systematic review of hundreds of publications in the cardio-thoracic surgery domain, only a limited number of 6 studies remained, showing quantitative improvements by using databases of registries for benchmarking. In addition to providing tangible evidence of benchmarking results, all studies provided additional instruments to come to best practices. Five out of 6 papers originated from the United States and Australia, and only one from Europe.

Chapters 3 and **4** are the results of 2 studies providing reports on the application of Mechanical Circulatory Support (MCS) in respectively adult and paediatric patients. EUROMACS, an EACTS registry for patients with MCS, provides a platform to accumulate baseline and follow-up data of these patients' therapy. Since it is the only European international registry of its kind, outcomes are provided on an international level and represents a comprehensive representation of European data.

In **Chapter 5** the Interagency (IMACS) report, a large worldwide database for patients receiving durable Mechanical Circulatory Support (MCS) devices is being presented. IMACS collects data from three major registries: EUROMACS, JMACS (Japan) and InterMACS (United States) as well as from individual hospitals in Australia and in

the Far-East (Australia, Singapore). By accumulating data from a growing number of countries, trends with respect to global developments such as use of axial, centrifugal and pulsating devices become apparent. The relevance of the IMACS report is that it offers multiple benchmarks with respect to device strategies, risk factors, adverse events and predictors of mortality for the worldwide "MCS-community".

In **Chapter 6** gender differences in indications, haemodynamics and outcomes are examined. The observed differences are evaluated. Gender-specific predictors for survival of women and men, undergoing MCS implantation are identified.

In **Chapter 7** we investigate the impact of a phenomenon that was unforeseen at the onset of MCS therapy: the rare occurrence of sufficient myocardial recovery resulting in explantation of the mechanical circulatory assist device. In this study we focus on the incidence of explantation and the long-term outcomes post explantation.

In **Chapter 8** we aimed to determine the association between concomitant tricuspid surgery and clinical outcomes. As tricuspid regurgitation is common after the implantation of a left ventricular assist device (LVAD), the controversy exists as to whether tricuspid valve surgery improves clinical outcomes in the early and late period after LVAD implantation.

In **Chapter 9** factors having an impact on the availability to patients of safe tissue and cell therapies are being investigated. To satisfy the clinical demands for human tissue allografts in cardio-thoracic surgery, as well as in other applications tissue establishments in general have a complex serious of tasks: to correctly estimate the demand for tissue transplants; plan the number of donations needed accordingly; purchase procurement and processing materials; and finally, produce transplantable allografts. The tissue and cell "market" is not subject to economic market dynamics given the fact that these are not typical commercial products. The tissue of donors is donated for altruistic reasons, and free of charge, while several process steps must ensure freedom of transmittable diseases as well as the compliance of the final "product" with clinical quality standards.

In **Chapter 10** we provide current and future perspectives and conclusions on the most common replacement tissues: cardiovascular, ocular, musculoskeletal tissue and skin. Different structures as well as incidence of donations and applications for tissue grafts are analysed.

Chapter 11 describes a conducted European survey to establish the level of cardiovascular tissue banking activities and the demand for allografts. For the first time different methodologies with respect to the use of decontamination protocols became discernible. These methods, as well as the de-selection of donors and discard of already donated cardiac tissues are determinants for the quality of the implanted allograft.

Thereafter, in **Chapter 12**, triggered by the observed differences in Chapter 12, we first conducted a quality round trial. In the trial tissue establishments received heart valve samples that were purposely contaminated with known micro-organisms. They were asked to carry out microbiology tests and decontamination protocols using their local methods. The applied methodologies should prove their effectiveness to decontaminate tissue allograft processes.

Following the data generated in the earlier chapters, and considering the fact that human cardiovascular tissues may be lifesaving and in high demand when it comes to patients with urgent etiologies such as endocarditis, it felt important to follow-up on a wide range of methodologies in **Chapter 13**.

Based on the previously published validations and publications it was decided to investigate in more detail the microbiological and decontamination protocols used in **Chapter 14**. The aims were to isolate and identify the micro-organisms present and to successfully decontaminate heart valve tissue.

Finally, in **Chapter 15**, we provide a general overview and discuss the most important findings of this thesis. In addition, the clinical implications and future perspectives will be discussed.

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CHAPTER 2

Consolidated Quality improvements following benchmarking with cardiothoracic surgery registries

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INTRODUCTION

The notion of measuring clinical outcomes in order to improve the results of the treatment provided is accepted as standard practice. Initiatives of data collections in clinical registries are available in many specialties around the world. The three main scopes for clinical registries are performance monitoring, quality improvement and clinical research.

Software-technology innovations have enabled health care organisations, authorities, professional societies, national or regional hospital groups to set up databases registering outcomes of cardiothoracic surgery procedures. The analyses of the data from these registries often revealed large differences in results, measured in parameters such as length of stay (LOS), morbidity and mortality between hospitals. The outcomes, being discussed at peer meetings and symposia prompted active discussions about techniques, patient selection and care approaches [1].

These days, it is widely recognised that registries are important tools for defining areas for improvement [2-9]. While this positive influence of registries is widely accepted as being evident, differences in quality continue to exist.

In this study we examined the causal relation between the use of cardiothoracic surgery-oriented registries, implementation and improvement of clinical outcomes. A systematic literature review was set up to identify studies providing evidence that benchmarking leads to consolidated quality improvement in cardiothoracic surgery units. The selected studies provide insight in how data was used to change processes, structures and outcomes in the organisations that were the subject of quality improvement initiatives.

METHODS

Definition

The following definitions were used. Firstly, a registry was defined as a clinical database in which cardio- thoracic centers systematically register some data on all patients with the purpose to use the data for the improvement of clinical outcomes. In the registry data are collected using standardised methods and data definitions, and patient intervention data are anonymised.[10]. As a result, the registry represents baseline data as well as outcomes of interventions, and is accessible for its contributors. Secondly, benchmarking was defined as a method of directly accessible, online quality assessment based on best practices, on which informed decisions can be made through the use of outcome related, validated statistics and trends from the registry.

Study outcome

The primary outcome of this review was the impact of cardiothoracic surgery registries on quality improvement in clinical practice. This impact had to be exhibited by data

before and after benchmarking, to prove the causal effect of the use of the registry. Such data includes improvement of outcome (survival, complications, surgery time, and morbidity) or health care utilisation (duration of stay, ICU stay, and re-hospitalisation). Secondary outcomes included any improvement of protocols or organisational processes.

Search strategy

In October 2018 a systematic literature search according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines was conducted [11,12]. Embase, MEDLINE, Web of Science, Cochrane and Google Scholar were searched for articles published after 1990 (search terms are provided in the supplementary Table 1.) [13]. Inclusion and exclusion criteria were defined a priori (Supplementary Table 2). The search was restricted to English language publications.

Studies were included if the study population consisted of cardiovascular patients in hospital care or community care setting. Furthermore, a registry needed to be used as a tool to produce changes in quality. This included: either assessments of changes in processes in the organisation as a result of benchmarking by means of registry data; validated scoring systems developed to predict outcomes based on registry data; identification or development of risk factors based on registry data and their quantification and application in clinical organisation practice.

Proposals, reviews, letters, case-reports, single center studies, and cost analyses were excluded. Studies were excluded if benchmarking by means of a registry data were not used to demonstrate tangible changes in outcome or quality with data from multi-center registries. Likewise, studies including quality improvement based on other interventions than registry data e.g. diagnostic tools, pharmaceuticals, surgery techniques were excluded. Also, studies limited to a specific population, technique, device, disease, or center were as well as studies and registries or databases, including data of a small number of centers (<5) were excluded. Two researchers (TdB and RM) independently extracted and reviewed abstracts and full texts in a blinded standardised manner. In case of a disagreement regarding the inclusion of a study an agreement was jointly negotiated. Finally, references were cross-checked for relevant studies.

Data extraction and statistical analysis

The data was extracted through a standardised form. The extracted data included year of publication, study design, population, data source used as registry, reporting technique and reporting mechanism, and feedback process. Additionally, the study outcome, intervention, impact of the registry on the processes of care, health service use and on clinical outcomes, and the study limitations were extracted. The individual study definitions were used to define the outcomes. Microsoft Office Excel 2011 (Microsoft Corp., Redmond, WA, USA) was used for data extraction. The ROBINS-I tool was used to assess bias in the individual study outcomes (Supplementary Table 3). A meta-analysis or pooling of the data was not possible due to the heterogeneity between studies and the use of words and text to summarize the findings. Therefore, a narrative synthesis

of studies meeting the inclusion criteria was conducted, and the authors made an inventory of the outcomes.

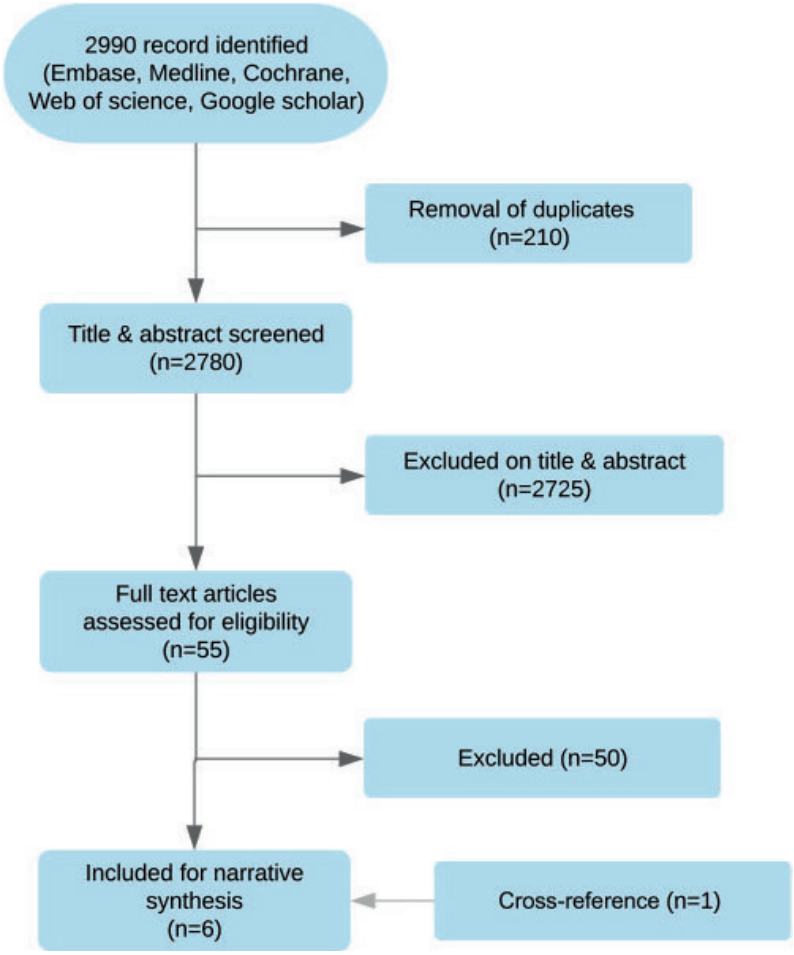

Figure 1. Literature search and inclusion flowchart

RESULTS

Search outcome

A total of 2990 records corresponded with the described search criteria. After removal of 210 duplicates, 2780 titles and abstracts were screened. Subsequently, 2725 studies did not match the inclusion criteria and 55 studies were selected for full text screening. Of these, another 50 full text articles were excluded for failing to match the definitions for inclusion. By means of cross-referencing, 1 additional article was selected, which made the total number for the narrative synthesis 6 studies (Fig. 1).

Six studies demonstrated that a registry was used for benchmarking at a local level, alleging that the results led to a change in improvement of outcomes [2,3, 14-17]. Three studies describe the method for the improvement of outcomes from the perspective of the cardiothoracic care unit and three studies describe the methodology from the perspective of the registry. Overall, there was a low risk of bias due to confounding according the ROBINS-I tool (supplementary material Table 1).

The included studies are summarised in Table 1 and broken down into demographics. Studies were mainly established in the USA (4), in addition to Sweden (1) and Australia (1). All included studies had a prospective design and were conducted between 2009 and 2018. One study was an internationally orientated registry, 3 studies were national registries, and 2 studies were regional registries. The median number of hospitals per study was 40 [minimum 30 - maximum 1150]. The median number of patients per study was 115500 [minimum 6720 – maximum 475000]. The main reporting mechanism was web-based (83%). Furthermore, the majority of the registries performed multiple audits (67%), logic checks (83%), error-checks (100%), and organised meetings to discuss subjects such as: registry outcomes, benchmarking of anonymous or unblinded data, and collaborative improvement initiatives (83%) (Table 2).

Impact of the individual registries on clinical result

An overview of consolidated outcomes, the impact of the selected registries on processes of care, health service use and clinical outcomes is depicted in Table 3.

Nayar *et al.*[14], conducted a quality improvement initiative linking the Society of Thoracic Surgeons Congenital Heart Surgery Database (STS-CHSD) and Infection Surveillance Database (ISD) with the local administrative data system. The combination of registry and in-house administrative data improved reporting and reduced the incidence of Surgical Site Infection (SSI). During a 24-month study period, the authors ascertained 1715 surgical cases. Through quality improvement initiatives, including: standardised clinical protocol changes; ameliorated communications, and reporting, corrective interventions were initiated in a rapid-cycle manner. By means of wound alert reports, focused actions were developed.

Table 1. Demographics of included studies

Reference	Year published	Scope	Design	Population	Number of hospitals (units)	Number of patients
D. Eccleston et al.[16]	2017	Australia	Prospective	Adult Cardiac population	40	6,720
R.S. D'Agostino et al.[17]	2018	USA, Canada & 7 other countries	Prospective	Adult Cardiac Surgery	1,150	224,724
T. Jernberg et al.[15]	2010	Sweden	Prospective	Cardiac Surgery	74	80,000
E.L. Hannan et al.[3]	2012	New York State	Prospective	CABG	30	57,187
V. Nayar et al.[14]	2016	USA	Prospective	Paediatric cardiac Surgery	1,061	475,000
R. Prager et al.[1]	2009	Michigan State	Prospective	CABG	33	151,000*

*151,000 is the annual average reported by Likosky et al.

CABG; Coronary arterial bypass grafting

Table 2. Data sources, characteristics and control mechanisms

				Control mechanisms			
Reference	Data source	Reporting region	Reporting mechanism	Audits	Logic checks	Error-checks	Meetings
D. Eccleston et al.[16]	Registry in real-time	National	Web-based	multiple	yes	yes	yes
R.S. D'Agostino et al.[17]	registry in real-time	International	Web-based	multiple	yes	yes	yes
T. Jernberg et al.[15]	Registry	National	Web-based	on site	yes	yes	yes
E.L. Hannan et al.[3]	Registry	Regional	Reports	on site	unknown	yes	yes
V. Nayar et al.[14]	Registry	National	Web-based	multiple	yes	yes	yes
R. Prager et al.[1]	Registry	Regional	Web-based	multiple	yes	yes	yes

Following the wound alert, a collaborative bedside review would take place, and in a multi-disciplinary manner a consensus decision would be made regarding the underlying cause. Through this method, compliance with the current guidelines and protocols would be assessed in order to determine the status of potential causes for the SSI development. This systematic approach resulted in a 59% SSI reduction in the Children's Hospital of Philadelphia over a year.

Jernberg *et al.* [15], described the functioning of the SWEDEHEART Registry as an online interactive reporting system functioning as a tool for continuous collaborative quality improvement projects in which all Swedish hospitals are engaged. Annual reports are openly published and outcomes of each hospital can be directly compared with others. All users are provided with online interactive reports concerning changes of processes of care. SWEDEHEART openly publishes quality comparisons and indexes reflecting the whole chain of patient care. 30-days mortality decreased from 1.9% to 1.1% between 1995 and 2008. Additionally, one year and in-hospital mortality after

an acute myocardial infarction decreased as well, while later reports show a continuous trend of decreased mortality in all cardiothoracic procedures [20].

Hannan *et al.* [3], studied the development of the New York State program to increase quality and improve outcomes in a historical perspective. Annual feedback reports, in which key performance indicators [30-day (risk adjusted) expected and observed mortality] were tools that were used to make cardiac surgeons, as well as interventional cardiologists, aware of their relative performance. Negative outliers received a letter from the NY State Department of Health (DOH).

This method and its impact on quality improvement in specific hospitals has been described by Chassin [2], who elaborated on the large influence of publicly disseminating cardiac surgery outcomes [2]. As in the study of Nayar *et al.* [14], administrative data suffered issues of data quality and were not appropriate for use for calculating necessary ratios to measure quality in New York State hospitals. For this reason, a patient-level clinical database was created to assess clinical outcomes for CABG surgery.

In all 3 hospitals, referred to by Hannan *et al.* [3], major changes in processes and structures were implemented. As a result, the 30-day mortality rate decreased from 26% to 0% (emergency cases only), from 9.2% to 2.3% and from 7.31% to 2.57% respectively over a 3- to 4-year period. After 5 years, the risk adjusted odds for short-time mortality was only 0.66 times the odds in the remainder of the country. Eccleston *et al.* [16], set up a registry to benchmark local practice against international standards. The feedback of the registry to the health care providers, either as individuals or as a group, is aimed at appropriate use of guidelines in cardiac therapy and to assess long-term medication compliance. Between the first and latest year of data collection there was significant improvement in the rates of statin therapy at discharge (92.1 vs. 94.4% $p<0.03$) and 12 months post-PCI (87.0 vs 92.2% $p<0.001$) and of antiplatelet therapy at 12 months (90.7 vs 94.3% $p<0.001$).

The Society of Thoracic Surgeons Adult Cardiac Surgery Database 2018 report goes beyond explaining the methodology of the registry in detail [4,17]. The 2018 publication reports on data quality improvement, reports to sites as well as voluntarily to the public and offers linking with other databases. Still, after 3 decades the 2016 outcomes show an overall decrease of in hospital mortality for the majority of procedures [17]. One of the additional instruments for quality improvement, on top of site-reports and other feedback is a task force on quality initiatives e.g. on the use of blood products resulting in 18% reduction of exposure to blood products in patients undergoing CABG, while similar trends are observed in aortic valve repair and mitral valve repair [17].

Prager *et al.* [1], report that parallel to the STS database, the Michigan State Collaborative Approach uses STS data, based on which quality meetings are organised in the state. From the onset in 2005, the provided data from state hospitals were anonymous. Practices were shared, sites visits and reverse site visits took place. Anonymous data were

gradually unblinded and peer to peer discussions took place in which an atmosphere of openness and trust prevailed. The initial results of this Collaborative Approach included: improvement of risk adjusted mortality rates; reduction of ventilation time; and an increase of the use of internal mammary arteries (IMA) as compared to the other STS national average. In its most recent study the Michigan State Collaborative proved that their methodology resulted in better outcomes in a project aimed at the reduction of pneumonia: a 3.23% reduction for Michigan State Collaborative hospitals versus a 1.96% reduction in STS hospitals [19].

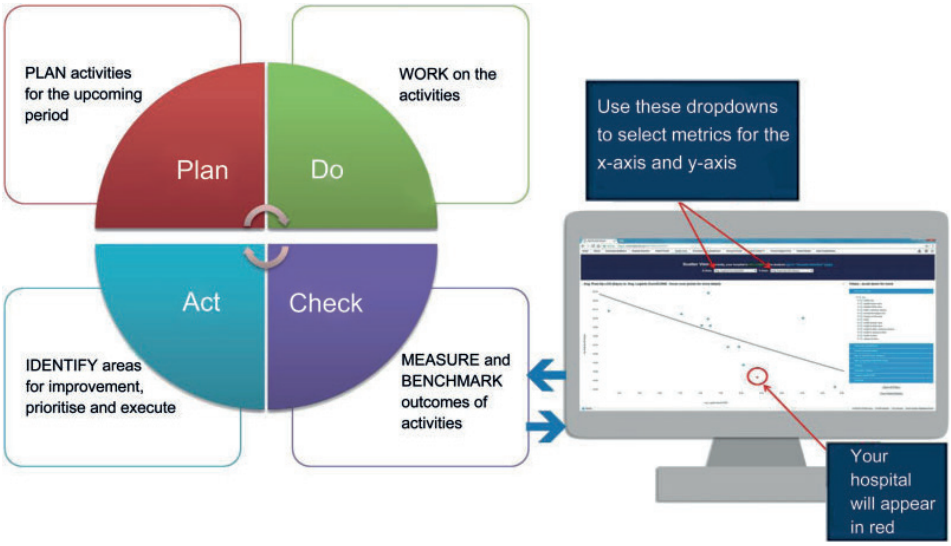

Figure 2. The Deming Circle, method of continuous quality improvement by Plan-Do-Check-Act with the application of registry data to benchmark local outcomes against registry data.

Table 3. Overview of consolidated outcomes and methods of quality improvement projects

Author	Time period	Primary outcome	Intervention	Registry impact on processes of care	Registry impact on health service use	Registry impact on clinical outcomes	Limitations
D.Eccleston et al.[16]	2010-2014	Improvement of compliance with guidelines	PCI Interventional cardiology practice	Streamlining of data collection over various departments	Robust feed-back of information on care processes to clinicians has led to improvement of practises	Mortality -0.4%, MI -0.26%, MACE -0.2%, Re-admission -0.4%	Devolution of responsibilities for centres performing less effectively than their peers. No governance principles to manage outliers
T.Jernberg et al.[15]	1995-2008	Online interactive reports, providing information on therapies outcomes lead to decreased mortality and morbidity	Heart surgery, angioplasty and angiography	Providing multi-disciplinary users with an array of online interactive reports, to continuously monitor care and compare outcomes	A continuous trend of decreased mortality in all cardiothoracic procedures over 13 years of time	30-days mortality decreased from 1.9 to 1.1%	Comparisons between hospitals can be difficult to interpret due to differences in base-line characteristics
E.Hannan et al.[3]	1992-2012	Major changes as a result of published data. Decreases of risk-adjusted mortality and morbidity. Closure of low-volume units. Data for studies	Cardiac surgery and angioplasty	Hospital-specific quality improvement initiatives. Insight in risk-avoiding behaviour of hospitals	Under-performing hospitals and surgeons discontinued practising CABG surgery within 2 years after publication of outcomes reports	Initially a decrease of risk adjusted mortality from 4.17 to 2.45% (-41%). In the period 1994-1999 the short time mortality odds was 0.66 times the odds in the remainder of the country.	Reports fail additional outcome measures more tailored to disease than to treatment. Should include process measures. Need to improve communication aspects
V.Nayar et al.[14]	2013-2014	SSI reduction. Systematic approach and Deming-Circle method. Resolving database discrepancies	Cardiac surgery and paediatric	Improvement of workflow and communication. Introduction of wound-alert reports & bedside reviews. Evaluations.	Administrative data alone are insufficient. Hospitals must be aware of statistical deviation/quality of administrative data. Combine registries and local medical data	59% reduction of SSI (surgical site infections) over a 2- year period of time	No correlation between each intervention assessed. Some communication issues caused flaws in coding but were resolved later on

Author	Time period	Primary outcome	Intervention	Registry impact on processes of care	Registry impact on health service use	Registry impact on clinical outcomes	Limitations
R.S. D'Agostino et al.[17]	2016-2018	Negative trends in blood product use. Renewed STS Risk Models. Composite performance indicators in most sub-specialties of cardiothoracic surgery (CABG, valves, etc.)	Cardiac surgery	The combination of high centre rate, large sample sizes, have made the STS ACSD a valuable resource for the study of cardiothoracic procedures penetrance, and a 10% annual centre audit and linkages to other databases makes STS the data-standard for US as well as beyond for benchmarking.	Data from the STS are used for research, public reporting, and quality improvement.	Mortality in rescue (Chu et al.)[30] -1.1% Risk-adjusted pneumonia rates declined by 1.96% (Likosky et al.)[19]	Participation is voluntarily. The results could be influenced by well-performing centres.
R.L. Prager et al.[1]	2005-2007	IMA use pre-operative intra aorta balloon pump usage. Reduced ventilation. Postoperative atrial fibrillation decrease and CABG mortality decrease	Cardiac surgery	Methodology creates opportunities for cardiac surgical leadership to participate in state health policy to lead the movement to quality.	Financial savings as a result of better outcomes. Health insurance organisations support changes.	Reduction of in-hospital mortality overall 24%. In 2 years 0.68 % risk adjusted mortality in aortic valve replacement 0.95 % as compared with other STS participating hospitals.	Participation is voluntarily. The results could be influenced by well-performing centres.

DISCUSSION

In this systematic review of the literature, the analysis of 2990 abstracts, the selection of many in-depth articles about databases, and about the importance, influence and use of registries, concluded that the number of publications regarding the use of registries runs into the thousands. Using the methodological limitations and inclusion criteria, of the systematic search-strategy, a very small quantity of six studies could be selected -though with an enormous number of patients- providing evidence that the use of a registry leads to quantifiable and consolidated improvements. Moreover, the selected studies deliver additional insights, and confirm what has elsewhere in research been published.

Several studies discuss the preferable sources of such data. While Siregar *et al.* stress that great caution is needed when using administrative data to measure cardiovascular events, Pagano and Gale state that both clinical as well as administrative sources of data have a qualitative restriction when considered separately [20,21]. Thus, the use of a combination of data sources is preferable.

The study of Nayar *et al.* demonstrates that the reliability of registry data is essential. Their applied methodology of using data from 2 robust clinical registries (STS and ISD) and an additional local administrative database to benchmark the level of Surgical Site Infection (SSI), enabled the researchers to obtain better insights. The execution of quality improvement initiatives at the Children's Hospital of Philadelphia (CHOP) is an example of the use of both internal databases and registries to benchmark outcomes and use these outcomes to improve patient care in the local hospital organisation. This resulted in changing infection incidences as a rolling 12-month rate for each data source. As expected, the in-house administrative data were sub-standard; using them in isolation would have resulted in limited insights and uncalibrated conclusions.

By means of a visualisation software tool, using statistical process control charts, the variations in trends were made visible and staff could concentrate on addressing the most influential causes for SSI through targeted quality improvement projects. As a logical spin-off process-flows were mapped and medical records were standardised from all perspectives, resulting in a 59% SSI reduction in the CHOP over a 2-year period of time. Additionally, the execution of the CHOP quality improvement initiatives shows all core characteristics of the innovation process that Deming described as "Plan, Do, Check, Act (PDCA)", and is shown in Fig. 2 [23]. According to Nayar *et al.* [14], the methodology can be applied to other disciplines within cardiac surgery: systematic continuous quality improvement based on observed differences between registry data and statistical deviation of the administrative in-house data must be the basis of every hospital's quality-strategy. The SWEDEHEART Registry encompasses all relevant baseline and follow-up data from all hospitals.

Several publications about the situation in Sweden appeared during the process of data analysis and selection in this study [15,20]. The report by Jernberg *et al.* was

selected because of meeting the criteria, yet it offers a 'tour d'horizon' with respect to structure, functioning and outcomes of the SWEDEHEART Registry. SWEDEHEART distinguishes itself by 'comparing not only the performance of participating hospitals but also different treatment modalities and medical devices'.

The proof that there's a causal relationship between the use of the registry and measurable quality improvement can be found in the additional features consisting of an array of interactive reports in which physicians, nurses and decision-makers can structurally follow the outcomes of their local processes of care in trend-analyses.

The public availability of outcome, and the aforementioned trends, have been correlated with wider professional attention. Subsequently, many hospitals have undertaken co-operative quality-improvement projects.

Although not similar to SWEDEHEART the New York State Cardiac Registries were initiated as an audit-oriented data collection to address inter-hospital variations in mortality and complications, and offer some comparable features. One of the earliest publications on the use of data to identify areas for improvement is the study of Chassin [2], on the New York State Cardiac Surgery

Reporting System (CSRS). Chassin describes the localised approach of several hospitals as well as individual surgeons and the publication of specifically negative outcomes and the publicity that followed [2, 24]. Ten years later Chassin questions whether "naming and shaming" is the preferable professional method of choice.

Hannan et al. [3] describe the further development of the New York State CSRS as a patient-level clinical database that was created to assess clinical outcomes for CABG surgery. Annual feedback reports in which key performance indicators [30-day (risk adjusted) expected and observed mortality] were tools that New York State Registries made available to cardiac surgeons as well as to interventional cardiologists. This demonstrates that through their life-cycle registries may develop into a multi-purpose tool from audit-oriented to the use for quality improvement or research and vice versa [25].

In the early 1990's, the New York State Cardiac Surgery Reporting System (CSRS) was a database, it wasn't a registry that functioned as a modern near real-time web-based tool though. Therefore, Chassin's publication wasn't selected for our study. However, the wider impact on health care of disclosing data for the public at large appears to be a necessity to induce quality improvement implementations. As surgeons were provided with trustworthy data concerning their performance, they, as well as hospital administrators, are stimulated to create effective quality improvement programs [3]. Hannan confirms that feedback reports to the general public are a trigger for hospital organisations and medical professionals to stimulate them to focus on how to minimize mortality in their institutions.

The selection method also excluded the study of the improvement project of the American College of Surgeons, described by Ingraham *et al.* [26]. Though the field of study is general surgery, rather than cardiothoracic surgery, the method recognises that data alone doesn't translate into improvement of outcomes. The authors identified 12 critical steps for implementing quality improvement on a local level. Measures through the entire care-process were taken, changing one or more of the steps in the pre-, peri- and post-operative working method(s) was evidence based, controlled and quantified. Moreover, the involvement of surgeons-champions, multi-area leadership support and a quality improvement team including trained and audited data abstractors, or Surgical Clinical Reviewers (SCRs), ensured the collection of consistent, robust and high-quality clinical data using precise definitions. Over a period of 15 years post-operative morbidity was reduced by 43% and mortality by 47%, though this may partially be attributed to the evolution of surgical techniques.

All publications that were selected for this study, and all that are considered relevant for the discussion concerning the best method to apply benchmarking to identify areas for improvement and to implement the results of quality enhancement initiatives, seem to follow a common pattern that consists of three components: structure, process and outcome: the 'Donabedian Model' [27]. Ingraham *et al.* [26] refer to this Model, in which health care is approached as being a system with processes that can be re-designed in order to improve quality. In the study of Ingraham *et al.* the Donabedian model is applied, overlapping with Deming's PDCA method, which results in a better:

1. Structure: consistent, reliable and uniform data collection. Assignment of roles of medical professionals in the quality-improvement projects (plan, do).

2. Process: evidence-based improvement in the working methods (check).

3. Outcomes facilitate the identification of process measures that are highly correlated with quality improvement resources (act).

As proof that the methodology can serve as an effective tool for achieving significant gains in surgical quality, the authors report a sharp decrease in morbidity and mortality.

While the availability of studies that demonstrate the relation between the use of registries in the cardiothoracic domain is very limited, the modest attainability of such evidence-based studies seems to be similar in other areas of medicine in which registries are used [28,29].

Our literature search resulted in a selection of registries in which the North American and Australian continents emerged predominantly. The limitation of our search to English language publications may have overlooked the existence of well-functioning national or regional registries in other geographical areas. With the exception of SWEDEHEART no other registries met the criteria set for this review. An early version of a European

Adult Cardiac Database (ACD) was abandoned because data were inaccessible online and did not offer the tools for multilayer comparison of data from individual hospitals with the database [30]. The comprehensiveness of the STS database and its ability to offer an adequate benchmarking tool led to the initiative of the European Association for Cardio- Thoracic Surgery EACTS) to accumulate data on a European level.

An incoming first report will present the first results and is expected to encourage Europeans to catch up participate in a European registry. It can be expected that aggregating and comparing outcomes on a larger than just national scale, and learning from successfully applied methods elsewhere, will lead to further quality improvement at an international level.

Limitations

This systematic review has limitations. consisting of the following elements: quality improvement methods and strategies are usually poorly indexed within bibliographical databases. Further, the selected studies show differences in size, in heterogeneity of the study cohorts, time frame, time-period, continuance and outcomes of interest in different quantification methods. These make it impossible to pool results. We found variation in follow-up, reporting mechanism, data management, quality assurance, and audit of data within the registry. In Table 3 (last column) we provided a breakdown of limitations per included papers.

A limitation of our review concerns the search strategy. The broadness of the search increased the number of irrelevant articles (we excluded more than 98% of all articles reviewed). However, it decreased the risk of systematically missing relevant studies. We believe that our findings haven't missed potentially relevant studies.

CONCLUSION

The perceived impact of the large quantity of medical registries is not denied, though often they're merely used as a reference. For a one-on-one relation between the use of registry data, measurements of outcomes of quality improvement is the key for determining the success of the quality improvement intervention. Registries do have limitations and clinicians who undertake quality improvement projects should seek to extract data from multiple resources if they provide reliable essential information. Feedback of outcomes does influence registry participants. However, several studies substantiate that providing data and feedback alone, contributes less to change processes focused on the improvement of outcomes. The application of additional tools such as collaborative meetings are provided and used. Subsequently, these tools result in the improvement of clinical outcomes.

This study demonstrates that the instruments to develop quality initiatives and methodologies can be supplied, and that a benchmarking platform can be created by the registry or by peer-associations, leading to quality improvement throughout the collaborative of cardiothoracic surgery units. Furthermore, this study shows that the comparison of consolidated results before and after quality improvement initiatives provide the tangible evidence that the use of registry data leads to clinical outcomes with decreased morbidity and mortality.

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CHAPTER 3

The European Registry for Patients with Mechanical Circulatory Support (EUROMACS) of the European Association for Cardio-Thoracic Surgery (EACTS): second report

De By TMMH, Mohacsi P, Gahl B, Zittermann A, Krabatsch T, Gustafsson F, Leprince P, Meyns B, Netuka I, Caliskan K, Castedo E, Musumeci, F, Vincentelli A, Hetzer, R, Gummert J.

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ABSTRACT

Objectives: The European Registry for Patients with Mechanical Circulatory Support (EUROMACS) was founded in Berlin, Germany. EUROMACS is supported fully by the European Association for Cardio-Thoracic Surgery (EACTS) and, since 2014, has functioned as a committee of the EACTS. The purpose of having the EUROMACS as a part of the EACTS is to accumulate clinical data related to long-term mechanical circulatory support for scientific purposes and to publish annual reports.

Methods: Participating hospitals contributed surgical and cardiological pre-, peri- and long-term postoperative data of mechanical circulatory support implants to the registry. Data for all implants performed from 1 January 2011 to 31 December 2016 were analysed. Several auditing methods were used to monitor the quality of the data. Data could be provided for in-depth studies, and custom data could be provided at the request of clinicians and scientists. This report includes updates of patient characteristics, implant frequency, mortality rates and adverse events.

Results: Fifty-two hospitals participated in the registry. This report is based on 2947 registered implants in 2681 patients. Survival of adult patients (>17 years of age) with continuous-flow left ventricular assist devices with a mean follow-up of 391 days was 69% (95% confidence interval 66–71%) 1 year after implantation. On average, patients were observed for 12 months (median 7 months, range 0–70 months). When we investigated for adverse events, we found an overall event rate per 100 patient-months of 3.56 for device malfunction, 6.45 for major bleeding, 6.18 for major infection and 3.03 for neurological events within the first 3 months after implantation.

Conclusions: Compared to the first EUROMACS report, the number of participating hospitals increased from 21 to 52 (+148%), whereas the number of registered implants more than tripled from 825 to 2947 (+257%). The increase in the number of participating hospitals led us to increase the quality control measures through data input control, on-site audits and statistical analyses.

INTRODUCTION

The purpose of the European Registry for Patients with Mechanical Circulatory Support (EUROMACS) registry of the European Association for Cardio-Thoracic Surgery (EACTS) is to accumulate clinical data on long-term mechanical circulatory support (MCS) and to enable scientific research to improve this method of treatment for patients with end-stage heart failure. The registry permits the retrieval of data on survival and morbidity rates so that clinicians and industry representatives can identify and learn from the factors that influence the results of MCS therapy. Various measures were taken to safeguard the completeness and correctness of the data that have been submitted by the participating centres to improve data quality. These methods include data input control, on- site audits and statistical analyses.

Data have been made available for several studies that resulted in publications [1, 2] or abstracts [3]. Upon the request of the participating centres, custom analyses of data could be provided. Of special interest, a paediatric study group has been established among the EUROMACS members to carry out studies on the treatment of children with MCS. The first article containing paediatric data from the EUROMACS will be submitted in 2017 to the *European Journal of Cardio-Thoracic Surgery (EJCTS)*. Several joint projects with other national and international registries to exchange or to accumulate data were initiated. Finally, a course for ventricular assist device (VAD) coordinators, including the EUROMACS registration modalities, has been conducted annually since 2015 [4].

The EUROMACS and the International Society for Heart and Lung Transplantation (ISHLT) have an agreement whereby the ISHLT participates in the Interagency Registry for Mechanically Assisted Circulatory Support (IMACS). IMACS enrolls and follows patients receiving durable MCS devices on a global basis. The first IMACS annual report, including data from EUROMACS, was published in the spring of 2016 [5].

METHODS

Hospitals that contribute baseline and follow-up clinical data from their consenting patients to EUROMACS agree to do so within 6 weeks after the patient receives an MCS device. Similarly, events are to be registered within 6 weeks after their occurrence. Hospitals register their patients with MCS online via a secured Internet connection, using an individual password, in an ongoing prospective manner, but also retrospectively to 1 January 2011. Although some centres have chosen to submit earlier records, only implants from 1 January 2011 are included in this analysis, which is consistent with the data included in the first annual report [6].

All paediatric and adult patients who received a long-term MCS device, designed for >_6 months support, were eligible for registration in the EUROMACS database (Table 1). A

provision has been made for devices that were implanted concomitantly (as a temporary right ventricular assist device) with a long-term device (see Table 1, 'Short-term devices').

QUALITY CONTROL

To safeguard the correctness and completeness of the data sub- mitted by the contributing hospitals, a set of tools and protocols has been developed. Primarily, the hospitals sign an agreement in which they consent to submit data from every patient who receives MCS on a long-term basis (support duration >_6 months), unless the patient refuses consent to participate. The procedure to obtain consent is based on national legislation, which varies in the different nations in which hospitals submitting data are situated. The hospitals agree to communicate data records to the registry in accordance with the structure of the EUROMACS data- base and ensure that all data have been correctly acquired, in accordance with the state of the art of medical procedures.

In addition, checks on data completeness and data consistency are carried out on a structural basis. Data managers are approached directly in case of specific issues. The participating hos- pitals are requested to confirm the completeness of their data on 30 June and 31 December each year. Thus, the consolidated data can be used for analyses and the annual report. For more details, see Supplementary Material.

On-site audits are conducted by the EUROMACS management team and comprise an overview of possible non-compliance re- ports using a random selection of patient files that are compared with the respective data files from the local hospitals.

Statistical analysis

In preparing the analysis for this report, we involved on-site data managers to achieve complete data with respect to the most important variables. Our goal was to increase the completeness of the survival data by assuming a patient's death if a date of death or a cause of death had been entered or if the patient's death was mentioned as an adverse event or as a type of discharge. We used the brand of the device to derive the type of pump in case this information was missing. No multiple data imputations were done. We checked for the chronological plausibility of the follow-up records and eliminated or corrected implausible re- cords by queries to on-site data managers.

The Kaplan–Meier estimates of cumulative probabilities were calculated for mortality, including 95% confidence intervals (CIs) as a measure of certainty, where we did not truncate the curves. A patient is considered at risk up to the date of his or her individual last follow-up information saying that the patient has received a transplant, has been weaned from the device, has died or is alive. For major adverse events other than death, we calculated event rates per 100 patient-months and constructed corresponding CIs that accounted for the Poisson distribution of event counts. Competing outcomes (ongoing device support or death or heart transplant or weaning) are presented for the

first 6 months after device implant. Percentages are calculated as the ratio of the number of subjects who experienced the mentioned outcomes divided by the total number of subjects in the data set multiplied by 100. To avoid any censored individuals, only patients with a follow-up period of at least 6 months were considered for the competing outcome analysis. All CIs and P-values were 2-sided. All calculations were made using Stata 12 (Stata Corporation LLC, College Station, TX, USA).

RESULTS

Since the publication of the first EUROMACS annual report, the enrolment of hospitals increased by 148%, from 21 to 52, and patients in the registry more than tripled from 741 to 2681 (262%) [6].

Table 1

MCS type	
Long term devices	
Continuous flow	Berlin Heart INCOR
	CircuLite Synergy*
	Heart Assist 5
	HeartWare HVAD
	Jarvik 2000
	MicroMed DeBakey
	Thoratec HeartMate II
	Thoratec HeartMate 3
Pulsatile extracorpreal	Berlin Heart EXCOR
	Thoratec pVAD
	Abiomed AB5000
Total artificial heart	SynCardia Cardiowest
Short-term devices	
	DeltaStream Medos**
	Levitronix CentriMag**
	Maquet CardioHelp**

*Withdrawn from the market in 2014.** These short-term devices can be used with an oxygenator as ECLS/ECMO. A provision has been made for devices which were implanted concomitantly (as a temporary RVAD) with a long term device.
CE: European conformity; MCS: mechanical circulatory support.

Centres

Table 2 presents the 52 hospitals in 18 countries (in 2013, 21 hos- pitals in 12 countries) [6] contributing data to the EUROMACS as of 31 December 2016. On the same date, the agreement in which the rules of engagement were defined was under consideration in 4 hospitals in 2 additional countries. In addition, the Spanish Registry for Mechanical Circulatory Support (ESPAMACS), which includes the collective data from almost all

Spanish hospitals that implant MCS devices, agreed to provide data to EUROMACS on a regular basis, whereas 1 hospital contributes its data separately [7]. At the end of 2015, an agreement was reached with the Societé Française de Chirurgie Thoracique et Cardio-Vasculaire (SFCTCV) [8] whereby the 18 hospitals in France that implant MSC devices will start contributing data in 2017.

Table 2. Participating institutions as of December 31, 2016.

Country	City, Hospital
Austria	Innsbruck, Universitätskliniken
Azerbaijan	Baku, Central Clinic Hospital
Belarus	Minsk, National Institute "Cardiology"
Belgium	Aalst, Onze Lieve Vrouwenziekenhuis
	Gent, Universitair Ziekenhuis Gent
	Leuven, Katholieke Universiteit Leuven
Czech Republic	IKEM (Institute for Experimental Cardiac Surgery)
	Brno, Center for Cardiovascular and Transplant Surgery
Denmark	Århus, Århus University Hospital Skejby, Copenhagen, Rigshospitalet
France	Le Plessis-Robinson, Centre Chirurgical Marie Lannelongue
Germany	Berlin, Deutsches Herzzentrum Berlin
	Lübeck, Universitätsklinikum Schleswig Holstein
	Bad Oeynhausen, Herz- und Diabeteszentrum Nordrhein-Westfalen
	Hamburg, Universitätsklinikum Eppendorf
	Freiburg, Universitäts Herzzentrum Freiburg - Bad Krozingen
	Jena, Universitäts-Herzzentrum Thüringen
	Karlsburg, Klinikum Karlsburg
	Köln, Universitätsklinikum Köln, AöR
Greece	Athens, Onassis Cardiac Surgery Center
	Thessaloniki, Aristotle University of Thessaloniki
Hungary	Budapest, Heart Center of the Semmelweis University
	Budapest, Gottsegen György Hungarian Institute of Cardiology
Italy	Bologna, Ospedale S. Orsola
	Rome, Ospedale San Camillo
	Milan, Ospedale Niguarda Ca'Granda
	Bergamo, Ospedale Papa Giovanni XXIII
	Naples, Ospedale dei Colli
	Palermo, ISMETT
	Rome, Ospedale Pediatrico Bambino Gesù
	Torino, Regina Margherita Children's Hospital
Kazakhstan	Astana, National Research Cardiac Surgery Center
Netherlands	Groningen, Universitair Medisch Centrum Groningen
	Rotterdam, Erasmus Medisch Centrum
	Utrecht, Universitair Medisch Centrum Utrecht
Norway	Oslo, Rikshospitalet
Poland	Warsaw, Childrens Memorial Hospital
	Zabrze, Silesian Heart Center
Spain	Pamplona, Clínica Universidad de Navarra
	ESPAMACS, collective of 7 hospitals

Country	City, Hospital
Switzerland	Bern, University Hospital Bern (Inselspital)
	Zürich, Kinderspital Zürich
Turkey	Izmir, Ege University School of Medicine
	Istanbul, Florence Nightingale Hospital
	Ankara, Bashkent University Hospital
	Ankara, Yüksek Ihtisas Hospital

EUROMACS, in turn, has come to an understanding with the ISHLT concerning its participation in IMACS.

Update per 31 December 2016

The analyses in this annual report are based on the data for implantation of MCS devices beginning 1 January 2011. Between 1 January 2011 and 31 December 2016, 2681 patients (mean age 51.7 years, median 55 years, range 0–86 years) were registered in the EUROMACS database (Table 3). The increase in the number of devices implanted, compared with the number in the first annual report, is 1856 (+225%).

Table 3. Demographic profile of 2681 patients

Patient characteristics	
Mean age ± SD (median, range), years	51.7 ± 15.3 (55, 0-86)
Gender (male/female)	2200 / 481
Ethnic origin	
Asian	217
Caucasian	2117
Other or unknown	347
Primary Diagnosis	
Idiopathic cardiomyopathy	926
Ischemic cardiomyopathy	1091
Restrictive cardiomyopathy	17
Hypertrophic cardiomyopathy	22
Toxin-induced cardiomyopathy	40
Postpartal cardiomyopathy	16
Myocarditis	136
Endstage valvular heart disease	45
Congenital heart disease	56
Neoplasia	7
Unkown	325

The aetiology of heart failure was primarily ischaemic cardiomyopathy (n = 1091, 40.7%) and idiopathic cardiomyopathy (n = 926, 34.5%) (Table 3). The distribution by ABO blood group type and gender is given in Table 4. Table 5 presents the types of VADs implanted stratified ac- cording to age in 2681 patients for whom exact data were available.

Table 4. Patient characteristics according to gender and blood groups

Blood Group	Male	Female	Total, N (%)
A	985	196	1181 (44.05%)
AB	119	30	149 (5.56%)
B	292	56	348 (12.98%)
O	803	199	1002 (37.37%)
Unspecified	1	0	1 (0.04%)
Total, N (%)	2200	481	2681 (100%)

Table 5. Type of VADs per age group in the 2695 implants of which data were available.

	<17	17-65	>65	Total
LVAD alone				
Continuous	37	1897	324	2258
Pulsatile	48	29	3	80
Unspecified	1	120	24	145
LVAD + RVAD				
Continuous	1	102	18	121
Continuous LVAD, pulsatile RVAD	0	4	0	4
Pulsatile LVAD, continuous RVAD	2	0	0	2
Continuous LVAD, unspecified RVAD	0	3	0	3
Unspecified LVAD, unspecified RVAD	0	1	0	1
BIVAD				
Continuous	2	20	4	26
Continuous LVAD, pulsatile RVAD	0	1	0	1
Continuous LVAD, unspecified RVAD	0	6	1	7
Pulsatile LVAD, continuous RVAD	0	1	0	1
Pulsatile	16	16	1	33
Pulsatile LVAD, unspecified RVAD	2	9	0	11
Unspecified LVAD, unspecified RVAD	0	2	0	2
All implants	**109**	**2211**	**375**	**2695**

An isolated left ventricular assist device (LVAD) was implanted in 2366 (88.3%) patients as a first implant. An LVAD with a temporary right ventricular assist device was implanted in 126 (4.7%) patients. Isolated right ventricular assist device s were implanted in 28 (1.0%) patients and total artificial hearts in 27 (1.0%) patients. Table 6 presents that, after the first implantation of MCS, 218 patients underwent a second device implantation and 37 patients received a third implantation, 9 patients a fourth implantation and 2 patients a fifth implantation.

Table 6. Primary and subsequently implanted devices (n=2947). (SVAD = VAD placement in single ventricle anatomy)

	Sequence of operation					
	1st	2nd	3rd	4th	5th	Total
BiVAD	80	1	0	0	0	81
LVAD	2366	122	22	5	0	2515
LVAD,RVAD	126	4	1	0	0	131
RVAD	28	72	10	3	1	114
SVAD	3	0	1	0	0	4
Total artificial heart	27	4	0	0	0	31
Unknown	51	15	3	1	1	71
Total	2681	218	37	9	2	2947

Strategy for ventricular assist device implantations

Table 7 presents the strategy for VAD implantations in 2947 implantations. VADs were implanted primarily as bridge to candidacy (possible bridge to transplant, *n* = 1052, 36%) or bridge to transplant (*n* = 813, 28%). VADs as a destination or a permanent therapy were implanted in 458 (16%) patients. We expected that, given the large numbers of patients on the heart transplant waiting lists in several countries, a relative increase would be seen in the number of patients older than 65 years on destination therapy compared to the numbers in other age categories [9].

Table 7. Device strategy at time of implantation, stratified by age categories, N (%)

	<50	50-64	65-70	>70	Total
Bridge to recovery	24 (2)	28 (2)	3 (1)	2 (1)	57 (2)
Bridge to candidacy	402 (42)	568 (39)	60 (18)	22 (12)	1052 (36)
Bridge to transplant	332 (34)	414 (28)	48 (14)	19 (10)	813 (28)
Destination therapy	22 (2)	170 (12)	157 (47)	109 (60)	458 (16)
Rescue therapy	68 (7)	105 (7)	19 (6)	18 (10)	210 (7)
Other	4 (0)	5 (0)	2 (1)	0 (0)	11 (0)
Unknown	112 (12)	176 (12)	45 (13)	13 (7)	346 (12)
Total	964	1466	334	183	2947

INTERMACS LEVELS

VAD implantation was performed primarily in Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) Levels 2 and 3 as presented in Table 8.

Table 8. INTERMACS levels of 2947 VAD implantations in 2681 patients

INTERMACS Patient Profile	N (%)
1 - Critical cardiogenic shock	424 (14)
2 - Progressive decline	896 (30)
3 - Stable but inotrope dependent	733 (25)
4 - Resting symptoms	472 (16)
5 - Exertion intolerant	104 (4)
6 - Exertion limited	49 (2)
7 - Advanced NYHA class 3	43 (1)
Unknown	226 (8)
Total	2947

OUTCOME OF VENTRICULAR ASSIST DEVICE IMPLANTATION

Types of ventricular assist devices implanted Figure 1 shows the types of VADs implanted in both paediatric and adult patients from 1 January 2011 to 31 December 2016, entered into the EUROMACS database.

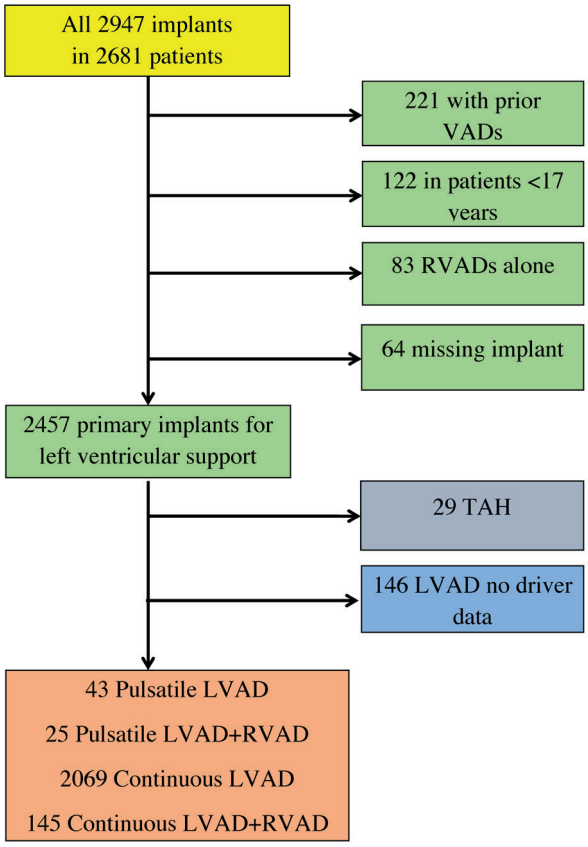

Figure 1. Types of Mechanical Circulatory Support Systems implanted from January 1, 2011 to 31 December 2015

Survival

The overall survival of 2268 adult patients (aged >17 years) with a continuous LVAD or a biventricular assist device (BiVAD) and a mean follow-up period of 379 days (median 236 days, range 1– 2098 days) was 86% (CI 85–88), 66% (CI 64–68), 53% (CI 51–56) and 42% (CI 39–45) at 30 days, 1 year, 2 years and 3 years, respectively (Fig. 2).

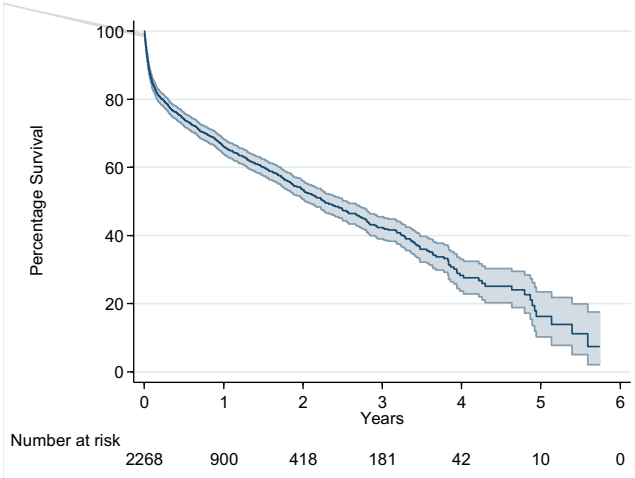

Figure 2. Survival of adult patients after primary LVAD or BiVAD implantation with continuous flow LVAD

Stratified according to the site of VAD implantation, the survival rate of 2113 patients with continuous-flow LVAD, either as a destination therapy or as a bridge to transplant, was 88% (CI 87–90), 69% (CI 66–71), 55% (CI 52–58) and 44% (CI 40–47) at 30 days, 1 year, 2 years and 3 years, respectively (Fig. 3). The survival rate of 141 patients with BiVAD was 61% (CI 52–68), 32% (CI 23–40), 27% (CI 19–35) and 21% (CI 13–30) at 30 days, 1 year, 2 years and 3 years, respectively (Fig. 3).

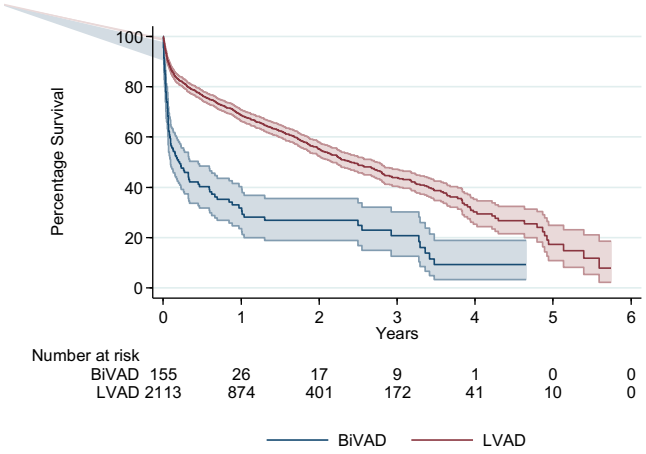

Figure 3. Survival of adult patient having continuous flow LVAD stratified by primary LVAD or primary BiVAD implantation

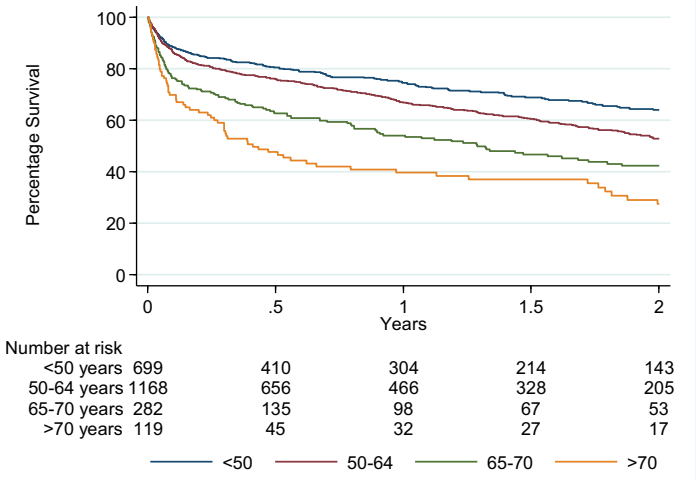

Figure 4. Survival of adult patient having primary LVAD or BiVAD implantation stratified by age cathegory with continuous flow LVAD

Figure 4 shows the age group-based survival rates of patients with primary LVAD and BiVAD support. At 2 years, the survival rate was 64% (CI 59–68), 53% (CI 49–56), 42% (CI 35–49) and 27% (CI 18–37) in patients aged <50, 50–64, 65–70 and >70 years, respectively. Figure 5 depicts the actuarial survival depending on device strategy. Bridge-to-transplant strategy revealed the best survival. Table 9 shows the causes of death of 1027 patients with VAD who were registered as deceased. The 2 main causes of death were multiorgan failure in 186 (18%) patients and infections and sepsis in 208 (20%) patients.

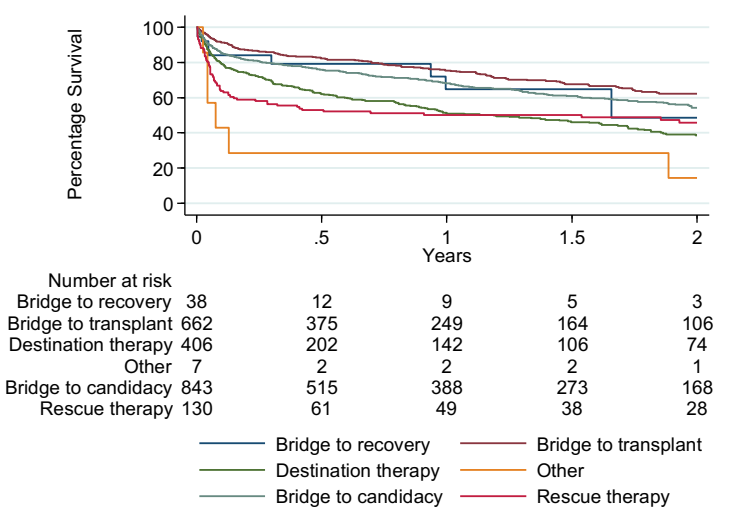

Figure 5. Survival of adult patients after primary LVAD or BiVAD implantation with continuous flow LVAD, stratified by strategy pursued with the device implantation

Table 9. causes of death

Causes of death	N (%)
Infection	208 (20)
Cerebro-vascular accident	132 (13)
Cardio-pulmonary failure	48 (5)
Multi-organ failure	186 (18)
Bleeding	50 (5)
Other cause of death	403 (39)
Total	1027

Adverse events (morbidity)

Major adverse events (Table 10) related to device malfunctions, such as accidental disconnection, wear or breaking of the driveline and pump thrombosis, were observed 454 times within the entire follow-up period, which corresponds to 0.037 malfunctions per patient year. For definitions of adverse events, we refer the reader to the corresponding INTERMACS definitions [10]. As other groups have reported, patients with continuous-flow assist devices had a higher risk for major bleeding [11]. In the EUROMACS database, major bleeding (requesting at least 1 unit of blood for transfusion) was reported 433 times, whereas 845 major infections caused by either the driveline or the assist device were observed. Neurological dysfunction (stroke) occurred in 319 of the adverse events, whereas 52 of the adverse events were a combination of one or more events. All major adverse events occurred more frequently within the first 3 months after implantation than later during the patients' course. The rate of device malfunctions and infections reached a stable state 1 year after implantation, whereas the rates of bleeding and neurological events decreased for the entire follow-up period.

Competing outcomes

Within 6 months after device implantation, 5.4% of the patients received a heart transplant and 27.9% died. Only 1.5% could be weaned from the device, and 65.2% had ongoing device support during this period (Fig. 6).

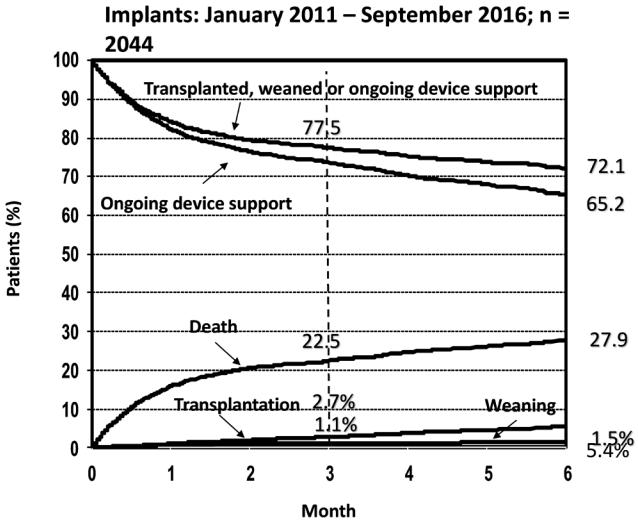

Figure 6. Competing risks of patients after assist implant who have been followed-up at Competing Outcomes in Patients with Assist Device Implants

DISCUSSION

Compared to the first EUROMACS report, the number of participating hospitals has increased from 21 to 52 (+148%), whereas the number of registered implantations more than tripled from 825 to 2947 (+257%). The 3-year survival rate of patients with continuous-flow LVAD and BiVAD implants, 44% and 21%, respectively, was far less favourable than the results of the seventh NTERMACS annual report (fig. 6 of that report), which was 58% and 40%, respectively [12].

There are major differences between the rate of morbidity in our current EUROMACS report and recent INTERMACS results, such as the occurrence of major infections, which is far higher in the INTERMACS cohort within the first 3 months after implantation (15.19 vs 6.18 events per 100 patient-months) but lower during the later course (4.03 vs 5.49) [6]. The same pattern can be seen with respect to neurological events (4.18 vs 3.03 events per 100 patient-months within 3 months after implant, 1.21 vs 1.87 in the later course).

What are the possible explanations for differences? (i) One rea- son might be differences in the quality of the data with respect to the completeness of reported events. INTERMACS

has a high level of completeness of collected data, mandated by the National Institutes of Health, though, similar to EUROMACS, INTERMACS has also periodic site visits, confirmation of case counts and frequent contact with sites to review adverse events (J.K. Kirklin, personal communication). On the other hand, EUROMACS, being an EACTS Committee, follows the same strategy of quality control as INTERMACS (see section 'Quality Control'). (ii) There might be some differences in definitions of events and different periods of observation times. (iii) There may be some real differences in outcomes related to different devices and management strategies or patient selection practices. These differences were discussed with IMACS before the 2 registries agreed to analyse aggregated anonymous EUROMACS data. An incoming study proposal intends to investigate the details of these differences. The growth in the number of participating hospitals precipitated the increase in quality control by means of statistical analyses.

Limitations

The registry continues recruiting to increase the numbers of contributing centres, the goal being to include as many European centres as possible. In contrast to the situation in the USA, participation in EUROMACS is not mandatory in Europe. Therefore, surveillance and improvement of data quality are ongoing efforts.

CONCLUSION

Because EUROMACS became an official committee of EACTS, the registry experienced an increase in the number of participating hospitals (+148%) and more than tripled the number of implants, representing European MCS data at the best achievable level and reached a unique comprehensive representation of European MCS baseline and follow-up data. In addition, the productive co- operation with IMACS permits the inclusion of worldwide data and important comparisons. Mortality and morbidity outcome data differ between the registries. It is of high importance to investigate the reasons for these differences.

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CHAPTER 4

The European Registry for Patients with Mechanical Circulatory Support (EUROMACS): first EUROMACS Paediatric (Paedi-EUROMACS) report

De By TMMH, Schweiger M, Waheed H, Berger, Hübler M, Özbaran, Maruszewski BI, Napoleone CP, Meyns B, Miera O.

Eur J Cardiothoracic Surg. 2018 Nov 1;54(5):800-808

Abstract

Objectives: EUROMACS is a registry of the *European Association for Cardio-Thoracic Surgery* (EACTS) whose purpose is to gather clinical data related to durable mechanical circulatory support for scientific purposes and to publish annual reports. Because the treatment of children with end-stage heart failure has several significantly different characteristics than the treatment of adults, data and outcomes of interventions are analysed in this dedicated paediatric report.

Methods: Participating hospitals contributed pre-, peri- and long-term postoperative data on mechanical circulatory support implants to the registry. Data for all implants in paediatric patients (<_19 years of age) performed from 1 January 2000 to 31 December 2017 were analysed. This report includes updates of patient characteristics, implant frequency, outcome (including mortality rates, transplants and recovery rates) as well as adverse events.

Results: Twenty-five hospitals contributed 237 registered implants in 210 patients (81 $, 129 #) to the registry. The most frequent diagnosis was any form of cardiomyopathy (71.4%) followed by congenital heart disease (18.6%). Overall mean support time on a device was 11.6 months (±16.5 standard deviation). A total of 173 children (82.4%) survived to transplant, recovery or are ongoing; 37 patients (17.6%) died while on support within the observed follow-up time. At 12 months 38% of patients received transplants, 7% were weaned from their device and 15% died. At 24 months, 51% of patients received transplants, 17% died while on support, 22% were on a device and 9% were explanted due to myocardial recovery. The adverse events rate per 100 patient-months was 0.2 for device malfunction, 0.05 for major bleeding, 0.06 for major infection and 0.03 for neurological events within the first 3 months after implantation.

Conclusions: The first paediatric EUROMACS report reveals a low transplant rate in European countries within the first 2 years of implantation compared to US data. The 1-year survival rate seems to be satisfactory. Device malfunction including pump chamber changes due to thrombosis was the most frequent adverse event.

INTRODUCTION

The use of durable mechanical circulatory support (MCS) in children in the form of a ventricular assist device (VAD) has increased dramatically over the years and has improved survival for paediatric patients on the waiting list for a heart transplant [1]. Paediatric patients receiving MCS is a unique area of study due to the physical size of the recipient, which not only requires careful selection of an appropriately sized device but also different management techniques than those used in adults. Children require specially adapted pharmacological treatment and the prevention of adverse events requires a very different clinical management from that in adults.

The EUROMACS Committee of the *European Association for Cardio-Thoracic Surgery* (EACTS) governs the registry, which was launched in 2009 and became operational in 2012. EUROMACS is the only European-based durable MCS registry for all devices with the CE Marking implanted in children and adults (Table 1). The purpose of the registry is to gather clinical data related to durable MCS for scientific purposes and to publish annual reports. From the outset, all possible options in MCS strategy with respect to devices on the market and to data on patients of every age and geographic area were included [2]. This approach enables the registry not only to select paediatric patients as a distinguished patient cohort for analyses of baseline data but also to follow them up even after they have passed the age of 19 years. EUROMACS collects data continuing through the period of VAD support; there are 3 end points: transplantation, weaning and death. The EUROMACS database has been designed in such a way that the patient and the device outcomes will be comparable with the Pedimacs and Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) databases.

So far, 2 EUROMACS reports have been published [2, 3] analysing the adult population. This EUROMACS report is the first to focus on patients <_19 years of age. Its goal is to report outcomes of children supported with MCS from a European perspective.

METHODS

As per 31 December 2017, 25 centres from 14 different countries (Table 2) submitted to EUROMACS data on patients <_19 years of age. The participating centres are advised to enter data (of the patients or of the parents who have given consent in writing) of the patients who received an MCS device since 1 January 2011. Thus, newly enrolled centres will retrospectively enter data through that date. Some centres have chosen to submit data from an earlier date, and 35 patients were registered before 1 January 2011.

Data quality checks and audits

To ensure the best quality of data and to exclude the under- reporting of suboptimal outcomes, the EUROMACS Registry applies several methods. Incoming data are analysed on a regular basis. Individual hospitals are approached, and guidance is offered to complete or correct their data. Entries are adapted to adhere to the standard. Twice a year, each centre receives a file in which an overview of patients whose statuses need to be updated and whose changes/answers have to be monitored is presented. Statistical consistency and plausibility checks are per- formed, and the records containing the inconsistent data of the participating centres are identified. Data that are not plausible re- quire checking and confirmation by the participating centres.

Table 1. Present CE-marked mechanical circulatory support systems registered in the EUROMACS database

MCS type	
Durable devices	
Continuous flow	Berlin Heart INCOR CircuLite SYNERGY[a] HeartAssist 5 HeartWare HVAD Jarvik 2000 MicroMed DeBakey Thoratec HeartMate II Thoratec HeartMate 3
Pulsatile extracorporeal	Berlin Heart EXCOR Thoratec PVAD Abiomed AB5000
Total artificial heart	SynCardia Cardiowest
Short-term devices	Medos DeltaStream[b] Levitronix CentriMag[b] Maquet CARDIOHELP[b]

[a]Withdrawn from the market in 2014.

[b]These short-term devices can be used with an oxygenator for ECLS/ECMO. A provision has been made for devices that were implanted concomitantly (as a temporary right ventricular assist device) with a long-term device.

CE: European conformity; ECLS/ECMO: extracorporeal life support/extracorporeal membrane oxygenation; EUROMACS: European Registry for Patients with Mechanical Circulatory Support; HVAD: HeartWare ventricular assist device; MCS: mechanical circulatory sup-port; PVAD: paracorporeal ventricular assist device; VAD: ventricular assist device.

Table 2. Participating paediatric units providing data for this report

Country	City, hospital
Austria	Innsbruck, Innsbruck University Clinics
Belarus	Minsk, Republican Scientific and Practical Center Cardiology
Belgium	Gent, Universitair Ziekenhuis Gent Leuven, Universitair Ziekenhuis UZ Leuven
Czech	Brno, Center for Cardiovascular and Transplant Surgery Republic Prague, Institute for Clinical and Experimental Medicine
France	Le Plessis Robinson, Centre Chirurgical Marie- Lannelongue
Germany	Bad Oeynhausen, Herz und Diabeteszentrum Nordrhein-Westfalen Berlin, Deutsches Herzzentrum Berlin Freiburg, University Heart Center Freiburg Bad Krozingen
Hungary	Budapest, Gottsegen Hungarian Institute of Cardiology
Italy	Rome, Ospedale Pediatrico Bambino Gesù Bergamo, Ospedale Papa Giovanni XIII Bologna, San Orsola Hospital Torino, Regina Margherita Children's Hospital
Kazakhstan	Astana, National Research Cardiac Surgery Center
Netherlands	Rotterdam, Erasmus Medisch Center Utrecht,Universitair Medisch Centrum Utrecht
Poland	Warsaw, Childrens Memorial Hospital
Spain	Madrid, Hospital La Paz
Switzerland	Zürich, Kinderspital Zürich Bern, University Hospital Bern (Inselspital)
Turkey	Ankara, Baskent University Hospital Izmir, Ege University Hospital Istanbul, Florence Nightingale University Hospital

The average number of follow-up records per patient is calculated on a per centre basis and serves as an indicator for homogeneity and completeness of recording. In addition, random on-site audits of participating centres are carried out.

Statistical analysis

We checked for the chronological plausibility of the records and eliminated or corrected implausible records by queries to on-site data managers. Data are presented as the mean ± deviation (SD) or frequency with percentage. To examine mortality after implant, Kaplan–Meier estimates of cumulative probabilities were calculated, including 95% confidence intervals as a measure of certainty, because we did not truncate the curves. Kaplan–Meier curves were censored at explantation due to trans- plant or recovery. A patient is considered at risk until explantation because the patient received a transplant, has been weaned from the device, has died or is alive. To determine these values, cumulative incidences were calculated using competing out- comes methods and are presented for the first 2 years after the device is implanted. To avoid any censored individuals, only patients with a follow-up period of 2 years were considered for the competing outcome analysis. The user-written programme 'STCOMPET' in STATA was used to calculate the cumulative incidence [4]. Statistical analyses and figures were constructed using Stata 15.0 (StataCorp, College Station, TX, USA).

RESULTS

Patient population

Between January 2000 and December 2017, 237 implants in 210 patients were registered (Fig. 1), 129 (61.4%) of which were male and 81 (38.6%) of which were female. The mean age was 9.3 years (±7.0 SD), and it ranged from 0 weeks to 19 years. Almost one-fifth of the patients were below 1 year of age, and half of the population was above 10 years of age. Baseline characteristics can be seen in Table 3. Primary diagnoses at admission included cardiomyopathy (including myocarditis) in 150 (71.4%), congenital heart disease in 39 (18.6%) and other in 21 (10%) (Table 4). VAD implantation was performed primarily in patients with INTERMACS levels 1, 2 and 3 with 44 (21.0%) patients at INTERMACS profile 1.

A total of 70.5% of all children were on inotropic support prior to VAD implantation. Extracardiac life support was used in 17.6% of the patients prior to VAD implantation. Twenty-two patients received a 2nd VAD implant after the 1st one, 3 patients a 3rd and 2 patients a 4th implant (Table 5). The majority of the patients (73.8%) were treated with the intention to transplant (i.e. bridge to transplant or possible bridge to transplant), and this was true for all age groups (Table 6).

A total of 46.8% of the patients were supported with the Berlin Heart Excor® (Berlin Heart, Berlin, Germany), 5.9% with the Heart Mate II® (Thoratec Corp., Pleasanton, CA, USA), 0.8% with HeartAssist5® (MicroMed, Houston, TX, USA) and 27.0% with HeartWare HVAD® (HeartWare Ltd., Framingham, MA, USA) (Table 7). In 67 patients, a concomitant cardiac procedure (21 congenital and valve procedures and 46 other procedures) was performed.

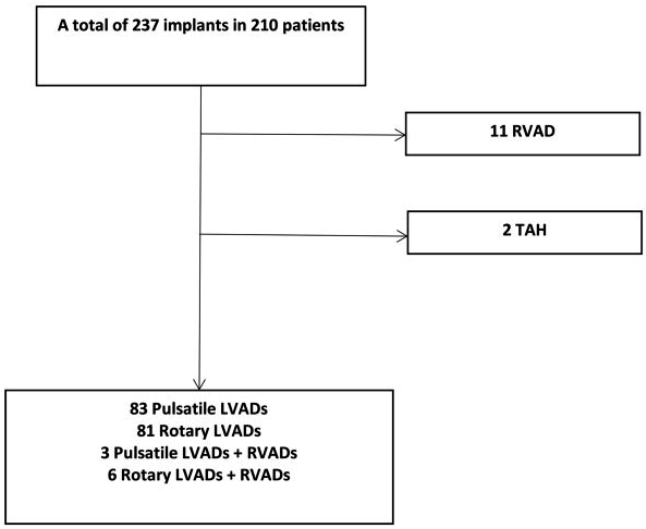

Figure 1. Paediatric patients registered in the EUROMACS Registry. LVAD: left ventricular assist device; RVAD: right ventricular assist device; TAH: total artificial heart.

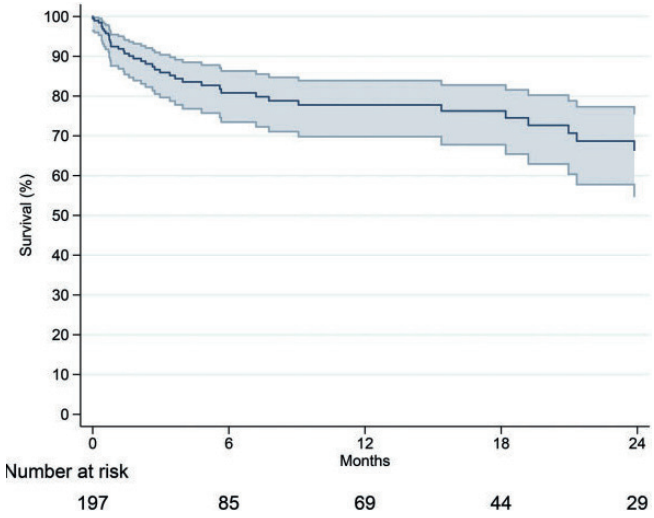

Figure 2. Survival of paediatric patients after primary left or biventricular assist device implantation.

Outcomes

The mean support time on a device was 11.6 months (±16.5 SD). The mean stay in the intensive care unit was 37.0 days (±54.5 SD). Ninety-three (44.3%) patients were discharged either to their homes or to a rehabilitation facility. A total of 173 children (82.4%) survived to transplant, recovery, or are on ongoing treatment until the last follow-up. At 6 months, 33% of the patients and at the 1st year 38% of the children received a transplant. This percentage climbed to 51% at 2 years post VAD implantation. Thirty-seven patients (17.6%) died while on support within the observed follow-up time (Table 8).

A total of 37 patients (17.6%) died, of which 24.3% died of cerebrovascular accidents. Five patients (13.5%) died of multiorgan failure. The primary cause of death was not specified for 14 patients (Table 9).

Survival

Event-free survival of all paediatric patients on MCS was 81% at 6 months, 78% at 12 months and 66% at 2 years with censoring at time of explantation for transplant or recovery (Fig. 2). When stratified by device type, i.e. left ventricular assist device (LVAD) or a biventricular assist device, 81% survival was observed in the 1st year for LVADs and 63% for biventricular assist devices ($P = 0.06$) (Fig. 3).

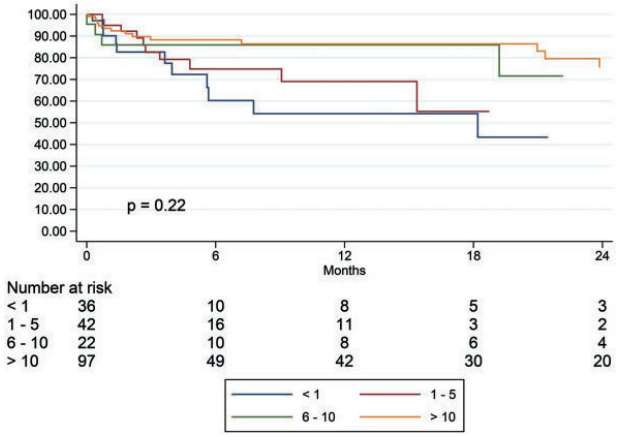

Figure 3. Survival of paediatric patients after primary implantation of a left ventricular assist device or a biventricular assist device, stratified by age.

When stratified by age, the oldest age group (11–19 years) had an 86% survival rate at the end of the 1st year and 76% at the end of the 2nd year; the age group 6–10 years had an 86% 1-year and 72% 2-year survival rate and the age group 1–5 years had a 69% survival rate at the end of the 1st year and 55% at the end of the 2nd year. Patients <_1 year old showed the poorest outcome: 54% had a 1-year and 43%, a 2-year survival rate (Fig. 4). However, the latter survival rates showed poor statistical significance (P = 0.22). Figure 5 shows the survival rate stratified by device strategy.

Table 3. Patient characteristics preimplant

Characteristics	**Total (*n* =210)**
Age (years), mean ± SD (median, range)	9.3 ± 7.0 (10.5, 0–19)
Preoperative creatinine level (mg/dl), mean ± SD (median, range)	0.83 ± 0.51 (0.70, 0.19 – 3.74)
Preoperative total bilirubin level (mg/dl), mean ± SD (median, range)	0.1 ± 0.1 (0.06, 0.001–0.9)
Body mass index (kg/m2), mean ± SD (median, range)	17.87 ± 5.08 (16.4, 9.78–37.65)
Age categories, *n* (%)	
<1 year	38 (18.1)
1–5 years	45 (21.4)
6–10 years	22 (10.5)
>10 years	105 (50.0)
Total	210
Gender, *n* (%)	
Male	129 (61.4)
Female	81 (38.6)

SD: standard deviation.

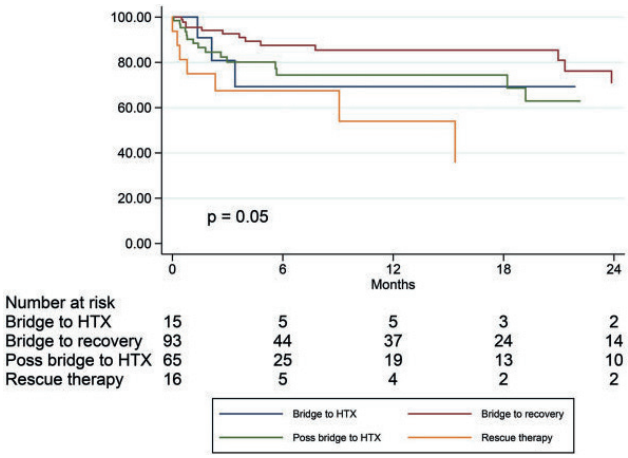

Figure 4. Survival of paediatric after primary implantation of a left ventricular assist device or a biventricular assist device, stratified by the device implant.
HTX: heart transplant.

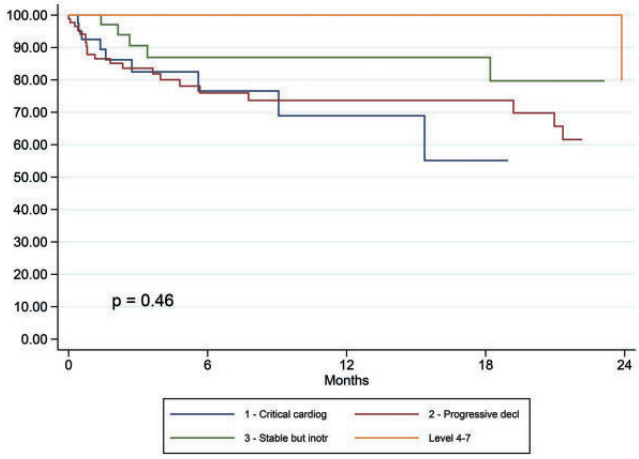

Figure 5. Survival of paediatric after primary implantation of a left ventricular assist device or a biventricular assist device, stratified by Interagency Registry.

Table 4. Primary diagnosis

Diagnosis	*n*	%
Cardiomyopathy	117	55.7
Myocarditis	33	15.7
Congenital heart disease	39	18.6
Coronary artery disease	1	0.5
Valvular heart disease	3	1.4
Cancer	1	0.5
Unknown/missing	16	7.6
	210	

Table 5. Primary and subsequently implanted devices

Devices	1st	2nd	3rd	4th	Total
BiVAD	36	2			38
LVAD	163	12	1		176
LVAD and RVAD	8	1			9
RVAD	1	6	2	2	11
Total artificial heart	1	1			2
Unknown	1				1
Total	210	22	3	2	237

Table 6. Device strategy at time of implantation, stratified by age categories

	<1	1–5	6–10	>10	Total
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Bridge to recovery	4 (8.9)	4 (8.0)	2 (7.1)	7 (6.1)	17 (7.2)
Bridge to transplant	18 (40.0)	23 (46.0)	9 (32.1)	51 (44.7)	101 (42.6)
Destination therapy	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.9)	1 (0.4)
Possible bridge to transplant	14 (31.1)	13 (26.0)	11 (39.3)	36 (31.6)	74 (31.2)
Rescue therapy	3 (6.7)	7 (14.0)	2 (7.1)	7 (6.1)	19 (8.0)
Unknown	6 (13.3)	3 (6.0)	4 (14.3)	12 (10.5)	25 (10.5)
Total	45 (100)	50 (100)	28 (100)	114 (100.0)	237 (100)

Table 7. Type of ventricular assist devices per age group

Lvad alone	1	1-5	6-10	10	Total
Pulsatile	32	30	7	14	83
Continuous	2	2	9	68	81
Unspecified		1	1	10	12
LVAD, temporary RVAD					
Continuous LVAD, continuous RVAD				6	6
Pulsatile LVAD, continuous RVAD	2	1			3
BiVAD					
Pulsatile	6	11	5	13	35
Continuous			3		3
Total artificial heart					
Pulsatile		2			2
Unknown				1	1
					237

BiVAD: biventricular assist device;
LVAD: left ventricular assist device; RVAD: right ventricular assist device.

Competing outcomes

Within 2 years after an implant, 51% of the patients received a heart transplant and 17% died. Only 9% could be weaned from the device and 22% had ongoing device support (Fig. 6).

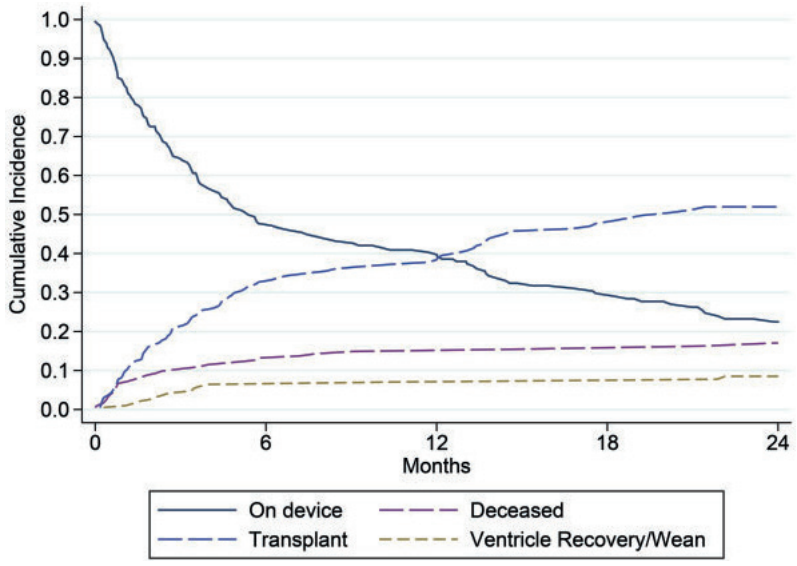

Figure 6. Competing outcomes.

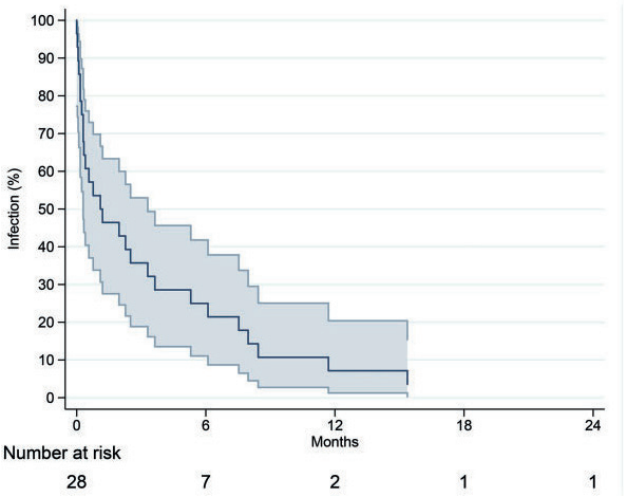

Figure 7. Freedom from infection.

Adverse events

Overall, 151 major adverse events were reported during VAD support. Major adverse events are defined using the INTERMACS definitions [5]. These included infection, device malfunction, bleeding and neurological events (Table 10). Within the first 3 months after VAD implantation, 38 events occurred whereas 113 occurred after 3 months.

The most frequent major adverse event was device malfunction, which included as per definition pump exchanges from extracorporeal devices due to pump thrombosis. Device mal- function occurred 20 times in the first 3 months. In the same period, the device malfunction rate was 0.2 per 100 person- months and 4.2 per 100 person-months after 3 months.

Infections were the 2nd most frequent adverse event (n = 31; event rate: 20.5%). Infections were divided into VAD-specific, VAD-related and non-VAD-related.

Major infection in paediatric patients occurred more frequently after the first 3 months post implantation (n = 23), i.e. 1.3 events per 100 patients. During the first 3 months, 8 cases, or 0.06 per 100 patients, were reported.

Major bleeding, defined as an episode of suspected internal or external bleeding that resulted in death, reoperation, hospitalization or major transfusion, but not including cerebral haemorrhage, occurred in 15 patients (event rate, 9.9%) with 0.05 events per 100 patients in the first 3 months and 0.5 events per 100 patients after 3 months. Two patients died (0.95%) of a bleeding event.

Neurological events were defined by the occurrence of an ischaemic or a haemorrhagic stroke. Eleven patients had a neuro- logical event (event rate: 7.3%). Whereas only 0.03 events per 100 patients occurred within the first 3 months after implantation, the majority occurred later (0.8 events per 100 patients after 3 months). Nine patients (24.3%) died of neurological events, making this the primary cause of death within the whole cohort (see also Table 9). Six patients had heart transplants or sere successfully weaned from the device after a neurological event.

DISCUSSION

This report is the first of children supported with durable MCS that has emerged from the EUROMACS database. The EUROMACS registry is the largest database monitoring children supported with VADs in Europe, and enrolment of centres and patients continues. The authors believe it is crucial to add information about the European cohort to the other paediatric MCS database of similar size, Pedimacs, which is restricted to North American data.

One of the most striking differences between the EUROMACS and the Pedimacs cohorts is the waiting time for a heart trans- plant. Whereas permanent support has long become a reality for adults, bridge to transplantation or transplantability still remains the highest percentage in intention to treat within the paediatric population. Whereas almost 50% [6] of the paediatric patients in North America had a transplant within the first 6 months after a VAD implant, in Europe, only 33% at 6 months had a transplant and 38% patients at 12 months. These numbers reflect the lack of suitable donor organs in Europe, which leads to significantly longer support times. Especially in small countries or in patients under 5 years of age [7, 8], times on the heart transplant waiting list have increased. In the registry of the Eurotransplant International Foundation, the percentage of paediatric patients who receive transplants is 48% at 6 months and 57% at 12 months (personal communication, J. Smits, Eurotransplant). In Switzerland, the number of paediatric heart transplant candidates between 2009 and 2013 increased by a factor of 4 compared to the previous period [9]. In Italy, the mean time on the waiting list is more than 11 months, and in Poland (all patients), the mean waiting time is 12 months. Especially for small countries, inter- national organ exchange among organ procurement organizations is essential. It has a direct positive impact on the possibility of patients receiving a timely, often life-saving, transplant [8]. The longer support times in Europe enable us to provide outcome data beyond 12 months of support.

One important finding of this report is that the cumulative competing incidence of death is 15% by the end of year 1 and 17% by the end of year 2. This result indicates a low mortality rate in the 2nd year of support and makes permanent support in children more feasible.

A total of 44.3% of the patients were discharged on the device. The methods used for quality checks do not indicate that serious infections are under-reported; in fact, the opposite is true. The percentage is high (20.5%), though the specificity with respect to the severity and location is low, which leads to the suspicion that different definitions may have been used.

The implantation strategy of bridge to recovery is low at 7.3%, which is relatively similar to the percentage published in the Pedimacs report (6.3%). In the group categorized as bridge to recovery (n = 16), 11 patients underwent successful explantation (69%). The others are either still on support (n = 2), have died (n = 2) or received a transplant (n = 1). For the whole cohort, 24 patients out of 210 had the device explanted due to weaning (see Table 8). The percentage of devices implanted with the intention to treat for bridge to recovery almost equals the number of devices explanted due to recovery. One reason why this number is so low might be the current lack of standardized guidelines for echocardiographic and haemodynamic criteria for LVAD removal in children [10], although children may have a greater potential for recovery [11] compared to adults.

Adverse events

Neurological events were the leading cause of death in our cohort as well in the North American cohort (24% vs 30%). Blume *et al.* [6] reported a higher stroke rate of 13 early events per 100 patient months and 2 late events per 100 patient months with paracorporeal devices compared to continuous flow devices (3 and 1 events per 100 patient months, respectively). Almond *et al.* [12] showed comparably high stroke rates for children on EXCOR VADs during the investigational device exemption trial of 15 events per 100 patient years with 29% of children affected. Although the stroke rate was not investigated within this 1st EUROMACS Paediatric Report, a recent study from the paediatric EUROMACS cohort reported low early and late stroke rates with intracorporeal continuous flow devices (0.03 and 0.4 events per 100 patient months, respectively), independent of body surface area [13]. The stroke rates in children on EXCOR and on continuous flow VADs reported in the EUROMACS registry are remarkably low. However, a recent survey addressing the antithrombotic protocols for children on EXCOR VADs in European centres revealed many modifications of the recommended Edmonton protocol with a trend towards more aggressive antithrombotic therapy [14]. Whether these modifications have contributed to lower stroke rates compared to the investigational device exemption trial is under investigation.

Another frequent adverse event was infection in 20.5%, which is clearly high (Fig. 7). One explanation could be that the definition of infection in the EUROMACS registry includes VAD- specific, VAD-related and non-VAD-related infections. The authors found that the major infection rate 3 months post implant was 1.3 per 100 person-months compared to 0.06 per 100 person-months within the first 3 months of implant. This result suggests that many of these infections are less likely to be related to implant surgery and occur while patients remain for pro- longed stays in the intensive care unit

or during hospitalization post implant. This result could be another effect of the lower transplantation rate and the longer support times in Europe.

Limitations

The present study does not include all European centres that are implanting MCS devices. Besides the contributing centres, 14 additional hospitals were invited to join EUROMACS and submit data. Considering their positive feedback, it is expected that, in a 2nd EUROMACS Paediatric report, the data from most of these 'additional' hospitals will be in the registry. Data collection by means of a registry has per se an important limitation: as in every database, despite all efforts to guarantee data quality and the implementation of audits, under-reporting of adverse events cannot be ruled out.

CONCLUSION

Because EUROMACS is supported by the EACTS, the registry can reach out to an increasing number of participating hospitals to collect baseline and follow-up data on MCS from both adults and children, thus representing European data at the best achievable level. The ability to specify the different factors contributing to the outcomes of MCS in patients enables paediatric medical professionals to benchmark their data against the results of this study. Many questions remain to be addressed, i.e. discharge, additional specifics in anticoagulation management, focus on congenital heart disease and much more, which were beyond the scope of this 1st paediatric EUROMACS report. Further, a comparison with the 2nd Pedimacs report shows that outcome data differ between the registries. Investigating the rea- sons for these differences may contribute to insights with respect to treatment modalities and thus provide leads to possible improvements both in Europe and elsewhere.

ACKNOWLEDGEMENTS

The authors would like to thank Jacqueline Smits of the Eurotranplant International Foundation for her advice.

Table 8. Current device strategy stratified by the end point

End point	On	Dead	Received	Weaned	Total device transplant
Missing		1	3	1	11
Bridge to recovery	2	2	1	11	16
Bridge to transplant	22	12	60	2	96
Possible bridge to transplant	10	16	38	6	70
Rescue therapy	2	6	5	4	17
Total	42	37	107	24	210

Table 9. Primary cause of death

Primary cause of death	*n*	%
Bleeding	2	5.4
Cardiopulmonary failure	2	5.4
Cerebrovascular accident	9	24.3
Device failure	1	2.7
Multiorgan failure	5	13.5
Other cause of death	1	2.7
Right heart failure	1	2.7
Sepsis	2	5.4
Unknown/missing	14	37.8
	37	

Table 10. Major adverse event rates

	Within 3 months after implant		More than 3 months after implant	
	Event counts	Events per 100 patient months (CI)	Event counts	Events per 100 patient months (CI)
Device malfunction	20	0.2 (0.1–0.3)	74	4.2 (3.4–5.3)
Major bleeding	6	0.05 (0.02–0.1)	9	0.5 (0.3–1.0)
Major infection	8	0.06 (0.03–0.1)	23	1.3 (0.9–2.0)
Neurological event	4	0.03 (0.01–0.09)	7	0.4 (0.2–0.8)
Total events	38		113	

CI: confidence interval

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CHAPTER 5

Second Annual Report from the ISHLT Mechanically Assisted Circulatory Support (IMACS) Registry

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The second annual International Society for Heart and Lung Transplantation Mechanically Assisted Circulatory Support (IMACS) Registry report includes over 14,000 patients from 35 countries. Survival, adverse events, and an updated risk model are presented. Continuous-flow pumps continue to dominate the world's experience. One- and 2-year survival remains at 80% and 70%, respectively. Congenital heart disease and biventricular support are the most prominent risk factors. The database is poised for major novel analyses.

The International Society for Heart and Lung Trans- plantation (ISHLT) Mechanically Assisted Circulatory Support (IMACS) Registry represents a global database for patients receiving durable mechanical circulatory sup- port (MCS) devices. The stated mission focuses on acquisition of international MCS patient data and generation of analyses and publications that benefit the field.[1,2] This second annual report focuses on an updated survival analysis, adverse events, and risk modeling.

Since the initiation of patient enrollment in January 2013, 14,062 patients have been enrolled through December 31, 2016, representing 35 countries (Table 1). Data sources include individual hospitals and the following collectives (large databases that have collected MCS data for entire countries or regions): the European Registry for Patients with Mechanical Circulatory Support (EURO- MACS, Europe)[3]; the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS, USA)[1]; the Japanese Registry for Mechanically Assisted Circulatory Support (J-MACS, Japan)[4]; and the UK Registry (UK). Collective data downloads occur in December of each year and are merged into one comprehensive IMACS data set for analysis.

Table 1. IMACS Participating Countries, January 1, 2013 to December 31, 2016 (n ¼ 14,062)

Australia	Finland	Japan	Spain Belarus
Austria	France	Kazakhstan	Sweden
Azerbaijan	Germany	Netherlands	Switzerland
Belgium	Greece	New Zealand	Turkey Canada
Brazil	Hong Kong	Norway	UK
Colombia	Hungary	Poland	USA
Czech Republic	Ireland	Saudi Arabia	
Denmark	Israel	Singapore	
Egypt	Italy	Slovakia	

IMACS; International Society for Heart and Lung Transplantation Mechanical Circulatory Support Registry

Patients' demographics and device types

Among device types represented in this database, left ventricular assist devices (LVADs) accounted for 13,102 (93%) implants, of which 99% were continuous-flow (CF) pumps. Total artificial hearts (TAHs) represented 2% (279 devices) of the experience and biventricular support 5% (refer to Supplementary Material Figure S1, available online at www.jhltonline.org/).

Patients' demographics indicate 79% males, with 60% of patients between 50 and 60 years of age (Table 2). At the time of implant, 51% of patients were in rapid decline or cardiogenic shock (Table 3). Only 16% of patients had ambulatory heart failure (Patient Profile 4 to 7). Nearly 60% of patients were actively listed or considered candidates for heart transplantation, whereas 41% received devices as long-term destination therapy (Table 4). The device strategies among various patient profiles1 at implant are listed in Table 5.

Table 2. Age Distribution at Implant, IMACS, January 1, 2013 to December 31, 2016 (n ¼ 14,062)

Age at implant	Number	Percent
19 to 29 years	713	5%
30 to 49 years	3,248	23%
50 to 60 years	8,447	60%
70þ years	1,654	12%
Total	14,062	100%

IMACS, International Society for Heart and Lung Transplantation Mechanically Assisted Circulatory Support.

Survival

Among all patients, survival at 1 and 2 years continued to be 79% and 70%, respectively (Supplementary Material Figure S2 online).[5] The 3-year survival with CF durable devices (Figure 1) was just over 60%. Figure 1 shows hazard function[6] had a rapidly decreasing risk, which merged with a constant phase at about 3 months. Survival was clearly superior for patients receiving isolated left ventricular support compared with biventricular support (Figure 2). The 1-year survival for isolated CF LVAD support was 81% vs 53% for biventricular support and about 48% for TAH. The rate of transplantation was rather low, 28% at 1 year, among patients with a CF LVAD listed for transplant (Supplementary Material Figure S3 online). The transplant rate was higher for listed patients requiring biventricular support (36% at 1 year), and highest for TAH patients (50% at 1 year) (Supplementary Material Figures S4 and S5 online). Patients implanted with a strategy of destination therapy continued to show worse survival compared with a strategy of bridge to transplant or transplant candidacy (Figure 3).

Causes of death

The most frequent primary causes of death were multi- system organ failure (21% of mortality), cardiovascular causes (primarily right heart failure) (20%), and stroke (19%) (Supplementary Material Table S1 online).

Table 3. Patient Profile Distribution at Implant, IMACS, January 1, 2013 to December 31, 2016 (n ¼ 14,062)

Patient profile at time of implant	Number	Percent
1. Critical cardiogenic shock	2,405	17%
2. Progressive decline	4,714	34%
3. Stable but inotrope dependent	4,558	32%
4. Resting symptoms	1,817	13%
5. Exertion intolerant	298	2%
6. Exertion limited	87	0.6%
7. Advanced NYHA Class III	66	0.5%
Unspecified	117	0.8%
Total	14,062	100%

IMACS, International Society for Heart and Lung Transplantation Mechanically Assisted Circulatory Support; NYHA, New York Heart Association.

Table 4. Device Strategy, IMACS, January 1, 2013 to December 31, 2016 (n ¼ 14,062)

Device strategy	N	%
Listed for transplant	3,984	28%
Bridge to candidacy	4,072	29%
Destination therapy	5,724	41%
Other	282	2%
Total	14,062	100%

IMACS, International Society for Heart and Lung Transplantation Mechanically Assisted Circulatory Support.

Adverse events

Infection and bleeding affected the most patients, occurring in 40% and 35% of patients, respectively; 19% had neurologic events (Table 6). Bleeding was the most frequent adverse event during the first 3 months post-implant, followed by infection (Table 7). During the later phase (beyond 3 months), infection and internal bleeding had the highest incidence. Among patients with CF devices, freedom from first infection was 68% at 6 months (Supplementary Material Figure S6). The likelihood of stroke (ischemic or hemorrhagic) was 14% at 6 months and 19% at 12 months (Supplementary Material Figure S7 online). The risk of respiratory failure was highest during the first month (Supplementary Material Figure S8 online).

Risk factors for mortality

A detailed multivariable analysis identified risk factors for early and midterm mortality for patients receiving CF devices (Table 8). The 2 most dominant risk factors for early mortality were a diagnosis of congenital heart disease (hazard ratio [HR] 5.2) and the need for biventricular support (HR 3.4). The adverse effect of congenital heart disease was only evident during the first 2 months, after which patients with congenital heart disease did as well as those with other diagnoses (Figure 4).

Older age was a risk factor both in the early and constant phases, particularly in patients 450 years old (Figure 5). Among patients 30 to 50 years old, the 2-year survival was 79% compared with 58% for patients 470 years old (p o 0.0001). The increased vulnerability of elderly patients is especially magnified when they are critically ill at implant or require biventricular support (Figures 6 and 7).

This global analysis quantifies the importance of. increased risk among patients who are critically ill (Profile 1 or 2) at the time of implant (Figure 8). Compared with stable but inotrope-dependent patients (Patient Profile 3) those presenting in cardiogenic shock had a 1-year survival of 71% vs 84% (p o 0.0001), respectively. The stratified Kaplan–Meier depiction in Figure 8 also shows the midterm survival benefit of patients who were less ill (Levels 5 to 7) at the time of implant. If this trend continues, and depending on associated post-implant morbidity in less ill patients, this may have implications regarding patient selection. A strategy of destination therapy was only a risk factor in the constant phase, with HR 1.14 (Figure 3 and Table 8). Concomitant surgeries also increased risk (Table 8, and Supplementary Material Figure S9 online).

Summary

1. The IMACS database now includes 14,000 patients with global representation.

2. CF pumps currently constitute 97% of device implants.

3. Patients with ambulatory heart failure account for only 16% of durable device implants.

4. Overall 1- and 2-year survival have continued at 80% and 70%, respectively, in this international database.

5. For the first time, we have identified a more favorable midterm survival among patients with ambulatory heart failure.

6. Among the elderly, survival is particularly poor among patients critically ill at implant or if biventricular support is required.

7. Bleeding and infection remain the most common adverse events.

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Table 6. Major Adverse Events, Continuous-flow LVAD/BiVAD, IMACS, January 1, 2013 to December 31, 2016 (n ¼ 13,618)

Adverse event type	Patient Experiencing event	Percentage of of all patients
Infection	5,439	40%
Bleeding	4,745	35%
Neurologic dysfunction	2,638	19%
Respiratory failure	2,205	16%
Device malfunction	233	2%
Arterial non-CNS thromboembolism	159	1%

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1. Kirklin JK, Cantor R, Mohacsi P, et al. First annual IMACS report: a global International Society for Heart and Lung Transplantation Registry for Mechanical Circulatory Support. J Heart Lung Transplant 2016;35:407-12.
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Pre-implant renal function had a dominant effect on survival (Table 8). Patients requiring dialysis within 2 days before implant had a high early mortality (Supplementary Material Figures S10 and S11 online), and higher blood urea nitrogen (BUN) (Supplementary Material Figure S12 online) and creatinine impacted early and later survival. Signs of hepatic dysfunction and tricuspid regurgitation as risk factors likely reflect worsening right heart failure. Poor nutritional status (lower albumin) impacted both early and longer term survival (Table 8).

Summary

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2. CF pumps currently constitute 97% of device implants.
3. Patients with ambulatory heart failure account for only 16% of durable device implants.

Causes of death

The most frequent primary causes of death were multi- system organ failure (21% of mortality), cardiovascular causes (primarily right heart failure) (20%), and stroke (19%) (Supplementary Material Table S1 online).

Table 3. Patient Profile Distribution at Implant, IMACS, January 1, 2013 to December 31, 2016 (n ¼ 14,062)

Patient profile at time of implant	Number	Percent
1. Critical cardiogenic shock	2,405	17%
2. Progressive decline	4,714	34%
3. Stable but inotrope dependent	4,558	32%
4. Resting symptoms	1,817	13%
5. Exertion intolerant	298	2%
6. Exertion limited	87	0.6%
7. Advanced NYHA Class III	66	0.5%
Unspecified	117	0.8%
Total	14,062	100%

IMACS, International Society for Heart and Lung Transplantation Mechanically Assisted Circulatory Support; NYHA, New York Heart Association.

Table 4. Device Strategy, IMACS, January 1, 2013 to December 31, 2016 (n ¼ 14,062)

Device strategy	N	%
Listed for transplant	3,984	28%
Bridge to candidacy	4,072	29%
Destination therapy	5,724	41%
Other	282	2%
Total	14,062	100%

IMACS, International Society for Heart and Lung Transplantation Mechanically Assisted Circulatory Support.

Adverse events

Infection and bleeding affected the most patients, occurring in 40% and 35% of patients, respectively; 19% had neurologic events (Table 6). Bleeding was the most frequent adverse event during the first 3 months post-implant, followed by infection (Table 7). During the later phase (beyond 3 months), infection and internal bleeding had the highest incidence. Among patients with CF devices, free- dom from first infection was 68% at 6 months (Supplementary Material Figure S6). The likelihood of stroke (ischemic or hemorrhagic) was 14% at 6 months and 19% at 12 months (Supplementary Material Figure S7 online). The risk of respiratory failure was highest during the first month (Supplementary Material Figure S8 online).

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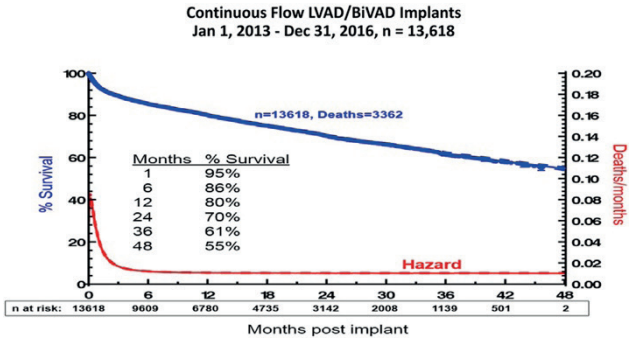

Figure 1. Parametric survival curve and associated hazard function with 70% confidence limit for survival after implantation of a continuous-flow left ventricular assist device (LVAD) or biventricular assist device (BiVAD), January 1, 2013 to December 31, 2016 (n ¼ 13,618). The number of patients at risk during each time interval is indicated below the diagram.

Older age was a risk factor both in the early and constant phases, particularly in patients 450 years old (Figure 5). Among patients 30 to 50 years old, the 2-year survival was 79% compared with 58% for patients 470 years old (p o 0.0001). The increased vulnerability of elderly patients is especially magnified when they are critically ill at implant or require biventricular support (Figures 6 and 7).

This global analysis quantifies the importance of increased risk among patients who are critically ill (Profile 1 or 2) at the time of implant (Figure 8). Compared with stable but inotrope-dependent patients (Patient Profile 3) those presenting in cardiogenic shock had a 1-year survival of 71% vs 84% (p o 0.0001), respectively. The stratified Kaplan–Meier depiction in Figure 8 also shows the midterm survival benefit of patients who were less ill (Levels 5 to 7) at the time of implant. If this trend continues, and depending on associated post-implant morbidity in less ill patients, this may have implications regarding patient selection. A strategy of destination therapy was only a risk factor in the constant phase, with HR 1.14 (Figure 3 and Table 8). Concomitant surgeries also increased risk (Table 8, and Supplementary Material Figure S9 online).

Table 5. Device Strategy by Patient Profile, IMACS, January 1, 2013 to December 31, 2016 (n ¼ 14,062)

	Device strategy at time of implant							
	Listed for transplant		Candidacy to transplant		Destination therapy		Other	
Patient profile at time of implant	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
1. Critical cardiogenic shock	526	13.2%	865	21.2%	885	15.4%	129	45.7%
2. Progressive decline	1,478	37.0%	1,315	32.2%	1,828	31.9%	93	32.9%
3. Stable but inotrope dependent	1,313	32.9%	1,212	29.7%	2,005	35.0%	28	9.9%
4. Resting symptoms	468	11.7%	534	13.1%	799	13.9%	16	5.6%
5. Exertion intolerant	107	2.6%	71	1.7%	115	2.0%	5	1.7%
6. Exertion limited	32	0.8%	23	0.5%	28	0.4%	4	1.4%
7. Advanced NYHA Class III	22	0.5%	20	0.4%	23	0.4%	1	0.3%
Unknown	38	0.9%	32	0.7%	41	0.7%	6	2.1%
Total	3,984	100.0%	4,072	100.0%	5,724	100.0%	282	100.0%

IMACS, International Society for Heart and Lung Transplantation Mechanically Assisted Circulatory Support; NYHA, New York Heart Association.

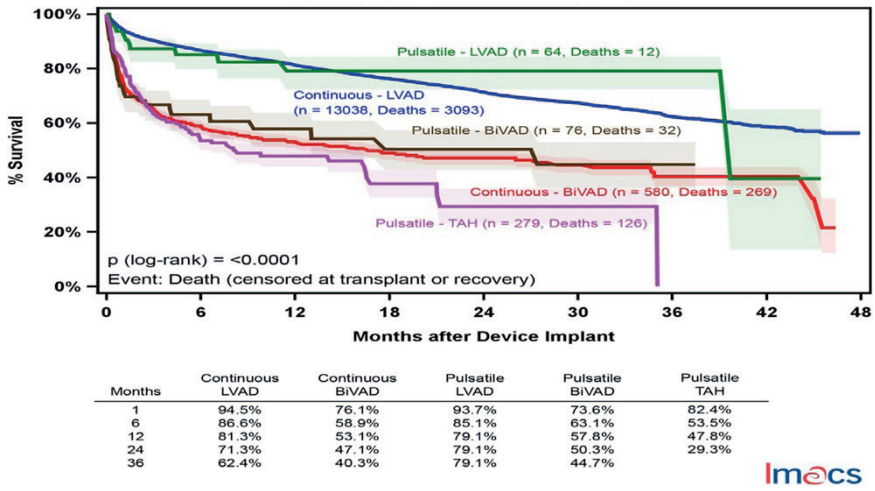

Figure 2. Actuarial survival curve for primary implants, stratified by flow type and device type, January 1, 2013 to December 31, 2016 (n ¼ 14,062). The shaded areas indicate ±1 standard error. BiVAD, biventricular assist device; LVAD, left ventricular assist device; TAH, total artificial heart.

Pre-implant renal function had a dominant effect on survival (Table 8). Patients requiring dialysis within 2 days before implant had a high early mortality (Supplementary Material Figures S10 and S11 online), and higher blood urea nitrogen (BUN) (Supplementary Material Figure S12 online) and creatinine impacted early and later survival. Signs of hepatic dysfunction and tricuspid regurgitation as risk factors likely reflect worsening right heart failure. Poor nutritional status (lower albumin) impacted both early and longer term survival (Table 8).

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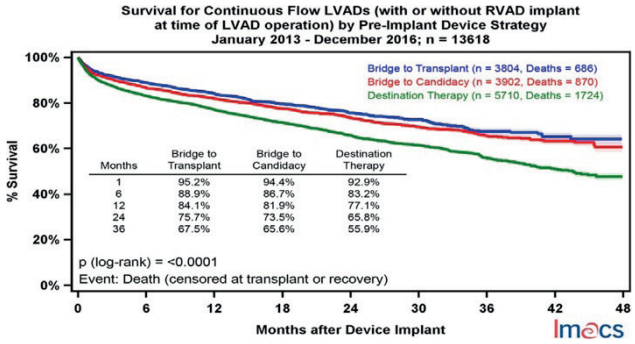


Figure 3. Actuarial survival curve for continuous-flow LVADs and BiVADs, stratified by pre-implant device strategy, January 1, 2013 to December 31, 2016 (n ¼ 13,618). The shaded areas indicate ±1 standard error. BiVAD, biventricular assist device; LVAD, left ventricular assist device.

5

Table 6. Major Adverse Events, Continuous-flow LVAD/BiVAD, IMACS, January 1, 2013 to December 31, 2016 (n ¼ 13,618)

Adverse event type	Patient experiencing event	Percentage of all patients
Infection	5,439	40%
Bleeding	4,745	35%
Neurologic dysfunction	2,638	19%
Respiratory failure	2,205	16%
Device malfunction	233	2%

BiVAD, biventricular assist device; CNS, central nervous system; IMACS, International Society for Heart and Lung Transplantation Mechanically Assisted Circulatory Support; LVAD, left ventricular assist device.

Table 7. Adverse Event Rates, Continuous-flow LVAD/BiVAD, IMACS, January 1, 2013 to December 31, 2016 (n ¼ 13,618)

Adverse event type	Early event (o3 months) count (n)	Early event (o3 months) rate (per 100 patient-months)	Late event (≥3 months) count (n)	Late event (≥3 months) rate (per 100 patient-months)	p-value
Bleeding	5,074	13.78	4,845	2.88	o0.0001
Infection	4,664	12.66	5,891	3.51	o0.0001
Respiratory failure	2,242	6.09	641	0.38	o0.0001
Neurologic dysfunction	1,536	4.17	1,943	1.16	o0.0001
Device malfunction	99	0.27	241	0.14	o0.0001
Arterial non-CNS thromboembolism	112	0.30	54	0.03	o0.0001

BiVAD, biventricular assist device; CNS, central nervous system; IMACS, International Society for Heart and Lung Transplantation Mechanically Assisted Circulatory Support; LVAD, left ventricular assist device.

Table 8. Continuous-flow LVAD/BiVAD, IMACS, January 1, 2013 to December 31, 2016 (n ¼ 13,618)

	Early hazard		Constant hazard	
Pre-implant risk factors for death	**Hazard ratio**	**p-value**	**Hazard ratio**	**p-value**
Demographics				
Older age (unit: 10 years)	1.44	o0.0001	1.23	o0.0001
Female	1.28	0.003	1.18	0.008
Higher BMI (unit: 5 kg/m^2)	1.12	o0.0001	1.04	0.021
Destination therapy strategy at time of implant			1.14	0.014
Not blood type O			0.89	0.013
Surgical complexities				
History of CABG	1.31	0.002	1.20	0.004
Concomitant surgery	1.34	o0.0001		
BiVAD	3.42	o0.0001		
Clinical status				
Patient Profile 1	1.77	o0.0001		
Patient Profile 2	1.51	o0.0001		
Not patient Profile 4 to 7			0.85	0.014
Primary diagnosis—congenital	5.23	0.002		
Peripheral vascular disease			1.41	o0.01
Intervention 48 hours pre-implant—ventilator	1.32	0.003		
BUN (unit: 10 mg/dl) higher	1.06	o0.0001	1.04	o0.0001
Creatinine (unit: 1 mg/dl) higher			1.08	0.004
Intervention with 48 hours pre-implant—dialysis	1.92	o0.0001		
Albumin (unit: 1 g/dl) lower	0.85	0.001	0.86	o0.0001
Sodium (unit: 10 mEq/liter) lower			0.86	0.004
AST (unit: 10 U/liter) higher	1.13	o0.0001		
ALT (unit: 10 U/liter) lower	0.94	o0.01		
Total bilirubin (unit: 5 mg/dl) higher	1.18	o0.0001		
Tricuspid regurgitation: moderate/severe	1.37	o0.0001		
Implantable cardioverter-defibrillator			1.21	0.004

CNS-Central Nervous System; LVAD-left ventricular assist device; BiVAD-biventricular assist device; CABG-Coronary Artery Bypass Graft; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Bi-VAD, biventricular assist device; BMI, body mass index; BUN, blood urea nitrogen; CABG, coronoary bypass artery graft; IMACS, International Society for Heart and Lung Transplantation Mechanically Assisted Circulatory Support; LVAD, left ventricular assist device.

- Age: calculated risk factor for 10 year range
- BMI-Body Mass Index (kg/m^2): 5 unit increase
- Total Bilirubin (mg/dL): 5 unit increase
- Sodium (mEq/L): 10 unit decrease
- BUN-Blood Urea Nitrogen (mg/dL): 10 unit increase
- Aspartate Aminotransferase/AST (u/L): 10 unit increase
- Alanine Aminotransferase/ALT (u/L): 10 unit decrease

[a]None.

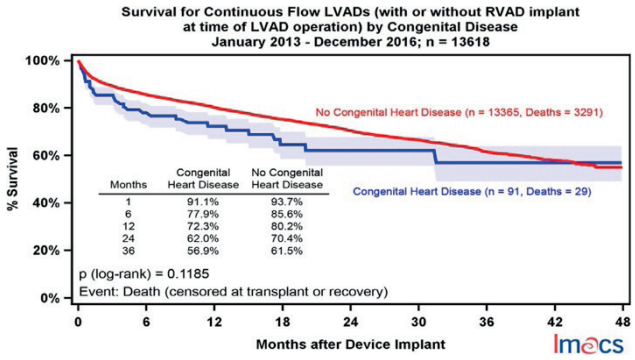


Figure 4. Actuarial survival curve for continuous-flow LVADs and BiVADs, stratified by pre-implant history of congenital heart disease, January 1, 2013 to December 31, 2016 (n ¼ 13,618). The shaded areas indicate ±1 standard error. BiVAD, biventricular assist device; LVAD, left ventricular assist device.

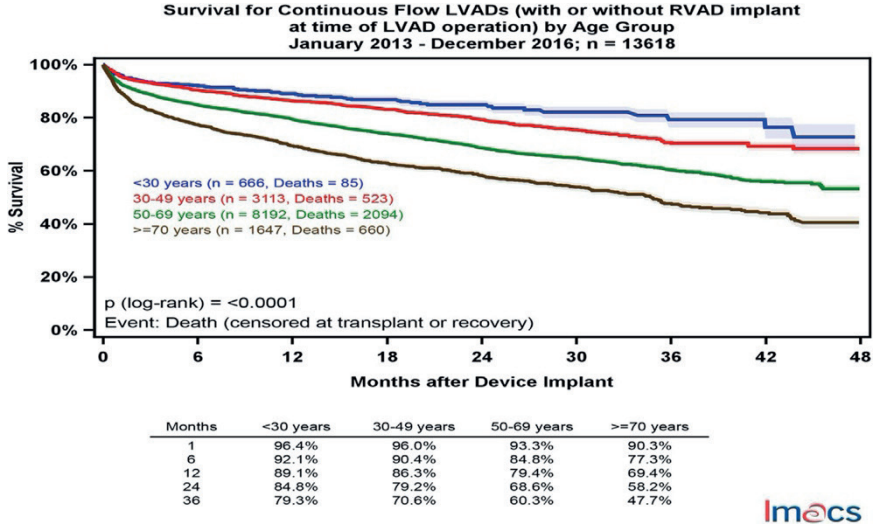


Figure 5. Actuarial survival curve for continuous-flow LVADs and BiVADs, stratified by pre-implant age group, January 1, 2013 to December 31, 2016 (n ¼ 13,618). The shaded areas indicate ±1 standard error. BiVAD, biventricular assist device; LVAD, left ventricular assist device.

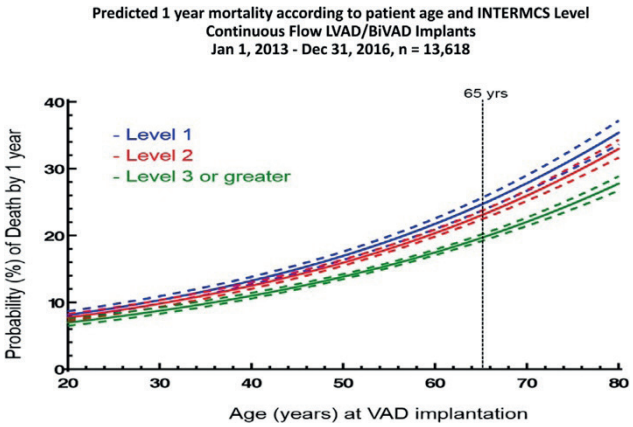


Figure 6. Nomogram showing the solution to the multivariable equation for death by 1 year, depicting the interaction between patient age and patient profile level at implant for continuous-flow LVADs and BiVADs, January 1, 2013 to December 31, 2016 (n ¼ 13,618).

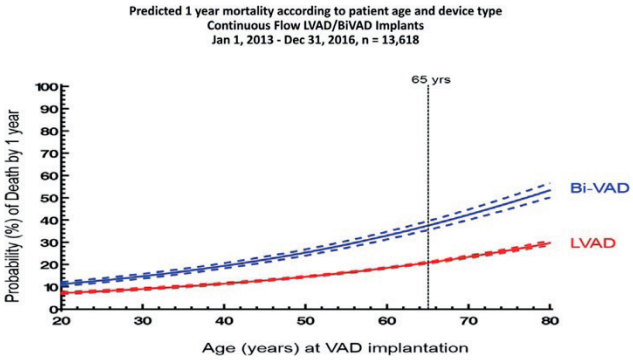
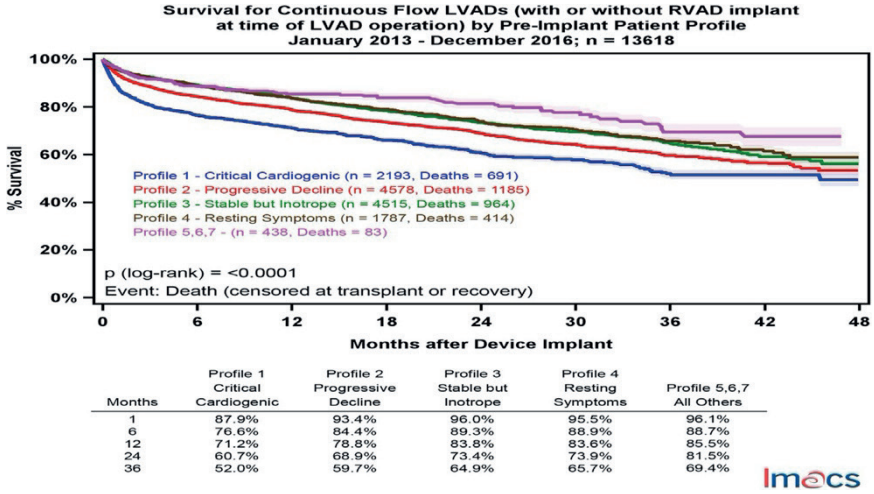


Figure 7. Nomogram showing the solution to the multivariable equation for death by 1 year, depicting the interaction between patient age and device type at implant for continuous-flow LVADs and BiVADs, January 1, 2013 to December 31, 2016 (n ¼ 13,618).



Months	Profile 1 Critical Cardiogenic	Profile 2 Progressive Decline	Profile 3 Stable but Inotrope	Profile 4 Resting Symptoms	Profile 5,6,7 All Others
1	87.9%	93.4%	96.0%	95.5%	96.1%
6	76.6%	84.4%	89.3%	88.9%	88.7%
12	71.2%	78.8%	83.8%	83.6%	85.5%
24	60.7%	68.9%	73.4%	73.9%	81.5%
36	52.0%	59.7%	64.9%	65.7%	69.4%

Figure 8. Actuarial survival curve for continuous-flow LVADs and BiVADs, stratified by pre-implant patient profile, January 1, 2013 to December 31, 2016 (n ¼ 13,618). The shaded areas indicate ±1 standard error. BiVAD, biventricular assist device; LVAD, left ventricular assist device.

Appendix A. Supplementary material

Supplementary data associated with this article can be found online version at www.jhltonline.org.

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CHAPTER 6

Gender differences and outcomes in left ventricular assist device support: The European Registry for Patients with Mechanical Circulatory Support

Magnussen C, Bernhardt AM, Ojeda FM, Wagner FM, Gummert J, de By TMMH, Krabatsch T, Mohacsi P, Rybczynski M, Knappe D, Sill B, Deuse T, Blankenberg S, Schnabel RB, Reichenspurner H.

J Heart Lung Transplant. 2018 Jan;37(1):61-70

ABSTRACT

Background: In contrast to the increasing use of ventricular assist devices (VAD), gender differences in indications, haemodynamics and outcome are not well understood. We examined gender differences and gender-specific predictors for perioperative outcome on ventricular support.

Methods: Multi-centre data of 966 patients (median age 55 years, 151 women) from the EUROMACS (European Registry for Patients with Mechanical Circulatory Support) registry were analysed. The median follow-up was 1.26 years.

Results: At the time of VAD implantation, women were more often in unstable condition (INTERMACS profile 1 and 2) (51.7% vs. 41.6% in men), suffered significantly more often from major bleeding (P=0.0012), arrhythmias (P=0.022) and right ventricular (RV) failure (P<0.001) with need for additional RV support. The survival of women on isolated LVAD support was significantly worse (1-year survival 69.3% vs. 78.3% in men). Age-adjusted Cox regression analyses showed significant associations with mortality for pre-operative inotropic therapy, percutaneous mechanical support, INTERMACS profile 1 and 2, RV dysfunction, major bleeding, cerebral bleeding, ischemic stroke, and RV failure. In women, pump thrombosis was more strongly related with mortality compared to men, while the direction of the association of renal dysfunction with mortality was different for women and men (P-Value interaction 0.028 and 0.023, respectively).

Conclusions: Women and men differ in peri-operative haemodynamics, adverse events and mortality after VAD implantation. A gender-dependent association of pump thrombosis with mortality was seen. The impact on treatment practice needs to be shown.

INTRODUCTION

Due to the growing organ shortage and technical progress, ventricular assist devices (VAD) are gaining importance in the therapy of end-stage heart failure (HF). Women and men differ in terms of HF aetiology, diagnosis, prognosis, and treatment.(1) Women have a higher HF incidence than men.(2) Women are hospitalised more frequently and die more often than men from the consequences of HF.(3) Although women are hospitalised in more advanced states of decompensated HF(4), VAD placement is far less likely.(5),(6) At time of implantation, women are more frequently in cardiogenic shock (INTERMACS level 1) (7) and receive devices with smaller pump sizes.(8) After VAD implantation, women require longer ventilatory and inotropic support resulting in prolonged intensive care stays.(7) Furthermore, women have a higher risk for neurological complications and perioperative right ventricular (RV) failure requiring additional RV support.(9),(6),(7) However, there is contradictory evidence regarding gender-specific outcome.(4),(7),(5),(6)

Therefore, in the largest European study sample of patients undergoing mechanical circulatory support to date, the EUROMACS registry (European Registry for Patients with Mechanical Circulatory Support), we aimed to further evaluate gender differences in adverse events. Additionally, we tried to identify gender-specific predictors for survival of women and men undergoing VAD implantation.

METHODS

Study population

Between January 2011 and June 2014, 966 patients were prospectively enrolled into the EUROMACS registry, an online database collecting anonymised data of demographics, device implantation and long-term follow-up of patients with ventricular support. (10) At present, more than 50 European and non-European centres from 15 countries are involved. Pre-implantation data regarding patient characteristics, social situation, HF medication at admission, pre-operative blood values, primary cardiac diagnosis and INTERMACS profile are recorded. Data on the following device strategies are available: bridge to recovery, bridge to transplantation (possibly bridged, currently listed), destination therapy, rescue therapy and others. Hemodynamic data from echocardiography and right heart catheter, intraoperative and procedural characteristics are stored. Adverse events including ischemic strokes, cerebral bleeding, arrhythmias, pump thrombosis, major bleedings, major infections, RV failure, renal and hepatic dysfunction are indicated. The definition of adverse events in the EUROMACS registry corresponds to the INTERMACS definition.(11),(10) Every follow-up visit and adverse events including death of a patient are reported. All contributing centres were contacted to confirm correctness of data by the end of follow-up. Paediatric patients were excluded.

Statistical methods

After exclusion of paediatric patients and one patient with missing device information, the dataset consisted of 966 patients undergoing long-term ventricular assist device support. Patients receiving an RVAD, BIVAD, total artificial heart, a CircuLite® Synergy™, HeartWare® MVAD® or not specified device brands were excluded from all survival analyses (including survival and incidence estimation, and Cox regression analyses).

Available-case analyses, also known as pairwise deletion, were used. That means that for each computation, only those without missing values on the variables involved in that particular analysis were used. For continuous variables, median, 25th and 75th percentile is given and the Mann-Whitney test is performed. For categorical variables absolute and relative frequencies and Fisher Exact test is computed. Gender-specific survival curves on LVAD and temporary RVAD therapy were drawn using the Kaplan-Meier method. The equality of the survival curves was tested using the Log-rank test. Cumulative incidence functions were computed for the outcomes transplanted, death and recovery for each gender using a competing risks approach. Equality of these functions was tested by Gray's test. Incident gender-specific adverse event rates are given. We performed Cox regression analyses to examine the extent to which selected hemodynamic parameters (analyses shown for pre-operative inotropic therapy, percutaneous mechanical circulatory support, INTERMACS profile 1 and 2 and RV function) and adverse events (analyses shown for major bleeding, cerebral bleeding, pump thrombosis, RV failure) associate with survival for each gender. All Cox models were adjusted for age, gender and LVAD device brand. Each adverse event was used as time dependent covariate in the respective Cox model. Besides the predictor of interest (hemodynamic parameter, adverse event) the models include age, device type, gender and an interaction term for gender and the predictor of interest. Cox models that do not include the aforementioned interaction term were also computed. Confidence intervals (CI) and P-values for the variable of interest were computed using the methods described in Figueiras et al.(12)

Analyses were performed using R version 3.3.3 (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/).

RESULTS

Gender-specific pre-operative characteristics

Selected baseline characteristics of the study sample are presented in Table 1 (for further baseline characteristics please see Supplementary Table 1a). N=966 patients (median age 55 years, 151 [15.6%] women, 84% European origin) underwent primary ventricular support (LVAD N=790; BIVAD N=52; LVAD with temporary RVAD N=99; isolated RVAD N=10; total artificial heart N=15). Ischemic cardiomyopathy was less frequent in women than in men with women exceeding men in prevalence of

dilated cardiomyopathy (Table 1). At the time of VAD implantation, women were more often in unstable condition (INTERMACS profile 1 or 2), although, preoperatively, no differences were seen in need for renal replacement therapy, ventilation or percutaneous mechanical circulatory support.

Table 1 Selected General Characteristics of the Study Sample

Variable	Men (n = 815)	Women (n = 151)	*p*-value
Age, years	56 (46.2, 62)	53 (40.3, 62)	0.088
Body surface area, m^2	2.0 (1.9, 2.1)	1.7 (1.6, 1.9)	<0.001[a]
Diabetes, *n* (%)	200 (25.2)	38 (25.7)	0.92
Ever smoker, *n* (%)	294 (69.2)	25 (34.7)	<0.001[a]
Chronic obstructive pulmonary disease, *n* (%)	88 (11.1)	10 (6.8)	0.14
Symptomatic peripheral vascular disease, *n* (%)	61 (7.7)	5 (3.4)	0.077
Carotid artery disease, *n* (%)	22 (3.2)	3 (2.2)	0.78
Positive history of neurologic event, *n* (%)	89 (11.6)	16 (10.7)	0.89
Dialysis, *n* (%)	20 (2.5)	5 (3.3)	0.58
Ultrafiltration, *n* (%)	49 (6.1)	6 (4)	0.44
Intubation, *n* (%)	118 (14.7)	26 (17.3)	0.39
Currently on intravenous inotropes, *n* (%)	501 (65.9)	105 (71.4)	0.21
Intra-aortic balloon pump, *n* (%)	97 (12.1)	27 (18)	0.063
Extracorporeal membrane oxygenation, *n* (%)	75 (9.4)	19 (12.7)	0.23
Primary diagnosed cardiomyopathy, *n* (%)			<0.001[a]
Congenital	12 (1.6)	2 (1.4)	1.0
Ischemic	369 (47.8)	39 (26.7)	<0.001[a]
Dilated	367 (47.5)	97 (66.4)	<0.001[a]
Restrictive	5 (0.6)	4 (2.7)	0.040[a]
Valvular	19 (2.5)	4 (2.7)	0.78
INTERMACS patient profiles, *n* (%)			0.30
1 and 2: unstable	334 (41.6)	77 (51.7)	0.025[a]
1: critical cardiogenic shock	90 (11.2)	24 (16.1)	0.099
2: progressive decline	244 (30.4)	53 (35.6)	0.21
3: stable but inotrope dependent	253 (31.5)	37 (24.8)	0.12
4: resting symptoms	177 (22.1)	32 (21.5)	0.91
5: exertion intolerant	28 (3.5)	3 (2)	0.46
6: exertion limited	7 (0.9)	0 (0)	0.60
7: advanced NYHA class III	3 (0.4)	0 (0)	1.0
Current device strategy, *n* (%)			0.80
Bridge to recovery	6 (0.7)	0 (0)	0.60
Bridge to transplantation			
Possibly bridged	366 (45.6)	72 (48)	0.59
Currently listed	243 (30.3)	43 (28.7)	0.77
Destination therapy	148 (18.4)	27 (18)	1.0
Rescue therapy	32 (4)	8 (5.3)	0.5
Other	8 (1.0)	0 (0)	0.62
Preoperative blood values			
Creatinine, μmol/liter	106.0 (82.0, 141.0)	94.0 (70.0, 132.0)	0.0097[a]
Hemoglobin, g/dl	11.9 (10.2, 13.6)	11.0 (10.1, 12.7)	0.0081[a]
Total bilirubin, mg/dl	1.3 (0.8, 2.1)	1.3 (0.8, 2.2)	0.83
Platelet count, $\times 10^9$/liter	189.0 (138.0, 241.0)	196.0 (149.0, 256.0)	0.28

For continuous variables, median (25th percentile, 75th percentile) is given and Mann-Whitney test is performed. For categorical variables, absolute and relative frequencies are given, and Fisher exact test is performed. For additional variables, see Table S1 (available in the online version of this article at www.jhltonline.org).

INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support; NYHA, New York Heart Association.

[a]statistically significant.

Gender-specific intra- and post-operative characteristics

More women had moderate or severe mitral and tricuspid regurgitation, but less aortic regurgitation resulting in less concomitant aortic valve replacement (Table 2 and 3; additional hemodynamic parameters and procedural characteristics are given in Supplementary Table 2 and 3). Choice of device brands was different in both genders

(Table 3). The HeartWare® HVAD® was significantly more often implanted in women. Women needed more additional RV support. In women, a longer post-operative ventilatory support and a trend towards a longer stay at intensive care unit was reported.

Table 2 Selected Hemodynamic Parameters

	Men (n = 815)	Women (n = 151)	p-value
Heart rate, beats/min	86 (74, 99)	88 (74.2, 102.8)	0.20
Systolic blood pressure, mm Hg	100 (90, 110)	96 (86, 107.3)	0.11
Mitral regurgitation, n (%)			0.0075[a]
None/trivial	51 (7.4)/60 (8.7)	15 (12.0)/6 (4.8)	0.11/0.16
Mild	233 (33.7)	26 (20.8)	0.0046[a]
Moderate	225 (32.6)	49 (39.2)	0.15
Severe	122 (17.7)	29 (23.2)	0.17
Tricuspid regurgitation, n (%)			<0.001[a]
None/trivial	58 (8.5)/97 (14.2)	17 (13.4)/10 (7.9)	0.095/0.063
Mild	267 (39.1)	28 (22)	<0.001[a]
Moderate	175 (25.7)	41 (32.3)	0.13
Severe	85 (12.5)	31 (24.4)	<0.001[a]
Aortic regurgitation, n (%)			0.020[a]
None/trivial	345 (56.7)/123 (20.2)	77 (71.3)/15 (13.9)	0.0042[a]/0.15
Mild	96 (15.8)	15 (13.9)	0.77
Moderate	38 (6.2)	1 (0.9)	0.020[a]
Severe	7 (1.1)	0 (0)	0.6
Left ventricular ejection fraction, n (%)	20 (15, 24)	20 (15, 25)	0.15
Right ventricular function, n (%)			0.49
Normal/mild	128 (19.3)/151 (22.8)	29 (25.0)/26 (22.4)	0.17/1.0
Moderate/severe	267 (40.3)/117 (17.6)	45 (38.8)/16 (13.8)	0.84/0.35
Pulmonary artery systolic pressure, mm Hg	50 (39, 64)	48 (36, 57)	0.017[a]
Cardiac index, liter/min/m^2	1.4 (0, 2.1)	1.3 (0, 2.0)	0.40

For continuous variables, median (25th percentile, 75th percentile) is given, and Mann-Whitney test is performed. For categorical variables, absolute and relative frequencies are given, and Fisher exact test is performed. For additional variables, see Table S2 (available in the online version of this article at www.jhltonline.org).

[a]statistically significant.

Gender differences in adverse events

After a median follow-up of 1.3 years (range 0.03-50.73 months; median follow-up 1.3 years in men vs. 1.2 years in women) and 987 patient-years, 309 deaths (247 among men and 62 among women) were reported. In women, more major bleedings (events per patient year [PY] in women: 0.3 vs. 0.14 in men, P=0.0012) (Figure 1) were reported. Women had a higher incidence of arrhythmias (events per PY in women: 0.08 vs. 0.03 in men, P=0.022) and RV failure (events per PY in women: 0.11 vs. 0.03 in men, P<0.001). No differences in ischemic stroke (events per PY in women: 0.08 vs. 0.06 in men, P=0.36) and cerebral bleeding (events per PY in women: 0.03 vs. 0.03 in men, P=0.84) were observed (Figure 1).

Survival and predictors for mortality

Women undergoing isolated LV support showed a significantly worse overall survival (Figure 2). On LVAD with temporary RVAD support, survival of women was even worse, but did not differ significantly from men (Figure 2). No gender differences in transplant rates were seen (Figure 3). Parameters mirroring hemodynamic compromise were shown to predict survival on VAD support. Pre-operative inotropic therapy, percutaneous mechanical support, INTERMACS profile 1 and 2, and highly reduced RV function,

respectively, were related to mortality (Supplementary Table 4) with a significant gender interaction for the association of percutaneous mechanical circulatory support and mortality (Table 4). In a refining analysis, which included these variables in a single model, INTERMACS profile 1 and 2 in women and preoperatively highly reduced RV function in both genders were no longer associated with mortality (Supplementary Table 5).

Furthermore, several VAD-related adverse events were significantly related to mortality. After adjustment for age, gender and device brand, major bleeding, cerebral bleeding, ischemic stroke, pump thrombosis, RV failure, and renal dysfunction were significantly associated with mortality in the overall cohort (Supplementary Table 6). In a refining analysis with gender interaction (Table 5), the associations persisted in women and men, with the exception of renal function in women. A significant gender interaction in the association of pump thrombosis with mortality was observed indicating a stronger association in women (Table 5). The associations remained statistically significant after further adjustment for body mass index, diabetes, systolic blood pressure, chronic obstructive pulmonary disease, and symptomatic peripheral and carotid artery disease (analyses not shown).

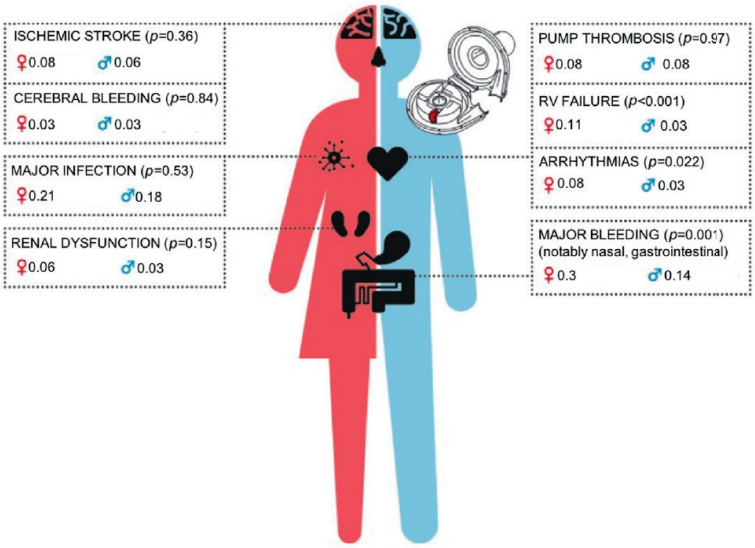
Table 3 Selected Intraoperative and Procedural Characteristics

	Men ($n = 815$)	Women ($n = 151$)	p-value
Device brand, *n* (%)			<0.001[a]
Berlin Heart Excor	9 (1.1)	3 (2)	0.42
Berlin Heart Incor	7 (0.9)	1 (0.7)	1.0
CircuLite Synergy	4 (0.5)	0 (0)	1.0
Heart Assist 5	4 (0.5)	1 (0.7)	0.58
Thoratec HeartMate II	339 (42.9)	36 (24)	<0.001[a]
Thoratec HeartMate III	1 (0.1)	0 (0)	1.00
Thoratec PVAD	10 (1.3)	5 (3.3)	0.075
HeartWare HVAD	411 (52)	102 (68)	<0.001[a]
HeartWare MVAD	0 (0)	1 (0.7)	0.16
Jarvik 2000	1 (0.1)	0 (0)	1.0
Other	4 (0.5)	1 (0.7)	0.58
Device type, *n* (%)			0.0094[a]
BIVAD	41 (5)	11 (7.3)	0.24
LVAD, temporary RVAD	73 (9)	26 (17.2)	0.0034[a]
LVAD	677 (83.1)	113 (74.8)	0.021[a]
RVAD	9 (1.1)	1 (0.7)	1.0
Total artificial heart	15 (1.8)	0 (0)	0.15
Cardiopulmonary bypass time, minutes	90 (68, 122)	83 (63, 117)	0.036[a]
Valve replacement, *n* (%)			
Aortic valve	53 (44.5)	5 (20)	0.026[a]
Mitral valve	7 (5.9)	2 (8)	0.66
Tricuspid valve	67 (56.3)	19 (76)	0.076
LVAD flow, liter/min	4.9 (4.2, 5.5)	4.4 (3.7, 4.9)	<0.001[a]
Ventilation time, hours	48 (16.9, 192)	89.0 (24, 248.7)	0.0068[a]
Intensive care stay, days	10 (5, 23)	11.5 (6, 29.6)	0.064
Patients discharged to rehabilitation, *n* (%)	154 (20.8)	29 (20.7)	1.0

For continuous variables, median (25th percentile, 75th percentile) is given, and Mann-Whitney test is performed. For categorical variables, absolute and relative frequencies are given, and Fisher exact test is performed. For additional variables, see Table S3 (available in the online version of this article at www.jhltonline.org).

BIVAD, biventricular assist device; LVAD, left ventricular assist device; RVAD, right ventricular assist device.

[a]statistically significant.



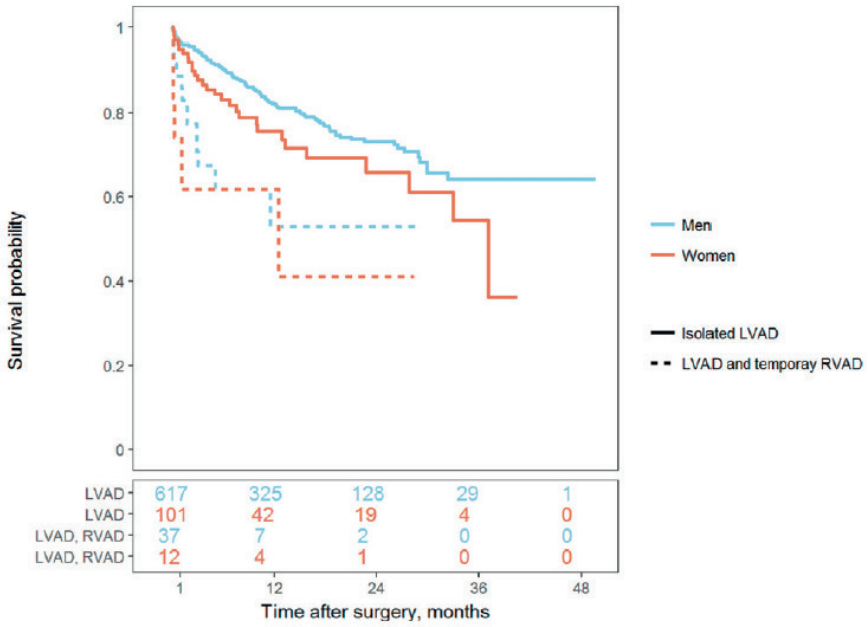
Adverse events in the first 30 days after ventricular assist device implantation.

	Observed events Men	N available Men	Event % 1 month Men	Observed events Women	N available Women	Event % 1 month Women	*p*-value
Ischemic stroke	11	739	1.55	3	136	2.5	0.54
Cerebral bleeding	3	738	0.45	1	135	0.84	0.66
Major infection	33	737	4.74	7	136	5.52	0.68
Renal dysfunction	10	739	1.4	5	136	3.73	0.024
Pump thrombosis	17	737	2.41	4	136	3.22	0.59
Right ventricular failure	14	739	1.93	11	136	8.33	<0.001
Arrhythmias	8	739	1.13	5	136	3.82	0.016
Major bleeding	48	738	6.7	18	136	13.86	0.007

Adverse events after 30 days of ventricular assist device implantation.

	Observed events Men	N available Men	Events per patient year Men	Observed events Women	N available Women	Events per patient year Women	*p*-value
Ischemic stroke	34	638	0.05	11	113	0.14	0.029
Cerebral bleeding	22	644	0.03	3	112	0.03	0.92
Major infection	106	644	0.19	18	108	0.26	0.60
Renal dysfunction	26	640	0.04	5	111	0.06	0.61
Pump thrombosis	50	634	0.08	8	111	0.09	0.85
Right ventricular failure	23	641	0.04	10	115	0.11	0.007
Arrhythmias	21	638	0.03	7	114	0.08	0.080
Major bleeding	92	617	0.16	24	108	0.32	0.021

Figure 1. Gender-specific adverse event rates.
Events per patient year are given for women and men. N=739 men, N=136 women.
Patients with RVAD, BIVAD, total artificial heart, CircuLite® Synergy™ and HeartWare® MVAD®, and not specified device brands were excluded from analysis.
Red: women. Blue: men.

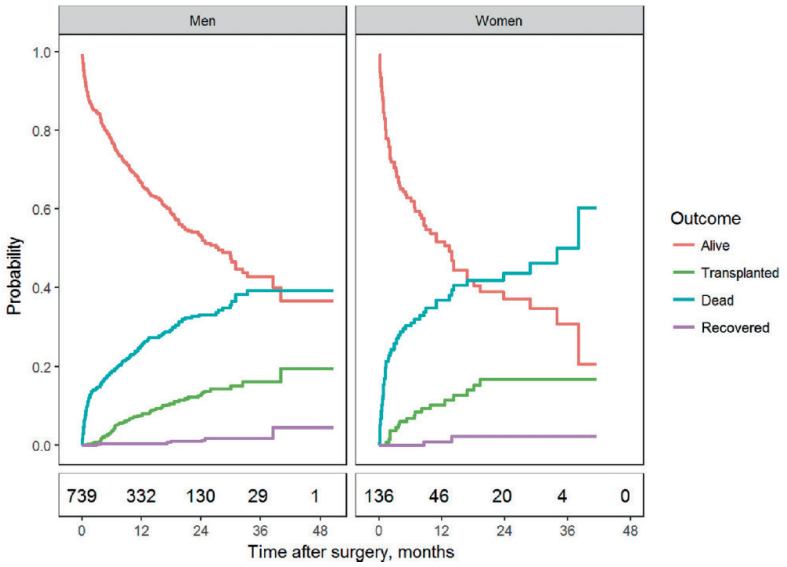


Abbreviations: LVAD: left ventricular assist device, RVAD: right ventricular assist device.

Probabilities of death:

	% at 1 year after surgery	% at 2 years after surgery
LVAD men	16.8	26.8
LVAD women	24.5	34.1
LVAD, RVAD men	38.2	47.0
LVAD, RVAD women	38.3	58.8

Figure 2. Kaplan-Meier survival curves by gender and assist types (with mortality as the endpoint). Patients who died or were censored in the first 30 days after surgery were excluded from analysis. The follow-up is censored at transplant or recovery.
Results of Log-rank test (men vs. women): LVAD: P=0.046; LVAD, temporary RVAD: P=0.50. Numbers at risk are given below the Kaplan-Meier curves. Patients with RVAD, BIVAD, total artificial heart, CircuLite® Synergy™ and HeartWare® MVAD®, and not specified device brands were excluded from analysis.
Abbreviations: LVAD: left ventricular assist device, RVAD: right ventricular assist device.



Competing risk analyses

	% at 1 year Men	% at 2 years Men	% at 1 year Women	% at 2 years Women
Alive	66.6	53.3	51.8	37.2
Transplanted	7.6	12.6	10.3	16.8
Dead	25.4	33.1	36.9	43.8
Recovered	0.4	1.0	1.0	2.2

Figure 3. Cumulative incidence functions for both genders with the outcomes *transplanted, death* and *recovery.* The probability of being alive without being transplanted nor recovering is also shown. Results of Gray's-Test (men vs. women): P=0.33; dead: P=0.004; recovery: P=0.81. Numbers at risk are shown below the curves. Patients with RVAD, BIVAD, total artificial heart, CircuLite® Synergy™ and HeartWare® MVAD®, and not specified device brands were excluded from analysis.

Table 4 Cox Regression Analyses for Selected Hemodynamic Parameters and Mortality With Interaction by Gender

Model	Gender	Hazard ratio (95% CI)	*p*-value	*N* events/ *N* individuals	*p*-value interaction
(1) Currently on intravenous inotropes					0.61
	Men	10.90 (7.59, 15.66)	<0.001	161/617	
	Women	13.19 (6.91, 25.19)	<0.001	39/108	
(2) Percutaneous mechanical circulatory support (IABP or ECMO)					0.034[a]
	Men	1.31 (0.93, 1.86)	0.13	204/723	
	Women	2.70 (1.54, 4.73)	<0.001	56/135	
(3) INTERMACS profile unstable condition					0.52
	Men	1.88 (1.42, 2.49)	<0.001	205/727	
	Women	2.29 (1.31, 4.01)	0.0036	54/134	
(4) Preoperatively highly reduced RV function[b]					0.5
	Men	2.69 (1.64, 4.44)	<0.001	176/613	
	Women	6.84 (2.14, 21.84)	0.0012	43/107	

All models are adjusted for age and LVAD brand. Patients receiving RVAD, BIVAD, total artificial heart, CircuLite Synergy, HeartWare MVAD, or not-specified device brands were excluded. Numbers vary slightly due to missing value information.
CI, confidence interval; ECMO, extracorporeal membrane oxygenation; IABP, intra-aortic balloon pump; INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support; RV, right ventricular.
[a]statistically significant.
[b]Hazard ratios for mildly and moderately reduced RV function are not shown.

Table 5 Cox Regression Analyses for Selected Adverse Events and Mortality With Interaction by Gender

Model	Gender	Hazard ratio (95% CI)	*p*-value	*N* deaths/ *N* individuals	*p*-value interaction
(1) Major bleeding					0.49
	Men	5.70 (4.14, 7.84)	<0.001	208/738	
	Women	4.55 (2.60, 7.95)	<0.001	56/136	
(2) Cerebral bleeding					0.22
	Men	12.06 (7.12, 20.42)	<0.001	208/738	
	Women	27.1 (8.95, 82.02)	<0.001	56/135	
(3) Ischemic stroke					0.86
	Men	3.65 (2.27, 5.84)	<0.001	208/739	
	Women	3.29 (1.18, 9.18)	0.023	56/136	
(4) Pump thrombosis					0.028[a]
	Men	3.27 (2.07, 5.16)	<0.001	208/737	
	Women	10.01 (4.44, 22.55)	<0.001	56/136	
(5) RV failure					0.36
	Men	8.39 (5.06, 13.91)	<0.001	208/739	
	Women	5.61 (2.77, 11.35)	<0.001	56/136	
(6) Renal dysfunction					0.023[a]
	Men	3.03 (1.71, 5.36)	<0.001	208/739	
	Women	0.67 (0.16, 2.74)	0.57	56/136	

All models are adjusted for age and LVAD brand. Patients receiving RVAD, BIVAD, total artificial heart, CircuLite Synergy, HeartWare MVAD, or not-specified device brands were excluded. Numbers vary slightly due to missing value information.
CI, confidence interval; RV, right ventricular.
[a]statistically significant.

DISCUSSION

We could demonstrate significant gender differences not only in the peri-operative but also in the long-term course after VAD implantation. Women were shown to receive less VAD support despite a more critical HF state at admission. Both genders differed for implanted device types with implantation of smaller device pumps in women. Women required temporary or permanent RV support more often due to a higher incidence of RV failure. The overall survival in women was significantly worse.

In contrast to prior smaller studies with a comparatively low number of women (6),(4), our sample represents the largest European registry and one of the largest cohorts worldwide that permits the investigation of gender differences in long-term mechanical support. In comparison to the earlier report on gender differences by Hsich et al. in the INTERMACS registry (13), our study is characterized by a longer follow-up time post VAD implantation and by a higher rate of continuous-flow devices (Heart Mate II® and HeartWare HVAD®). The latter have almost completely replaced pulsatile systems. Thus our study mirrors current daily clinical practice with a state-of-the-art technology.

Gender-specific peri-procedural characteristics

Despite a higher probability of hemodynamic compromise,(4),(7) women still are less likely to undergo assist device support.(4, 5) Women are underrepresented in large multi-center trials as stated by the ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult.(14) Accordingly, only a small part of 15.6% in our study population were women, which is slightly lower than in

comparable international registries like the INTERMACS registry. If this is due to European referral strategies or preferred device types cannot be answered by our data. Women presented significantly more often in INTERMACS level 1 and 2. This may be explained by two reasons: First, women are more likely to be transferred for VAD implantation in a later and more critical clinical state. Second, the smaller intrathoracic volume of women is not suited for larger pump sizes. We and others have shown, that the smaller HVAD® pump was preferred in women.(8) Despite the more unstable condition at admission in women, there were no differences in inotropic therapy or percutaneous mechanical support. Consistent with prior reports,(7) women needed longer ventilatory support. They showed a trend towards a longer stay at intensive care unit. Additionally, women and men differed in HF aetiology. Ischemic cardiomyopathy is less frequent in women undergoing VAD support,(6) explained by the fact that women have coronary artery disease less often than men(15).

Gender-specific adverse events

In line with the report by Boyle et al., who could show that women have a higher risk for bleeding complications on continuous-flow assist devices(16), women had a higher rate of major bleeding after VAD implantation in our cohort. The assumption that differences in blood coagulation or gender-specific pharmacokinetics and -dynamics of anticoagulant medication are driving the bleeding risk, is pathophysiologically plausible, but cannot be answered by our current data. In addition, women and men differed in RV haemodynamics. In line with prior studies(6),(7), we could show that women had a higher incidence of peri-operative RV failure requiring more additional RV support, although no difference in pre-operative RV function was seen. With respect to the observed higher incidence of arrhythmias in women, RV failure may partly be explained by the occurrence of arrhythmias. In particular ventricular arrhythmias have the potential to induce RV failure under LVAD support.(17)

A possible relation of device type and specific adverse events has to be considered. The HeartWare® HVAD®, which was significantly more often implanted in women, has been related to a higher rate of cerebrovascular events.(18) The centrifugal flow of the HVAD® pump was associated with a hypercoagulable state,(19) which renders thromboembolic events more plausible. Prior investigations could show, that anticoagulation, antiplatelet therapy and blood pressure management affected stroke rate after HVAD® implantation. (20) In our study, after adjustment for LVAD device brands, the association of cerebral bleeding, ischemic stroke and pump thrombosis with mortality did not change markedly. Therefore, the association of these adverse events with mortality cannot be explained by the device choice.

Survival and predictors for mortality

There is conflicting evidence for survival differences between genders undergoing VAD therapy across heterogeneous studies with mostly smaller numbers of participants and different device types. In studies performed with several device types, continuous-flow systems were shown to be related to better survival rates.(21) Morgan et al. found a

significantly worse survival for women undergoing pulsatile-flow support.(4) In contrast to other studies, which did not see differences in short-term survival,(9, 13),(6),(7) women had a significantly worse 1- to 3-year survival in our study population. Our data provide evidence for three possible reasons for our findings: First, women were shown to present to hospital in more advanced heart failure states.(4) Second, women suffered significantly more often from peri-operative RV failure. Third, women are more likely to suffer from arrhythmias and major bleeding complications. In line with Shah et al.(22), we could show that need for percutaneous mechanical support was associated with peri-operative mortality in both genders. INTERMACS profile 1 and 2, which represent a clinically unstable condition, were identified to predict survival. We could directly link RV haemodynamics with mortality in both genders. In women with need for LVAD and temporary RVAD support, the probability of death was 81% at 1 year compared to 31% for women undergoing isolated LVAD support. That means, once temporary RVAD support is needed, the survival is significantly worse. But as soon as women undergo additional RV support, no differences in outcome were seen compared to men under biventricular support. If a better pre-operative evaluation of the right ventricle and a stricter determining of indication will improve the outcome on mechanical circulatory support needs to be further investigated.

In addition, the significant association of pump thrombosis with mortality was stronger in women. Prior studies could show that women suffer more often from thromboembolic events under VAD support and may therefore need more intensive anticoagulation.(9),(23) Prospective studies are needed to verify if gender-specific anticoagulation regimens will reduce thromboembolic complications and improve survival of women on long-term assist device support.

Limitations

Data quality of a large registry relies on the data input of the participating centers. Therefore, the possibility exists that not all adverse events were reported. Furthermore, the very nature of a registry allows no analyses on center-specific trends and device choices.

CONCLUSIONS

In summary, we were able to demonstrate in a large European sample that women and men undergoing VAD support differ in pre-operative condition, peri-operative haemodynamics, adverse events and survival. Women were shown to have a higher incidence of major bleeding, arrhythmias, perioperative RV failure and a worse early and long-term survival. Several hemodynamic parameters and adverse events predicted survival in both, women and men. The association of pump thrombosis with mortality was stronger in women. Whether changes in referral strategies, implant timing and gender-specific out-patient aftercare may improve outcome for women on VAD support needs to be investigated.

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CHAPTER 7

Long-term outcome of patients after successful LVAD explant: A EUROMACS Study

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Abstract

Aims: Sufficient myocardial recovery with the subsequent explantation of a left ventricular assist device (LVAD) occurs in approximately 1-2% of the cases. However, follow-up data about this condition is scarcely available in the literature. This study aimed to report the long-term outcomes and clinical management following LVAD explantation.

Methods: An analysis of the European Registry for Patients with Mechanical Circulatory Support (EUROMACS) was performed to identify all adult patients with myocardial recovery and successful explantation. Pre-implant characteristics were retrieved and compared with the non-recovery patients. The follow-up data after explantation were collected via a questionnaire. A Kaplan-Meier analysis for freedom of the composite end-point of death, heart transplantation (HTx), LVAD-reimplantion or heart failure (HF) relapse was conducted.

Results: A total of 45 (1.4%) cases with myocardial recovery resulting in successful LVAD explantation were identified. Compared with those who did not experience myocardial recovery, the explanted patients were younger (44 vs. 56 years, $p<0.001$), had a shorter duration of cardiac disease ($p<0.001$) and were less likely to have ischemic cardiomyopathy (9% vs 41.8%, $p <0.001$). Follow-up after explantation could be acquired in 28 (62%) cases. The median age at LVAD implantation was 43 years (IQR: 29-52) and 23 (82%) were male. Baseline left ventricular ejection fraction was 18% (IQR: 10-20%), and 60.7% of the patients had INTERMACS profile 1 or 2. Aetiologies of HF were dilated cardiomyopathy in 36%, myocarditis in 32%, ischemic in 14% of the patients, and 18% had miscellaneous aetiologies. The devices implanted were: HeartMate II in 14 (50%), HVAD in 11 (39%), Heartmate 3 in 2 (7%), and one unknown with a median duration of support of 464 days (range: 59–1286). The median follow-up after explantation was 26 months (range 0.3 –73 months) and 82% of the patients were in NYHA class I or II. β-blockers were prescribed to 85%, ACE-inhibitors to 71% and loop-diuretics to 50% of the patients, respectively. Freedom from the composite end-point was 100% after 30 days and 88% after two years.

Conclusions: The survival after LVAD explantation is excellent without the need for HTx or LVAD reimplantation. Only a minority of the patients suffer from a relapse of significant HF.

INTRODUCTION

Continuous flow left ventricular assist devices (cf-LVADs) have become an important modality in the treatment of end-stage heart failure (HF) as a bridge to transplantation (BTT), bridge to candidacy (BTC) or as destination therapy (DT). This has led to a significant improvement of the quality of life and overall survival of patients once all other therapeutic options have been exhausted[1].

A small percentage of these patients experience significant myocardial recovery under LVAD support and can therefore undergo LVAD explantation, defined as actual bridge-to-recovery (BTR)[1]. The eighth Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) annual report and the second European Registry for Patients with Mechanical Circulatory Support (EUROMACS) report, reported that 1-2% of the patients implanted with cf-LVADs recovered, allowing successful LVAD explantation[1, 2]. Similarly, in a pooled Heartmate II cohort with 1.108 patients enrolled, the rate of myocardial recovery was 1,8%[3].

Several case series and small cohort studies report patients having sufficient recovery of left ventricle (LV) function that allowed LVAD explantation. These studies show higher rates of myocardial recovery after LVAD explantation and survival ranging from 78.3-100%, with varying rates of HF recurrence[4-8].

These studies report encouraging survival outcomes, however, much remains unknown concerning adverse events (AEs) and HF management after explantation and most studies are based on single centre experiences. Little is known about long-term outcomes and AEs, such as ventricular tachycardia or thromboembolic complications, given the fact that the inflow cannula is not (always) extracted and data about its management is usually lacking. Furthermore, the follow-up of these patients is limited, details on specific medication are lacking and not consistent due to a small number of patients. There is no evidence-based knowledge regarding the long-term treatment of these patients in case of recurrence of HF or other complications. Such inconsistencies prompt a need for a complete long-term follow-up in these patients, with a special emphasis on chronic medication, AEs and longer-term survival after successful LVAD explantation

Objectives

The aim of the study is to evaluate long-term outcomes and patient management after a successful LVAD termination due to the recovery, including survival, complications, relapse of HF and specific medical treatments.

METHODS

Study design

A retrospective study was conducted in all patients in whom a LVAD was successfully explanted after myocardial recovery as registered in the European Registry of Mechanically Assisted Mechanical Circulatory Support (EUROMACS)[2, 9]. Inclusion criteria were: successful explantation of an cf-LVAD (as a stand-alone VAD system, not RVAD) as captured in the follow-up of EUROMACS. Exclusion criteria included an explantation due to any other reason than myocardial recovery (e.g. infection, device malfunction) or patients aged <18 years.

Data collection

From January 2011 until March 2018, a total of 45 patients in the EUROMACS registry were identified as being successfully explanted after myocardial recovery, from now on named recovery patients. Baseline characteristics before LVAD implantation including age, sex, aetiology of HF, preoperative condition and comorbidities, electrocardiogram (ECG), echocardiogram and blood chemistry values were collected for adult patients in EUROMACS. Furthermore, perioperative data on device strategy, device type, concomitant surgical procedures and cardiopulmonary bypass (CPB) time, time in operating theatre (OR), intensive care unit (ICU) and hospital stay were retrieved. Finally, time on LVAD, type and number of AEs and hospitalizations were collected from the follow-up.

Subsequently, a detailed questionnaire was sent to involved centres to attain the follow-up of these patients after LVAD explantation. These data are currently not captured in the EUROMACS registry. Since data entry into EUROMACS is anonymized for external reviewers, the executive director (TdB) approached the centres asking to provide the follow-up of these patients. Data collected included the primary outcome: survival, HTx, reimplantation of LVAD and data on re-hospitalizations due to HF. Secondary outcomes consisted of the following parameters: presence of the inflow cannula, the occurrence of cerebrovascular accidents (CVAs), New York Heart Association (NYHA) class at last follow-up, oral anticoagulation and HF medication. Finally, data of ECG, echocardiography and blood chemistry values were requested.

Statistical Analysis

Continuous parameters are expressed as mean and confidence interval or median and range or interquartile range(IQR). Categorical parameters are expressed as number and percentage. For categorical parameters Chi-square test or Fischer's exact test were applied as appropriate. For continuous parameters Student's t-test or Wilcoxon rank sum test were used. A comparison of baseline characteristics was performed to assess differences in patients with VAD-explantation and without VAD explantation. Furthermore, baseline characteristics of patients whose LVAD was explanted with and without follow up were compared to assess a potential reporting bias. Finally, a Kaplan-Meier curve was constructed to evaluate freedom of the composite end-point of death, HTx, LVAD

reimplantation or relapse of HF to ≥NYHA III after LVAD explantation. Statistical analysis was performed using SPSS, version 25.0 for Windows (IBM Inc, Armonk, NY, USA).

RESULTS

Baseline characteristics

A total of 45 patients in whom the LVAD was explanted due to myocardial recovery were identified in the EUROMACS registry, representing 1.4% of the patients registered at the time. A complete follow-up of 28 (62.2%) recovery patients post-explantation was obtained. In these patients, median age at implantation was 43 years (range 29-52) and 23 patients (82.1%) were male (Table 1A). Predominant aetiologies of HF were myocarditis in 9 (32.1%) patients and dilated cardiomyopathy in 10 (35.7%) patients. Most patients had a short history of cardiac disease, with 14 (50%) patients having had their first cardiac diagnosis less than one month prior to the LVAD implantation and 20 (71.4%) within one year prior to the implantation. Patients were almost evenly distributed over INTERMACS patient profiles I to III, only 3 patients had INTERMACS patient profile IV-V. Median LVEF was 18% (IQR: 10-20%),whereas 5 patients exhibited ≥moderate mitral regurgitation. At the time of implant, 23 (82.1%) patients had inotropic support and 14 patients (50%) experienced any form of mechanical circulatory support by either extra-corporeal life support (ECLS) or an intra-aortic balloon pump (IABP). There were no significant differences between patients with or without an obtained follow-up after LVAD explantation (Table 1A).

A comparison between patients whose LVAD was explanted and patients with another outcome (ongoing, heart transplantation or death) reveals that recovery patients were significantly younger (45 vs. 53.5 years ($p < 0.001$), had a shorter duration of cardiac disease ($p <0.001$), less ICD's implanted (8.9% versus 61.4% ($p < 0.001$) and were more often in INTERMACS patient profile 1 ($p = 0.01$; (Table 1B). Furthermore, the predominant aetiologies of heart failure were myocarditis and dilated cardiomyopathy for recovery patients, while ischemic cardiomyopathy was the main cause of heart failure in the non-recovery group.

Perioperative characteristics

The indication designation was BTT in 22 (78.5%), DT in 2 (7.1%) and rescue therapy in 4 (14.3%) (Table 2). Implanted devices included the HeartMate II (n=14) (Abbott, Lake Bluff, IL, USA), HeartWare HVAD (n=11) (Medtronic, Minneapolis, MN, USA), HeartMate 3 (n=2) (Abbott, Lake Bluff, IL, USA) and one unknown device. Concomitant cardiac surgery was performed in 5 patients: 3 patent foramen ovale repairs, 1 tricuspid valve repair and 1 aortic valve replacement. Two patients received a temporary right ventricular assist device. Median time in the operating room was 208 minutes (range:130-683), with a median CPB time of 75 minutes (95-147). Post-operative ICU stay ranged from 4 to 147 days with a median of 17 days and a median

hospital stay of 30 days (17-165). Patients with a follow-up after LVAD explantation had significantly different device strategies (p <0.001) (less BTR and more BTT patients) and a shorter duration of CPB (p = 0.034) compared to patients without a follow-up.

Table 1A. Baseline characteristics of all patients with LVAD explantation due to myocardial recovery with and without follow-up after explantation

	With follow-up (28)	No follow-up (17)	p-value
Age (y)	43 (29-52)	53 (41-65)	0.053
Male	23 (82.1)	13 (76.5)	0.711
BMI	26.9 [25.1-28.6]	25.7 [23.2-28.2]	0.182
BSA	2.02 [1.92-2.12]	1.98 [1.87-2.10]	0.395
Aetiology			0.579
Myocarditis	9 (32.1)	3 (17.6)	
Dilated cardiomyopathy	10 (35.7)	4 (23.5)	
Ischemic cardiomyopathy	4 (14.3)	5 (27.1)	
Peripartum	1 (3.6)	1 (5.9)	
Valvular heart disease	2 (7.1)	2 (11.8)	
Hypertrophic cardiomyopathy	0 (0)	1 (5.9)	
Toxic	1 (3.6)	0 (0)	
Restrictive cardiomyopathy	0 (0)	0 (0)	
Congenital heart disease	0 (0)	0 (0)	
Other/Unknown	1 (3.6)	1 (5.9)	
Time since first cardiac diagnosis			0.453
<1 month	14 (50)	6 (35.3)	
1 month-1 year	6 (21.4)	5 (29.4)	
1 year or more	4 (14.3)	5 (29.4)	
Unknown	4 (14.3)	1 (5.9)	
Current ICD in place	2 (7.1)	2 (11.8)	1.000
INTERMACS profiles			0.918
INTERMACS 1	8 (28.6)	6 (35.3)	
INTERMACS 2	9 (32.1)	6 (35.3)	
INTERMACS 3	8 (28.6)	3 (17.6)	
INTERMACS 4 – 5	3 (10.7)	2 (11.8)	
INTERMACS 6 – 7	0 (0)	0 (0)	
Echocardiography			
LVEF	18 (10-20)	15 (15-20)	0.612
LVEDD(mm)	68 (63-70)	66 (62-73)	0.771
Aortic regurgitation ≥moderate	1	0	1.000
Mitral regurgitation ≥moderate	4	9	0.038
ECG rhythm			0.389
Sinus	18 (64.3)	14 (82.4)	
Atrial fibrillation/flutter	5 (17.9)	3 (17.6)	
Paced	1 (3.6)	0 (0)	
Other/Unknown	4 (14.3)	0 (0)	
Heart rate (bpm)	97 (89-121)	98 (74-111)	0.455
Blood pressure			
Systolic	105 (92 – 115)	106 (94-114)	0.919

	With follow-up (28)	No follow-up (17)	p-value
Diastolic	61 (60-70)	67 (50-70)	0.942
Mean arterial pressure	74 (72-84)	78 (70-82)	0.965
Diabetes	2 (7.1)	0 (0)	0.519
Inotropic support			
Intravenous Inotropes	23 (82.1)	14 (82.4)	0.333
1-2 inotropes	18 (64.3)	8 (47.1)	
≥3 inotropes	5 (17.9)	6 (35.3)	
IABP	4 (14.3)	3 (17.6)	1.000
ECLS	10 (35.7)	7 (41.2)	0.715
Mechanical ventilation	8 (28.6)	6 (35.3)	0.637
Blood chemistry			
Creatinine (μmol/L)	105 (79-114)	114 (91-141)	0.142
ALAT (U/L)	76 (39-177)	46 (34-520)	0.892
ASAT (U/L)	180 (42-592)	73 (27-184)	0.147
LDH (U/L)	469 (308-1189)	407 (338-992)	0.859
Total Bilirubin (mg/dL)	1.5 (0.8-2.5)	1.5 (0.8-2.1)	0.525
Haemoglobin (g/dL)	11.8 (10.2-13.4)	10.9 (10.4-13.7)	0.971
White blood cell count (x 10^9/L)	10.6 (9.3-14.3)	10.7 (8.7-13.4)	0.819
Thrombocytes (x 10^9/L)	164 (75-241)	191 (104-266)	0.479

Values are median(IQR), mean[confidence interval] or, n(%) BMI: body mass index; BSA: body surface area; ICD: implantable cardioverter-defibrillator; INTERMACS: Interagency Registry for Mechanically Assisted Circulatory Support); LVEF: left ventricular ejection fraction; LVEDD: left ventricular end-diastolic diameter; ECG: electrocardiogram; IABP: intra-aortic balloon-pump; ECLS: extra-corporeal life-support.

Table 1B. Baseline characteristics of all patients with and without LVAD explantation due to myocardial recovery.

	Explanted	Not explanted	p-value
Age (y)	44 (32 – 54)	56 (47 – 62)	<0.001
Male	36 (80.0)	2568 (83.6)	0.542
BMI	26.4 [25.0 – 27.8]	26.1 [26.0 – 26.3]	0.691
BSA	2.00 [1.93 – 2.08]	1.97 [1.96 – 1.98]	0.313
Aetiology			<0.001
Myocarditis	12 (26.7)	125 (4.1)	
Dilated cardiomyopathy	14 (31.1)	995 (32.4)	
Ischemic cardiomyopathy	9 (20)	1285 (41.8)	
Peripartum	2 (4.4)	14 (0.5)	
Valvular heart disease	4 (8.9)	45 (1.5)	
Hypertrophic cardiomyopathy	1 (2.2)	30 (1.0)	
Toxic	1 (2.2)	51 (1.7)	
Restrictive cardiomyopathy	0 (0)	20 (0.7)	
Congenital heart disease	0 (0)	28 (0.9)	
Other/Unknown	2 (4.4)	480 (15.6)	
Time since first cardiac diagnosis			<0.001
<1 month	20 (44.4)	302 (11.1)	
1 month-1 year	11 (24.4)	356 (13.1)	
1 year or more	9 (20)	1857 (68.4)	

	Explanted	Not explanted	p-value
Unknown	5 (11.1)	198 (7.3)	
Current ICD in place	1633 (61.4)	4 (8.9)	<0.001
INTERMACS profiles			0.01
INTERMACS 1	14 (31.1)	412 (13.4)	
INTERMACS 2	15 (33.3)	996 (32.4)	
INTERMACS 3	11 (24.4)	781 (25.4)	
INTERMACS 4 – 5	5 (11.1)	637 (21.6)	
INTERMACS 6 – 7	0 (0.0)	123 (4.2)	
Echocardiography			
LVEF	17 (15 – 19)	19 (19 – 19)	0.125
LVEDD(mm)	65.6 (62.2 – 69.0)	71.3 (70.1 – 72.6)	0.270
Aortic regurgitation ≥moderate	1 (3.4)	92 (3.8)	1.000
Mitral regurgitation ≥moderate	13 (38.2)	1221 (50.9)	0.141
ECG rhythm			0.005
Sinus	32 (71.1)	1359 (51.5)	
Atrial fibrillation/flutter	8 (17.8)	424 (16.1)	
Paced	1 (2.2)	663 (25.1)	
Other/Unknown	4 (8.9)	192 (7.3)	
Heart rate (bpm)	100 (92 – 107)	87 (86-87)	<0.001
Blood pressure			
Systolic	106 (100 – 112)	100 (100 – 102)	0.048
Diastolic	64 (59 – 69)	65 (64 – 65)	0.725
Mean arterial pressure	78 (73 – 83)	77 (76 – 77)	0.519
Diabetes	2 (4.4)	2105 (73.5)	0.001
Inotropic support			
Intravenous Inotropes	37 (82.2)	2495 (89)	0.547
1-2 inotropes	26 (57.8)	2149 (76.7)	
≥3 inotropes	11 (24.4)	346 (12.3)	
IABP	7 (15.9)	297 (11.3)	0.342
ECLS	17 (37.8)	303 (10.5)	<0.001
Mechanical ventilation	14 (31.1)	406 (15.4)	0.004
Blood chemistry			
Creatinine (μmol/L)	111 (83 – 123)	111 (85 – 150)	0.465
ALAT (U/L)	29 (54 – 177)	29 (18 – 70)	0.001
ASAT (U/L)	135 (31 – 410)	33 (23 – 74)	<0.001
LDH (U/L)	434 (314 – 1173)	308 (238 – 452)	<0.001
Total Bilirubin (mg/dL)	1.78 (0.78 – 2.26)	1.29 (0.80 – 2.10)	0.591
Haemoglobin (g/dL)	11.9 (10.3 – 13.6)	11.8 (10.2 – 13.5)	0.844
White blood cell count (x 109/L)	10.7 (8.9 – 14.0)	8.4 (6.7 – 11.0)	<0.001
Thrombocytes (x 109/L)	173 (80 – 247)	199 (150 – 250)	0.030

Values are median(IQR), mean[confidence interval] or, n(%) BMI: body mass index; BSA: body surface area; ICD: implantable cardioverter-defibrillator; INTERMACS: Interagency Registry for Mechanically Assisted Circulatory Support); LVEF: left ventricular ejection fraction; LVEDD: left ventricular end-diastolic diameter; ECG: electrocardiogram; IABP: intra-aortic balloon-pump; ECLS: extra-corporeal life-support.

Table 2. Implantation and post-implantation characteristics for recovery patients

	With follow-up (28)	No follow-up (17)	p-value
Device strategy			<0.001
BTT	22 (78.5%)	7 (36.9%)	
DT	2 (7.1%)	2 (10.5%)	
Rescue Therapy	4 (14.3%)	2 (10.5%)	
Bridge to recovery	0 (0%)	8 (42.1%)	
Device type			0.204
HeartMate II	14 (50%)	6 (31.6%)	
HVAD	11 (39.3%)	9 (47.4%)	
HeartMate 3	2 (7.1%)	0 (0%)	
PVAD	0 (0%)	2 (10.5%)	
Other/Unknown	1 (3.6%)	2 (10.5%)	
Concomitant cardiac procedures			
PFO/ASD closure	3 (10.7%)	2 (10.5%)	
Tricuspid repair	1 (3.6%)	4 (21.1%)	
Tricuspid replacement		1 (5.3%)	
Aortic repair		1 (5.3%)	
Aortic valve replacement	1 (3.6%)		
Mitral repair		1 (5.3%)	
CABG		1 (5.3%)	
Concomitant temporary RVAD implantation	2 (7.1%)	2 (10.5%)	1.000
Time in OR(minutes)	208 (130-683)	276 (95-375)	0.600
Cardiopulmonary bypass time(minutes)	75 (95-147)	124 (50-235)	0.034
ICU stay(days)	17 (4-147)	27 (2-66)	0.377
Hospital stay(days)	30 (17-165)	39 (14-144)	0.328

Values are median(range) or, n(%)
ASD: atrial septal defect; BTT: bridge to transplantation; CABG: Coronary artery bypass grafting; DT: destination therapy; ICU: intensive care unit; RVAD; right ventricular assist device; OR: operating room; PFO: patent foramen ovale.

Outcomes during VAD support

The median support time of the patients was 465 days (59-1286). Within this time frame the following key AEs were captured: major infection, major bleeding and device malfunction and haemolysis (Table 3). 8 (28.6%) patients remained free of any AEs during LVAD support, while 10 patients (35.7%) encountered 3 or more AEs. Forty-eight (73.8%) captured AEs required a hospitalization. There were no significant differences to the patients with missing follow-up after explantation.

Table 3. Time on support and adverse events during mechanical circulatory support for recovery patients

	With follow-up (28)	No follow-up (17)	p-value
Time on support(days)	410 (59-1286)	231 (10-1425)	0.06
Type of adverse events			
Major Infection	21 (32.3%)	4 (14.3%)	
Major Bleeding	8 (12.3%)	1 (3.6%)	
Device malfunction	6 (9.2%)	5 (17.9%)	
Haemolysis	4 (6.2%)	2 (7.1%)	
Cardiac Arrhythmia	3 (4.6%)	1 (3.6%)	
Stroke	2 (3.1%)	3 (10.7%)	
Renal Dysfunction	2 (3.1%)	4 (14.3%)	
Right heart failure	0 (0%)	1 (3.6%)	
Other	19 (29.2%)	7 (25%)	
Number of AEs per patient			0.889
0	8 (28.6%)	5 (29.4%)	
1-2	10 (35.7%)	8 (47.1%)	
3-4	7 (25.0%)	3 (17.6%)	
≥5	3 (10.7%)	1 (5.9%)	
Hospitalizations required for AE	48 (73.8%)	26 (92.9%)	
Hospitalizations per patient			1.000
0	10 (35.7%)	6 (35.3%)	
1-2	11 (39.3%)	7 (41.2%)	
3-4	5 (17.9%)	3 (17.6%)	
≥5	2 (7.1%)	1 (5.9%)	

Values are median(range) or, n(%)
AE: adverse event

Outcomes after LVAD explantation

Median follow-up time after LVAD explantation is 26 months (0.3-73). Freedom from death, LVAD reimplantation, HTx and relapse of HF ≥NYHA III was 100% at 30 days and 88% at 24 months after explantation (Figure 1, Table 4). Two patients encountered a HF relapse, which is in part attributable to new onset of degenerative mitral regurgitation in 1 patient. One patient required reimplantation of an LVAD after 32 days. Finally, one patient died 302 days after LVAD explantation due to sepsis. Until 48 months this percentage remained unchanged (88%), however follow-up was only available for six patients for this duration of follow-up

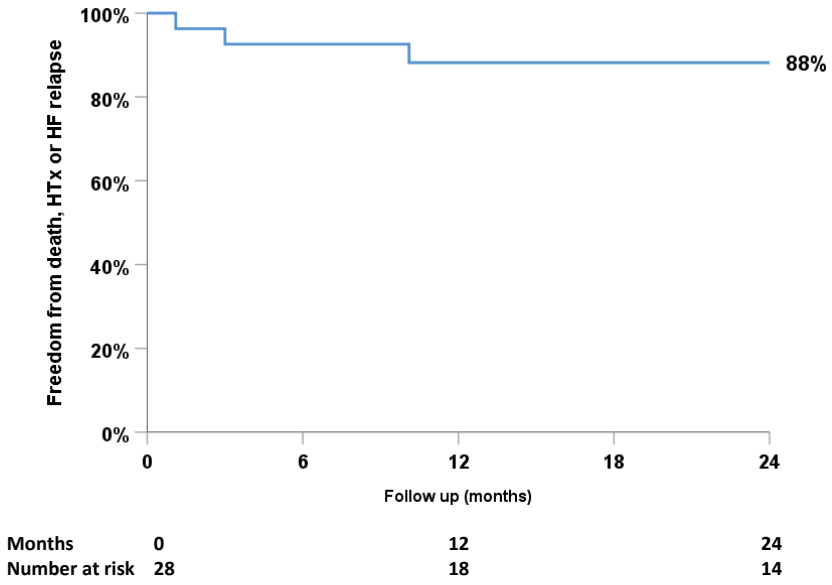

Figure 1. Freedom of death, LVAD reimplantation, heart transplantation and significant heart failure relapse after LVAD explantation.

The inflow cannula remained in situ after explantation in 3 patients (11%). Of these patients, 2 were on both warfarin and aspirin, one patient only used warfarin. The inflow cannula was not *in situ* in the 25 other patients, 2 of them used both warfarin and aspirin. Long-term anticoagulation treatment was aspirin in 43% of the patients, while in 39% warfarin was used. Four patients were prescribed both aspirin and warfarin. In 8 (29%) patients no antiplatelet or anticoagulation therapy was used. No CVA was reported in any patient.

Table 4. Long-term outcome post-LVAD explant for recovery patients

Follow-up time(months)	**26 (0.3-73)**
Primary outcome	
Ongoing after explant	26 (92.8%)
HF recurrence	3 (10.7%)
LVAD reimplantation	1 (3.6%)
Death	1 (3.6%)
BMI	27.6 [25.4-29.7]
Blood pressure	
Systolic	113 (88-160)
Diastolic	77 (51-98)
Mean arterial pressure	90 (68-113)

Follow-up time(months)	26 (0.3-73)
Echocardiography data	
LVEF	40 (15-60)
LVEDD(mm)	54 (41-74)
LVESD(mm)	43 (27-63)
MR grade≥3	2
ECG	
Heart rate	73 (48-105)
Rhythm	
Sinus	21 (75%)
Atrial fibrillation/flutter	3 (10.7%)
Other	4 (14.3%)
QRS width (ms)	98 (74-188)
QTc duration (ms)	435 (374-593)
Blood chemistry	
Creatinine (μmol/L)	97 (62-248)
Bilirubin (mg/dL)	0.6 (0.3-2.8)
Functional status at last FU	
NYHA class I	7 (25%)
NYHA class II	16 (57.2%)
NYHA class III	3 (10.7%)
Unknown	2 (7.1%)
HF medication	
β-blockers	24 (85.7%)
ACE inhibitors	20 (71.4%)
Loop diuretics	14 (50%)
Inflow cannula in situ	3 (10.7%)
Anticoagulation/antiplatelet therapy	20 (71.4%)
Acetylsalicylic acid	12 (42.9%)
Vitamin K antagonist	11 (39.3%)
Both	4 (14.3%)
None	8 (29%)
Number of patients with CVA	0 (0%)

Values are median (range), mean [standard deviation] or, n (%) ACE; angiotensin converting enzyme; BMI: body mass index; BSA: body surface area; CVA: cerebrovascular accident; ECG: electrocardiogram; ECLS: extra-corporeal life support; HF: heart failure; IABP: intra-aortic balloon pump; ICD: implantable cardioverter-defibrillator; INTERMACS: Interagency Registry for Mechanically Assisted Circulatory Support); LVAD: left ventricular assist device; LVEF: left ventricular ejection fraction; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end systolic diameter; MR: mitral regurgitation; NYHA: New York Heart Association,

DISCUSSION

This study provides a multi-centre, mid- to long-term follow-up of patients whose LVAD was explanted due to myocardial recovery. The mid- to long-term outcomes appear to be encouraging with 88% of the patients surviving without HTx, LVAD reimplantation or relapse of HF at 24 months of follow-up. Furthermore, the majority of patients suffered from mild HF-symptoms only (NYHA class I-II). The number of patients with sufficient myocardial recovery for LVAD explantation is comparable to other registries such as INTERMACS, which reported a successful weaning rate of approximately 1% in the latest annual report[10, 11].

A comprehensive review of single-centre studies reporting on myocardial recovery allowing LVAD explantation found weaning rates ranging from 4.5% to 63% and thus contrasts with lower recovery rates reported in the INTERMACS or EUROMACS registries[12]. Some of these studies have reported higher rates of successful weaning. Interestingly, some showed a successful explantation rate of 12 in 19 (63%) patients supported by a HeartMate II[13]. However, this population was young (mean age 35.2 years) and patients with ischemic heart disease were excluded. Moreover, they received aggressive pharmacotherapy with maximum HF medication combined with clenbuterol (β_2-agonist).

These high rates of recovery may partly be explained due to the commitment of some centres resulting in specific clinical and scientific focus on the recovery. This might result in advanced, aggressive strategies to identify potential patients eligible for LVAD explantation and thus treating those patients with targeted and strictly regulated HF medication. Furthermore, studies that included patients with non-ischemic heart disease as aetiology of HF and patients with recent onset of HF tend to have higher rates of successful myocardial recovery. These observations correspond well with our study in which the majority of patients had their first cardiac disease diagnosis less than 1 year ago and ischemic cardiomyopathy represented an uncommon aetiology of HF. Indeed, the baseline characteristics that are significantly different in patients with LVAD explantation are very similar to the variables used in the INTERMACS Cardiac Recovery Score (I-CARS) of Wever-Pinzon *et al.*[11].

In the literature, several case series and small cohort studies report on patients having recovery of left ventricle (LV) function that allowed LVAD explantation. Studies such as Dandel *et al.* report that LVAD (Novacor) explantation was successful in 32 of 131 patients with idiopathic dilated cardiomyopathy, with a 5-year survival of 78,3% and 31,1% HF recurrence rate in 3 years, after being supported with a LVAD for a mean duration of 4,6 months (SD ± 4,4)[4]. Birks *et al.* reported that out of 15 patients with severe HF due to non-ischemic cardiomyopathy, 11 had LVAD explantation after a mean of 320 days LVAD support. 88,9% of the surviving patients were free from HF at 4 years after explantation[5]. Another study showed survival after explantation of HeartMate II was 83.3% after 3-year follow-up[13]. A study of 14 patients revealed

that after a mean follow-up time of 3.6 years (± 1.9) after explantation, no patient had died and had a functional NYHA class of I[6]. Frazier *et al.* achieved explantation in 27 patients out 657 patients supported by a LVAD, with 25 of them surviving after a mean follow-up of 3.2 years (± 2.6) all of them with NYHA class I with medical therapy[7]. In comparison, we showed a similar excellent survival of 88% without HTx, LVAD reimplantation or significant HF relapse in a European multi-centre registry, with the majority of patients only suffering from mild HF symptoms after explantation. This highlights that, in carefully selected patients, excellent results can be achieved after LVAD explantation and are not restricted to one centre.

Pathophysiology and clinical implications of LV recovery

The pathophysiology of HF is complex and multifactorial. Systolic HF is accompanied by left ventricular (LV) remodelling, which is characterized by three categories of changes in the heart: myocyte defects, myocardial defects and abnormal LV geometry [14]. In contrast, to achieve myocardial recovery, the heart has to undergo cardiac reverse remodelling. Support by cf-LVADs results in left ventricular pressure and volume unloading as well as increased cardiac output[15, 16] and has shown to promote certain forms of reverse remodelling[17]: reduction of cardiac myocyte hypertrophy[18, 19], changes in gene expression[20, 21] and normalization of β-adrenergic receptor and inotropic responsiveness[22]. Concerning restoration of the ECM, conflicting studies exist, with some studies reporting an increase in total ECM collagen[23, 24], while others report a decrease[25]. Finally, studies have shown an improvement in cardiac myocyte contractility after LVAD implantation[26, 27]. There are some excellent reviews covering this topic in much more detail[14, 28]. On the clinical side, the criteria used for the decision of LVAD explantation differ between centres[8, 13, 29]. Clinical parameters that indicate LV remodelling and might indicate myocardial recovery often include an increase in LVEF, decreases in end-diastolic left ventricular diameter, (partial) reversal of functional mitral regurgitation, normalization of cardiac filling pressures and cardiac sinus rhythm with a normal heart rate. Unfortunately, studies linking pathophysiological findings with these clinical outcomes are scarce.

Future perspectives

A combination of data on clinical and biological findings for patients undergoing LVAD implantation is currently lacking robust data with both studies conducted separately of each other. Preferably, one would collect histological/biological data and clinical data before LVAD implantation, during LVAD support and after explantation (if applicable). This holistic approach would provide much needed insight in the changes that are induced by VAD therapy and would enable us to link and understand pathophysiological changes to clinical changes and *vice versa*.

Finally, because the number of patients that have sufficient myocardial recovery to enable LVAD explantation is limited, it is critical that researchers and clinicians cooperate in large registries such as EUROMACS and INTERMACS, by adding data fields for centres willing to capture data on the follow-up of these patients. As a consequence, the

EUROMACS board set out the goal that follow-up of after explantation of VAD devices due to recovery should also be captured in the near future.

Limitations

This study has certain limitations that should be considered while interpreting the results. Data were gathered retrospectively and the number of data fields captured is limited. Furthermore, it is possible that not all patients whose LVAD was explanted due to recovery were captured in the EUROMACS registry. It is also possible that the follow-up of patients who actually have been explanted due to recovery, has not been registered yet. This might result in a relative underestimation of the number of patients with myocardial recovery, however the EUROMACS registry regularly checks and audits participating centres for data quality and completion. Finally, we only received follow-up data on 62% of those patients weaned from LVAD support which may constitute a potential selection bias resulting in favourable outcomes. However, the baseline characteristics and follow-up during LVAD support of patients with and without follow-up were, apart from two variables, not significantly different (Table 1A).

CONCLUSION

To our knowledge this is one of the first multi-centre studies to review mid- to long-term follow-up after LVAD explantation due to myocardial recovery. Although LVAD explantation remains rare, outcomes after explantation are excellent with a majority of patients ongoing without HTx or LVAD reimplantation while having limited HF symptoms only. Large, prospective registries and/or studies are required to generate pertinent data in order to better understand this challenging population.

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CHAPTER 8

Outcomes after tricuspid valve surgery concomitant with left ventricular assist device implantation in the EUROMACS registry: A propensity score matched analysis

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ABSTRACT

Objective: Tricuspid regurgitation (TR) is common in patients receiving a left ventricular assist device (LVAD) and controversy exists whether concomitant is tricuspid valve surgery (TVS) is beneficial in currently treated patients. Therefore, we aimed to investigate the effect of concomitant TVS during LVAD implant.

Methods: The European Registry for Patients with Mechanical circulatory Support (EUROMACS) was used to identify adult patients. Matched patients with and without concomitant TVS were compared by using a propensity score matching strategy.

Results: In total, 3323 patients underwent LVAD implantation of which 299(9%) with TVS). After matching 258 patients without TVS were matched to 258 patients with TVS. In the matched population hospital mortality, days on inotropic support, temporary right ventricular assist device implant and hospital stay were comparable, whereas intensive care stay was higher in the TVS cohort (11 vs. 15 days, P=0.026). Late mortality (P=0.17), cumulative incidence of unexpected hospital readmission (P=0.15) and right heart failure (P=0.55) were comparable between patients with and without concomitant TVS. In the matched population probability of moderate-to-severe TR immediately after surgery was lower in patients with concomitant TVS compared to patients without TVS (33%vs70%, P=0.001). Nevertheless, the probability of moderate-to-severe TR decreased more quickly in patients without TVS (P=0.030), resulting in comparable probabilities of moderate-to-severe TR within 1.5 year of follow-up.

Conclusion: In matched patients concomitant TVS during LVAD implant does not seem be associated with better clinical outcomes. Concomitant TVS reduced TR significantly early after LVAD implant, however, differences in probability of TR disappear during follow-up.

INTRODUCTION

Implantation of a left ventricular assist device (LVAD) improves survival, functional status and quality of life in patients with end-stage heart failure (1, 2). In these patients tricuspid regurgitation is common (3) and current guidelines recommend consideration of tricuspid valve surgery when moderate to severe tricuspid regurgitation is present (4). Nevertheless, controversy exists whether concomitant tricuspid valve surgery is associated with better outcomes, as contemporary studies are hampered by small sample size and biased due to baseline differences (5). In this study, we investigate the clinical outcomes after concomitant tricuspid valve surgery during LVAD implantation compared to a propensity score matched controls using the European Registry for Patients with Mechanical Circulatory Support (EUROMACS). Furthermore, we aimed to assess the postoperative course of tricuspid regurgitation in patients with and without concomitant tricuspid valve surgery (TVS).

METHODS

Study design

The EUROMACS is a registry of the European association for Cardio-Thoracic Surgery. In this registry all relevant clinical, echocardiographic hemodynamic and laboratory parameters of patients who require mechanical circulatory support are collected prospectively since January 2011. Participating centers were allowed to enter data before 2011 retrospectively, making this study an ambispective cohort study. A detailed description of the database and collection procedure are described previously (6).

Patients

All patients operated between 1995 and 2018 were identified. Patients <18 years old and with planned right ventricular -, biventricular- and assist device were excluded from analysis. Additionally, patients with single ventricle physiology were excluded (Supplementary Figure 1).

Study outcome

The main outcomes that were assessed were early (both 30-day and hospital mortality separately) and late mortality. Late mortality was defined as mortality after 30 days, regardless of hospital admission status. Furthermore, unplanned hospital readmission and right heart failure were assessed. Right heart failure was defined according to the INTERMACS adverse event definitions (7). Patients were censored at heart transplant, death and lost to follow-up. Lastly, the course of the probability of moderate-to-severe tricuspid regurgitation was evaluated in patient with and without TVS.

Missing values

Multiple imputation by chained equations using the statistical "MICE" package in R was used to impute missing values (8). Selected baseline variables with <55% missing, were imputed, above 55% missing was considered excessive missingness (Supplementary Table 1). Nevertheless, 51 out of the 67 imputed variables (76%) had less than 30% missings. An exception was made for the variable Tricuspid annular plane systolic excursion (TAPSE) (62% missing), since this variable is highly important in the setting of TVS, and it was reasonable to assume it could be imputed based on observed variables, such as right ventricle ejection fraction (missing mechanism: missing at random). Imputations were done based upon the other baseline variables. In case of highly correlated variables the variable with highest clinical value was chosen as predictor (Supplementary Table 2). Correlation was tested with Pearson R or Spearman rho, as appropriate. Five imputed datasets were generated using this method using 5 iterations each. The imputations were visually checked by strip plots and density plots, and no major deviations are noted between imputed data and complete data (Example TAPSE: Supplementary Figure 2). Analyses was done on each dataset separately and pooled according to Rubin's rules (9). In baseline comparisons of the matched groups, continuous data was transformed to approximate Gaussian distribution and was pooled according to Rubin's rules.

Statistical analysis

Continuous data is presented as mean ± standard deviation (Gaussian distribution) or median (interquartile range[IQR]) (non-Gaussian distribution). Categorical data is presented as frequencies (percentage). Comparisons among continuous variables were made with the Student T-test or Mann-Whitney test, as appropriate. Continuous data outside 3 standard deviations was considered erroneous and removed (Supplementary Table 3). Comparisons of categorical variables were made with the Chi-squared test or with the Fisher exact test, as appropriate. Propensity score matching was used to balance baseline differences, since the main interest of this study is the treatment effect in a typical treated patient instead of a population level treatment effect (10). The parsimonious propensity score model was developed using least absolute shrinkage and selection operator (LASSO) regression (11). This machine learning analysis technique shrinks unimportant covariates to zero. The parsimonious model consisted out of all non-zero covariates. In total, 62 variables were offered to the Lasso model, which selected 15 variables (Supplementary Table 4). Thereafter, 9 variables were added due clinical significance and to achieve satisfactory balance (Supplementary table 5). The final propensity score model contained 24 variables (Supplementary table 5/6). One-on-one matching without replacement was performed and the caliper was set at 0.15. For the main outcome a sensitivity analyses was performed with caliper set at 0.001. Standard mean difference prior and after matching was used to assess covariate balance. Late survival was calculated and visualized with the Kaplan-Meier method and both cohorts were compared with log-rank test. Since some patients had no recorded follow-up, a sensitivity analyses was performed to test the robustness of the log-rank test under different missing mechanisms. Unplanned hospital readmission and right heart failure was considered a competing risk with mortality and Fine and Gray competing risk

models were used to calculate cumulative incidences. Gray's tests were used to quantify significant differences among cohorts. Generalized mixed-models were used to analyze repeated echocardiograms. Further details regarding the mixed-models are provided in Supplementary Text 1 Follow-up completeness was calculated using the modified Clark C (C*) (12). All analyses were done in R (R core team 2017, Austria, Vienna) with the use of statistical packages "glmnet", "Matching", "survival", "cmprsk", "splines" and "lme4".

RESULTS

In total, 3323 procedures were included (3024 [91%] without TVS and 299 [9%] with TVS). In the TVS cohort 292 (97%) patients had a tricuspid valve repair and 7 (3%) patients had a tricuspid valve replacement (6 mechanical and 1 biological). After propensity score matching, 258 procedures without TVS surgery were matched to 258 procedures with additional TVS. Density plots of the propensity score in the unmatched and matched cohorts are presented in Figure 1. In patients that survived 30-days and had recorded late follow-up the mean follow-up time was 1.7 ± 1.5 years with a completeness of 86% (*C).

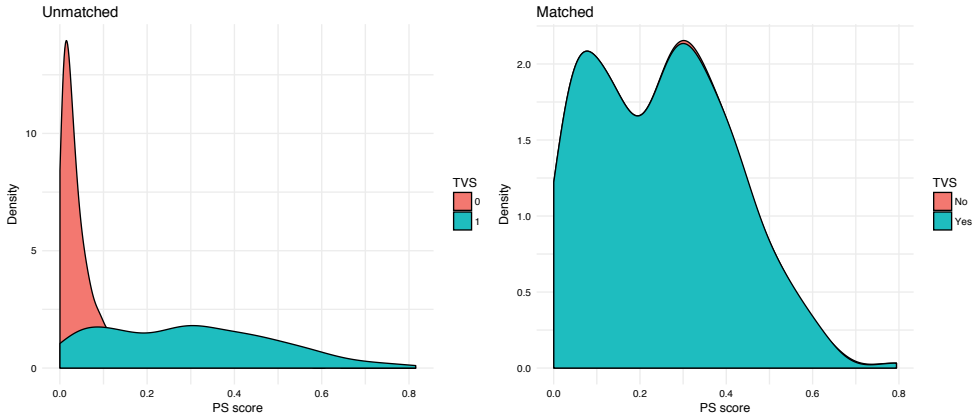

Figure 1. Density of propensity score in the unmatched and the matched cohorts.

Patient characteristics

Patient characteristics are presented in Table 1. In the unmatched cohort, patients who did not undergo TVS had, among others, significantly less tricuspid regurgitation, more ischemic cardiomyopathy and better kidney and liver function. In the matched cohort, patients no significant differences in baseline characteristics were noted. In addition, overall absolute standard mean difference before matching was 18.7 and after matching 4.9 (Supplementary Table 7).

Table 1. Patient characteristics of patients with or without concomitant tricuspid valve surgery in matched and unmatched cohorts. [1]Data and tests on complete cases. [2]Data from first imputed dataset , and p-values from tests are derived from the pooled analyses.

	Unmatched groups[1]			Matched groups[2]		
	No TVS	**TVS**	**p-value**	**No TVS**	**TVS**	**P-value**
n	**3024**	**299**		**258**	**258**	
Age, y	56.00 [47.00, 62.00]	57.00 [47.50, 63.00]	0.044	56.00 [47.00, 64.00]	57.00 [47.25, 63.00]	0.74
Male sex, n (%)	2519 (83.3)	235 (78.6)	0.048	205 (79.5)	202 (78.3)	0.83
Body surface area, m2	1.96 [1.81, 2.12]	1.96 [1.85, 2.12]	0.80	1.94 [1.79, 2.11]	1.96 [1.84, 2.11]	0.75
Caucasian, n (%)	2271 (87.4)	248 (95.8)	0.003	247 (95.7)	245 (95.0)	>0.99
Etiology (%)			**<0.001**			**0.77**
Coronary artery disease	252 (10.0)	24 (9.3)		20 (7.8)	26 (10.1)	
Ideopatic	614 (24.5)	100 (38.8)		95 (36.8)	97 (37.6)	
Ischemic	1011 (40.3)	62 (24.0)		66 (25.6)	65 (25.2)	
Other	632 (25.2)	72 (27.9)		77 (29.8)	70 (27.1)	
≥2 years since first diagnosis	1546 (63.5)	188 (75.5)	0.001	190 (73.6)	192 (74.4)	0.90
Destination therapy	467 (16.9)	47 (15.9)	0.72	42 (16.9)	43 (16.8)	>0.99
Ascites	198 (10.3)	36 (18.0)	<0.001	55 (21.3)	56 (21.7)	0.90
Rhythm, n (%)			**0.084**			**0.99**
Sinus	1337 (55.4)	119 (47.8)		128 (49.6)	120 (46.5)	
Atrial fibrillation	397 (16.4)	44 (17.7)		45 (17.4)	49 (19.0)	
Paced	613 (25.4)	80 (32.1)		82 (31.8)	82 (31.8)	
Other	68 (2.8)	6 (2.4)		3 (1.2)	7 (2.7)	
INTERMACS class, n (%)			**<0.001**			**0.90**
1	427 (15.0)	19 (6.4)		17 (6.6)	20 (7.8)	
2	942 (33.2)	118 (40.0)		101 (39.1)	93 (36.0)	
3	738 (26.0)	92 (31.2)		80 (31.0)	80 (31.0)	
≥4	733 (25.8)	66 (22.4)		60 (23.3)	65 (25.2)	
IABP, n (%)	287 (11.3)	17 (6.6)	0.030	24 (9.3)	15 (5.8)	0.34
ECMO, n (%)	306 (10.9)	22 (7.5)	0.097	18 (7.0)	19 (7.4)	>0.99
Ventilator (%)	377 (14.8)	19 (7.5)	0.002	18 (7.0)	26 (10.1)	>0.99
Medication, n (%)						
Loopdiuretics, n (%)	1886 (80.5)	218 (86.9)	0.018	213 (82.6)	224 (86.8)	0.82
Use of ≥3 inotropes, n (%)	198 (10.5)	23 (11.2)	0.87	51 (19.8)	33 (12.8)	0.79
Laboratory values						
Serum creatinine, mg/dL 1	107.00 [83.00, 150.00]	115.00 [90.50, 150.00]	0.035	109.50 [84.00, 152.75]	114.00 [88.00, 150.00]	0.51
ASAT, U/L	33.00 [23.00, 75.00]	35.00 [25.00, 57.00]	0.41	34.00 [24.00, 67.75]	34.00 [25.00, 55.00]	>0.99
Total bilirubin, mg/dL	1.20 [0.78, 2.00]	1.69 [1.14, 2.50]	<0.001	1.50 [0.90, 2.55]	1.53 [1.05, 2.28]	0.92
Albumin, g/dL	507.15 [420.21, 579.60]	507.15 [449.91, 574.16]	0.54	507.15 [405.72, 579.60]	507.15 [434.70, 579.60]	0.82
Hemoglobin, g/dL 1	11.80 [10.20, 13.60]	11.40 [10.07, 13.03]	0.11	11.70 [9.83, 13.20]	11.40 [10.00, 13.28]	0.65

	Unmatched groups[1]			Matched groups[2]		
	No TVS	**TVS**	**p-value**	**No TVS**	**TVS**	**P-value**
n	**3024**	**299**		**258**	**258**	
Hemodynamic						
RA pressure, mmHg	10.00 [7.00, 15.00]	13.00 [9.50, 17.00]	<0.001	12.00 [8.00, 16.00]	13.00 [9.00, 16.00]	0.63
PCWP, mmHg	24.00 [18.00, 30.00]	25.00 [20.75, 29.25]	0.085	24.00 [18.00, 30.00]	24.50 [20.00, 29.00]	0.21
PVR	231.50 [137.00, 354.75]	267.00 [166.75, 372.50]	0.11	262.00 [177.00, 368.00]	276.50 [160.00, 372.50]	0.71
SVR	1262.00 [896.25, 1676.50]	1446.50 [1102.75, 1908.00]	0.001	1317.00 [1021.00, 1590.00]	1300.00 [1062.50, 1858.00]	0.38
PAP, systolic, mmHg	51.00 [39.00, 64.00]	49.50 [40.00, 63.00]	0.71	52.00 [40.00, 63.00]	52.00 [40.00, 65.00]	0.66
Echocardiographic						
TAPSE, mm	14.00 [12.00, 17.00]	15.00 [12.00, 18.00]	0.28	14.00 [11.00, 17.00]	14.00 [12.00, 17.00]	0.63
No aortic regurgitation, n (%)	1469 (63.5)	151 (55.7)	0.060	146 (56.6)	148 (57.4)	0.98
Severe mitral regurgitation, n (%)	392 (17.4)	77 (30.4)	<0.001	76 (29.5)	66 (25.6)	0.83
Tricuspid regurgitation			**<0.001**			**0.79**
None	286 (11.4)	4 (1.4)		8 (3.1)	4 (1.6)	
Trivial	504 (20.1)	14 (4.8)		15 (5.8)	15 (5.8)	
Mild	907 (36.2)	34 (11.7)		39 (15.1)	37 (14.3)	
Moderate	564 (22.5)	113 (38.8)		96 (37.2)	112 (43.4)	
Severe	243 (9.7)	126 (43.3)		100 (38.8)	90 (34.9)	
LVEF (%)	19.00 [15.00, 23.00]	20.00 [15.00, 25.00]	0.029	20.00 [15.00, 24.00]	20.00 [15.00, 23.00]	0.85
RVF			**<0.001**			**0.89**
Normal	400 (22.1)	21 (10.7)		37 (14.3)	31 (12.0)	
Mild	460 (25.4)	44 (22.3)		45 (17.4)	52 (20.2)	
Moderate	700 (38.6)	96 (48.7)		124 (48.1)	114 (44.2)	
Severe	252 (13.9)	36 (18.3)		52 (20.2)	61 (23.6)	

IABP: intra atrial balloon pump, ECMO: Extracorporeal membrane oxygenation. ASAT: Aspartate Aminotransferase, RA: Right atrium, PCWP: pulmonary capillary wedge pressure, PVR: pulmonary vascular resistance, SVR: systemic vascular resistance, PAP: pulmonary atrial pressure, TAPSE: Tricuspid annular plane systolic excursion, LVEF: left ventricular ejection fraction, RVF: Right ventricle function.

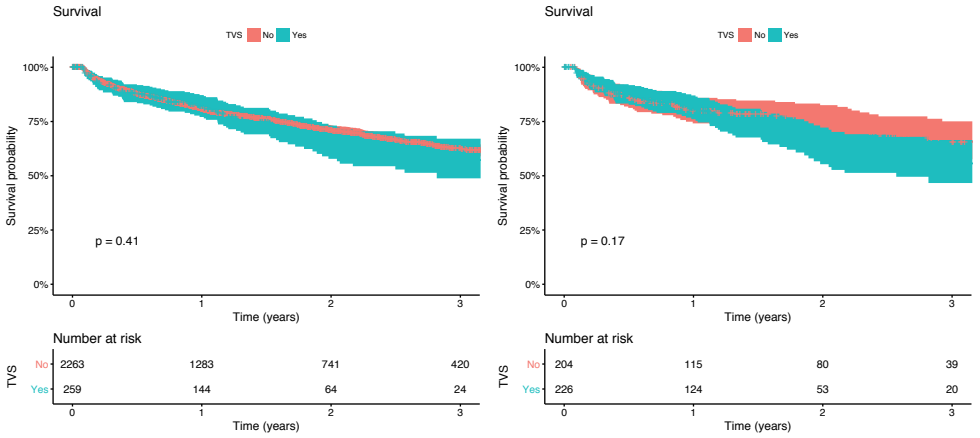

Figure 2. Kaplan-Meier curve of patients that survived 30-days in the unmatched cohort and the matched cohort.

Hospital outcome

Hospital outcomes are presented in Table 2. In the unmatched cohort, cardiopulmonary bypass time (80 vs 118 minutes, p<0.001), ICU stay (10 vs 15 days, P<0.001) hospital stay (30 vs 34, p=0.001) and days on inotropic support (>14 days: 24.7% vs 32.4%) were longer in the patients that underwent TVS. In the matched cohorts these were all comparable, except for cardiopulmonary bypass time (85 vs 116 minutes, p<0.001) and ICU stay (11 vs 15 days, p=0.026) (Table 2). Additionally, in the matched groups, the 30-day mortality (13.6%, 95%CI [9.5–18.6] vs 10.0%, 95%CI [6.5–14.4], p=0.27) and hospital mortality (20.2% 95%CI[14.7–24.7] vs 16.5% 95%CI[13.0–22.6], p=0.41) was comparable between the patients with and without concomitant TVS. Sensitivity analyses with caliper at 0.001 did not change point estimates considerably (Supplementary Table 8).

Table 2. Hospital outcomes of patients with or without concomitant tricuspid valve surgery in matched and unmatched cohorts.

	Unmatched groups			Matched groups		
	No TVS	**TVS**	**p-value**	**No TVS**	**TVS**	**P-value**
n	**3024**	**299**		**258**	**258**	
CPB time (min)	80 [58, 111.5]	118 [94, 157]	<0.001	84.50 [61.00, 114.50]	115.50 [92.25, 157.75]	<0.001
Device brand			**<0.001**			**0.93**
HeartMate II	776 (27.4)	120 (40.4)		102 (39.5)	96 (37.2)	
HeartWare HVAD	1481 (52.3)	117 (39.4)		112 (43.4)	113 (43.8)	
HeartMate III	414 (14.6)	58 (19.5)		42 (16.3)	47 (18.2)	
Other	160 (5.7)	2 (0.7)		2 (0.8)	2 (0.8)	
Hospital death n (%)	452 (15.2)	55 (18.8)	0.58	50 (20.2)	45 (16.5)	0.41
30 – day death	306 (11.9)	32 (11.0)	0.72	32 (13.6)	25 (10.0)	0.27

	Unmatched groups			Matched groups		
	No TVS	**TVS**	**p-value**	**No TVS**	**TVS**	**P-value**
n	**3024**	**299**		**258**	**258**	
Temporary RVAD support	138 (4.5)	23 (7.7)	0.024	22	16	0.40
Days of intropic support			**0.013**			**0.29**
1 -7 days	993 (56.6)	92 (48.2)		11 (7.0)	13 (7.7)	
8 - 13 days	321 (18.3)	37 (19.4)		85 (53.8)	85 (50.6)	
14 - 27 days	276 (15.7)	48 (25.1)		27 (17.1)	41 (24.4)	
>27 days	158 (9.0)	14 (7.3)		33 (20.9)	29 (17.3)	
Ongoing	6 (0.3)	0 (0.0)		2 (1.3)	0 (0.0)	
ICU-CCU stay	10 [5, 23]	15 [6, 53]	<0.001	11.00 [5.00, 24.00]	15.00 [6.00, 31.00]	0.026
Hospital stay	30 [21, 46]	34 [25, 53]	0.001	33.00 [22.00, 54.00]	34.50 [24.75, 52.25]	0.38

RVAD: Right ventricular assist device, ICU: Intensive care unit, CCU: Cardiac care unit.

Late outcome

In total, 2522 patients had recorded late follow-up and did not die within 30 days (no TVS: 2263 and TVS: 259 patients), of which 819 patients died during follow-up (no TVS: 736 and TVS: 73) (Supplementary Figure 3). Kaplan-Meier curves of survival are shown in Figure 2a. Unmatched patients with and without concomitant TVS had comparable late survival (p=0.41). Additionally, cumulative incidence of unplanned hospital re-admission from any cause (No TVS: 764 and TVS: 102) and cumulative incidence of right heart failure (No TVS: 138 and TVS: 25) was higher in the TVS cohort (Figure 3a and 4a), p=0.006 and p=0.011, respectively.

In the matched cohort, 226 TVS patients survived 30 days and had recorded late follow-up versus 204 matched controls, of which 128 died during follow-up (no TVS: 62 and TVS: 66). Late survival was comparable between patients with and without TVS (p=0.17) (Figure 2b). Notably, the curves diverged after approximately 1 year of follow-up with 2-year survival estimates of 75.6% (95% CI[69.3 – 82.5]) in the no TVS cohort and 63.2% (95% CI[55.3 – 72.2]) in the TVS cohort, but still with overlapping confidence intervals. In total 22 patients in the matched control group and 7 patients in the TVS cohort, did not have recorded follow-up. Sensitivity analyses revealed that only in the scenario that all missing patients in the no TVS cohort survived and in the TVS cohort all died the log-rank test was significantly differed (Supplementary Table 9). Sensitivity analyses with caliper set at 0.001 did not change point estimates considerably (Supplementary Table 8).. In the matched cohorts, cumulative incidence of unplanned hospital re-admission (No TVS: 80 and TVS: 92 patients, p=0.15) and right heart failure (No TVS: 16 and TVS: 20 patients, p=0.55) were comparable between patients with and without concomitant TVS (Figure 3b and 4b).

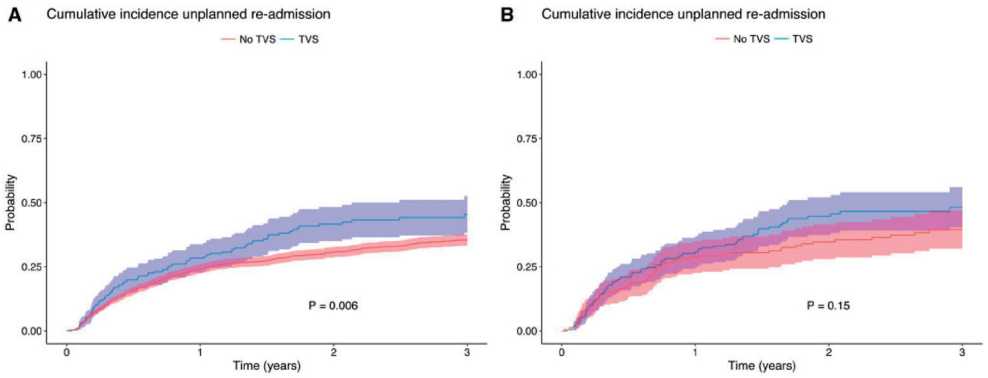

Figure 3. Cumulative incidence estimated by Fine and Gray model with death as competing risk of unexpected hospital readmission in the unmatched and the matched cohort.

Evolution of tricuspid regurgitation

In total, 1219 patients had 3956 recorded echocardiograms during follow-up (mean: 3.2 echocardiograms, range: 1 – 28). Figure 5a presents the probability of moderate-to-severe tricuspid regurgitation over time in the unmatched cohorts. In the matched cohorts 224 patients had 725 recorded echocardiograms (mean 3.2, range: 1 – 21), which could be used in the mixed models. Immediately after LVAD implantation, patients that underwent TVS had significantly lower probability of moderate-to-severe tricuspid regurgitation (33% vs 70%, p=0.001). Nevertheless, during follow-up probability of moderate-to-severe tricuspid regurgitation decreased more quickly in the no TVS cohort compared to the TVS cohort (p=0.030), resulting in comparable probabilities within one year of follow-up.

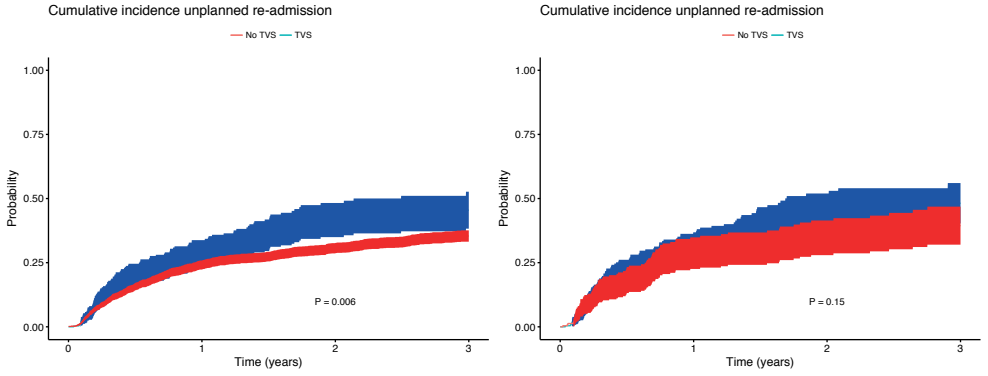

Figure 4. Cumulative incidence estimated by Fine and Gray model with death as competing risk of right heart failure in the unmachted and matched cohort

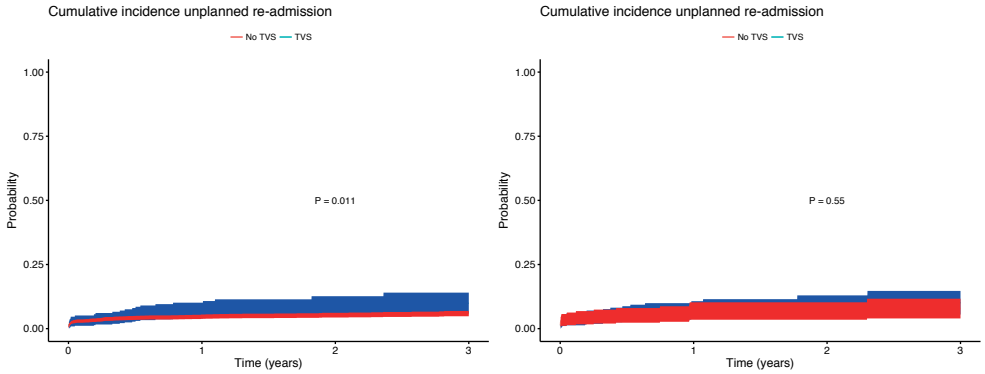

Figure 5. Course of the probability of moderate-to-severe tricuspid regurgitation over time in the unmatched and matched cohort estimated by the mixed model.

DISCUSSION

In this study we evaluated outcomes of concomitant TVS during LVAD implantation in the largest European LVAD registry. In a matched cohort comparable risks and rates of mortality, days on inotropic support, cumulative incidence of unexpected readmission and right heart failure were noted. Not surprisingly, cardiopulmonary bypass time was longer in the TVS cohort. Furthermore, patients that underwent concomitant TVS stayed longer in the ICU compared to patients that did not underwent TVS. Immediately after surgery the probability of moderate-to-severe TVS is significantly lower in the TVS cohort, however, this difference disappeared to during follow-up.

Patients undergoing TVS are significantly different compared to patients without concomitant TVS. Patients undergoing TVS presented as less acute patients with a longer history of cardiac diagnosis and less ischemic etiology (among others), which is also illustrated by different densities in propensity scores (Figure 1). Hence, patients undergoing TVS seem to be a select subgroup in the overall LVAD population. It has to be noted that conclusions regarding treatment effect in this study only apply this subgroup and may not apply in other subgroups within the LVAD population.

Prior analyses of the society of Thoracic surgeons (STS) database and the INTERMACS database noted comparable results compared to this study (13, 14). Patients receiving TVS that were recorded in the STS database stayed longer in the ICU. RVAD implant and hospital mortality was comparable in this cohort (13).

The investigators of the INTERMACS database noted comparable late survival in patients with preoperative moderate-to-severe TR with and without concomitant TVS (14). Moreover, a recent systematic review, pooling mostly small retrospective studies,

found no differences early and late survival (5). Interestingly, both in retrospective studies and INTERMACS database it was noted that pre-LVAD moderate-to-severe TR was associated with a poorer late survival (3, 14, 15). Regarding the latter observation, it seems peculiar that eliminating TR does not result in better outcome. Two hypotheses may explain these paradoxical results. Firstly, TVS may not sustainably reduce post-LVAD TR. Song et al. found a relatively high rate of recurrent TR in patients that received concomitant TR. Additionally, there are reports that observe that LVAD support exacerbates TR due to a leftwards shift of the interventricular septum and increased venous return (16, 17). Nevertheless, our results support that TVS does reduce TR from early on postoperatively, but that in patients with patients without concomitant TVS TR does also decrease in the following months. Secondly, it may be possible that TR is not a causing factor of mortality in most cases. It is known that TR is frequently caused by right ventricular dilatation in response of elevated pulmonary pressures (18). Therefore, TR may merely be a symptom, or a marker of, RV damage secondary to longstanding pulmonary hypertension or primary damaged RV due the underlying ischemic or cardiomyopathic diseases. By treating TR one may be treating the symptom rather than the causing factor of mortality and morbidity (e.g. right ventricle dysfunction). To some extent our findings do support this theory, since favorable right ventricular remodeling is observed in patients with a LVAD implantation without concomitant TVS (19, 20). This would inherently be paired with a reduction of TR, even without an intervention, assuming the TR is functional in nature.

In this respect, the cause of TR (primary or secondary) is important. Primary TR, caused by structural valve damage or interfering pacemaker/ICD leads will certainly not reduce by itself and may even cause right ventricular dysfunction (21). Therefore, we propose this aspect be taken into account in the decision process whether to perform concomitant TVS. Roberston et al. suggested that the decision to perform concomitant TVS should not be solely based on pre-LVAD TR grade (13). Our data and current literature support this suggestion, as our results and multiple other studies were unable to show any benefit from concomitant TVS with current guidelines suggesting TVS in all patients with pre-LVAD moderate-to-severe TR may not be necessary. Nevertheless, following the trends in concomitant TVS for functional TR during left sided valve surgery it has become clear that TR in some cases does not reduce, or even worsens (22, 23). The remaining challenge is now to adequately identify these patients in the LVAD population.

Strengths and Limitations

The strength of this study is the relatively large sample size compared to current literature. Additionally, the EUROMACS registry records serial echocardiograms which made it possible to analyze the change of TR over time. In contrast to previous studies, we accounted for the within-patient correlations in our analyses of postoperative course of TR over time using advanced statistical modelling. This study has several important limitations. First of all, this database is not designed to specifically address concomitant TVS in patients with LVAD implantation. Therefore, important factors, such as the

cause of TR or reasons for intervention are not collected. This may introduce selection bias, as these could not be captured in the propensity model. Furthermore, surgeon and institutional preferences can introduce selection bias. Although the majority of variables of interest had below 30% missing, we accepted up to 55% missingness. On the other side, the EUROMACS database collects many variables, making it more plausible missing data could be predicted on other observed variables and, therefore, strengthening the missing at random assumption. Additionally, since last year the EUROMACS investigators intensified their quality control measures to reduce missingness in the future (24). Furthermore, assessing TR remains challenging and TR is subject to loading conditions, which means TR severity is highly dynamic (25). Unfortunately, it was impossible to analyze patients receiving a tricuspid valve replacement compared to a tricuspid valve repair due to small numbers. Due to multiple testing some differences could be due to chance. Propensity score matching reduces the sample size, and therefore may reduce power of tests. Nevertheless, we utilized a matching technique because the main interest of this study was the effect if treatment in a typical treated patient. Some patients in the matched population had no recorded follow-up. Notwithstanding, sensitivity analyses did not change the direction of conclusions in most hypothetic missing scenarios.

CONCLUSIONS

Patients undergoing concomitant TVS differ significantly from patients without TVS. In matched patients concomitant TVS during LVAD implant does not seem be associated with better clinical outcomes. Concomitant TVS reduced TR significantly early after LVAD implant, however, differences in probability of TR disappear during follow-up. Using current selection criteria, TVS does not seem beneficial

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CHAPTER 9

Economic landscapes of human tissues and cells for clinical application in the EU. Horizontal Aspects of economic factors in tissue and cell banking

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HORIZONTAL ASPECTS OF ECONOMIC FACTORS IN TISSUE AND CELL BANKING

Tissue establishments, regardless of whether they focus on replacement tissues, assisted reproductive tissues and cells, or hematopoietic stem cells, share some common, horizontal economic aspects.

It is widely accepted and encouraged in the EU Member States that donation of human tissues and cells for transplantation is unpaid. According to the EU Directive on quality and safety of human tissues and cells (2004/23/EC), the philosophy of voluntary unpaid donation (VUD) should be a guiding principle, and Member States are urged to take steps to encourage a strong public and non-profit sector involvement in the provision of tissue and cell application services (2004/23/EC:18).

However, it is important to note that economic factors do have an impact on the broad range of tissue banking activities. Every activity undertaken comes with a cost, regardless whether they are undertaken within a public or private setting. As such these factors have an impact on the availability to patients of safe tissue and cell therapies.

This section focuses on costs and incomes for tissues and cells produced and delivered by tissue establishments.

If tissues or cells are needed for a patient in a hospital or clinic, either that organisation or the appropriate health (insurance) institution[1] is charged by a tissue establishment.[2] Although tissue was donated altruistically and free of charge, costs of labour and services necessary to transform the donor material into a usable and safe transplant add up, and need to be covered. For tissues aimed at autologous treatments after processing or storage in the tissue establishment, costs are equally applicable.

Throughout the European Union, the process of establishing the fees necessary to cover the costs incurred in tissue and cell banks is regulated and influenced in many ways. This chapter provides an indication of which costs are generated in the different steps of the process from donation to transplantation.

INVESTMENT AND FUNDING OF TISSUE ESTABLISHMENTS

Investment

The demand for tissue or cellular allografts can be intermittent (incidental) or structural. For example, if a hospital or clinic uses donor tissue only a few times per year, it may not be feasible to invest in the construction of a tissue bank. If the demand is structural (e.g. daily use in orthopaedics or ophthalmology), setting up a tissue or cell bank can guarantee a continuous flow of tissue, of which the specifications should correspond with the demand from one or more hospitals. Many tissue and cell banks have been set

up to cover a specific local demand. The bone/musculoskeletal sector is a good example of this. In the EU, there are hundreds of local tissue establishments providing femoral heads, procured in the OR, for the benefit of recipients in the same hospital. However, in parallel larger tissue establishments supply bone (and other multiple tissues) on a regional or national level or even across borders.

Initiating a tissue establishment compliant with the requirements of EU Directives (Directive 2003/94/EC), particularly with relation to the processing facility requirements, requires investment. Not only for the construction of a cleanroom and adjacent service rooms, but also for special instruments, education and training of personnel, quality system and certification, as well as for putting in place the appropriate ICT solutions. The table below shows the following range of costs per m^2 of a GMP laboratory.

Table 1. Classification of cleanroom levels

	Maximum permitted number of particles/m^3 equal to or above				Recommended limits for microbial contamination			
Grade	**At rest****		**In operation**		**Air sample cfu/m^3**	**Settle plates cfu/4 hours**	**Contact plates cfu/plate**	**Glove print 5bfingers cfu/glove**
	0.5µm	5µm	0.5µm	5µm				
A	3,500	0	3,500	0	<1	<1	<1	<1
B*	3,500	0	350,000	2,000	10	5	5	5
C*	350,000	2,000	3,500,000	20,000	100	50	25	-
D*	3,500,000	20,000	not defined	not defined	200	100	50	-

Source: Camfil Farr (via Advantage Business Media, Controlled Environments, 2013)
*(a) In order to reach the B, C and D air grades, the number of air changes (i.e. a measure of how many times the air within a defined space per hour is replaced) should be related to the size of the room and the equipment and personnel present in the room. The air system should be provided with appropriate filters such as HEPA for grades A, B and C.
**(b) The guidance given for the maximum permitted number of particles in the "at rest" condition corresponds approximately to the US Federal Standard 209E and the ISO classifications as follows: grades A and B correspond with class 100, M 3.5, ISO 5; grade C with class 10 000, M 5.5, ISO 7 and grade D with class 100 000, M 6.5, ISO 8.

Indeed, one of the main drivers for initial investment relates to the level of environmental air quality and related cleanroom conditions required to process tissues and cells. Depending on the tissue grafts that will be processed, different levels of airborne particulate classification can be defined, based on the EU guide to GMP, which is also used by the Directive 2006/86/EC to define the requirements of the environment during processing of tissues and cells in a tissue establishment.[3] These so called cleanrooms are necessary to prevent microbial contamination of the tissues during processing. The requirements for a certain level also depend on the processing steps (debridement, cutting, sizing) after the work in the clean room has been completed. To achieve this, a

certain level of absence of particles in the air must be maintained (see table below). For a Class A clean room, a level of maximal 3 500 particles of a size of 0.5 µm and 0 (zero) particles of 5 µm is permitted (Commission Regulation (EC) No 1234/2008).[4] A Class A environment requires continuous monitoring of particle counts (FDA 2004; 91/356/EEC Annex 1 Revised 2008). Under Class D these values are 3 500 000 and 20 000 per m3 respectively.

Although legislation, guidelines and standards exist, in general best practice translates into aseptic tissue processing within environmentally controlled facilities, as it is up to each establishment to determine and establish the qualification of their facility in relation to cleaning and sanitation (AATB, 12th Edition; Nair et al, 2012). As such, musculoskeletal and cardiovascular tissues may be processed in Grade A clean rooms (A laminar Flow Cabinet against in a grade B environment), but a lower grade (A with C or D background) is also still common practice. Skin and corneal grafts, depending on the final graft that is to be produced, may be processed in a Grade A Laminar Flow Cabinet, with an environment of Grade C or D. HPC processing usually takes places in Grade C or D environment in a Grade A Laminar Flow Cabinet. If the grafts are not processed in a Grade A clean room, and the tissue allows it, terminal sterilisation is applied or required by local authorities. The investment for a clean room facility can be considerable as shown in the table below.

Table 2. Investment cost of GMP cleanrooms

	€ price m² Minimum	€ price m² Maximum
Class A	12,331	14,061
Class B	9,730	11,178
Class C	7,452	8,746

Source: Advantage Business Media 2013

As indicated, since facilities with Grade A clean rooms must be entered from Grade B, and assuming that the minimum Grade A surface area is 16m², and the Grade B room is 8m², the minimum investment in this facility (without service and office space) would be about €275 000. Assuming a ten-year depreciation period, the annual costs would be €27 500. If the tissue establishment in this example were to distribute 1,000 tissue grafts per year, a cost of €27.50 per graft could be included (in addition to other costs, see below) in the calculation of the final fee to the end user, in order to recover this investment. It might be assumed therefore, that it is in the interest of tissue establishments with high investment costs to process as many grafts as possible to achieve an economy of scale in which these costs per tissue are as low as possible.

In a typical processing unit, multiple environmental Grades are required. For instance, a clean room complex can consist of 20 m² Grade A, 100 m² Grade B, 20 m² Grade C

and 800 m² Grade D. A typical investment in a cleanroom facility therefore costs easily over €1 million.

Maintaining and running clean rooms also incur high operational costs, including:
- Electricity to provide clean air and temperature control
- Regular change of hepa-filters
- Sterile personnel suits
- Microbiological control and environmental monitoring
- Sterile materials and agents
- Cleaning after each donor

Additionally, before the tissue establishment can start using its clean rooms for processing tissues, there will be a non-productive, preparatory, testing and validation period required for the qualification of the facility. The cost of this non-productive period and the investment in construction of the tissue establishment during a phase without income should be taken into account.

Funding

There are multiple options for financing a new, state of the art tissue establishment:
- Donation such as a subsidy by a government, a charity or the general public
- Grant or financial injection by the hospital or the university, primarily to create a solution for the demand for tissues or cells in that specific clinic/hospital
- Bank loans (from financial institutions)
- Private party investment
- A combination of two or more of the above.

The source of investment may very well influence the fees that tissue establishments ask for their services, either within or outside the location where they are situated.

In the case of subsidies, grants or injections by public actors, charities or governments, there is usually no necessity for a return on investment. So it may very well be that the investment and operational costs are not reflected in the fee or price. When banks or private investors have contributed, standalone or in a combination with other modalities, there is a necessity for a return on investments through interests, and/or a dividend to the investor(s) and eventually return of the investment itself.

The modality of investment also influences the legal entity under which the tissue establishment is operating. The nature of the legal entity may influence the fees tissue establishments are charging. Tissue establishments are set-up in different ways. As a result, the following broad funding models can be observed in tissue establishments in the EU.

1. Public sector: centrally allocated operating budget; tissues and cells provided to the user without charge

This was the typical model for a hospital based tissue bank, supplying only to users in its own hospital, and fully hosted and financially supported by the hospital. Although common in the past, this model is now much less frequently seen. It does however still exist in a number of Member States for some tissue and cell types. It avoids any kind of competitive market, any disincentive for hospitals to use tissues and cells, and any pressure on tissue establishments to distribute a particular volume of tissues or cells for clinical use. However, it is often challenging for centrally-funded tissue establishments to obtain the funding needed for investment in new or improved facilities or for preparation process developments (depending on who is the centrally funding entity, e.g., a hospital or government). In this model, many essential internal steps are carried out by public sector players also without charging, e.g. donor testing or microbiological testing of tissues or cells, and total service costs are often not well defined, which can be performed by other horizontal hospital services. Salaries and benefits of employees are tightly controlled as in all public sector health service units.

2. Public Sector: cost recovery from user hospitals, clinics, patients or health insurance schemes

This health service 'internal market' model is increasingly common in the field of tissue and cell services, as is it is in the entire public health sector. It requires a full costing activity that includes identification and quantification of fixed and variable costs, including capital depreciation and the costs of all third party contracts. The fee charged for each unit of tissues or cells distributed should equal the total unit cost so that income from tissues or cells supplied provides adequate funds for a sustainable service. The fee charged might include a small percentage for service development and for contingency planning in the event of emergency. Salaries and benefits of employees are also controlled in this model, as in all public sector health service units.

In this model, there is a 'break-even' point when the tissue establishment is supplying a sufficient number of finished tissues or cells to cover all costs; a so-called critical mass. The need to achieve a 'break-even' point could be seen as putting pressure on the tissue establishment to compete with other (private or public) tissue establishments for orders; their service might not be economically sustainable otherwise and hospitals might become dependent on commercial providers. However, in this model, there is usually more opportunity for leading tissue establishments to carry out thorough cost- assessments and to obtain adequate funds for service developments and facility improvements. Commonly, this model exists in the context of tissue establishments that are established within other public sector health organisations such as blood transfusion services. In some cases, tissue establishments operating in this funding model might obtain an injection of 'start-up' funding from the central health service or cross-subsidisation from the parent organisation for an initial period until well established.

3. Independent: non-profit; cost recovery from user hospitals, clinics, patients or health insurance schemes

The establishment of independent foundations or other types of non-profit organisations has been observed in this sector over a number of years. This model is characterised by cost-recovery based on a full-cost analysis as in the previous model and the same pressure exists to achieve a threshold level of supply activity to ensure adequate income to cover all costs. The independence of these organisations, however, means that there is less (or no) health service financial governance and aspects such as employee salaries, and benefits and the margins included for developments or other contingencies, can be much higher. The greater flexibility provided in this model often allows a tissue establishment to achieve a high level of efficiency and rapid development but the model raises concerns regarding the sustainability of services if the owners choose to move to another activity. Many independent non-profit organisations achieve success through supplementing their income by achieving charity status and organising fund-raising programmes; this applies notably to a number of independent bone marrow donor registries.

4. Independent: for profit companies

This purely commercial model involves accurate costing of activities but no requirement to ensure that fees or prices equal costs. In the EU, this model exists in the field of assisted reproduction (private ART establishments), private cord blood banks for family use and some replacement tissues, particularly bone. In the case of commercial bone banking and supply, most commercial actors are subsidiaries of US companies.

Traditionally, tissue banking in the EU has been organised under one of the models described above. Some commercial players are quoted on the stock exchange, bringing the added feature that their strategy for tissue and cell processing and supply can be determined by shareholders, not in any way involved in the field. The levels or sources of the investments were not studied for this report.

Operational costs

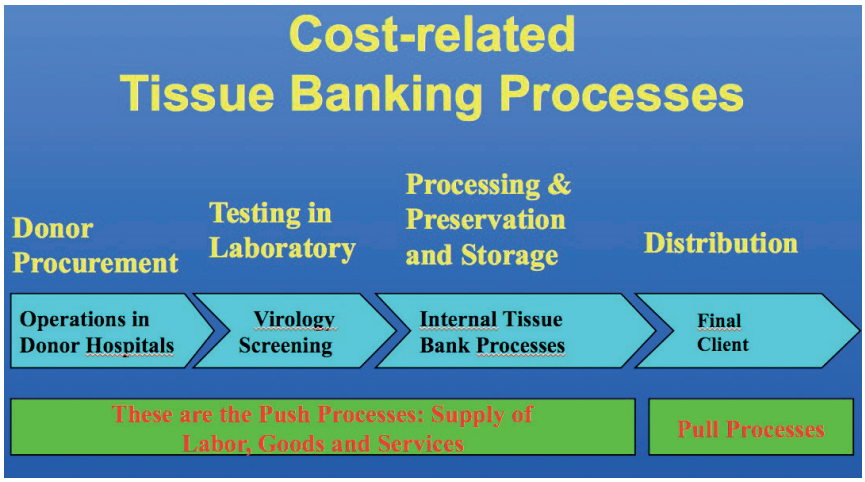
In order to understand how tissue establishments have calculated their costs, four main activities (processes) are recognised in any tissue establishment. In terms of micro-economics, these may be indicated as cost-centres. This division in four major activities doesn't differ from similar structures in any productive organisation:

- Donor recruitment and selection; tissue procurement or cell collection (acquisition of raw materials)
- Testing and quality control (quality management)

- Processing and storage of tissues or cells (production into end products)
- Distribution (customer relations and physical distribution to end users - hospitals, health professionals or recipients directly).

Overhead costs such as management costs, public relations, software, etc. are allocated over the four main activities by means of 'activity-based costing'. This means that these costs are divided according to a factor representing the level of activity (work) taking place in these four activities.

Figure 1. Core processes and cost in tissue establishments



Source: Theo de By (2013)

The costs, generated in these four main processes, add up to the total costs of the tissue establishment. The tissue establishment management needs to recover these costs from the revenue for the number of finalised tissue/cell grafts that are distributed during one year, in order to financially break-even. Also, the investment costs might also need to be recovered from these revenues, over a period of years of depreciation, depending on the funding model.

It is therefore important that tissue establishments have a good understanding of their cost-structure. Cardiovascular and ocular tissue establishments were asked in research for this report whether they have cost calculation information, specified for the four main processes. Replies are summarised in the table below.

Table 3. Number of tissue establishments with cost calculation

	Cost calculation made	No cost calculation made	No answer	Total
Cornea Banks*	12	3		15
Cardiovascular Banks**	6	5	1	12
Total	18	8	1	27

Sources: * Survey to ocular tissue establishments (2015), data from 2012 ** Survey to cardiovascular tissue establishments (2014), data from 2012

Respondents who did not calculate the costs, were mostly tissue establishments where the price is determined by either the government or by another authority. Only three cornea banks, and no cardiovascular tissue establishments, specified the costs in line with the core processes.

Donor recruitment and selection, and tissue procurement costs

Direct and indirect costs occur when a tissue establishment recruits donors and donations. The direct costs are the costs of the procurement that can vary significantly in complexity. Obtaining sperm cells from a living donor is relatively simple, while obtaining heart valves from a deceased donor requires invasive surgery. Appropriately-trained staff, sterile instruments and materials for tissue retrieval, and transportation of tissues and/or retrieval teams are the main direct costs.

Frequently, tissue has to be discarded after procurement due to quality and safety reasons such as medical contraindications to transplantation, microbiological contamination, and tissue quality. For example, in cornea banking the percentage of discarded tissues is approximately 40% on donor related, serological, microbiological or morphological grounds (EEBA Annual Directory, 22nd edition). In cardiovascular tissue banking, many valves are rejected because of calcification, fenestrations or other anatomical abnormalities.

Thorough donor selection based on the donor history, professional procurement techniques, as well as donor testing (see Chapter 2) is therefore essential. Obviously these are easier with living donors. A set of well thought-out questions, with cross references and an assessment by qualified and trained healthcare professionals is therefore of upmost importance in order to pre-select donors well and minimize later discard rates.

The donor retrievals that result in discarding of tissue grafts are still a direct cost burden that contributes to the overall running cost of a tissue establishment.

The indirect costs include maintenance of an infrastructure for recruitment and initial selection of donors. In the case of regional post-mortem donation, the infrastructure includes such necessities as: maintenance of a stock of materials, trained staff and a 24/7 duty desk.

Tissue establishments providing bone marrow, Peripheral Blood Stem Cells (PBSC) or sperm present a specific situation of donor recruitment and banking. These activities are based on an inventory of potential donors. Having a register of potential donors is an essential asset for providing an adequate number of donations for medical purposes. The effort that goes into recruiting donors, but also in retaining and keeping track of them, is a substantial part of the costs in these establishments. For stem cell registries, it also means that because of the unique HLA typing, an enormous number of potential donors is needed to provide for a limited number of effective donations and eventual grafts (0.01-0.1% of the registered donors actually donate per year) (WMDA report, 2012).

Costs of testing for safety

To minimise the risk of serious adverse reactions (SARE) due to the infection of the recipient with contaminated material from the donor, various tests are carried out in all tissue establishments. EU legislation lays down a set of minimum testing requirements (Directive 2006/17/EC Annex II), while in several countries, there are additional tests designed to address the local epidemiological situation.

The composition of the test regimen may therefore be different from country to country and from bank to bank, however a minimum set of tests to be performed is mandatory. In addition to blood tests for markers of transmissible diseases, tissue establishments sometimes include blood cultures or the determination of absence of malignancies by a pathologist. Emerging infectious diseases or changes in the prevalence or incidence of existing viruses require that new tests be added to the existing regimen.

Testing techniques are continually developing. Contaminant micro-organisms that could not previously be detected may be detectable in the future; e.g. nucleic acid technology (NAT) tests, which enable tissue establishments to determine the presence of specific donor-related viruses, and/or to confirm the negative outcome of other tests. The existing regimen and the necessity to add additional tests can influence the final fee for the tissues.

Costs of processing and storage

Processing is the activity in which the donated tissue is prepared as a graft for storage and subsequent transplantation. Processing can be more or less intensive, depending on the kind of tissue as well as on the chosen decontamination method. For example, the

preparation of corneas involves cleaning and disinfection of the whole eye, dissection of the cornea, placement in storage medium (with or without subsequent microbiological testing) and an assessment of tissue quality. On the other hand, the processing of bone may involve different steps, including terminal sterilisation by gamma-irradiation. Many tissue establishments perform microbiological testing during processing and and/or apply methods aimed at eliminating any micro-organism. These activities generate high costs. Processing requires trained personnel with appropriate expertise who process the tissue in cleanrooms.

Depending on the kind of tissue and the packaging and storage method, the storage time after processing can vary from a few days to many years. Short-term storage has the risk that tissue or cell grafts expire more quickly, while long-term storage has the risk that revenues may be obtained years after the costs have been incurred, possibly leading to financial liquidity problems.

The costs for equipment and organisation of storage also depend on how the tissue is stored; e.g. by room temperature on shelves (glycerol-preserved skin); in incubators (cornea); at -80°C (musculoskeletal tissue); or in liquid or vapour phase nitrogen (heart valves). Monitoring storage temperatures by continuous logging and alarm systems add to the cost of storage.

Costs of distribution

In the distribution process, which aims to provide finalised tissue/cell grafts to the end- user (surgeon, patient or hospital) of the tissue establishment, there are direct and indirect costs. The direct costs include packaging and transportation. The indirect costs consist of the administration of logistics in relation to final destination, recipient data and costs to be charged.

Distribution costs vary significantly. There are tissue establishments which process tissue/cells mainly for local use, for example, tissue establishments related to a hospital. At the other end of the scale are tissue establishments that ship tissues/cells to clinics/healthcare professionals across borders, as is often the case in the HPC sector.

Allocation of financial surpluses

In order to guarantee long-term continuity, all organisations, both for-profit and non-profit, must at least generate sufficient income to cover their costs. Financial surpluses occur when there is more income than costs.

Whereas for-profit organisations focus at maximising profits and will use (part of) these surpluses to compensate shareholders or other providers of risk capital, organisations that are not aimed at making profit might allocate any financial surplus as 'reservations' to the financial balance, or might transfer them to the centrally funding entity. Where reservations are made, these can be earmarked to compensate future losses, or investments, for example. As in tissue banking, donations fluctuate from year to

year, and therefore income fluctuates accordingly, such reservations are important to guarantee the continuity of the services provided.

MANAGING VOLUMES AND THE RELATED COST FOR TISSUE ESTABLISHMENTS

The main purpose of banking human materials for transplantation is to satisfy the clinical demand for tissues and cells. In many cases, a tissue/cell transplant is the best or only therapeutic option for patients. Tissue establishments in general have a complex serious of tasks: to correctly estimate the demand for tissue transplants; plan the number of donations needed accordingly; purchase procurement and processing materials; and finally, produce transplantable allografts.

Tissue establishments have an additional challenge: they depend on the willingness of the general public to donate tissues and cells before or after death. The return on investment of donor recruitment is uncertain. Donor shortage is frequently reported (WHO, 2014); NTS, 2014), and can be absolute or relative (insufficient donors of a specific type of tissue). In a situation of absolute donor shortage, the tissue establishment cannot satisfy the medical demand, nor can it cover its projected costs. The nationally defined donor consent system plays a role in this, and is often related to organ donation, although consent systems fall outside the scope of this report.

A different scenario is when tissue establishments are very successful in recruiting donors. In such situations the stock of grafts, for which no recipients can be found (yet), becomes a financial burden. For the management of the tissue or cell bank, it might be difficult to explain why, after many years of publicity about the need to donate, donations from the public are refused because there is a surplus.

In situations where tissue establishments distribute relatively few tissues, it may be that the total financial proceeds received by distributing tissues to the end-users (hospitals or surgeons) is not enough to compensate exploitation and investment costs. In such cases, without other financial resources, the tissue establishment may have insufficient 'critical mass' for innovation and investments for improvements and ultimately, the continuity and sustainability of the tissue establishment may become uncertain (as was demonstrated in the case of *Bayerische Gewebebank* and *Bislife* in the Netherlands). The 'critical mass' is difficult to estimate however, since tissue establishments are organised in different ways, as described above.

In conclusion, the supply and demand in banking of human materials is a management task in which medical, financial and additional social aspects must be constantly balanced in order to strive for the continuity of the organisation and its acceptance in society.

VALUE AND FEES CHARGED FOR TISSUES AND CELLS

There is a great variety in the way prices or fees of tissues are calculated. Moreover, in many instances the fee charged to the end-user is determined on grounds other than the costs generated in the tissue establishment. Referring to the 'fee' for tissues rather than to the 'price' is therefore more appropriate.

EU Directive 2004/23/EC states: "As a matter of principle, tissue and cell application programmes should be founded on the philosophy of voluntary and unpaid donation, anonymity of both donor and recipient, altruism of the donor and solidarity between donor and recipient. Member States are urged to take steps to encourage a strong public and non-profit sector involvement in the provision of tissue and cell application services and the related research and development".

Since the tissue of donors is donated for altruistic reasons, and free of charge, some state that the tissue has a socio-medical value before it gets a financial value. Some speak of the production of bio-value in this respect (Waldby, 2002). The value of altruism is the result of people being concerned for the welfare of others (Rodriguez and Leon, 2002). The socio-medical value cannot be expressed in monetary terms, since it is literally the value of life. If only the economic value of donated tissue or cells is appraised, serious concerns can be raised that, through the creation of a market for body parts, there is the potential to devalue human life. It would imply that an individual's worth is based on the material value of their body rather than that of a human being (Boo et al, 2011).

Donation of tissue and cells is estimated to be cost-effective and potentially cost saving, since the final resulting therapies are often live-saving and/or enabling patients to return to work and therefore reduce the reliance on social or medical support (Committee on Increasing Rates of Organ Donation Board on Health Sciences Policy, 2006). In other areas of healthcare, for example, pharmaceuticals, this value is often estimated through dedicated bodies working on health technology assessments (HTA). Such value-based pricing-mechanisms do not explain how the prices or fees for tissues and cells are determined, however. Tissue and cell therapies are relatively low priced on a cost-basis, or sometimes even below costs in a public funding model. It is said that fees just 'cover the costs', but this does not entirely explain the differences between the fees charged by tissue establishments. Fees partly depend on the cost of procurement, and take into account the additional tests required to safeguard the potential recipient (Epstein, 2009). These fees may differ per laboratory (Hoeyer, 2013).

In order to protect the system of altruistic donation and the socio-medical value of the cells and tissues that is based on principles of subsidiarity and solidarity, many countries have installed (semi, or quasi) governmental organisations to regulate tissue and cell banking. The tasks of these organisations are among others, to prevent financial over-compensation or the commercialisation of tissue and cell grafts, and to safeguard the

interest of the general public from mishandling donations of human tissue and cell grafts (WHO Guiding Principles on human cell, tissue and organ transplantation).

The work of the tissue establishments in executing the four major processes as explained earlier in this chapter results in an economic value of the grafts.

CROSS-BORDER EXCHANGE, IMPORT AND EXPORT

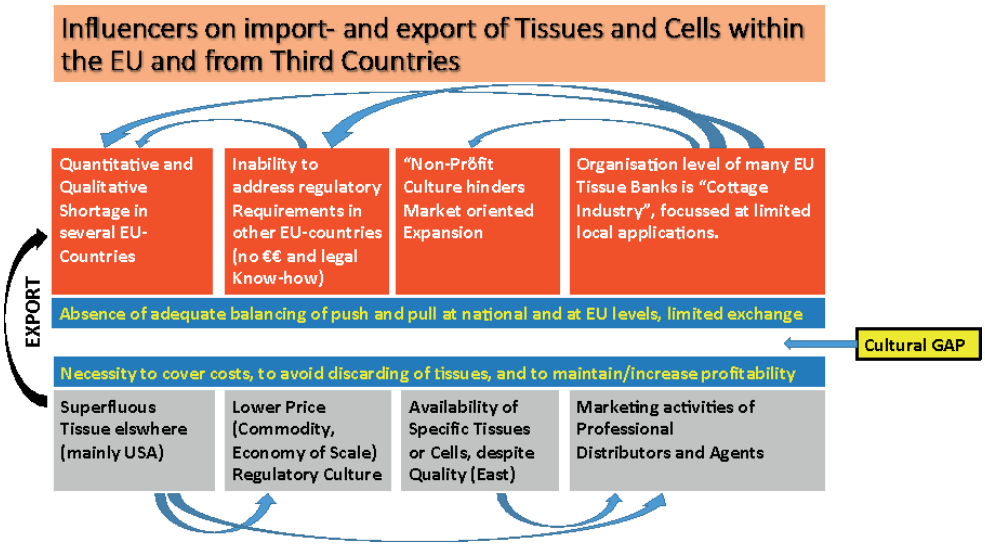
A market is a physical or a virtual place where the demand and supply of goods or services meet to trade. In free markets, the economic reality is that given a high demand and a low supply of a certain product, the price of that product will undergo an upward pressure. However the tissue and cell "market" is not fully subject to these dynamics given the fact that these are not typical products and authorities often determine their price or the fee charged by the tissue establishment. Contrary to free markets for other goods, the flow of tissues and cells is controlled by authorities. In some countries, policies are in place to prevent tissues being the subject of free market forces in order to ensure that no profit is made from the donated material itself. Only in a few instances is a higher fee charged to international (including non-EU) recipients.

The cross-border distribution (within the EU), as well as import and export of tissues from/to third countries, are influenced by a number of factors (see also figure 2 below). One factor could be the introduction by some Member States of more stringent quality and safety requirements (than those laid down in the EU Directives), which may lead to the fact that a tissue establishment in one Member State does not necessarily meet the more stringent requirements in another Member State. An important obstacle for tissue establishments in freely distributing tissue grafts throughout the EU, relates to the different national implementations of EU legislation, and consequent differences in administrative and regulatory procedures which have to be overcome to get access to another Member State. EU legislation lays down only minimum requirements, and many Member States add a significant number of requirements (and procedures). An overview of these additional requirements is not available for the actors in the field (unless costly consultants can be paid for). It is therefore very difficult for a single tissue establishment in one Member State, in particular with limited resources, to address demands in other EU Member States. From this point of view, it may be considered that the European 'market' for tissues and cells is restricted.

Imports from third countries, specifically from the USA, seem to be needed to cover the needs of European patients. However, since most EU Member States do not have a sufficiency policy and/or do not collect data on cross-border distribution (within the EU) and import/export of tissues and cells from/to third countries, it is impossible to understand if these imports are absolutely needed or could be covered from other sources (e.g. EU tissue establishments). In some countries the import takes place on a structural basis.

Compared to EU, US developed a completely different model. Donor recruitment is well organised and organ and tissue procurement organisations in USA are rewarded for tissue donor referrals, which results in a sufficient quantity of tissue. For US tissue establishments, Europe is an attractive market where they can distribute their surpluses and generate additional income. In particular musculoskeletal tissues and cornea are imported from the US.

Figure 2. Cross-border distribution, import and export



Source: Theo de By (2013)

Alliances between US tissue establishments and European partners have been created to ensure the continuity of the flow of tissues to Europe. These alliances also allow getting the necessary know-how to address (national) administrative procedures in EU Member States. As these US tissue establishments are usually larger, they also have resources to acquire insights into regulations and administration of different Member States, and hence facilitate access. Some US tissue establishments have assigned managers to facilitate distribution (in the US the term 'sales' is used) of their tissues in Europe. A few Member States report these imports to be important in order to address local shortages.

Some EU countries have organised their tissue banking structure in such a way that self- sufficiency is enabled. In some countries (IT, UK, NL) a mandatory allocation system is in place. This guarantees the supply to cover the national demand. In other countries, such as Germany (DE), Spain (ES) and France (FR), voluntary networks strive to achieve optimal allocation of available grafts.

The situation of being able to satisfy the national need for tissue grafts, which is the result of this structure, prevents dependency on other countries. As we have seen over time, this dependency could interrupt the availability of tissue grafts for patients, e.g. in situations where import from areas with a high incidence of viral contamination is prohibited. Such was the case when an epidemic of SARS (Severe Acute Respiratory Syndrome) broke out in April 2003. The result of the epidemic was that tissue establishments outside the geographical area of contamination could not accept tissues from tissue establishments in that area. Moreover, donors from Europe, who had travelled in those areas, could (if they died after return) not be accepted as tissue donors in Europe. Safety criteria are also the reason why, for many years, the donor-acceptance criteria of tissue establishments forbid import of tissue originating from donors in the UK and Ireland (IE). The reason for this was the risk for variant Creutzfeldt-Jakob disease transmission. For example, IE does not procure from IE donors, nor does it accept UK donors. Therefore IE is strongly reliant on US import.

The factors mentioned above have a significant impact on the underlying discussion whether the EU should strive for self-sufficiency, and whether the exports to countries outside the EU could have (partly) compensated for the imports from the US.

IMPACT OF TAX AND VAT REGULATIONS

Increasingly, non-profit organisations active in the field of donation, banking and distribution of human tissue for transplantation, are confronted with contradictory policies between healthcare authorities and finance authorities.

In the EU, in line with the EU and national legislation and WHO and Council of Europe recommendations, health care authorities consider tissue banking as an activity which should take place in non-profit organisations. In some countries this principle is strictly enforced via different instruments, such as centrally made price decisions (e.g. by the Ministry of Health or other governmental bodies), and control measures (e.g. inspections/audits and border-control, etc.). Moreover, the non-profit character of tissue banking is comparable to that of other health services such as the provision of blood. If not exempted, then a zero VAT tariff would be compatible with the non-profit approach.

In EU treaties, and subsequent Directives with respect to Value Added Tax (VAT), human organs, blood and breast milk are exempted from being charged with VAT. Even though human tissues and cells are not mentioned, taking into account also the WHO guiding principles (where it is emphasised that the term 'organ' has to be interpreted widely and therefore include any type of human tissue), it is the authors' view that tissues and cells should also be exempted from VAT. Several Member States used this globally accepted understanding when they transposed the provisions of the VAT Directive 2006/112/EC into their national law and mentioned concretely tissues and cells for therapeutic

purpose as to be exempted from VAT to avoid any misinterpretation of the "exemptions for certain activities in the public interest" and to clarify the meaning of the written words "organs, blood and milk" (Article 132 (1)d of Council Directive 2006/112/EC of 28 November 2006 on the common system of value added tax).

Recently, finance authorities in some national regions and EU countries have considered that human tissues should be subject to VAT. VAT charging Member States include DE, IT, ES and PT, while for example NL has a 0% VAT. Charging tax on human tissue may conflict with the principles in the EU Directive and also seems contradictory to the interpretation by these same finance authorities in the past. Besides decisions of tax authorities to charge VAT, it has been reported by some non-profit organisations that tax authorities charge them for profits and first national finance courts confirmed such decisions with the argument, that fair competition to commercial companies that could offer comparable activities would be harmed.

In parallel, local and national health authorities request from tissue establishments to continue to strive for and to stay close to state-of the-art methods (e.g. for screening and processing methods with the aim of further developed safety for patients) and to do clinical studies and research to bring evidence of such activities. The financial burden of such requests from health authorities can only be financed by making surpluses in advance or finding other resources. Taxes on these surpluses directly limit the cash ability of tissue establishments and their capacity to fulfil the above mentioned requests from health authorities.

In conclusion, according to declarations of tissue bankers as provided in the context of this study, the application of VAT seems to be against the principle of non-commercialisation of the human body as laid down in the EU Charter of Fundamental Rights. Currently, various interpretations of the VAT Directive 2006/112/EC within the EU create inequality between regions or countries, with tissue establishments required to charge VAT, while others do not. This inequality may disrupt the level playing field, putting in difficulty those who have to charge taxes to the end-user. At the same time, end-users, usually non-profit hospitals, are confronted with higher costs. This may give the general public the impression that altruistically donated tissues are commercialised, which in turn could potentially harm future willingness to donate organs and tissues.

CONCLUDING REMARKS AND SUMMARY HORIZONTAL ASPECTS

- The set-up of a tissue establishment requires equipment, know-how and appropriate facilities. These call for significant investment, in particular to build clean-rooms offering the environmental conditions to safely process tissues and cells, as required by EU-law. Building a high-grade clean-room (grade A in B) at least costs €250,000, and more regularly require amounts of over €1 million.

- The consequent operational costs of tissue establishments are defined along four major activities needed to transform a tissue from a donor into a therapy for a recipient:

- Donor procurement: to identify a donor, obtaining consent, verification of suitability to donate and obtain the tissues or cells.

- Testing: to identify and avoid the risk of transmitting diseases

- Processing and storage: to transform the procured tissue/cell into a substance ready to be applied as treatment

- Distribution: to ship the final graft towards the clinician or hospital where the tissue/cell will be applied on a patient.

- The relative importance of each of these activities varies between the 3 sectors and along tissue type. E.g., for bone marrow transplants (HPC) processing and storage is relatively limited, but a reliable and fast distribution mechanism is of utmost importance.

- General overhead costs, such as management, or the need for a 24/7 presence, can add significantly to the budget. The high discard rates for quality reasons, like for heart valves, and the often short shelf-lives, like for corneas, add further challenges and significant costs. The stock of transplantable tissues should be high enough to cover direct demand, but low enough to prevent expiration, this is a challenge in itself.

- The surveys performed within this study indicate a limited cost-awareness on the real costs made by tissue establishments. The legal structure and funding model of the tissue establishment may play a role when it comes to this, in particular where tissue establishments are hosted in public hospitals, or where funding is based on charity.

- This limited cost-awareness is also reflected by the high variability in prices/fees charged for tissue and cells over the EU. These prices are often fixed at national level, and do not necessarily reflect costs, but rather reflect a national policy like ensuring self-sufficiency or avoiding commercialization of the human body. They do not really take account of the real cost or the volumes needed to cover all costs and come to a financial break-even.

- All these factors led to the conclusion that there is no real single EU market for tissues and cells, which is the reason why the authors prefer to title this study rather as an 'economic landscape'.

- Four funding models can be seen for tissue establishments in the EU. Tissue establishments can either have (1) a public model, with all costs carried by a public budget and the eventual tissue/cell provided without charge to the users. Alternatively, (2) public, (3) non-profit or (4) for-profit tissue establishments do recover their costs by charging a fee to the users. The (3) non-profit and (4) for- profit tissue establishments do also aim to obtain a financial surplus for investments, for building a reserve or for obtaining a profit in case of (4). Public tissue establishments can usually rely on public budgets or on charity to make investments.

REFERENCES

1 In research undertaken as part of this study, three cases were found where the tissue establishment provided grafts free of charge. These tissue establishments were located in a university hospital which compensates the total costs of these tissue establishments.

2 In the UK, hospitals currently pay no processing fee for corneas or sclerae from the Bristol and Manchester Eye Banks. There is a charge levied by NHSBT to cover transportation and a contribution towards the cost of the NHSBT Eye Retrieval Scheme, which provides funds for eye retrieval staff in 10 hospitals around the UK. The eye banks run as NHSBT-commissioned services with the salary and other running costs funded through an agreed budget with NHSBT. This is all changing and full cost recovery is due to be introduced by NHSBT starting April 2015.

3 Directive 2006/86/EC. Annex D. "3. Unless specified in point 4, where tis-sues or cells are exposed to the environment during processing, without a subsequent microbial inactivation process, an air quality with particle counts and microbial colony counts equivalent to those of Grade A as defined in the current European Guide to Good Manufacturing Practice (GMP), Annex 1 and Directive 2003/94/EC is required with a background environment appropriate for the processing of the tissue/cell concerned but at least equivalent to GMP Grade D in terms of particles and microbial counts.
A less stringent environment than specified in point 3 may be acceptable where: (a) a validated microbial inactivation or validated terminal sterilisa-tion process is applied; (b) or, where it is demonstrated that exposure in a Grade A environment has a detrimental effect on the required properties of the tissue or cell concerned; (c) or, where it is demonstrated that the mode and route of application of the tissue or cell to the recipient implies a signifi-cantly lower risk of transmitting bacterial or fungal infection to the recipient than with cell and tissue transplantation; (d) or, where it is not technically possible to carry out the required process in a Grade A environment (for ex-ample, due to requirements for specific equipment in the processing area that is not fully compatible with Grade A).

4 Commission Regulation (EC) No 1234/2008 of 24 November 2008 concern-ing the examination of variations to the terms of marketing authorisations for medicinal products for human use and veterinary medicinal products

CHAPTER 10

Economic landscapes of human tissues and cells for clinical application in the EU. Replacement Tissues

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REPLACEMENT TISSUES

This chapter starts with a description of the field of replacement tissues, followed by the organisational structures and collaborations; it provides current and future perspectives and conclusions on the most common replacement tissues: cardiovascular, ocular, musculoskeletal tissue and skin.

FIELD DESCRIPTION (INCLUDING ECONOMIC ASPECTS)

Tissue grafts that replace a recipient's existing tissue(s) in order to take over the functionality of damaged tissue are generally referred to as replacement tissues. In the large majority of cases, such tissue grafts are obtained from a donor who is different from the recipient, through an allogeneic donation. Although the donor and recipient can, in some cases, be the same person (autologous use), this chapter focuses on allogeneic donation.

Replacement tissues are mostly procured after death, but can also be donated by living donors (e.g. femoral heads removed during primary hip replacement). They consist of a wide variety of types. In this chapter the focus will be on the tissues that are used most commonly:

- cardiovascular tissue
- ocular tissue
- musculoskeletal tissue
- skin

Besides these, many other tissues can be transplanted, such as pancreatic islets, amnion, fascia and nerves, but the numbers donated and transplanted are much lower compared to the four categories listed above. Composite tissues such as hands or faces are transplanted on very rare occasions and are not addressed in this chapter. The procurement and transplantation of organs is beyond the scope of this study.

The substances, processes and therapies described below are largely established practice in the tissue sector. However, these substances, processes and therapies are subject to continuous innovations in processing or clinical application, some of which might lead to a classification as Advanced Therapy Medicinal Products (ATMPs). In these cases, the products are not only subject to the tissues and cell legislation for the donation, procurement and testing steps but also to the pharmaceutical legislation (ATMP Regulation) for all subsequent steps in the process. The legal classification and the different legal requirements fall outside the remit of this study, but for tissue establishments that are active in those fields, the economic implications are important.

This chapter describes the different types of replacement tissue grafts and tissue establishments for replacement tissues and mentions ATMPS where they may, in the future, have a significant impact on the demand, and therefore the economic landscape, for a particular tissue as a starting material for ATMP manufacture.

Cardiovascular tissues

Cardiovascular tissues include heart valves (pulmonary and aortic), conduits, arterial vessels (in different forms), veins and pericardium.

Clinical application

Surgical heart valve replacement is an effective treatment for patients with damaged heart valves. The first clinical reports on the transplantation of cardiovascular tissue date back to 1962. While artificial heart valves were still in development and industrial engineering failures emerged, the human tissue allograft turned out to be an alternative for those patients suffering from severe and irreparable regurgitation, congenital heart diseases or endocarditis. For replacement of the damaged valve, current options include a prosthetic (synthetic) valve or a biological valve (of human or animal origin). As innovation has led to better artificial heart valves with fewer disadvantages and the availability of human hearts has decreased, the surgical use of human donor heart valves has declined and is now important for two main indications: children and adolescents suffering from congenital heart defects and patients suffering from endocarditis (TRIP Annual Report 2012, Biovigilance). Transplantation of human heart valves requires specific surgical skills, which not all thoracic surgeons have acquired during their training.

The use of vascular tissue in surgery also developed in the 20th century. Autografts and allografts, as well as artificial vessels, are used for repairing and replacing damaged or defective vessels. Vascular allografts are used to repair or replace large and medium-sized arteries or veins. They are used in cardiovascular, reconstructive and solid-organ transplantation surgery. Vascular allografts are sometimes used to create a shunt to enable access for dialysis (Inston, 2015). For transplant purposes, mostly arterial vessels and conduits are used. They are indicated for aortic disease that leads to slackening of the vessel wall (aneurism) and in patients who suffer from vascular infection or infected synthetic blood vessel prostheses.

Patches are prepared from the pulmonary artery or aortic artery and are used for reconstructions of congenital malformations in paediatric cardiac surgery.

Recently, development of endovascular stent-grafts has reduced reliance on conventional grafts to replace diseased arteries and it is likely that this trend will continue in the future. Aortic grafts are recently also used as a biological matrix for extensive airway reconstruction (Martinod et al, 2013).

Pericardium is a membrane tissue that can be used for cardiovascular anastomosis, dental and ocular replacement procedures (Fehily et al, 2012).

Activity level in the EU

In 2012 in the European Union, a total of 77 cardiovascular tissue establishments were active. In 2012, Member States reported the donation of 1,974 hearts, processing of 3890 heart valves and discard of 1008 heart valves, resulting in 2,882 heart valves issued for transplantation.

Table 1. Volumes of heart valves

	Donation	Recovered	Tissues available 1-1-2012	Processed	Discarded	Total Import	Total Export
AT							
BE	274	549	170	549	12		188
BG							
HR	19	9	12	31	10	1	
CY							
CZ	53	90	407	72	42		
DK							
EE							
FI		231					
FR	326	368	494	272	196	76	1
DE	296		141	277	124	128	
EL							
HU		20	14	14	6		
IE							
IT	231	366		364			
LV							
LT							
LU							
MT							
NL	220			452			47
PL	179	358	145	230	78		5
PT	22	54	75	10	35		
RO							
SK	11	15		15			
SI	1	1					

	Donation	Recovered	Tissues available 1-1-2012	Processed	Discarded	Total Import	Total Export
ES	192	350		350	102		65
SE	150	323		323	136		
UK		985	814	931	267	37	37
Total	1,974	3,719	2,272	3,890	1,008	242	343

Source: EUROCET 2012, Economic landscape survey NCATC (2015), data from 2012

The import and export is the total of cross-border distribution and to/from third non-EU countries.

Besides valves, depending on the demand, and the 'defined graft-range' of the tissue establishment, several other vascular and non-vascular tissues can be procured from a single donor.

Procurement, processing and storage of cardiovascular tissues

Donations of cardiovascular tissues originate from three different sources:

- Organ donors, where the heart is not suitable for heart transplantation;
- Domino donors, i.e. living recipients of transplanted hearts where the removed heart can provide transplantable tissues;
- Deceased non-organ donors, from whom multiple tissues can be removed.

For the procurement of heart valves, the complete human heart is procured and subsequently the heart valve establishment performs dissection and preparation of heart valve grafts. The valves used for transplantation are the pulmonary and the aortic valve. Besides valves, depending on the demand, and the 'defined graft-range' of the tissue establishment, several other vascular and non-vascular tissues can be procured from a single donor.

Processing of cardiovascular tissues takes place in cleanrooms which are often Grade A in a Grade B background. Minimum requirement is Grade A in Grade D. Valves are prepared from the heart and macroscopically evaluated (e.g. measured, tested for function, examined for defects). The valves are temporarily stored in a mixture of antibiotics and cryopreserved in a container with medium that contains cryoprotectant. Vessels are similarly treated (Fehily et al, 2012).

Cardiovascular tissues are cryopreserved and stored in liquid or vapour phase nitrogen. This preservation technique has enabled the storage of donated cardiovascular allografts in heart valve establishments for years in order to create a stock of different sizes.

Pericardium can be freeze-dried before storage.

Heart valves are matched between donor and recipient based on type and size. In general, pulmonary valves are in much higher demand compared with aortic valves. This has partly to do with a specific technique for the treatment of congenital valve abnormalities called the Ross operation in which pulmonary allografts are needed. Small size valves for newborns are rare and require donation by infants that die young. Larger sizes also have a limited availability.

Cost structure

Three cardiovascular tissue establishments provided a breakdown of costs over different activities for this study, including staffing dedicated to these processes, such as costs for donor procurement, additional testing, processing, and distribution. Unlike the removal of an eye, or a cornea from an eye, a cardiectomy requires a small operation team.

The differences in cost structure of these three cardiovascular tissue establishments (TEs) find their origin in the way they organise their processes. TE 1 receives the donor hearts free of charge from organ donation teams or from organ donor organisations.

Costs of the surgical removal of the heart (cardiectomy) are therefore not invoiced. TE 3 has outsourced the donor screening to a commercial laboratory. The different aspects contributing to the costs of cardiovascular tissue banking are elaborated below.

Table 2. Cost divisions in cardiovascular tissue establishments

	TE 1	TE 2	TE 3
Donor procurement	2%	15%	23%
Testing	14%	2%	30%
Processing	70%	75%	47%
Distribution	14%	7%	Not specified*
Total	100%	99%	100%

Source: Survey to cardiovascular tissue establishments (2014), data from 2012
*Distribution costs of TE 3 are separately charged to the requesting hospital

The cost of procurement of cardiovascular tissue is mainly dependent on the donor source (organ donors, domino donors or non-organ donors).

An important factor, negatively contributing to the cost/benefit ratio, is the high discard rate of retrieved donor hearts. This high discard rate finds its origin in the morphology of the donor hearts as well as in the incidence of contamination of the tissue when it arrives in the tissue establishment. As a result, discard rates for reasons of morphology of 44% and microbiology up to 9.7% have been reported (De By, May 2013). One tissue establishment reported a total of 68% and 70% discard rate respectively for 2012 and 2013 (Heart Valve Bank Rotterdam, Annual Report 2013).

Another important factor to take into account is the cost of donor operations, specifically those in non-organ donors with cardiac arrest. To enable these donations a well-trained team and accompanying logistics must be available.

The cryopreservation method enables cardiovascular tissue establishments to extend shelf life over many years. However, to safeguard preservation in the cryo-containers, the levels of liquid nitrogen must be constantly monitored and kept on a level sufficient to keep the temperature of the tissue grafts at an adequate level. Other costs relate to the maintenance and daily operations of clean room facilities.

The costs for retrieval, transport, materials and initial testing for all procured hearts must be calculated into the fee of the limited number of eventual grafts for transplantation.

A final aspect to be mentioned relates to financial liquidity: the relatively long shelf-life for cardiovascular grafts means that, at least for some (less frequently used) outlier graft sizes, it may take several years before the costs can be recuperated by a fee. In some instances, tissues have to be discarded after the shelf life expiration date.

Fee structure

The table below reflects the fees that cardiovascular tissue establishments charge to local recipient hospitals. It may be the case that different fees are charged to different hospitals as opposed to its 'own' hospital, where the tissue establishment is located, in the same country, or other countries. Where the costs of tissue establishments are compensated by the public health system, no fees are charged to hospitals, e.g. in Italy and in France. When tissues are distributed to other Member States, the fees in the table apply.

Table 3. Fee of cardiovascular grafts

Fee charged to local recipient hospitals							
Country	**Heart- valves**	**Conduits**	**Arteries**	**Aortic bifurcations**	**Veins**	**Peri- cardium**	**Po patch**
Belgium	3,895	2,269	2,647	2,647			
Germany	3,500						
Italy CVB 1	2,800						
Italy CVB 2	2,800		1,200	1,200	1,200		
Italy CVB 3	3,200	1,750	1,250	2,300		8	
Netherlands	5,230		2,820				1920
Poland CVB 1	2,250					160	
Poland CVB 2	1,823		939			944	
Poland CVB 3	1,900						
Portugal	1,925		1,405		1,405		
Spain CVB 1	0						
Spain CVB 2	3,300						
Spain CVB 3	940						
Spain CVB 4	1,336	1,336	1,336	1,336	1,336		
UK	3,691	2,541	2,541				1,452
Average fee	2,573	1,974	1,767	1,871	1,314	371	1,686

Source: Survey to cardiovascular tissue establishments (2014), data from 2012

The table shows a remarkable difference in the fees charged to local hospitals. The one cardiovascular tissue establishment that does not charge anything nevertheless incurs costs; this can be an indication that the hospital management may have chosen not to work with cost-centres. It also has to be mentioned that this cardiovascular tissue establishment processes only three heart valves per year; the quantitative costs are thus a minimal part of the total university hospital budget.

It needs to be noted that in the Netherlands, as for corneas, the fees charged to the local and national hospitals cover more than just the processing in the tissue establishments; it includes overhead charges of the National Transplant Foundation, and other costs.

The difference in pricing between the cardiovascular tissue establishments has several causes:

- The fee to the end user is often determined by outside parties (authorities, insurance) and not based on calculations in which all costs are integrated into a price or fee that covers the real costs.

- A systematic difference in organisational embedding. Some tissue establishments are "stand alone", meaning that they are responsible for balancing the costs and the revenues. Others are part of a larger structure, usually a (university) hospital, in which this larger structure carries (part of) the costs.

- Fee differences may also occur due to differences in the level of salaries and of other costs in the EU countries.

Fees determined by the tissue establishment itself are higher than those determined by a Ministry of Health or by a regional government though this is not to imply that in those cases, fees are too high. As explained elsewhere in this report, the economics of tissue banking are complex and depend on many factors.

Cross-border exchange and import/export of cardiovascular tissue

Cardiovascular tissues are occasionally exchanged between EU Member States. In some countries, specifically with 'opting in' donation systems, there is a shortage of donations (or procurements) rather than a shortage of donors. Organising permission and consent for the donation requires additional efforts, and always entails the possibility of a refusal to donate by the next of kin. To cover the shortage, tissues are distributed from other Member States and imported from the USA.

In the experience of the tissue establishments there are several hurdles to be overcome when tissues are to be distributed cross-border to other EU Member States.

- Verifying that tissues, selected for cross-border distribution or export, are not needed for national patients. Such a system is present in some Member States (like NL and IT), but lacking in most.

- Complying with (often unclear) additional donor selection and testing criteria in the country of destination or importing country, other than required in the exporting country.

- Complying with administrative and regulatory requirements, in particular when tissues are to be distributed to Germany, where there is the greatest demand.[1]

- Complying with criteria requires extended knowledge, sometimes about pharmaceutical regulations for those countries such as Germany where tissues are classified as medicinal products. Such knowledge isn't available in most tissue establishments, and only those who can afford to pay consultancy fees to supporting experts, can – after going through an approval process that may take several years - achieve compliance with the criteria and regulations of some countries.

- Differences in price or fee structure and of reimbursement systems.
- Differences in VAT rates, if applicable.

Many of the alliances mentioned earlier aim to create mutual support between tissue establishments financially (sharing the costs of regulatory consultants, for example) and also by sharing expertise. Such alliances include: BISlife (NL)-TSF (now Banco de Tejidos) (ES)-Homograftlabor DHZB; and EHB (BE) with the Homograftlabor in Zagreb (HR) (see also page 215 for international alliances).

Ocular tissues

Ocular tissues include corneas and sclera. The cornea is the clear anterior part of the eyeball which permits light to enter into the eye. A corneal transplant is indicated when eyesight is decreased due to corneal disease and provided that other parts of the eye are functional. The sclera is the outermost part of the eyeball and is partly visible as the whites of the eyes.

Clinical application

The most common indications for corneal transplant are opacity, deformation or scarring following infection or injury of the cornea. Often a transplant is the only available option for improving eyesight in these patients. In a small sub-group of patients the matching of HLA type between donor and recipient is indicated when the recipient has an atopic constitution or had previous rejection of a cornea graft. A large number of cornea donors need to be HLA typed in order to identify a suitable match a single patient because of the low probability of finding a match. Sometimes the HLA typing costs can be shared with the organ transplantation organisation in the case of an organ and tissue donor.

The affected cornea is (partly) replaced by the cornea of a deceased donor. Two types of corneal transplant techniques are used: penetrating and lamellar keratoplasty. In penetrating keratoplasty (PKP) the full thickness of the cornea is replaced by a donor cornea. There are some disadvantages of the PKP technique such as graft rejection, slow healing and irregular astigmatism. In lamellar keratoplasty (LKP) only the affected layer is replaced. Lamellar keratoplasty is subdivided according to the replaced layer, anterior or posterior. The disadvantages described for PKP are fewer. But the lamellar technique requires specific skills of the technician in the TE or the surgeon. Besides that, additional investments have to be made in devices to assist the cutting of the lamellas. The preparation of the lamellas can either be done in the operating theatre or at the cornea TEs (pre-cut lamella).

Donor sclera is used in reconstructive surgery of eyes and eyelids. Sclera can be preserved and stored for several years.

Activity level in the EU

In Europe, in 2012, 141 corneal tissue establishments were active.

The table below shows the recovering of 40,185 corneas. From these, 30,428 corneas were distributed.

Table 4. Volumes of corneas

	Donors	Recovered	Distributed in own MS	Distributed to other MS	Exported to third country	Received from other MS	Import from third country
AT*	200	NA	188	3	0	0	0
BE	413	973	906	0	0	45	0
BG	73	145	119	0	0	0	0
HR	178	374	133	0	0	1	53
CY	0	0	0	0	0	0	0
CZ	502	998	676	46	81	0	0
DK*	202	NA	356	0	207	23	0
EE	42	48	46	0	0	0	0
FI	134	273	NA	0	0	4	0
FR**	9,893	9,918	4,372	145	0	0	0
DE	NA	8,435	5,458	405*	68*	188	0
EL	NA	NA	NA	NA	NA	NA	NA
HU	287	302	536	0	0	0	0
IE***	0	0	NA	0	0	0	175
IT	7,529	15,071	6,575	400*	178*	12	0
LV	13	26	NA	0	0	0	0
LT	29	58	53	0	0	0	0
LU***	2	0	0	0	0	0	0
MT	NA	NA	NA	NA	NA	NA	NA
NL	1,635	3,239	2,603	291	43	1*	0
PL	559	1,104	855	0	0	0	0
PT	448	905	NA	0	0	136	0
RO	48	48	NA	0	0	89	0
SK	99	186	118	0	0	0	0
SI	56*	146	110	0	0	0	0
ES	2,735	4,808	2,743	10	0	70	0
SE	516	859	829	21*	0	0	0
UK	3,031*	7,040	3,752	0	0	74	615
Total	37,059	40,185	30,428	1,321	577	643	841

Sources: EUROCET 2012 unless stated otherwise:
*The European Eye Bank Association, EEBA Directory, Twenty-Second Edition, January 2014;
**Agence de la Biomédecine, Rapport Annuel 2012
*** Data by IE NCATC. No procurement in IE due to vCJD disease

Procurement, processing and storage of ocular tissues

Ocular tissues are obtained from deceased donors. Retrieval of ocular tissue is performed in the mortuary, in the hospital or at home. For cornea and sclera the complete eyeball is procured. For corneas only, the corneal disc can be removed at the donor procurement site; processing is done in ocular tissue establishments (Bredehorn-Mayr et al, 2009).

The preservation of the (living) endothelial cells is significant in cornea banking. If the corneal disc has not been removed at the procurement site, the eyeball is washed and in the cleanroom the cornea is removed. After evaluation and decontamination, the cornea is stored in preservation media. Preventing contamination is extremely important and antibiotics are used and microbiological cultures taken. Storage in organ culture medium is preferred for the endothelial cells as these will be more vital and there is more time for obtaining the results of donor testing and of microbiological cultures and, where relevant, HLA typing (Fehily et al, 2012). Some cornea banks distribute pre-cut lamellar cornea for LKP. The cornea is cut with a microkeratome by hand. New laser technics however can also be used and even enable cutting multiple lamellas from one cornea.

The shelf life of corneas is limited to four weeks for storage in organ culture medium at 30-37°C. When stored under hypothermic conditions cornea's can be stored up to 14 days (Wilhelmus et al 1995), though in practice tissue establishments usually store grafts up to 5 days as endothelial cells will decrease in viability during storage. After evaluation of cell quality and quantity, the grafts can be released for transplantation. For reasons of quality, storing corneas in culture medium at body temperature is the most common technique applied in Europe, contrary to the US where cold storage is the standard method.

Sclera are stored separately in 70% ethanol. Distribution may be in segments of 10x15 mm, in quadrants or complete sclera (TRIP Annual Report, Biovigilance, 2012; Haase-Kromwijk et al, 2007; Rijneveld, 2010).

Cost structure

In an additional survey, performed in the context of this study, three cornea tissue establishments (TEs) provided a breakdown of the costs of the major processes, while one cornea establishment could provide its processing costs. They have calculated the cost division per process (note: all TEs reported that donor procurement includes a ±40% discard rate). In this overview, while cornea TE1 had specified its costs in great detail, cornea TE 2 has not separated processing costs from distribution costs.

Table 5. Cost division in cornea tissue establishments

	TE 1	TE 2	TE 3
Donor procurement	24%	25%	30%
Testing	22%	10%	8%
Processing	30%	65%	38%
Distribution	24%	NA	23%
Total	100%	100%	99%

Source: Survey to cornea tissue establishments (2014), data from 2012

One of the cornea tissue establishments indicated that the total costs couldn't be recovered from the income; the difference is covered by fundraising. The other two tissue establishments indicated that National Health Insurance compensated for most of the costs. An example mentioned elsewhere is the investments in the Lions Cornea Banks (Germany), which are mainly provided by the Lions service clubs.

An important cost factor in these calculations, recognized by all cornea tissue establishments, is the high discard rate of donor corneas (Bredehorn et al, 2009). Reports show that the discard rate can reach levels of over 50% (BIS Foundation, Annual Report 2007). This discard rate has several causes and is a challenge to address. One of the main reasons is that many suitable donors are of older age, and, the older the eye, the higher the rate of idiopathic endothelial cell loss. This can however only be diagnosed in the tissue establishment after completion of procurement. Thus, many donated corneas do not fulfil the minimum cell count per mm^2 of 2 000-2 500 according to the criteria of the European Eye Bank Association and have to be discarded.

Sundmacher and Reinhard (2009) reported that, to provide 300 transplantable grafts, an annual budget of almost €600 000 is needed to achieve a break-even between expenses and revenues (Sundmacher and Reinhard, 2009). The fixed expenses include a minimum of two technicians and two MDs on call to cover a 24/7 service. In the study of Sundmacher and Reinhard, 600 corneas had to be recovered to provide 300 transplantable grafts to hospitals. To cover the total costs of €600 000, each distributed cornea would cost around €2 000. If the number of distributed grafts were higher than the necessary 300, the economy of scale could cause a favourable effect on the fee charged to the recipient hospitals, or to the health insurance responsible for reimbursement.

In our survey organized within the context of this report, two tissue establishments that indicated distribution of over 1 000 corneal grafts per year, processing cost per graft are shown to be lower as compared to those which processed fewer than 1 000 grafts per year. A decrease in number of corneas processed would mean that such a cornea tissue establishment would have to find financial support to cover the expenses, increase the price charged per cornea or else reduce its service level, which may lead to being unable to address donor calls or other service cut-backs.

The table below shows the correlation between the number of distributed grafts and the processing costs of four cornea tissue establishments that could provide data with respect to their processing costs. It demonstrates the effect of the economy of scale.

Table 6. Number of corneal grafts and cost

	Number of distributed grafts	Processing costs per graft in €
Cornea TE 1	59	825
Cornea TE 2	989	643
Cornea TE 3	1386	471
Cornea TE 4	2702	315

Source: Survey to cornea tissue establishments (2014), data from 2012

The cost for a cornea also differs per preservation technique. The use of the preservation method should have no influence on the costs of procurement, testing, or distribution.

However there are large differences in costs when it comes to comparison of the two storage methods. When using the organ culture method, there's need for a clean room environment in which establishments usually process under a class A cabinet with a background of class B. Moreover, the need to operate an incubator, the necessary change of medium before transportation and the regular microbiology tests of that medium contribute to additional costs. It can therefore be assumed that the culturing method generates more costs than the alternative 'cold storage' method. For cold storage industrially provided closed systems are available, negating the need for clean rooms. This can be a cost-effective solution where economic resources are limited

On the other hand, the culture medium method has a significant positive impact on discard rates due to the longer shelf life, thus contributing to the number of transplantable grafts and a better balance between costs and revenues. Overall, most European cornea tissue establishments have chosen to apply this preservation method based on quality arguments. This method enables evaluation of the quality of the cells, the presence of micro-organisms in the culture, as well as extended possibilities to screen donor material for the presence of transmittable diseases and viruses.

Fee structure

Table 7 reflects the fees TEs charge for different tissue grafts to local end-users. Different fees may be charged to hospitals outside the region. Again, different fees may be invoiced to hospitals in another EU country or to countries outside the EU.

Table 7. Fee of ocular and amnion tissues to local end-users

	Cornea PKP	Cornea emergency	Anterior cornea lamella	DMEK[2]	Sclera	Amnion
AT	1901	1901	2480	2319	479	210
BE	1349	1349			95	1092
BG	500*					
CZ	790	790	1160	1160	790	190
DK	2078		2480		2078	429
DE	1875	1875	1875	1875	560	560
HU	250					
IE	2988	2988	2988	2988	523	497
IT	3465*	875*	3130*	3130*	300	500
NL	2250*	2250*	2250*	2250*	100*	400*
PL	663*		840*			
PT	671					
RO						
SK						
SI						
ES	1030*	1670*	825*		464*	203*
SE	1235	1235			107	
UK	738					
Minimum	250	790	825	1160	95	190
Maximum	3465	2988	3130	3130	2078	1092
Average	1420	1659	2003	2287	567	453

Source: Survey to cornea tissue establishments (2014), data from 2012; Economic landscape survey NCATC (2015), data from 2012

The difference in pricing, as shown in the table, leads to the following observations:

- Fees for all types of ocular tissue vary remarkably from one TE to another. For PKP corneas the range is €250-3465. Though this may partly be explained by differences in the technique used. When considering the differences in degree to which real costs are taken into account for price-setting as well as the difference in healthcare wages in the responding EU-countries, some of these fees include more than the costs of tissue establishment processes. In the Netherlands, for example, the fee includes the costs of HLA-typing of the recipient, but most importantly it must cover the overhead of the National Transplant Foundation and the imbalance between costs and income of several tissue establishments.

- The differences in fees, specifically for those tissue establishments that have the freedom to determine the fee to the end-user, may be explained from the point of 'critical mass'. The greater the number of distributed tissue grafts, the better

that fixed costs, such as infrastructure and personnel, can be covered by income (Reinhard and Sundmacher, 2009). As mentioned, the break-even point would be expected at 300 transplantable corneas.

- Emergency corneas are grafts which are in the tissue establishment to be allocated in urgent cases (rather than scheduled operations) in which the patient may lose an eye if he/she doesn't receive a graft. These emergency cases often occur at night or during weekends. Most cornea establishments have a small stock that is ready and prepared (to save time) for immediate shipping. Usually, the quality of these emergency corneas is lower than grafts for elective operations when it comes to the number of viable cells.

- The fee of sclera, which are in fact a by-product from cornea processing, varies from €95 to €2078. In some countries, sclera are considered to be a medicinal product (e.g. in Germany), the registration of which requires intensive legal- administrative application processes, but the observed differences cannot solely be explained from that point of view.

- While sclera are procured from deceased donors, amniotic membranes are donated by living donors. They serve the same purpose in ophthalmology surgery, namely providing a natural support to the eye globe that can easily be sutured. It may be expected that the cost structure for obtaining and processing amnion membranes are quite different from the collection of sclera. The observed differences in fees can otherwise not be explained.

Authorities and health insurance organisations may influence the fee for the end-user. Table 8 provides an overview of legal status and fees in which the decision-makers are listed per EU country. Given the fact that only 15 out of 60 cornea TEs responded to the questionnaire for this research, no conclusions can be drawn concerning the correlation between legal status, the determination of the fee and the fee to the end-user.

Table 8. Fee determination of cornea

	Legal status of organisation	Determination of fee by	Fee of cornea PKP
Hungary	Private foundation	TE itself and insurance	250
Czech Republic	University Hospital	Insurance	790
Italy	Private Foundation	TE itself	1100
Sweden	University Hospital	Regional Council	1140
Belgium	University Hospital	Government	1349
Spain	National Health	TE itself	1425
Germany, TE 1	University Hospital	Hospital	1850
Germany, TE 2	Private foundation	TE itself	1900
Germany, TE 3	University Hospital	TE itself	2000
Austria	University Hospital	Hospital	1901
Denmark	University Hospital	TE itself	2078
Ireland*	National Health	TE itself	2988
Netherlands TE 1	Private Foundation	Insurance	2250
Netherlands TE 2	Private Foundation	Insurance	2250

Source: Survey to cornea tissue establishments (2014), data from 2012
* Ireland imports all corneas from the US (because of vCJD in Ireland and the UK)

Cross-border exchange, and import/export of corneas

For cornea TEs, the international exchange shows different dynamics of cross-border distribution and import/export. In some Member States there is a surplus and corneal grafts are distributed to other Member States or exported, while in other countries there is a shortage and numerous grafts are ordered from other Member States or imported. Imports are mostly from the USA.

Table 9 shows that there is only one tissue establishment (in Hungary) that clearly indicates that there is no donor shortage. One cornea tissue establishment from Germany remarked that there shouldn't be a donor shortage if donor referral and procurement were well organised. One tissue establishment from Germany reported an estimated national shortage of 5 000 corneas per year. One indicator for measuring a donor shortage is whether there is a waiting list for cornea recipients. Such a waiting list could also report the average and maximum waiting time for patients to receive a transplant.

Clearly, there is a donor shortage in different EU countries. Several respondents (BE, DE) reported that in the absence of such waiting lists, it is hard to determine the real level of this shortage.

The table below shows the exchange of tissue grafts, as reported by 14 responding tissue establishments. The reporting cornea TEs distributed 919 grafts to other EU countries, and exported 367 to third countries. All in all, more corneas were imported (772) from

outside the EU than exported (367) to these third countries. Greece has 5 ocular tissue establishments but as shown in the table, no data is available on number of donors, distribution and import and export. The donated cornea from donors in Luxembourg are processed and stored in another Member State.

Table 9. Cross-border distribution, import, export and shortages of cornea

	Shortage of donors	Cross-border from MS	Cross-border to MS	Import from third country	Export to third country	Countries
Austria	yes	1	11			
Belgium	yes		45			
Czech Republic			47		78	
Denmark	300	23		207		from USA
Germany TE 1	5,000					
Germany TE 2						
Germany TE 3	yes	188		405	68	from USA
Hungary	no		80			to RO
Ireland				160		from USA
Italy		12	400		178	
Netherlands	yes	1	291		43	
Portugal	yes	136				
Spain	100	70	10			
Sweden	150		35			to DK
Total	>5,550	431	919	772	367	

Source: Survey to cornea tissue establishments (2014), data from 2012

At the same time, the table also shows that there are substantial imports from outside the EU. The reporting cornea tissue establishments purchased 772 grafts from the United States; in this survey this represents a large part of the distributed volume reported.

The export to countries outside the EU may be, partly, explained by the fact that during European holiday periods (e.g. Christmas–New Year) there is a reduced activity in ophthalmology clinics. As the preserved corneas in the tissue establishments have a short expiration period of about four weeks, corneas may expire if they are not transplanted. Corneas, which would otherwise expire, are therefore offered to countries that do not observe the same holiday periods. Often, these corneal grafts are offered at reduced fees.

Some EU countries have organised their tissue banking structure in such a way that self- sufficiency is enabled. In some countries (IT, UK, NL) a mandatory allocation system is in place. This guarantees the supply to cover the national demand. In other

countries, such as Germany, Spain and France, voluntary networks strive to achieve optimal allocation of available grafts.

Musculoskeletal tissues

Musculoskeletal tissues include bone and related soft tissue such as tendons and ligaments.

Clinical application
The need for bone grafts is determined by a number of medical indications:

- To substitute the loss of a patient's own bone caused by trauma
- To replace a patient's resected bone or joint because of malignancy
- To fortify the host's bone mass to enable the placement of an artificial joint, usually in re-operations of the (replaced) hip or acetabulum
- To fortify the spine after trauma, for correction of spinal defects caused by congenital disease (scoliosis, lordosis), or severe wear of the vertebrae for other reasons
- To reconstruct dental or maxillofacial bone loss, and to support artificial dental implants by filling the cavity around the implant-base with demineralized bone powder or gel.

The main indications for tendons and ligaments are injuries, such as tearing, of the patient's own tendon, usually the patella or Achilles tendon. Typically in the case of a revision of a previous reconstruction with an autologous tendon (Anterior Cruciate Ligament (ACL) and Posterior Cruciate Ligament (PCL) reconstruction with the patients' own hamstring or other hemi tendon), allografts are needed (Robertson and Nutton, 2006). Other musculoskeletal tissues include cartilage, fascia and menisci from deceased donors. In exceptional cases a graft coming from a donor can replace a ruptured meniscus.

Chondrocytes are mainly used in autologous settings. This treatment involves in vitro culturing of cartilage cells after a harvesting procedure; the cultured cartilage cells are transplanted in a second procedure. The cartilage cells adhere to the bone surface and start forming new cartilage. The in vitro growing of cartilage is a sophisticated, recently developed technique (Bugbee et al, 2015). This cultured cartilage can be classified as ATMP; it was the first authorised product under this regulation.

Demineralized Bone Matrix (DBM) is produced from cortical bone. Cortical bone is harder than the above-mentioned cancellous chips, which, because of their

open structure, allow faster ingrowth of the host bone. Thus, cortical bone is less in demand for orthopaedic surgery. DBM is frequently used in dental applications, specifically to support the base of implants in the mandibular or in the upper jaw. Tissue establishments have developed DBM into several products, such as bone-gel with glycerol or hyaluronic acid, also called hydrogel, bone-flex, bone-putty etc. Bone-gel is often used for implantation along spinal fusion devices. The osteo-induction, caused by the DBM, results in a more rapid bone growth in the recipient the spinal implant/ the spine will obtain stability more rapidly. By removing the minerals from the cortical bone, the tissue becomes osteo- inductive, rather than osteo-conductive. The absence of minerals exposes the endogenous growth hormones which then stimulate the host bone to grow into the DBM. Since the removal of minerals causes the loss of weight bearing capacity of the bone structure, DBM is not suitable for grafting in recipient sites that need weight-bearing capacities. DBM is used as bone filler in cavities that need fast restoration.

During the last 10 years there is a clear trend of combining, in a package, CE-marked orthopaedic hardware, such as surgical instruments and/or industrially produced implants with bone tissue. The combination of tissue and hardware is offered to the surgeons to increase the efficiency of their operations. By making use of innovative technologies, musculoskeletal tissue can be combined with antibiotics. The argument for this approach is that it can prevent or eliminate the contamination of bone tissue, respectively prophylactic (in trauma patients with high risk for infection) or in patients who undergo a re-operation because of the failure of an industrial or of a tissue implant.

In the past, combinations of bone tissue grafts with growth factors, bone morphogenetic proteins (BMPs), have been seen. Although this combination has not resulted in successful clinical application, it may be expected that in the future, new combinations of bone and cells, and/or pharmaceuticals, aimed at stimulating bone healing, will be introduced.

Activity level in the EU

In 2012 there were 400 authorised musculoskeletal tissue establishments within the EU. There are several types of bone tissue establishment. Hospitals and orthopaedic centres often operate a bone tissue establishment to provide the required donor bone from their own patients. There are tissue establishments that focus on living bone donation and those that focus on processing deceased donor bone and other musculoskeletal tissues. Other tissue establishments focus on import of musculoskeletal tissue and/or storage and distribution.

Table 10 shows an overview over donation and distribution of musculoskeletal tissues. Though the number of living donors (of femoral heads) is 8 times as high as the number of deceased donations, the yield of tissue grafts coming from living donations is limited as compared to deceased donations. Depending on the number and size of donated bone tissues from a deceased donor, these can be cut and shaped into many units

of transplantable grafts. Specifically when grinded into small particles of DBM, the number of units can reach 100 or more. 87% of the distribution of musculoskeletal tissues processed by EU based tissue establishments takes place in the own Member State. In addition musculoskeletal tissue is imported with a quarter of all tissues coming from third countries. The import from third countries, outside the EU, is about ten times as high as the cross border exchange between Member States, inside the EU.

Table 10. Donation, distribution, import and export of musculoskeletal tissue

	Deceased Donors	Living Donors	Total Distri-buted	Distributed in own MS	Cross- border to other MS	Exported to third country	Cross-border from other MS	Import from third country
BE	192	5595	14653	14243	275	1		135
BG	137							
CZ	121	111	3344	3344				
DE*		21372	64358	64358		144373		41648
EE	3	27	98	102			4	
FR	60		31310	30130	1184		3796	
HR	75	165	156	156				
HU	20	458	623	623				
IT	304	3540	16824	16824				
LV	18		0					
LT	4	82	67	67				
LU	2							
NL	128		36649	10027	19977	6645	257	110
PL	242	123	9657					
PT	28	20	121	121			91	
RO	10	127	89	89				
ES	672	1451	14630	13731			900	1
SE	3	1893	1201	1164			58	
SK	309	102	294	127	167			
UK			28851	38384		2452		11985
Total	4326	35076	222925	193490	21603	153470	5106	53879

Source: EUROCET 2012. Please note that total numbers don't add up as not all countries reported
*The total distribution in Germany has been interpreted as "Distributed in own Member State". The import and export from third countries refer to a relation of one single tissue establishment.

Procurement, processing and storage of musculoskeletal tissues

Bone is obtained both from deceased and from living donors, who may donate a femoral head at hip replacement surgery. The femoral heads donated during hip replacement can only be used for non – structural purposes due to the brittleness of the bone (TRIP Annual Report 2012, Biovigilance).

It must be collected aseptically into a suitable container and frozen. The donor must have been screened for mandatory markers using traditional serological tests plus either a repeat test 180 days after donation (unless Nucleic Acid testing (NAT) for the mandated markers was performed at donation). Testing for bacterial and fungal contamination must also be performed on samples taken from the tissue itself.

Surgically removed femoral heads are usually provided to the clinical user without further processing.

A significantly larger quantity of bone, and range of bone types, can be procured from deceased tissue donors. It can be processed to prepare a wide range of shaped bone allografts (e.g. ground bone, cubes, demineralised bone paste and putty etc.). From 50-300 units of bone/tendon can be prepared from one donation (Gillan et al, 2014). Often the bone donation is part of a multiple tissue donation including other musculoskeletal tissues, such as tendons and meniscus, and also other tissues such as skin and heart valves. The processing of bone is performed in a clean room suite to GMP standards, usually Grade A. The bone is then terminally sterilised using irradiation.

Procurement of musculoskeletal tissues requires a surgical approach. Preventing contamination is one of the main challenges. Cleaning, disinfecting and working in a local sterile field is necessary for procuring the grafts. Culturing samples for microbiological contaminants is an important step in guaranteeing the safety of the bone. Procurement of musculoskeletal tissues therefore usually takes place in an operating theatre (OR), particularly in those programmes where the tissue is not subsequently terminally sterilised. Depending of the number of grafts taken, an operation on a deceased donor may take up to three hours or more in which the OR cannot be used for other purposes. Some tissue establishments have therefore invested in dedicated operation theatres at their site, while others use the hospital facilities outside working hours.

Processing of bone may consist of several steps depending of the type of material and the use of the grafts. In general the following steps are applied (Veen, 1994):

a. Cleaning and removal of debris
b. Washing or rinsing to remove debris and bacteria
c. Cutting and shaping
d. Disinfection with different kind of disinfectants or by heating
e. End sterilisation by Gamma or E beam radiation
f. Freeze drying or freezing for storage.

While cleaning and washing with desinfective agents (processing steps a and b) are aimed at reducing the level of micro-organisms, the techniques used in the process steps d and e, are intended to remove or destroy all living micro-organisms. In order to achieve elimination of micro-organisms, the following sterilization and decontamination methods can be applied by musculoskeletal tissue establishments (Veen 1994):

- Heat-sterilization
- Gas-sterilization
- Gamma-irradiation
- Ethanol
- Beta-propiolactone (Lo Grippo et al, 1956)
- Merthiolate (Arde, 1956)

Some musculoskeletal tissues (e.g. femoral heads from living donors) are frozen directly after procurement and distributed after microbiological cultures and donor tests are confirmed negative.

Decalcification (demineralization) of cortical bone is widely used to remove the nonorganic matrix and expose the organic matrix, with subsequent release of osteoinductive growth factors. This kind of bone implant stimulates renewing of bone and is primarily used where bone is implanted in a site that does not have bone mass, e.g. in dental implantation.

Bone can be cut and shaped into a large number of forms and sizes. Some of the most commonly used musculoskeletal tissue grafts are: femoral heads (from living and from deceased donors); cancellous chips, patella tendon whole, and patella tendon or hemi patella tendon); proximal tibia or distal femur frozen, cortical struts or rings demineralized bone powder and osseous gel (putty).

Cost structure

Depending on the donor source (living or deceased), the cost structure of bone banking varies considerably. The response to the survey into cost structures of musculoskeletal tissue establishments didn't result in any information, either because the tissue establishments didn't have the required data, or they didn't want to provide it. Only one reliable source was available (table 11). Depending on factors such as organisation of the procurement teams, the distance to the donor site, the required virology and microbiology testing regiment, the method of processing, the costs of a post-mortem donation can reach a level of around €5000 in some tissue establishments, while other tissue establishments may be able to carry out these tasks at more reduced cost levels. The procurement of a femoral head from a living donor occurs additional costs such as swabbing, microbiology testing, packaging, labelling and transportation to the tissue establishment. Depending on organisational factors the total costs are a few €100.

The following division of processes and related costs can be recognized.

Table 11. Cost division in a musculoskeletal tissue establishment

	Post mortem donors	Living donors
Donor procurement	16.20%	39.00%
Testing	4.30%	49.20%
Processing	42.00%	0.00%
Distribution	37.50%	11.80%
Total	100%	100%

Source: BIS Foundation Annual Financial Report 2008 and Netherlands Bone Bank Foundation 2008. Percentages based on financially audited costs of 111 deceased donors and 1145 femoral heads. Survey to musculoskeletal tissue establishments (2014)

Procurement from living donors

As a femoral head is removed from the donor to place an artificial implant, there are only costs of the surgical recovery procedure (which takes place anyway, despite of the donation). After training and instruction of the staff, donor screening of medical suitability is executed in the hospital. The orthopaedic surgeon signs the screening form. Packaging of the graft is done in the operating room. Some tissue establishments offer compensation for administration and packaging. The fees, known to the authors, vary from €40 to €70 per donated femoral head. Additionally, there are transport costs, depending on distance from the tissue establishment, and of transport circumstances (cooled, frozen or otherwise).

Procurement from deceased donors

Deceased tissue donors can be organ or non-organ donors. In both cases, there is a limit with respect to the time during which musculoskeletal tissues can be removed after cardiac arrest. In case of a donor referral, a team with on average three trained professionals (MDs and/or technicians) travels to the donor site and carries out the donor operation. In this process, a variety of costs are incurred:

Compared to other tissue procurements (cornea, skin) the costs of musculoskeletal tissue donations are much higher. This is caused by the higher quantity of team members, more sterile materials for packaging of the tissues, a larger instrument set and a

multitude of microbiology swabs for the procured tissues. A 24/7 on-call service, executing an initial screening, requires many elements to be organised including:

- A well trained donor team which is on stand-by
- Instruments and sterile materials to operate and package the tissues
- Stock of tubes/swabs for virology and microbiology testing
- Travel to the donor site and back

- Using the OR of the donor hospital or dedicated OR from Tissue establishment

- Carrying out the donor operation. The time depends on the circumstances (waiting for organ donation to finish y/n) and experience of the operating team.

- Administration of donor clinical details and operating report

- Transport costs, depending on distance from the tissue establishment, and of transport circumstances (cooled, frozen or otherwise)

- Virology and bacteriology testing. Virology testing is done with the post mortem blood (preferably also with the pre-mortem blood) of the donor. Bacteriology as well as virology tests are carried out by contracted laboratories, which are certified for these tests;

Donor detection and family interviews is organised in different ways throughout the EU Member States. In some Members States there are no costs reported for such activities, which may be related to an opting-out system, while in other Member States these services are part of the organ network structure and no costs are forwarded to the tissue establishments. In some countries hospitals have dedicated officers for donor recognition and in-house coordination of procedures.

Since bone donation comes with a large number of contra-indications, and to avoid procurements resulting in discards, thorough donor selection (before starting the procurement) is of utmost importance to avoid unnecessary costs. Discard rates of over 1.3% have been reported (EUROCET 2013). These discards are caused e.g. by contra- indications of the donor, by virology and microbiology results as well as by the morphology of the donated tissue.

Further processing and distribution

- Cutting the bones and tendons into shapes and sizes matching with the expected demand from surgeons.

- Processing of the bone. The costs are dependent on the method which is chosen, and which leads from almost no costs (when the bone is not cut, not macroscopically cleaned, e.g. by removing cartilage) to hundreds of euros per bone piece if intensive cleaning, cutting and processing is applied. Depending on the (terminal) sterilization method, and whether tissues are cryopreserved or freeze-dried, the processing costs can be higher or lower.

- Without processing, grafts are released after negative virology and bacteriology tests and approval of the donor's medical screening.

- Cleaning of the bone by removing attachments such as cartilage, sawing and soaking in an antiseptic such as alcohol. Usually irradiation afterwards.
- Sterilisation by means of irradiation.
- Cleaning by means of critical CO^2 method, irradiation afterwards.
- Freeze drying or freezing, packaging and labelling.
- Storage and administration.
- Distribution.

Additional cost factors to consider relate to the maintenance and daily operations of clean room facilities. The freeze-drying and cryo-preservation methods enable tissue establishments to extend shelf life over many years. However, to safeguard preservation in the cryo-containers, the levels of liquid nitrogen must be constantly monitored and kept on a level sufficient to keep the temperature of the tissue grafts at an adequate level.

In the case of musculoskeletal tissue establishments it is a challenge for the management to balance demand and supply. Because of the fact that grafts are applied in a great variety of specific interventions, depending on the different shapes and forms into which they have been processed. Management should be aware of the demand in such medical-specialist areas such as orthopaedic surgery, traumatology, neurosurgery, maxillofacial surgery and plastic surgery. The difficulty for the tissue establishment management is to optimize and process the donations in such a way that the demand is completely covered, while stock size is adequately kept and expiration of grafts is prevented.

Fee structure

The fee determination for musculoskeletal tissues reflects the relationship between the legal status of the organisation, determination of the fee and the fee charged to local end-users. As explained elsewhere in this chapter and in this report, the economics of tissue banking are complex and depend on many factors. This is one of the reasons for the observed differences in fees. On top of the factors already mentioned, the fee charged to the end-users may be influenced by specific situations such as the mix between living and deceased donations in a particular tissue establishment (the latter usually being more expensive than the first).

Table 12. Fee determination of musculoskeletal tissues

Country	Legal status of organisation	Determination of fee by	Fee in € charged to local hospitals	
			Cancellous chips 30cc	Achilles tendons
Belgium 1	Part of private hospital	National insurance authority		
Belgium 2	Part of university hospital	National insurance authority	340	
Bulgaria	Not for profit shareholder company	Tissue establishment	400	500
France	Part of university hospital	Public body		
Germany 1	Part of university hospital	Not applicable: the grafts are free for all the patients, tissue establishment receive lump sum by social insurance company directly, income is independent from production		
Germany 2*	Not for profit shareholder company	Tissue establishment	350	750
Greece	For profit shareholder company	Public body (not CA)		
Hungary	Independent public foundation	Public body (not CA)		
Italy	Not for profit shareholder company	Competent authority	893	1200
Netherlands	For profit shareholder company	National insurance authority	485	1466
Spain 1	Part of public blood bank	Regional health authority	695	1200
Spain 2	Part of public blood bank	Regional health authority	440	486
UK 1	Part of national health hospital	Tissue establishment		
UK 2	Part of national health hospital	Tissue establishment		
Average fee			515	934

Source: Survey to musculoskeletal tissue establishments (2014)
The not-for profit shareholder company is a legal entity in some EU Member States. Usually the shares are owned by other not for profit institutions such as universities..

By distributing DBM, tissue establishments are able to reduce the surplus cortical bone stock, while addressing clinical needs, thus generating additional income for their organisations.

Cross-border exchange and import /export of musculoskeletal tissue

Only a small number of musculoskeletal tissue establishments responded to the survey conducted as part of this study and hence the information with respect to donor shortage and import-export provides a limited view on the economic landscape of musculoskeletal tissues in Europe. Table 13 shows a 25% import from third countries, while two US tissue establishments are responsible for about half of these imports (table 21); it may be expected that the relative share of imports from third countries will increase over time.

Many distributors of orthopaedic instruments and other hardware companies, which have qualified as tissue establishments, do import from different US tissue establishments.

Such companies advertise allografts on the websites or in catalogues. This observation was reason for the researchers to look at US imports.

Table 13. Import, export and reported shortages of musculoskeletal tissues

Country	Donor shortage L/D*	Cross-border to EU	Cross-border From EU	Import from third country	Export to third country	Countries
Belgium 1	No answer	No	Yes	No	No	FR
Belgium 2	No	No	No	No	No	
Bulgaria	No	Yes	Yes	No	Yes	NL DE IT EL and Turkey
France	Yes	Yes	No	No	No	Not specified
Germany 1	No	No	No	No	No	
Germany 2	No	Yes	Yes			Not specified
Greece	50/50	Yes?		Yes		ES and US
Hungary	No	Yes	Yes	No	No	Not specified
Italy	No		Yes		Yes	UK DE
Netherlands	200/10	Yes	Yes	No	Yes	UK AT and Turkey
Spain 1	No	No	Yes	No	No	Not specified
Spain 2	No	No	No	No	No	
UK 1	No	No	No	No	No	
UK 2	No	No	No	Yes	No	US

Source: Survey to musculoskeletal tissue establishments (2014)
*L = living donors, D = deceased donors

All over the European Union musculoskeletal tissue grafts are imported from the US. There are two major reasons for the import of these tissue grafts:

- There is a shortage of tissue donors in the EU, compared to the demand in different EU Member States. EU Tissue establishments, through alliances with US tissue establishments, but also orthopaedic companies registered as tissue establishments, strive to cover the shortage.

- Many tissue establishments in the US have a surplus of tissue grafts. The possibility to export tissues to other countries enables them to reduce their surplus stock while obtaining a better financial coverage of the costs they incurred to recover and process the donations or increase their profits if they work on a 'for-profit' basis.

For the purpose of this study, two US (non-profit) tissue establishments have been willing to provide data with respect to their export to the EU in 2012 and 2013.

Table 14. Export of musculoskeletal tissue from US to EU in units

	US TE 1		US TE 2		Total	
	2012	**2013**	**2012**	**2013**	**2012**	**2013**
Bone tissue	4,857	5,572	2,894	2,806*	7,751	8,378
Soft tissue	813	1,070	38	52	851	1,122
DBM	12,156	12,265	1,333	2,318	13,489	14,583
Total	17,826	18,907	4,265	5,176	22,091	24,083

Source: Survey to musculoskeletal tissue establishments (2014)
*US TE 2 indicated that, because of a change in regulations in Italy, there has been a temporary decrease. In 2014 the level of bone tissue export to the EU was 4,286

This table reflects the increase (by 9%) of the number of musculoskeletal tissue grafts imported in the EU from 2012 to 2013. Both organisations reported an intention to strive for further growth. The table gives a breakdown of three different categories of tissue grafts:

- Bone. Unfortunately no particular information was received for a more detailed overview of categories (chips, struts, intercalary etc.). The US sources confirmed that more than 50% of the bone tissues were cancellous chips, since this specific graft is mainly used for hip revisions.

- Soft tissue. This includes primarily patella and Achilles tendons. In recent years an increase of tendon grafting has been observed in sports medicine (Leys et al, 2012; Persson et al, 2014).

- DBM (demineralized bone matrix). The result of the research for the report into the exchange of musculoskeletal tissue shows a mixed picture. Looking at the substantial import form the United States, it would be possible to conclude that there are general and specific shortages in the EU. General shortages refer to the number of donors for replacement tissues. Specific shortages refer to 'products' such as DBM, which are in general not produced by European tissue establishments. Clearly, also in the area of distribution of bone tissue grafts, the dependency of the supply from the United States (25% and growing, in relation to the total number of allografts distributed (tables 13 and 14) is noted.

Skin grafts

Clinical application

Skin tissue grafts are mostly used to cover chronic wounds or burn wounds. Skin consists of a thin outer layer called epidermis, and a thick inner layer called dermis. The benefit of a skin graft is that it forms a natural layer (as opposed to bandages) over the exposed tissue of the recipient. The term 'biological bandage' is often applied to skin grafting.

Thus, donor skin prevents the recipient from drying out, but skin grafts also protect the patient against the ingress of microbes. Additionally, human donor skin can form a scaffold for the recipient's newly generated autologous skin. Autologous skin is harvested and applied in one procedure and used for limited covering. Large burn wounds are covered with allografts.

In second degree burn wounds a significant reduction in pain can be achieved very soon after grafting of the donor skin. In the first instance the donor skin is adherent and remains in place like a supple scab. In general, a very rapid high-grade epithelialization takes place under the skin grafts, after which the dried-out scab comes loose.

Both in second and in third degree burn wounds, the donor skin can be perforated (meshed) before being grafted. The advantage is that a larger surface of the recipient can be covered. In third degree burn wounds, after operative removal of the necrotic tissue, the wound is, if possible, covered with an autograft. This graft can then be covered with donor skin (so-called sandwich graft). This provides a covering for the areas of the wound left exposed by the distribution of the autograft. Donor skin also promotes rapid epithelialization (Verween et al, 2012).

The standard treatment of burn wounds by applying (donor) skin does not often lead to acceptable functional and cosmetic outcomes and leads to development of scar tissue and skin contractions. By growing skin cells (keratinocytes) in vitro to be applied with a meshed split skin graft, the burn will heal faster with less scarring. Although a well-established process in many tissue establishments, this is now classified as an ATMP with significant cost implications associated with achieving marketing authorisation as a medicine.

In chronic wounds as well as in burn wounds, glycerol-preserved donor skin has a cleansing and granulation-promoting effect on the wound bed. Significant pain reduction is an important side effect. Once the donor skin adheres to the wound bed, the latter is suitable for auto grafting.

Other clinical applications of skin grafting are reconstructive and cosmetic plastic surgery.

Activity level in the EU

In 2012 there were 57 authorised skin tissue establishments within the EU. Table 15 shows an overview of donation and distribution of skin tissues.

Based on the EUROCET 2013 report, 80% of the distribution would take place in the own Member State. Unfortunately the 2013 EUROCET report has no data with respect to distribution activities from the Netherlands. In an interview the Euro Skin Bank indicated that for distribution in the own Member State about 20-25 donations per annum would be necessary. This means that more than 90% of the donor tissue, equivalent to about 1 500 000 cm^2, from the Netherlands is distributed to other Member States and to third countries. In the same interview it was stated that procured skin from all donations in Bulgaria is processed in the Netherlands and from there distributed by the Euro Skin Bank (Interview 2014).

Table 15. Skin graft donations, distribution, import and export

	Donations	Total Distributed	Distributed in own MS	Cross-border to other MS	Export to third country	Cross-border from other MS	Import from third country
BE	106	198427	198427				
BG	166						
CZ	44	926		1019			
DE	26	35500				2	
FI	32	0					
FR	370	295905					
HR	8	1250					
HU	4	0					
IT	339	845017	845347			330	
LV	0	0					
LT	0	0					
LU	0	0					
NL	495	0		8099	1952		
PL	39	65513	65513				
PT	1	9193	10067			74	
RO	4	500	500				
ES	94	214711	214691	20			
SE	83	0	0				
SK	3	19310	19319				
UK	0	1225					1225
Total	1822	1684252	1353864	9144	1952		1225

Source: EUROCET 2012. Please note that total numbers don't add up as not all countries reported.

Procurement, processing and storage of skin

Deceased donor skin grafts are procured after shaving, washing, disinfecting and oiling the skin of the back, side and legs up to 24 hours after death. With an electric dermatome, a layer of 0.2 to 0.8 mm can be removed. Samples should be uniform of thickness and preferably have large dimensions, suitable for treating patients with major burns. Procurement takes place in mortuaries or operating theatres. If musculoskeletal tissue is also donated, it is preferred that skin is procured first.

The retrieved skin is placed in transport medium (e.g. glycerol solution) and transported in refrigerated containers to the tissue establishment for processing. Alternatively, skin can be placed on sterile gauze soaked with saline solution.

Skin from living donors after abdominoplasty operation is procured to obtain full thickness skin grafts for the production of de-epidermilised dermis. The tissue, complete with adipose layer, is stored in sterile containers for transport to the tissue establishment.

The processing of skin takes place in cleanrooms. Every skin tissue establishment uses specific graft processes, according to standard operating procedures and mandatory regulations, and depending whether sterilised or non-sterilised grafts are processed.

Fresh skin grafts and skin for cryopreservation are processed immediately to maintain cell viability (Leung 2009). After receipt, the skin is decontaminated and processed into fresh, glycerol-preserved, cryopreserved or freeze dried allografts. Glycerol preservation, a method in which the skin is preserved in glycerol. As glycerol has virucidal and bactericidal characteristics, the skin can be processed in a laminar flow cabinet (Grade A) placed in a Grade D cleanroom. By using the glycerol method, the skin matrix is kept intact while cells are not viable. With cryopreservation all cells for the donor skin are kept viable. To apply this method of conservation, processing must take place in a Grade A flow cabinet placed in a Grade D clean room. When skin cells are preserved by using cryopreservation, their function is maintained after grafting on the recipient. A disadvantage is that the recipient may undergo an immunological reaction and reject the allogeneic skin. Both glycerol preserved skin and cryopreserved skin are only temporary grafts; the final closure of the wound always takes place with autologous skin.

Freeze dried and glycerol-preserved skin can be stored at room temperature. Glycerol-preserved skin is a non-viable skin-graft that is considered a safe product due to the antibacterial/antiviral properties of high concentrations of glycerol and is less immunogenic than cryopreserved skin. It is used in partial thickness burns and other types of skin loss, or within the sandwich grafting technique, in which meshed human skin allograft is used as an overlay over widely meshed autograft (Vloemans et al, 2002).

Fresh skin has a safety risk, as donor screening and microbiological testing is mostly not completed. Therefore most physicians prefer cryopreserved skin to fresh skin.

Also the ease of storage and availability is an advantage of cryopreserved skin. Cryoprotectants such as glycerol or dimethylsulfoxide are used to maintain cell viability during cryopreservation. Despite this procedure, cell viability is negatively affected by cryopreservation in comparison with fresh skin.

Depending on the recipient's wound site, allogenic donor skin can be grafted as "full thickness" as well as "split-thickness". A full thickness grafts consists of the entire epidermis and a dermal component of variable thickness if the entire thickness of the dermis is included (Wax et al, 2015). The interaction of the autologous dermal and epidermal cells ensures a secure secretion of chemokines, growth factors and cytokines into a non- healing wound bed. Wound reactivation and re-epithelialization occurs through the direct secretion and cover of the wound bed (A-skin, Amsterdam). A split-thickness skin graft (STSG) is a skin graft including the epidermis and part of the dermis (Barret-Nerin et al, 2004).

By making openings (meshing) in the graft, the recipient cells are able to "bridge" the gaps toward the allogeneic graft. Thus, the wound site heals by re-epithelialisation from the dermis and surrounding skin and requires dressings.

For many decades, skin banks have also cultured autologous keratinocytes for burned patients. An autologous skin biopsy is taken and cells are cultured during some weeks to form skin sheets. These are often grafted together with allogeneic skin on burn wounds and chronic wounds. They are not rejected and stimulate the wound bed to provide faster healing and definitive coverage of the wound with less scars and contractions.

This method has advantages and disadvantages. The advantages are that, specifically in autologous grafts, the immunological reaction of the recipient is avoided. When used as allograft, cultured skin may function as temporary dressing releasing growth factors that can stimulate wound healing. When skin culturing could be done on a large scale, the necessity to recover skin from deceased donors would disappear. The consequent advantage would be that the extended logistics to maintain a 24/7 donor recovery team and associated costs would become obsolete.

The disadvantage of the autologous skin culture techniques is the time lapse between the start of the culturing process and the final results, which can be at least several days. In urgent situations, such as third degree burn wounds, cultured skin can't bring immediate relief. The second disadvantage is that the laminar flow cabinet (Grade A), in which the culturing (both autologous and allogeneic) takes place, must be placed in a clean room Grade B. The investment and maintenance costs may be 60% higher than when a Grade D background is applied (like for deceased donor skin processing). However, many skin banks anyhow process cryopreserved skin in a grade A/B environment. Also the maintenance costs of a Grade B clean room are considerably higher.

Although a well-established process in many tissue establishments, skin culture techniques are now sometimes classified as an advanced therapy medicinal product (ATMP). The classification of these two products as ATMPS implies significantly higher costs due to investment in obtaining a marketing authorisation as an ATMP (Pirnay et al., 2013).

Cost structure

For the purpose of this study, two skin TEs were investigated in depth: Human Cell and Tissue establishment, Queen Astrid Military Hospital, Brussels, Belgium, and the Euro Skin Bank, part of the Euro Tissue Bank Foundation in Beverwijk, The Netherlands.

Euro Skin Bank is the larger. Annually, 400-500 donors are reported to this foundation, providing about 1 700 000 cm2 of skin. Most of these donors originate from the Netherlands. The Human Cell and Tissue Establishment Queen Astrid Military Hospital reports about 100 donors annually, while its distribution is around 200 000 cm^2.

No specific data were available with respect to the four cost categories: Donation, testing and screening, processing and distribution. Information which was provided during the interviews for this research learned that the costs of virology tests are often shared with the tissue establishments that procure corneas and/or the heart from the same donor.

Procurement costs are estimated to be 33%, and processing costs are estimated to be about 50% of the costs (source: Euro Skin Bank).

To enable post mortem donations a well-trained team and accompanying logistics must be available. Each donation generates expenses for logistics, salary costs for procurement personnel, virology testing etc. Specifically at a post mortem skin donation it is important to procure sufficient cm^2 skin to cover those costs by the distribution of allografts after processing; For example the Euro Skin Bank indicated that they strive for 4000 cm^2 per donor to make the donation feasible.

The need for a clean room environment in which establishments usually process takes place under a class A cabinet with a background of class B.

The cryopreservation method is used by some skin tissue establishments, while a glycerol preservation method is used in others. Both methods guarantee a shelf life of several years. However, the investment in hardware for cryo-preservation and to safeguard preservation, the levels of liquid nitrogen must be constantly monitored and kept on a level sufficient to keep the temperature of the tissue grafts at an adequate level.

The culturing of skin requires surgical procurement techniques from the living donor. After a culturing process which takes place under class A clean room conditions, and which may take several weeks, the cultured cells are placed into the wound bed of

the recipient. Again, this takes place under class A conditions. Although calculations couldn't be provided, this entire process results in higher costs per cm^2 than other skin grafting methods.

Fee structure

From the interviews for this research, it was reported that the following fees for glycerol preserved skin to the end-user per cm^2 were applicable in 2014: Belgium EUR 1.39; Netherlands EUR 1.20 (when exported, e.g. to Belgium, the fee is adjusted to the local price in Belgium: EUR 1.39).

Cross-border exchange and import /export of musculoskeletal tissue

Since distribution of tissue grafts is done all over Europe, the Euro Skin Bank developed and maintains several donor sites over the continent. Thus, the organisation strives for a balance between national and international activities. In 2012 a total of 1 125 000 cm^2 was distributed by the Euro Skin Bank, of which 11% was used nationally, 71% was sent to other Member States and 18% was exported to third countries.

Of all tissues distributed, only 13 units of cultured skin were produced and transplanted (TRIP Annual Report 2012).

Research for this report provided little information about the need for donor skin in the EU Member States. The table below shows the national need in five Member States.

Table 16. Need for donor skin in EU Member States

	Year	cm^2/year	cm^2 per 106 inhabitants
Belgium	2011	221,040	0.02
Croatia	2012	295,905	0.06
France	2012	2,960,000	0.04
Netherlands	2012	123,750	0.008
Poland	2012	601,000	0.01

Source: Economic landscape survey NCATC (2015), data over 2012

The number of tissue donors in the Netherlands is more than sufficient to cover the demand (495 in the year 2012, with an estimated yield of 1,980,000 cm^2). The low volumes of distributed skin in the Netherlands is due to the fact that, compared to other Member States, there are relatively few severe burns in that country.

Amniotic membrane

The amniotic membrane or amnion is one of the foetal membranes and is used for various applications in ophthalmic surgery as well as for chronic ulcers, skin burns

and other skin wounds. Amnion can be used fresh (Adds et al, 2001) but is mainly cryopreserved or freeze dried and stored (TRIP Annual Report, 2012, Biovigilance;).

Within the EU there were 18 authorised tissue establishments for procurement, processing and distributing amnion, often linked to a cornea tissue establishment.

Pancreatic Islets

Langerhans' islets beta cells can be prepared from donor pancreas. These cells are injected into the liver of a patient suffering from diabetes; the beta cells will synthesise insulin. At this point the procedure is only indicated for patients with type 1 diabetes who have severe complications. In the future, it can be extended to patients with fewer complications.

In order to produce grafts composed of these cells, human donor Langerhans' islets are provided by organ donor centres. Since the recipients are mostly in need of a kidney transplant as well, islet cell donation and transplantation is often considered to be part of the organ transplant network. The processing of grafts, however, is part of the tissue banking framework from a regulatory point of view. After receiving a pancreas in the tissue establishment, grafts with selected size, composition and function are produced.

The biological properties of beta cell grafts should be characterized and adjusted to the metabolic and immunological status of the recipient. This type of graft should increase the efficacy of accompanying measures aiming at graft survival and/or metabolic correction (Gaba et al, 2012).

The supply of Langerhans' islets to use for human beta cell grafting is insufficient. To produce a graft which matches the recipient's requirements, at least one organ is needed. Cases of up to 5 organs being used to process one beta cell graft have been reported (Qi et al, 2009).

From the economic landscape survey to NCATC (2015), Poland reported 13 pancreas donations and 10 transplantations of beta cells in the year 2012. In 2012 there were 8 authorized pancreatic islet tissue establishments in the EU.

Other tissues and cells

A variety of tissues and cells can be categorised in this group, including adipose tissue, nerves and hepatocytes. Autologous adipose tissue is sometimes used in plastic and cosmetic surgery, and was identified as a source of pluripotent stem cells (up to now only in experimental therapy (TRIP Annual Report, 2012).

Nerve tissue can be transplanted when nerves are damaged by trauma or resection and to reduce neuropathic pain. Preferably autologous tissue is used but this is not always possible. In those cases an allogeneic nerve graft can be transplanted. Nerve tissue can be radiated, freeze-dried or cryopreserved (Nunley et al, 1996; Houston, 2001).

The grafting of hepatocytes has barely left the stage of clinical experiment (Guha et al, 2000). No hepatocyte implantations were reported in the EU as part of the research survey. The donor tissue for hepatocyte grafts origins from the liver. The liver is recovered in organ donation procedures. In some European centres, the liver is provided to a US-based firm (Cytonet), which processes these livers into hepatocyte grafts.

ORGANISATION OF THE SECTOR

Tissue establishments are organised in a variety of different legal structures. The table below contains an overview of the total number of tissue establishments per tissue group and a division in public and private establishments for replacement tissues.

The majority of tissue establishments are (part of) public organisational structures or 'private' foundations that operate on a non-profit basis. Hospital-based tissue banks can be categorised as public or as private, depending on whether they are part of a publicly or privately funded hospital. Supply of tissue by for-profit, shareholder-oriented banks also occurs in Europe, although not on the scale that is practiced in the US.

Initiated by surgeons in need of certain tissues, many tissue establishments are hospital based and associated with the surgical department they supply. The majority (about 75%) of the 653 banks focus on one type of tissue (e.g. skin, bone), or one category of surgeons (e.g. ophthalmologists who need corneas and amnions). Most of the single-tissue banks are hospital-based bone banks where femoral heads are stored. The size of these single-tissue establishments is small (50-250 donations per annum) and the tissue they handle is often only distributed in the own hospital and sometimes to hospitals in the region. The cost for these activities is usually difficult to distinguish from the budget of the hospital in which they are located. Given the similarity of most of the processes, sometimes tissue banks are part of the local or national blood establishments.

Over time, some of these single-tissue establishments have developed into larger independent establishments handling a higher volume of donations. These establishments supply tissue to many hospitals on a supra-regional or national level, and may also exchange or export material internationally. Examples of these are the FBOV (Fondazione Banca degli Occhi del Veneto Onlus - Veneto Eye Bank Foundation) (>2 000 donations a year) and Euro Skin Bank in the Netherlands (>80% of skin distributed internationally).

Table 17. Number of tissue establishments for replacement tissues

	Number of TEs*	CV*	MSK*[3]	OC*	SKIN*	Amnion*	Pancr. Islets*	Other*	Multi TEs*	All TEs public**	TEs mainly public >75%**	TEs partly public and private*	All TEs private**	Unclear public or private**
DE15	154	6	119	24	2	30		41	10			x		
FR	76	14	26	17	10	15		2	16		x			
ES	74	26	38	22	13	1	4	15	29		x			
UK	65	3	45	3	2			53	41			x		
NL	33	1	27	2	1	1	1	2	5		x			
SE	30	2	18	9	3	1	1	8	7	x				
AT	28	1	7	5	1	2		24	17			x		
FI	27	1	22	3	1	1		1			x			
IT	27	5	7	14	5	2	2		7	x				
BE	20	1	15	4	3	4	1	16	7	x				
CZ	20	3	18	5	3	1			5					x
DK	18	1	12	1		1		3	1	x				
PT	11	1	3	6	1				1					x
BG	9		3	3	3									x
HU	9	2	4	1	2				1		x			
IE	8	2	2	1		1		2	3			x		
PL	8	2	4	5	2			1	6			x		
SK	7	1	3	3	2	1		2	2			x		
HR	6	1	4	1	1				1	x				
SI	6		3	1				5	2			x		
GR	5			5										x
EE	3	3	3	2	1	2			3	x				
LV	3	1	1	1	1	1			1	x				
RO	3		2							x				
CY	2			2									x	
LT	1			1		1			1	x				
LU	0													
MT	0													
Total	653	77	386	141	57	35	9	137	166	9	5	7	1	4

Sources: *EUROCET128 ** Implementation survey by DG SANTE (2013)

Public and private tissue establishments

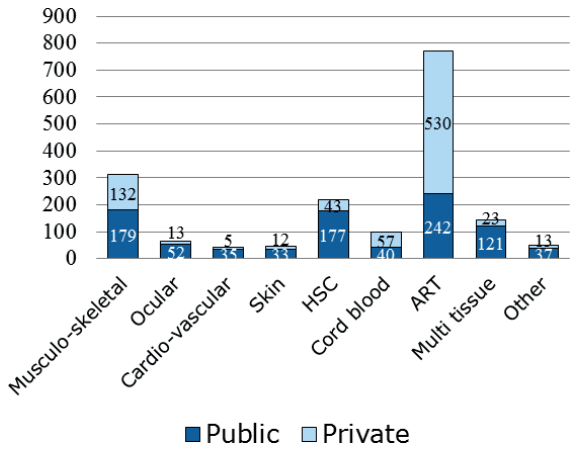
In the common law, a public organisation is an organisation that is directly or indirectly legally related to a governmental body, such as a ministry or a ministerial agency. For tissue establishments, the distinction between public and private tissue establishments is a gross one.

In the survey to tissue establishments as part of this research, tissue establishments indicated that they were either:

- Part of a public (university) hospital
- (Part of) a national health organisation
- For profit shareholder company
- Not for profit shareholder company
- Independent private foundation
- (Part of) a private hospital

From the 27 responding tissue establishments 12 (44%) reported they are part of a university hospital, being a public body, while 5 (19%) belonged to a national health organisation. 10 tissue establishments (37%) indicated that they are a private foundation. The Implementation survey, carried out in 2011, demonstrated that from 461 tissue establishments concentrating on replacement tissue activities, 299 (65%) is considered as a public establishment.

Table 18. Status of EU tissue establishments



Source: European Commission Implementation Survey (2013), data 2011

Multi-tissue establishments are in most cases active in the procurement of tissues from deceased donors, and are usually stand-alone, i.e. independent of the surgical units in the hospitals. Given the source of the tissues, some of the multi-tissue establishments are associated with pathology departments. Examples of large multi-tissue establishments include the FBTV (Fondazione Banca dei Tessuti di Treviso) in Treviso, Italy; BST Blood and Tissue bank in Barcelona, Spain; DIZG in Berlin, Germany and the NHSBT Tissue Services in Liverpool, UK.

Associations and foundations of replacement tissue establishments

Given the scattered landscape in Europe with many small establishments that operate locally and a small number of large (multi-tissue) establishments, collaborations are important in this sector. These collaborations find their origin in the limited availability of tissues on the one hand and the regular need for matching/characterisation to find grafts that meet the needs of specific recipients on the other hand. The following sections include an overview of the associations active in the field of replacement tissue banking, the international alliances between tissue establishments in EU Member States and between tissue establishments in the EU and organisations in relevant third countries.

Associations

Two main associations are active in replacement tissue banking. These are the European Eye Bank Association (EEBA) and the European Association of Tissue Banks (EATB). Both associations have been active for over two decades and concentrate their efforts on sharing of information to support endeavours to improve the quality and quantity of tissue donation and tissue banking. The EATB has been actively involved in the development of Good Tissue Practices; a set of GMP (good manufacturing practices) adapted to the specific needs of the different replacement tissue sectors. Both associations hold annual congresses to provide a forum for scientific, ethical and clinical discussions relating to tissue banking and to provide a forum for presentation of research and collaborative working. The associations contribute to formal consultations and seek to actively involve their members in all their activities.

Another organisation, the Foundation of European Tissue Banks (SEGB), was founded in Germany when new national legislation, based on the EU Tissue Directives, was introduced. The aim is to assist tissue establishments in Germany and in Europe with fulfilling legal criteria, by supporting projects focused on the improvement of quality, and to increase the number of tissue donors in Germany and elsewhere in Europe. The Foundation organises meetings focused on specific aspects of tissue banking, mainly of cardiovascular tissue establishments, as well as on the advancement of tissue donation in forensic medicine institutions.

National alliances between tissue establishments

In order to cover the need on a national level, cooperation between tissue establishments is an important tool to ensure allocation and best use of donor tissue, which is available in one tissue establishment, to hospitals in the regional working area of another tissue establishment. Such networks exist in one form or another in Belgium, France, Germany, Italy, Spain, The Netherlands and the UK. In some Member States, such as Italy and the Netherlands, there are regulatory requirements to ensure that tissue grafts originating from national donors are allocated to recipients from that Member State before export is allowed.

In the Netherlands, two large tissue establishments, BISLIFE and the Euro Tissue Bank have agreed to share with other tissue establishments all information with respect to multi-tissue donors. The donation and procurement teams for skin, corneas, cardio- vascular and musculoskeletal tissue are complementary, and procedures are synchronized. Test results for different tissues that are recovered from the same donor, are shared. The evaluation methods of test results and the process to interpret outcomes takes place through consensus between the participating organisations and the tissue establishments, that will carry out the processing of the different procured tissue types. Such shared efforts therefore have a direct cost-reducing impact.

In Germany, there are two active national networks in the field of cornea banking. One consists of a group of Lions Cornea Banks, which exchange tissues on request. This network is, as the name suggests, supported by regional Lions Service Clubs. The Lions have a specific focus on blindness and usually express their support by financial donations that enable the cornea establishments to purchase capital investment goods such as incubators or laminar flow cabinets. The other network consists of a group of cornea establishments that operate under the umbrella of the Deutsches Gesellschaft für Gewebetransplantation (DGfG) (German Society for Tissue Transplantation). This society finances, partly or entirely, mainly cornea establishments, and strives for standardising processing methods and quality management. By optimal allocation of the available tissue graft within the network of 15 tissue establishments, the use of available tissue grafts is optimised for specific patient groups and maximised in order to prevent expiration of grafts in stock.

International alliances between tissue establishments

Mainly for reasons of balancing the supply and demand in different countries, some larger tissue establishments have created alliances to provide tissue to one another, across borders within EU, but also with non-EU countries. These alliances include the following:

BISLIFE (NL) and Musculoskeletal Transplant Foundation (MTF, USA)
The cooperation is based on an agreement that came into effect at the inception of both foundations in 1989. The agreement has been renewed from time to time. Initially, BISLIFE (then BIS Foundation) distributed MTF bone and soft tissue (tendons) whenever BISLIFE couldn't provide tissues from their national donors.

Since 2010, the agreement includes processing of bone tissue from two-thirds of the deceased donors in the Netherlands at MTF's facility in the USA. After processing, the grafts are sent back and are distributed by BISLIFE (until 2010, BISLIFE had a similar processing agreement with Osteotech Inc. in the USA). Since MTF has distributors and marketing experts focused on different European countries, the agreement with BISLIFE limits distribution by BISLIFE to the BENELUX countries.

Euro Tissue Bank (NL) and the Tissue Bank of the University Hospital Brno (CZ)
The Euro Tissue Bank receives donor skin from different sources in Europe. One of these sources is the Tissue Bank of the University Hospital Brno. Donor skin is recovered in the Czech Republic. After medical screening of the donor and negative test results, the skin is processed at the Euro Skin Bank (part of the Euro Tissue Bank) in the Netherlands and distributed to different EU Member States and to third countries whenever the stock assigned for use in European hospitals allows.

Euro Tissue Bank (NL) and Tissue Bank Bulgaria (TBB, BG)
One other source for skin donation is the TBB. Donor skin is recovered in Bulgaria, though tested in the Dutch national blood bank Sanquin. After medical screening of the donor and negative test results, the skin is processed at the Euro Skin Bank and distributed to different EU Member States and to third countries whenever the stock assigned for use in European Hospitals allows.

Euro Tissue Bank (NL) and Banc de Sang I Teixits Barcelona (BST, ES)
As with TBB (above, under 3) donor skin is recovered in Catalunya and processed in Euro Tissue Bank's facility in the Netherlands. After processing, the tissue grafts are distributed to different EU Member States and to third countries whenever the stock assigned for European hospitals allows.

European Cell and Tissue Bank (ECTB, AT) and European Medical Contract Manufacturing (EMCM, NL)
EMCM uses a process, which uses critical CO2 to remove all soft tissue blood and bone marrow bone tissue, reducing viruses or other micro-organisms to the sterility assurance level of log 10^{-6}.

The ECTB sends bone tissue from femoral heads of living donors to EMCM's facility in the Netherlands. After processing, the tissue is sent back to ECTB in Austria. Superfluous tissue, not needed for Austrian recipients, is distributed in other EU Member States. This distribution is always done within the regulatory limits of the respective countries.

Tissue Bank Osteocentre Bulgaria (TBOCBG[4], BG) and Deutsche Institut für Zellen und Gewebeersatz (DIZG, DE)
Bone tissue of deceased donors is recovered in Bulgaria and processed at the DIZG in Germany. Tissue grafts are distributed in Germany, while the fulfilment of need for tissue in Bulgaria is guaranteed.

BISLIFE (NL) and Banc de Sanq I Teixits Barcelona (BST, ES)
Since the early 1990s, BISLIFE has functioned as the distributor for cardiovascular tissues of *de Banc de Sang i Teixits in Sant Boi* (near Barcelona). Tissue grafts are imported mainly into the Netherlands and Germany.

In 2015, the relationship was extended to the processing of bone tissue. Donor tissue from the Netherlands is shipped to BST for processing, after which the tissue is returned to BISLIFE for distribution in Europe.

BISLIFE (NL) and DIZG (DE)
Bone tissue of deceased donors is recovered in the Netherlands, one third is shipped to DIZG and, after processing, distributed by DIZG mainly in Germany.

DIZG (DE) and MTF (US)
DIZG is a full subsidiary of MTF through Biocon Corporation, its controlling affiliate. DIZG and MTF exchange (patented) knowledge on processing techniques for processing of tissue including bone and dermis and MTF provides DIZG with base material with the help of US procurement partners.

European Homograft Bank (EHB, BE)
The European Homograft Bank (EHB) is a non-profit association according to the laws of Belgium. The association operates a cardiovascular tissue establishment, and has international members throughout the EU. The members provide donor tissues to the bank. In return they receive processed tissue grafts. The EHB has been instrumental in setting up a cardiovascular tissue establishment in Zagreb (HR) which uses the same methods as the EHB Brussels facility.

University Tissue Bank Leuven (BE) and France
The University Tissue Bank has a regular contact with French tissue banks in which demand and supply are balanced in case there are shortages in either Member State.

Tutogen Medical GmbH (DE) and RTI (USA)
Tutogen Medical GmbH is a subsidiary of RTI (formerly Regeneration Technologies International). The acquisition took place in 2008. It is known that in the past, Tutogen processed donor bone tissues procured in Estonia, Latvia, Czech Republic, Hungary and Slovakia. In 2012 Tutogen terminated import from tissues from deceased donors from Ukraine. Tutogen distributes the final tissues to Europe and exports them to the USA.

Tutogen and TBB (BG)
TBB provides bone tissue to Tutogen. TBB declares that these donations take place according to the legislative rules and regulations of Bulgaria. No other relations are known.

This list includes some well-known alliances in the sector, but is not exhaustive.

FUTURE PERSPECTIVES IN REPLACEMENT TISSUES

Cardiovascular tissues
Although a few years old, the surveys regarding the use of cardiovascular tissue allografts show no increasing trend in the number of implantations (Foundation of European Tissue Banks, directories of European Cardiovascular Tissue Banks, 2012, 2013). Several meetings of cardiovascular tissue bank experts discussed these trends and their probable causes (consensus meeting 2011). Our recent surveys confirm this finding. The reasons are three:

- The improvement of mechanical (industrially produced) valves and bio- prostheses;
- The limited availability of cardiovascular allografts has lead surgeons to use alternatives;
- The so called Ross operation, in which the own pulmonary valve of the congenital heart disease patient is placed in the aortic position and a pulmonary allograft is placed in the place of the relocated pulmonary graft of the patient, is considered to be extremely difficult. Given the alternatives, residents seem to be less commonly trained to perform the Ross operation. As a result, the use of human valves is reduced.

Alternatives for allograft transplantations are improving, their availability is increasing compared to allografts, and fewer surgeons are trained to apply the complicated transplant procedures; as such it is expected that the number of cardiovascular graft transplants will remain low (1 to 2% of all valve replacements) and even diminish in the coming years. However, there is a continuing demand for human valves for young children to avoid multiple replacement operations as the child grows.

There is a trend to research the possibilities and the long-term effect of decellularisation of homograft valves. The theory is that decellularisation diminishes the immune response of the tissue recipient and would allow the colonisation and growth of host tissue on the graft in vivo. Decellularisation is so far undertaken by a small number of tissue establishments and might be a development that can add to the success of cardiovascular tissue in the future. Long-time results are not yet known but a number of clinical studies are underway and some results are promising (Da Costa et al, 2010).

Cardiovascular tissue can be decellularised and cultured with autologous stem cells to create tissue engineered valves. There is currently no authorised ATMP product of this kind on the market but if one were to be developed and approved it might have a significant impact on the need for cryopreserved heart valves, possibly replacing them entirely at some time in the future (De Jonge, 2013).

In conclusion, it is expected that the use of traditional human cardiovascular tissue allografts will slowly decrease over the next 5 years. Yet the application of homografts will not entirely disappear, especially not for young patients. Research into methods for decellularisation and tissue engineering of both heart valves and vessels may result in grafts with a better recipient compatibility and extended survival as compared to those preserved with traditional methods, and therewith into a new increase of demand.

Ocular tissues

Given the ageing population in the EU, there will be a gradual increase in the demand for corneal tissue. No innovative scientific developments seem to be promising short term alternatives.

What can be observed, however, is the increase of the use of lamellar tissue grafts. These grafting procedures, contrary to the traditional penetrating keratoplasties (PKPs), reduce surgical scarring and the complications of immunological reaction. It is expected that the number of anterior lamellar implants will, because of poor long-term results, be considerably reduced, or almost disappear. At the same time, the posterior lamellar implants are not only replacing the performance of PKPs, they also enable treatment of patients that were not suitable for PKP, and thus increase the demand for cornea donations and grafts.

One side effect for the tissue establishments of these shifts is an increase in costs. The equipment to process a cornea into a lamellar graft, and the instruments used, are still in development. Demands for greater precision using new techniques will require investments at tissue establishments.

A more recent addition to the options to treat corneal damage (especially with limbal deficiencies in cases of burns to the eye), comprises the culturing of limbal stem cells into cell sheets that can be transplanted into the damaged limbal region of the eye. Rama et al. reported long-term corneal regeneration using autologous cultivated limbal stem cells (CLET). They showed that permanent restoration and a renewal of the corneal epithelium were achieved in 76.6% of 107 damaged eyes (Rama et al, 2010). Many centres are providing these cells with good results in the hospital and TE setting (EEBA 2015), while one such technique has been authorized as ATMP in 2015 (Holoclar, see also EMA 2015).

Musculoskeletal tissues

With the increased possibilities presented by alliances between several tissue establishments in the European Union to complement each other's activities and to increase efficiency by making use of investments made, one would expect an increase in the exchange of processed bone grafts within the EU.

These European alliances though, will have to be able to compete with other EU tissue establishments, which have, in their turn, an existing alliance with one or more tissue establishments in the United States. As the latter make use of orthopaedic distributing firms and sales representatives in their working areas, they will certainly remain a very important factor for providing musculoskeletal tissues in the EU.

As the exchange and distribution by the aforementioned organisations increases, a reduction in the number of local or regional bone tissue establishments may be expected, as these cannot cope with the increased needs for investment such as in GMP-level facilities, quality management, software development, training of employees and innovation. Performance under a critical mass of an estimated 150 post-mortem, or 1,000 living (femoral head), donations per year is no longer sustainable[5]. As a result, the number of tissue establishments is likely to decrease.

In the next 5 years, the quantity of exchanged and distributed musculoskeletal tissues is expected to grow. Tissue establishments continue to demonstrate the superiority and safety of allografts over alternatives such as bovine bone products (Amini et al, 2012).

While technologies such as, for example, 3D printing of materials which could be equal, or better, than the present tissue grafts, have a long way to go before they can offer an affordable alternative for human tissue allografts, they do not present a viable alternative to tissue grafts in the short term. Specifically for tissue grafts such as tendons, there is no alternative to human tissue on the horizon.

What will be seen in the next five years is the increase of combinations of allografts and medical devices and pharmaceuticals (such bone tissue impregnated with antibiotics or growth factors).

Skin

In a five-year forward looking assessment of the present techniques, it is expected that skin grafts will remain the first choice for patients with burn wounds and other dermatological diseases which require skin grafting. Yet, there will be a shift toward autologous skin culturing either as an alternative, but mostly in addition to grafting of allografts. In the next 5-10 years, it is likely that grafts of autologous cells will be used to complement meshed allografts by placing cultured autologous grafts in the openings of the allografts. The use of dermis (as a decellularized skin graft), which enables enhanced return of the recipient's epidermis at the wound site, is a trend that is already evident.

Further increase in its application is to be expected.

CONCLUDING REMARKS AND SUMMARY REPLACEMENT TISSUES

Category	Cardiovascular	Ocular	Musculoskeletal	Dermatological
Indications	Congenital, endocarditis, & alternative for mechanical valves	Idiopathic cell loss, cut- & burn-accidents	Bone loss, trauma, cancer, tear & break of tendons	Burns, reconstructive surgery
Tissue types	Heart valves (vascular graft)	Cornea (Sclera)	Bone Demineralised Bone Matrix (DBM) Tendons	Skin grafts
Donors	Deceased	Deceased	Deceased + Living	Deceased
Main costs	Much discard	Much discard, easy expiry	High procurement costs with high yield for DD, end-irradiation reduces processing costs	Easy procurement, some storage cost
Average price/fee (min-max) reported by sample of TEs	2600€ (950€-5250€) for a heart valve	1420€ (250€-3500€) for a cornea graft	520€ (340€-900€) for a 30CC cancellous chip	1.2-1.4€ per cm^2 skin
Tissue establishments	77, small scale, mainly public	141, small scale, mainly public	386, half are private, from local to large international scale	57, mainly local and public, few large international player
Import, export and cross-border exchange	I/+, E/- Informal networks for cross- border exchange	I/+, E/+ Informal networks for import/export	I/ +++, E/ - International partnerships	I/ - E/ - Some international partnerships

Replacement tissues allow to replace some damaged tissues and therewith their biological functions. The majority of EU tissue establishments focus on four categories of replacement tissues: cardiovascular, musculoskeletal, ocular and skin.

In addition there are 181 tissue establishments authorized to process other tissue grafts like amniotic membrane or pancreatic islets, and 166 to process multiple tissue types.

Price comparisons for the different types of replacement tissues reveal strong difference between Member States. These differences do not reflect real costs made, but rather are a consequence of different factors including the price-setting process, policy objectives (non-commercialisation, self-sufficiency), the relative high presence of public tissue establishments, limited cost-awareness and intra-EU differences in salaries and purchasing power.

The absence of a single EU 'market' for tissues and cells is in particular true for the replacement tissue sector and driven by some additional thresholds at national borders:

- Several Member States have set-up prior authorization schemes for export and (outward) cross-border exchange, in order to ensure local supply and self- sufficiency.

- Many Member States require additional national requirements on safety and quality including donor screening, testing or processing (in addition to EU legal requirements). These are not always clear for tissue establishments.

- Some Member States apply national legal frameworks for pharmaceuticals or medical devices on replacement tissues, which can for example lead to the need for prior administrative authorizations to supply tissues to that Member State.

- Administrative procedures in different Member States are to be addressed in different languages.

- Fees are set at different levels by different national authorities, often reflecting a more general policy objective (non-commercialisation, self-sufficiency).

- Some Member States recently changed VAT taxation policies for tissues and cells distributed within their borders, which impacts the fees to be charged.

All these factors hinder a free distribution of grafts across borders of EU Member States. Also, only few Member States have an overview of the distribution and cross-border exchange of different tissue grafts. More importantly, these thresholds also lead to situations where surpluses in some EU Member States exist in parallel to shortages in other EU Member States. Eventually, clinicians/surgeons cannot access the optimal replacement tissue graft matched to their patients' individual needs.

- Overcoming these thresholds at the border, and supplying tissues to multiple countries, requires dedicated regulatory know-how, which is expensive and usually not available for small-scale, public-funded tissue establishments with limited resources. This prevents many EU tissue establishments from supplying hospitals/ clinicians in a larger area and from growing their activities. This puts an important limitation to their development as, for reasons of efficiency a larger scale of activities might be increasingly needed to obtain economies of scale, and eventually to break-even or remain profitable.

- Over time, a few larger tissue establishments in the EU have built alliances cross borders to overcome these barriers. Several of them are described in the report. These agreements allow for more efficient procurement, processing or distribution.

In addition, some US-based tissue establishments seem to be able to overcome that barrier as well. They are usually private and well-funded, sometimes even through public listing on the financial markets, and therewith can acquire the resources and know-how to overcome these barriers and supply multiple EU countries. High quantities of surplus musculoskeletal tissues, some corneal grafts and specific sizes of heart valves, are exported to, and distributed into Europe. They often do this by building partnerships with or acquiring major EU- based tissue establishments to do so. This can raise some competitive challenges for the local/smaller-scale establishments. It also brings a need to verify equivalency of safety and quality of the imported substances and to reflect on EU self-sufficiency and dependency on imports.

- Exports out of the EU to third countries seem to be limited. It mainly concerns cornea grafts, which have a limited shelf life and are therefore rather exported than expired, usually during seasonal holiday periods in the EU.

- When it comes to the long term continuity of individual tissue establishments in Europe, there are signs that, given the fact that many of them are small entities, there may be a consolidation or shake-out of tissue establishments in the next five years. It is expected that not all of the small entities will be able to continue processing and supplying a critical mass of tissues large enough to cover the increasing costs of regulatory quality requirements, and many of them may therefore not be able to survive.

- A strengthened collaboration between Member State authorities to facilitate cross- border exchange of tissues within the EU might therefore be a key factor for success for the future of many EU based (replacement) tissue establishments. The fragmented availability of activity data and the need for a common vision on optimal demand and supply for replacement tissues at EU level are priorities to be addressed.

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1 For the import of tissues and/or certain tissue preparations an import permit pursuant to Section 72b (1), AMG, from the competent regional authority is required. Also, a certificate from the state of origin pursuant to Section 72b (2), AMG, that confirms that the standards observed during the extraction and processing of the tissue/tissue preparations are at least equivalent to the standards of good practice laid down by the European Union (Good Manufacturing Practice - GMP). Pursuant to Section 21a (9) of the AMG, a certificate from the Paul-Ehrlich-Institut is required for first introduction of tissue preparations from Iceland, Liechtenstein or Norway, to Germany.

2 DMEK = Deep lamellar endothelial keratoplasty

3 DE authorities report 614 sites for MSK, however these do include the procurement sites for chondrocytes

4 Note TBOCBG and TBB are different organisations

5 Internal business plan of BIS Foundation, based on 10 years of procurement, processing and distribution of musculoskeletal allografts, 2004

CHAPTER 11

Cardiovascular Tissue Banking in Europe

De By TMMH, Parker R, Delmo Walter EM, Hetzer R.

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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[1,2,5,6-13,15]. The use of these allografts and the number of cardiovascular tissue banks and their respective techniques increased[3,4].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.

Materials and Methods: In 2011, the Foundation of European Tissue Banks collected data from 19 different cardiovascular tissue banks in 11 European countries.

Results From 2007 to2010 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.

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 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.

MATERIALS AND 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.

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 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 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 4° C to 37° 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 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.

CONCLUSIONS

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-year period.

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 view-points 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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TABLES

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

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

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	Antibiotics: concentration	Duration of culture	Temperature	mg/L	Medium
Barcelona, BST	Cefoxitin	240µg/mL	24hrs	5ºC (2-8ºC)	240	
	Vancomycin	50µg/mL			50	
	Polymyxin B	120µg/mL			120	
	Clindamycin (Lyncomicin)	100µg/mL			100	
	Amphotericin B	5µg/mL			5	
Barcelona, TSF	Penicillin	50 U / ml	24 hrs	5o C (+/- 3oC)	50 U	
	Vancomycin	50 µgr /ml			50	
	Streptomycin	50µgr /ml			50	
	Amphotericin B	10µgr /ml			10	
	in medium 500 ml RPMI	w/o L-glutamine				RPMI
Bad Oeynhausen	Mefoxitin	0.024% (m/V)	18-24 hrs	6o C	240	
	Lincocin	0.012% (m/V)			120	
	Colistin	0.0099% (m/V)			99	
	Vancomycin	0.005% (m/V			50	
Berlin	Amikacin	1.2mg/2ml Syringe	18-24 hrs	5o C (+/- 3o C)		
	Metrodinazol	1.2mg/2ml,				
	Flucytosin	3.0mg/2ml,,				
	Vancomycin	1.2mg/2ml,,				
	Ciprofloxacin	1.2mg/2ml,,				
Bristol	Amphotericin	0.05mg/ml	21-24 hrs	22o C	50	
	Ciprofloxacin	0.20mg/ml			200	
	Vancomycin	0.05mg/ml			50	
	Gentamicin	4.00mg/ml			4000	
	in Hanks' BSS					HANKS
Brussels	Lincocin	120µg/ml	48 hrs	4o C	120	
	Vancocin	50µg/ml			50	
	Polymixine B	124µg/ml			124	
	in medium 199					M199
Cracow	Gentamicin	100mg/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	

Valve bank	Antibiotics	Antibiotics: concentration	Duration of culture	Temperature	mg/L	Medium
London	Cefuroxime	250ug/ml	24 hours	37oC	250	
	Gentamicin	80ug/ml			80	
	Ciprofloxacin	200ug/ml			200	
	Vancomycin	500ug/ml			500	
	Colistin	1000 IU/ml			1000 UI	
	Amphotericin	100ug/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	250ug/ml	24 hours	5oC (+/- 3o C)	250	
	Ketokonazol	100ug/ml			100	
	Colistin	200ug/ml			200	
	Vancomycin	500ug/ml			500	
	Gentamicin	500ug/ml			500	
Milano	Polimyxine B sulphate	100 µg/ml in RPMI1640 medium	24 hours	4°C	100	
	Vancomycin	50 µg/ml i n 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 - 30o C	1000	M199
	Cefuroxime	500mg/L			500	
	Vancomycin	1g/L			1000	
	Timentin	3.2g/L			3200	
	Polymixin B	10,000,000iu/L				
	Nystatin	1440,000iu/L				
Paris	Vancomycin	500mg/L	18/24 h	4°C	500	
	Gentamicin	320mg/L			320	
	Clindamycin	600mg/L			600	
	In RPMI medium					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	

Valve bank	Antibiotics	Antibiotics: concentration	Duration of culture	Temperature	mg/L	Medium
	Amphotericin B 0.1 for NHBD in medium 199	0.1			100	
Rotterdam	Amikacin (as sulphate)	0.6 mg/mL	5-6 hours	37oC	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 di medium RPMI 1640	72 hrs	+ 4° C	100	RPMI
	Polimyxine	100 mg/ml (= 1 Mio mIU/ml) di medium RPMI 1640			100	
	Ceftazidima	240 mg/ml di medium RPMI 1640			240	
	Lincomycin	120 mg/ml di medium RPMI 1640			120	
Warsaw	Tazocin (Piperacillin/ Tazobactam)	0.5 mg/ml	24 hrs	20o C (+/- 2o C)	500	
	Gentamicin	0.05 mg/ml			50	
	Nystatin	2 500 j./ml				
	Vancomycin	0.5 mg/ml			50	

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

CHAPTER 12

Validation of microbiological testing in cardiovascular tissue banks: results of a quality round trial

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ABSTRACT

Objectives: Surgeons needing human cardiovascular tissue for implantation in their patients, are confronted with cardiovascular tissue banks using different methods to identify and decontaminate micro- organisms. To elucidate these differences, we compared the quality of processing methods in 20 tissue banks and 1 reference laboratory. We did this to validate the results for accepting or rejecting tissue. We included the decontamination methods used and the influence of antibiotic cocktails and residues with results and controls. The minor details of the processes were not included.

Methods: To compare the outcomes of microbiology testing and decontamination methods of heart valve allografts in cardiovascular tissue banks, an international quality round was norganised. Twenty cardiovascular tissue banks participated in this quality round. The quality round method was validated first and consisted of sending purposely contaminated human heart valve tissue samples with known micro-organisms to the participants. The participants identified the micro-organisms using their local decontamination methods.

Results: Seventeen of the 20 participants correctly identified the micro-organisms organisms; if these samples were heart valves to be released for implantation, 3 of the 20 participants would have decided to accept their result for release. Decontamination was shown not to be effective in 13 tissue banks because of growth of the organisms after decontamination. Articles in the literature revealed that antibiotics are effective at 36°C and not, or less so, at 2–8°C. The decontamination procedure, if it is validated, will ensure that the tissue contains no known micro-organisms.

Conclusions: This study demonstrates that the method of sending contaminated tissues and assessing the results of the microbiological cultures is an effective way of validating the processes of tissue banks. Only when harmonization, based on validated methods, has been achieved, will surgeons be able to fully rely on the methods used and have confidence in the consistent sterility of the tissue grafts. Tissue banks should validate their methods so that all stakeholders can trust the outcomes.

INTRODUCTION

Since the founding of the heart valve bank at the National Heart Hospital in London[1], the practice bywhich cardiovascular tissue banks receive cardiac human donor organs and tissues from deceased orliving donors has persisted for more than 50 years[2,3]. The demand from cardiac surgeons for safe tissue grafts of consistent quality for implantation in their patients, has been the impetus for continuity among cardiovasculartissue banks. Whereas graft quality in general is guaranteed by directives and legislation4, the processingmethods of tissue banks often differ considerably. Such is the case in cardiovascular tissue bankprocesses[5].

In short, the practical tissue banking process is as follows: The donor source of the received cardiac tissuesis screened for transmittable diseases and contraindications for using these tissues for implantationinto human recipients[6]. When no contraindications are present, the tissues are treated and tested formicrobiological sterility, processed and stored (with a final sterility control) until needed fortransplantation.

Subsequently, implantation of tissue grafts takes place in the operating theatres of the recipients' hospitals. In each of the processes, there is a risk that microbiological contamination may occur[7]. The idea for a quality round, whereby cardiovascular tissue banks compare results of microbiological tests during their tissue processing procedures, finds its origins in the fact that microbiological decontamination protocols in European tissue banks differ significantly, partly due to differences in endemic microflora and patented processes. Even when these methods have been validated, the results may differ[8].

Organising quality rounds, in which standardised samples are sent to different laboratories for testing and resulting comparisons, is an accepted method to measure the quality of the outcomes[9,10].

This study provides an inventory of methods of 20 participating cardiovascular tissue banks worldwide, most of which are in Europe. In this way, the entire process related to microbiological decontamination is checked. Additionally, the purpose of the study is to demonstrate the possibility of executing quality rounds comparing the differences in methods used by tissue banks.

METHODS

Validation of the Quality Round method

Sending the same contaminated heart valves to tissue banks, following their standard procedures of testing, decontamination and control and comparing these results with those from other tissue banks and the reference laboratory is a proven method to validate all procedures of all participating banks. Validation of the quality round method took place in 2 stages of testing.

The first validation test took place in January 2014. The Centraalbureau voor Schimmelcultures of the Royal Netherlands Academy of Sciences validated the method for the quality and transport of freeze-dried bacteriological samples.

These freeze-dried samples were sent to 7 different European cardiovascular tissue banks and to 1 reference microbiology laboratory, all of which scored above average[11].

The results and organisational findings from the two validation tests were used to improve and finalise the protocol for the quality round with donated Human Tissue, as described in the 'Quality round with donated human tissue' section below.

Quality round with donated human tissue

In September 2015, the third quality round was executed. Donated tissues, consisting of 6 cardiac valvular homografts, were received from one of the participating cardiovascular tissue banks [The donated tissue was made available according to the provisions of the law of the Federal Republic of Austria:

Bundesgesetz über die Festlegung von Qualitäts- und Sicherheitsstandards für die Gewinnung, Verarbeitung, Lagerung und Verteilung von menschlichen Zellen und Geweben zur Verwendung beim Menschen (Gewebesicherheitsgesetz-GSG) StF: BGBl. I Nr. 49/2008 (NR: GP XXIII RV 261 AB 343 S. 40. BR:AB 7823 S. 751.) and StF: BGBl. Nr. 1/1957 (NR: GP VIII AB 164 S. 22. BR: S. 121.)]. These grafts were washed to remove any residues of antibiotics, and post-wash sterility testing was performed. Subsequently, these tissues were cut into 2 sets of 20 samples to be sent to the participating laboratories and 2 sets of 2 samples for the reference laboratory. The 2 x 22 samples were contaminated and marked as follows:

- Tissue sample A (22): Escherichia coli (Ec) and Enterococcus faecalis (Enco).

- Tissue sample B (22): Due to insufficient stock, 50% of the tissue banks received tissues inoculated with coagulase-negative Staphylococcus and Streptococcus anginosus, and 50% of the tissue banks received tissues inoculated with coagulase-

negative Staphylococcus, Escherichia coli (Ec) and Streptococcus anginosus (Sa) (Table 1).

The strains were isolated from the donated cardiac tissue, cultured and checked twice by a reference laboratory.

After contamination, the samples were packed in a sterile containe with saline. Samples were then packed and dispatched to

the participating tissue banks according to the European Directives (94/55/EC) for Transportation of Biomaterials.

Logistics

Transportation temperatures were validated at 3 different levels by the reference laboratory: 32°C, refrigerated at 6°C and at room temperature +20°C. In September 2015, the samples were sent to the 20 participating tissue banks. They were sent midweek to avoid weekend delays. The time between contamination and delivery at the address of the tissue banks was within 24 h. The start of the investigations, i.e. the culturing of the samples, occurred 6 h to 168 h after delivery. There were no differences in the culture results between those that were started early and those that were started late.

Instructions for the laboratories on how to process the samples were given on an insert. After the specimens were received, they were processed and tested in accordance with the procedures of the individual tissue banks. All results were sent back to the reference centre and were received by December 2015. Tissue banks described the essentials of their processing: which antibiotic cocktail they used, how long they incubated the tissue, at which temperature and if they rinsed or neutralized the antibiotics and checked to see whether any residuals were left.

Reference laboratory

For sample A, the reference laboratory correctly identified both micro-organisms—*Enterococcus faecalis* and the *Escherichia coli*. For 50% of Sample B, coagulase-negative *Staphylococcus* was identified, while in the other 50% of the samples, *Streptococcus anginosus* , coagulase-negative *Staphylococcus* and *Escherichia coli* was correctly ientified. The reference laboratory isolated the *S. anginosus* from only 1 out of the 2 samples tested. Therefore, *S. anginosus* was excluded from the score.

RESULTS

The results of all the 20 participating cardiovascular tissue banks were collated (Table 1): Seventeen tissue banks scored tissue sample A correctly. Three tissue banks either missed the *Enterococcus* (2) or the *E coli* (1).

Seventeen tissue banks scored tissue sample B correctly. Two tissue banks missed the coagulase-negative Staphylococcus and 1 bank received a sample contaminated with Enterococcus, probably due to cross-contamination with Sample A. All tissue banks used a cocktail of 3 to 6 different antibiotics to decontaminate the tissue. The antibiotic cocktails that were in use are as follows: 17 tissue banks used vancomycin, 11 used gentamicin and 10 tissue banks used antifungal drugs (mainly amphotericin or nystatin).

Specific anaerobic coverage (such as metronidazole, cefoxitin, sulbactam and tazobactam) was used by 11 tissue banks; 9 tissue banks did not cover anaerobic micro-organisms. Similarly, 10 banks did not cover fungi (Table 1, column 4) and 2 banks did not cover enterococci (which was one of the contaminants in the trial).

The goal of the decontamination protocol that the tissue banks use is to disinfect the tissue by immersion and incubation in mixture of the aforementioned antibiotics. Most (60%) contain vancomycin and aminoglycoside (Table 1, column 4). The tissues were incubated for a minimum of 6 h in 1 bank, and a maximum of 48 h in 4 banks. All other banks used time periods between 6 and 48 h (Table 1, column 5). Sixteen tissue banks immersed the tissue during decontamination at a temperature of 2°C to 8°C, and 4 tissue banks decontaminated the samples at room temperature or 37°C (see Table 2). Of these last 4 tissue banks, 2 had a negative culture after decontamination. As can be seen in Table 2, only 7 tissue banks had a negative microbiological culture after decontamination with antibiotics.

Five tissue banks found only 1 of the 2 samples to be negative (4 times in Sample B). Seven tissue banks found the last-sterility-check culture to be negative, but only 3 tissue banks would release the tissue for implantation; the other 4 rejected the tissue. All other tissue banks (13) would reject the tissues. So, most tissue banks discarded the tissue, whatever the results of their controls.

Only 6 tissue banks rinsed the tissue or neutralized the tissue to get rid of antibiotic residues after decontamination. They did not indicate whether they tested the tissue for antibiotic residues and/or whether their methods were validated.

Table 1. Results of the quality round of cardiovascular tissue banks, September-December 2015

Bank No.	Isolation[a]		Decontamination			Acceptance		Rejection	
	Sample A	Sample B	Antibiotic cocktail[b]	Duration	Temp. °C	Rinsing Neutral"	Post growth	Sample A	Sample B
1	Ec	Ec,CNS, Sm	Ce,Co,Li,V	18—24	6		+/+	Rej	Rej
2	Ec,Enco	CNS,Sa	Cl,G,V	18-24	4		-/-	Rej	Rej
3	Ec,Enco	CNS	G,Ny,Po,V	18-24	2-8		-/-	Rej	Rej
4	Ec,Enco	Ec,CNS	Am,V	24-32	2-8		+/+	Rej	Rej
5	Ec	Ec,CNS	A,Ce,G,V	12-24	2-8	1x	-/-	Rej	Rej
6	Ec,Enco	Ec,CNS	F,G,V	24	4	1x	+/+	Rej	Rej
7	Ec,Enco	Ec,CNS, Ssp	G,Ny,Pi,V	24	20-21	No	+/-	Rej	Rej
8	Ec,Enco	Ec,CNS	A,As,Ax, Cef,Fl,	24	20-30		-/-	Acc	Acc
9	Ec,Enco	Ec,CNS	C,G,V	6-24	4		+/+	Rej	Rej
10	Ec,Enco	CNS,Sa	Ce,Li,Po,V	48	4	1x	+/+	Rej	Rej
11	Ec,Enco	CNS	Li,Po,V	24-48	4	3x	+/-	Rej	Acc
12	Ec,Enco	Ec,CNS, Sa	A,Cip,G,Mt.V	24	4	3x	-/-	Rej	Rej
13	Ec,Enco	CNS,Sa	A,Co,G,V	24	4		-/-	Acc	Acc
14	Enco	Ec	A,Cip,Co,Cx,G,V	24	37	No	-/-	Acc	Acc
15	Ec,Enco	CNS,Sa, Enco	Fl,G,Mt	24	4		+/-	Rej	Rej
16	Ec,Enco	Ec	G,Mt,V	24-48	4	1x	+/+	Rej	Rej
17	Ec,Enco	CNS,Sa	Fl,G,Mt	24	4		+/-	Rej	Rej
18	Ec,Enco	CNS,Sa	Li,Po,V	48	4		+/+	Rej	Rej
19	Ec,Enco	CNS,Sa	A,Cl,Co,Pi,V	24	4		-/+	Rej	Rej
20	Ec,Enco	CNS,Svir	Am,Cx,Ny,Po,T,V	18-24	25	.	+/+	Rej	Rej

[a]CNS=Coagulase-Negative *Staphylococcus*, Ec=Escherichia coli, Enco=*Enterococcus faecalis*,
Sa= *Streptococcus anginosus*, Sm=*Streptococcus mitis*, Ssp= *Streptococcus* species, Svir= Streptococcus viridans
[b]A= amphotericin, Am= amikacin, As= amoxicillin, C= cotrimoxazol, Ce= cefoxitin, Cef= cefoperazone,
Cip= ciprofloxacin, Cl= Clindamycin, Co= Colistin, Cx= Cefuroxime, F= Fungizone,
Fl= Flucloxacillin, G= Gentamicin, Li= Lincomycin, Mt= Metronidazole, Ny= Nystatin,
Pi= Piperacillin, Po= Polimyxim, T= Tazocin, V=Vancomycin

Table 2. Temperature of decontamination and post-antibiotic growth

Temp. (°C)	Number of banks	Negative/total tissuesa	No. of tissues accepted or rejected
2-8	16	14/32 = 44%	3 accepted and 29 rejected
20-30	3	3/6 = 50%	2 accepted and 4 rejected
=36-37 ?	1	2/2 = 100%	2 accepted

[a]Each Tissue Bank cultured 2 tissues: A and B. 20 Tissue Banks cultured together 20 tissues

DISCUSSION

Decontamination of cardiovascular allografts is a step in the tissue banking process aimed at eliminating microorganisms from the donor or contamination from cardiectomy team members. Thus, this step in the process contributes to the safety of the allograft required by the implanting surgeon.

In this study, decontamination was shown not to be effective in 13 tissue banks because of growth after decontamination. The literature revealed that antibiotics are effective at 36°C[6,12,13] and not, or less so, at 2°C to 8°C. If microbes were found and identified, almost all tissue banks would reject the tissues, even when micro- organisms were not isolated from the tissue after decontamination. If a sample was already positive for micro-organisms at the first control, which is mostly the transport fluid, it would be rejected anyway.[14]

However, tissue banks do proceed the entire process because cultures usually become available several days after processing. This means considerable work with associated additional costs.

The reason why tissue banks, using a validated method, reject tissue that is negative for micro-organisms in the final control, is probably the risk of sampling error. Samples taken during processing consist of a biopsy from the valve and fluid samples from the irrigating and immersion solutions used to decontaminate and rinse the valves. The more fluid and tissue they check, the more reliable are the results. A second reason to reject could be the methods of culturing, which should be up-to-date according to microbiological quality handbooks for clinical specimens. Comparing the sampling and culturing methods could be a subject for the next quality round. Those tissue banks that would still deviate after a second quality round need to seriously consider a change in their procedures in this regard.

One-hundred percent sterilisation would only be achieved by applying rigorous methods, such as radiation or heat- sterilisation. However, the use of such methods would damage the valve tissue and make cardiovascular grafts unfit for implantation. The decontamination procedure, if it is validated, would ensure that the tissue contained no known micro-organisms. If there were no other contra-indications, the tissue could

be used for implantation. There are no recent reports of recipients of donated tissues developing infections due to the transplants they received, although 1 study showed that 9.5% of the cultures taken from thawed valves just before implantation were positive for bacteria[15] but no adverse reactions were found in recipients. An infection in recipients who receive bone tissue or corneal transplants is also rare.

It is advisable to validate procedures and methods by always taking cultures from homografts at the time of implantation or during a specific period of time. Most tissue banks use extremely high concentrations of antibiotics for decontamination, much higher than those used in clinical situations. After decontamination, the tissues should ideally be rinsed, or even better, the residual antibiotics on the tissues should be neutralised. If not, the presence of antibiotics can induce bacteriostasis of micro-organisms, which will lead to false-negative results in all the tested samples [16, 17]. Perhaps even worse, there is a risk that the antibiotics can cause severe allergic or toxic reactions in tissue recipients [18, 19]. There were no current studies that reported on this issue and how serious the clinical consequences may be. There is a risk that recipients who have an allergy to specific antibiotics could develop allergic reactions after transplantation as a result of extremely high concentrations of antibiotics in the tissues [20, 17]. Because recipients are treated with antibiotics prophylactically, it would be difficult to prove, and there are no known recent clinical reports of a reaction caused by an antibiotic residue on the tissue allograft. Rinsing of grafts is standard practice in most tissue banks as well as clinically before implantation.

Also, residues of dimethyl sulphoxide, a cryoprotective fluid used for valve preservation, can cause renal and hepatic dysfunction and cardiovascular complications locally [21]. Soquet et al. describe the acute rejection of a cryopreserved arterial homograft of unknown origin that could be caused by the residual contents of antibiotics, dimethyl sulphoxide or damaged tissue proteins [22].

Many antibiotics (such as beta-lactam) do not work better in higher concentrations. On the contrary, the higher the dosage, the more toxic their effects may be on the recipient; the effect on graft survival is unknown.

Antibiotic cocktails disturb the intrinsic properties of tissues, enzymatic activities and immunological events. The presence of antibiotic lipids has been described in skin, cornea and amniotic membranes [17]. Furthermore, most antibiotics need multiplying micro-organisms to eliminate them, and cell replication does not happen at 4°C. If the tissue is immersed at a temperature of 4°C, the antibiotics will only be absorbed by the tissue, giving a false negative result in the post-antibiotic incubation tissue culture.

However, after weeks of incubation, the tissue might contain a slow-growing microbial population that could cause a chronic problem at the implantation site of the tissue recipient [12]. It is recommended that one use a temperature that allows bacteria to

grow well (36° C) and then use the antibiotic cocktail at a low clinical dose for only 6 h, because immersion for more than 6 h does not provide better results [8, 16].

The use of antibiotics means that there is an obligation to rinse the tissue after the decontamination procedure. The European and the US Pharmacopoeias recommend eliminating from samples any factor that may interfere with microbial growth during sterility testing [18]. As was shown previously, there are a number of ways to achieve this goal [23, 24].

Limitations

Cardiovascular tissue banks use concentrations of antibiotics that differ considerably in composition and concentration. An earlier survey showed that concentrations of, e.g. vancomycin vary from 50 mg/ml to 1000 mg/ml and of gentamicin from 50 mg/ml to 4000 mg/ml, which is up to 400 times more than what is clinically used in patients [5]. The composition and concentration of antibiotics contribute to the level of toxic effect on the tissue cells. So, one question that remains unanswered in this study is the influence of these different concentrations of antibiotics on structural and biomechanical properties of the tissue matrix and of the valve leaflets. Although this unanswered question is a limitation of this study, the study of biomechanics requires a different group of specialists.

CONCLUSIONS

A surgeon who wishes to implant a cardiovascular tissue graft from a reliable source is confronted with cardiovascular tissue banks that have their own, often locally validated, methods of tissue sampling and processing and their own regimens for decontamination of antibiotics [8]. In addition, at this time, no consistent, quality parameter for microbiological decontamination exists. By using quality rounds to validate the practices of the various cardiovascular tissue banks, we can identify those cardiovascular tissue banks whose tissue processing methods (nontoxic) deviate from appropriate standards. Quality rounds will identify where the processing methods used are insufficient and need to be changed in conjunction with an agreed minimal protocol, accepted by the entire tissue bank community, for validation of sampling and testing, taking into consideration all of the different parameters used in those tissue banks.

In the first trial with the freeze-dried tissues, the microbiological culture results of the tissue banks were assessed and the scores were above average. However, there was some room for improvement [12]. In this study, the entire processing chains of 20 participating tissue banks were evaluated, including the antibiotic decontamination regimens, tissue rinsing methods, postantibiotic incubation (if any) and the sampling technique.

This method of sending contaminated tissues to be processed by the tissue bank according to local standard procedures and testing them pre- and post-decontamination, and assessing the microbiological culture results, is an effective way of validating the processes of the participating tissue banks. It also demonstrates that the majority of tissue banks, 17 of 20, can save costs and manpower if they rely totally on the outcomes of their own procedures.

By repeating this method over time, it may be expected that the differences in processing, the interpretation of the results and, finally, the decision to release tissues for transplantation may be harmonized by the individual tissue banks [25].

Before harmonization has been achieved, surgeons must be aware of the techniques applied in the cardiovascular tissue bank that provides the allografts they use, especially when a graft comes from a supplier whose methods are unknown. Sampling at implantation is advised.

To prevent discarding scarce allografts, tissue banks should alter their procedures such that they rely on their own method of decontamination and not reject grafts if a non-specified micro-organisms, which could be neutralized, is found.

Specifically, this argument gains additional weight when the decontamination method has been validated.

Differences between methods used by tissue banks may lead to incorrect outcomes of microbiological testing. Only when harmonization and validation of methods are achieved will surgeons and colleagues from cardiovascular tissue banks be able to rely fully on one another's methods and have confidence in the consistent sterility of the tissue grafts and uniform quality criteria in the event that tissues for implanting are exchanged across borders.

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CHAPTER 13

Significant Variation in Heart Valve Banking Practice

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ABSTRACT

Purpose: The European Blood Alliance (EBA) Tissue and Cells annual benchmarking exercise identified that in 2014 the heart valve (HV) discard rate in tissue establishments (TEs) run by EBA members was between 19% and 65%. Given this significant discard rate a decision was taken to carry out a worldwide data-gathering exercise to assess the processing methodology in different TEs.

Method: In collaboration with the Foundation of European TEs a questionnaire asking for the details on HV processing was sent to TEs worldwide. Nineteen questionnaires were received back from 15 European TEs and 4 non-European TEs.

Results: The data provided confirmed a significant discard rate of HVs with 43-50% of aortic valves and 20-32% of pulmonary valves being discarded in 2015. The causes of HV discard varied, with microbiology contamination, anatomical and medical reasons being the main causes.

Conclusion: This data-gathering exercise highlighted significant variations in practice in different TEs including how donor suitability is assessed, critical timings for heart retrieval and processing, heart rinsing, HV decontamination protocols and methods of microbiological testing. To reduce the discard rates, there are several aspects of HV banking that could be validated and standardised. Here we report the findings of this data-gathering exercise. We consider this a first step that will help lead to standardising HV banking.

Key Words: Heart valves, Discard, Microbiology, Transplantation

INTRODUCTION

The European Blood Alliance (EBA) Tissue and Cells Working Group (T&C WG) carries out an annual benchmarking exercise. The results of the 2014 EBA T&C WG benchmarking exercise identified that within the EBA TEs that provided data for 2014, there was a HV discard rate ranging from 19% to 65% with a median of 41% (unpublished data, dr. S Zahra).

HVs are precious resources that may be life-saving [1] and which may be in short supply in a number of countries. It was therefore felt important to gather further information to establish the causes of HV discard in the different TEs. Furthermore many tissue banking and processing methodologies are not based on hard evidence. It was therefore felt important to try to gather as much information as possible about HV banking practice around the world. To this end a detailed data-gathering exercise to map the process of HV banking was carried out in May 2016, with the aim of improving practices that could be shared with all TEs.

In order to maximise the number of participating TEs the EBA T&C WG carried out this data-gathering exercise in collaboration with the Foundation of European TEs.

METHOD

A detailed questionnaire covering all aspects of HV banking was developed which included questions on types of donors (live, multi-organ and tissue-only donors), age of donors, how donor assessment is carried out, details of retrieval, donor testing, tissue transport and HV processing. Each TE was also requested to provide data on the number of donors, number of HVs discarded and reason for discard.

The questionnaire was sent out at the end of August 2016 to TEs in 15 European countries and an additional 11 countries world-wide. There was a very good response rate of 73% with 15 European and 4 non-European TEs returning results. The responses received were anonymised.

RESULTS

HV Donors

From the responses received all 19 TEs retrieved HVs from multi-organ donors (MODs). Of these 19, 15 also retrieved HVs from tissue-only donors (TODs) and 8 TEs also retrieved HVs from live donors (LDs) (the latter are patients undergoing a domino-donor operation during a heart transplant).

The number of HV donors in each TE is shown in figure 1. The TEs vary significantly in size, ranging from as few as 6 donors in 2015 to a maximum of 320 donors in the same year. The majority of HV donors are MODs, followed by TODs and a smaller number of LDs.

Donor Age Limit for HV Donation

The upper age limit for HV donation varied from as low as 40 years for both aortic valves (AVs) and pulmonary valves (PVs) in one TE, to a cut off of 70 years for both AVs and PVs in two TEs, with the rest of the TEs having a cut off of 60 years or 65 years for AVs and/or PVs. HVs were not collected from donors above these age limits from any of the TEs that took part in this survey.

Proportion of HVs Discarded

The TEs reported a significant discard rate (for all reasons) of both AVs and PVs, with a median discard rate for AVs of 43% from TODs and 50% from MODs and a median discard rate for PVs of 32% from TODs and 20% from MODs.

Causes of HV Discard

The main causes (data not shown) of HV discard included microbiological contamination (i.e. bacterial and/or fungal contamination – variable type of contaminants are identified from the skin, airway, gastrointestinal tract or the environment, including *Coagulase Negative Staphylococci, Streptococci, E. Coli* and *Pseudomonas* amongst others), anatomical abnormalities and medical deferrals. Other less frequent causes of discard included damage at retrieval, damage at dissection, blood borne virus (BBV) positivity and other miscellaneous causes.

The discard rate due to microbiological (bacterial and/or fungal) contamination of HVs from TOD and HVs from MOD in the same TE has been compared – Figure 2. While the microbiological contamination rate is higher in TOD in TEs C, H, I, N and O, the reverse is true in TEs A, B, L, M and R with no difference in TE G; caution is however required in interpreting this data as some TEs are very small with very few donors in a given year (refer to Figure 1) so that results could easily vary from one year to another.

Donor Suitability Assessment

The TEs were asked to provide information as to whether potential tissue donor suitability is assessed pre- or post-donation. Not all TEs provided information for this part of the questionnaire. Of the 13 TEs that did provide information, 12 carried out some form of pre-donation assessment. One TE did not perform detailed pre-donation assessment relying instead on Blood Borne Virus testing and post-mortem results to confirm suitability of the donor.

The TEs were asked whether they would routinely go through the donor medical notes, contact the donor family doctor and obtain post-mortem results as part of the donor suitability assessment. Not all the TEs provided responses for these questions, however

of the responses received, 8 TEs reviewed the medical notes while 4 did not; 7 TEs contacted the donor family doctor while 5 did not and 10 TEs reviewed the post-mortem results while 1 did not.

Blood Borne Virus Testing

All TEs carried out testing for BBVs although there was variation between the different TEs – Table 1.

Heart Retrieval for Valve Donation

The TEs indicated that heart retrieval for HV donation from MODs was always carried out (or supervised) by a medical doctor in a hospital theatre, in almost all cases at the end of other organ retrieval.

On the other hand heart retrieval from TODs was carried out by varying groups of staff including doctors, nurses and/or laboratory staff; a doctor was involved in the retrieval process in the majority of TEs although in one TE the heart was retrieved by a team of trained nursing and laboratory staff and in another TE heart retrieval was carried out by trained laboratory staff only. In both organisations a responsible medical doctor was on call and could be consulted 24/7. When the donor was a TOD, the heart tended to be retrieved as the first or one of the first tissues during the tissue retrieval process by almost all TEs.

The venue for tissue retrieval from TODs varied and included mortuaries, hospital theatres, undertakers, and also dedicated tissue retrieval suites. In this data-gathering exercise there was no correlation between the venue used for tissue retrieval and the discard rate secondary to microbiological contamination (data not shown).

Heart retrieval from MODs took place as part of the organ donation process and as such was done very soon after the time of cardiac arrest/cross-clamp. This was different to heart retrieval from TODs. In the case of TODs retrieval tended to take place several hours after the time of cardiac arrest with different TEs having different criteria for acceptable time limits - Table 2. From the information provided there was no clear correlation between the retrieval timings and the rate of microbiological discard rate.

Ten TEs rinsed the heart at retrieval, six did not, while one TE had a variable practice, rinsing the heart from LDs but not from MODs. The rate of discard secondary to microbiological contamination was similar between TEs that rinsed the heart at retrieval (microbiological discard rate 2-23%) and those that did not (microbiological discard rate 0-20%), although there was a trend for a lower microbiological discard rate for TEs that did not rinse the heart at retrieval.

HV Processing

Practice varied as regards how quickly after retrieval the HVs were processed: six TEs processed the HVs immediately, seven TEs had a 24 hour cut off and the rest had a

variable longer cut off for HV processing up to a maximum of 72 hours. All TEs indicated that the time cut off indicated for HV processing was the maximum acceptable, with the HVs being processed as quickly as possible. The microbiological discard rate for the three groups was broadly similar with no significant difference: 0-25% (TEs processing the HVs immediately), 0-20% (TEs processing the HVs within 24 hours) and 3-23% (TEs processing HVs beyond 24 hours).

HV Decontamination

All the TEs decontaminated HVs, however practice was very variable. The antibiotic cocktails used varied significantly; combinations of 2, 3, 4 or even 5 antibiotics were used and most but not all included antifungals (data not shown).

The protocol followed during HV decontamination also varied significantly. The majority of TEs carried out decontamination at 4°C, five TEs decontaminated at room temperature and only two TEs carried out decontamination at 37°C – Table 3.

The duration of antibiotic decontamination was also very variable, from as short as 6 hours in some TEs to a maximum of 48 hours in other TEs.

Fourteen of the TEs rinsed the HVs after decontamination while five did not. This aspect of HV decontamination was subject to a detailed separate analysis.

Microbiological Testing During HV Processing

The samples taken and the culture methods used to detect microbiological contamination of the HVs varied significantly between different TEs – Table 4.

All TEs took a tissue sample for culture, eleven cultured the transport fluid, less then half cultured a sample of the rinse fluid, and fewer still took swabs, or cultured a sample of the cryoprotectant or the antibiotic solution.

The culture techniques used were very variable, varying from culturing in TSB (Tryptic Soy Broth; a nutritious medium for detecting aerobic bacteria) and Thioglycolate (a broth suitable to detect anaerobic/facultative anaerobic bacteria) for 2 weeks at room temperature or 32°C, to culturing in Medium 199 (a medium that is considered unsuitable to identify the presence of contaminants) for 18 hours only at 4-7°C, and a significant variation in between.

Only 8 of the 19 TEs cultured the HVs for the presence of tuberculosis (TB). The duration of TB culture varied significantly from as short as 40-48 hours (one TE), to 2 weeks (one TE), 2 months (five TEs) and one TE culturing for TB for 3 months.

Ten TEs carried out environmental monitoring during HV processing while nine did not.

DISCUSSION

This data-gathering exercise was an excellent collaborative venture between EBA T&C WG and the Foundation of European Tissue Banks (FETB). Despite the level of detail requested in the questionnaire, there was an excellent response, indicating that individuals involved in HV banking were keen to examine the process being followed.

The upper age limit for HV donation varied significantly in the different TEs. When the donor age limit was compared to the individual TE's discard rate for anatomical reasons and medical reasons there was no correlation (data not shown). The TEs with the lowest and the highest age limits did not have a significantly different discard rate for either anatomical or medical reasons. The TE with the lowest age cut off for HV donation may wish to consider increasing the age cut off used if there is an unmet clinical demand. Clinical feedback on the suitability of HVs from older donors is required; this may allow the donor age limit to be safely increased.

Not all TEs provided information on the type of donor assessment carried out. Some TEs were in fact not responsible for the pre-donation part of the process. From the data provided all but one of the TEs indicated that there was an assessment of donor suitability done prior to proceeding with tissue retrieval. Further detailed comparisons of the type/level of detail of pre-donation donor assessment that is carried out is required to establish whether this has a significant impact on HV discard rate.

The detail of post-donation donor assessment also varied between the different TEs, with some TEs reviewing the medical notes and contacting the donor family doctor and practically all TEs indicating that they also reviewed the post-mortem results. Only one TE indicated that rather than carrying out a detailed donor history they relied on BBV testing and post-mortem results to establish suitability of potential HV donors.

What is unclear from the information available is whether there was any difference in HV safety assessment between TEs that carried out very detailed potential donor assessment and those TEs that carried out a less detailed one, in particular if post-mortem results were routinely reviewed. Further information as regards any serious adverse events/ reactions in recipients is needed to establish whether the level of donor assessment detail that is carried out has an impact on product safety.

Further it is unclear whether the acceptance criteria between different TEs varied. It is quite possible that some TEs may be declining donors and/or discarding HVs that may be accepted by other TEs. Certainly it would be important to try and compare acceptance criteria between different TEs with the aim of standardising them. This will require further detailed work.

From the data provided the main reasons leading to HV discard included microbiological (bacterial and/or fungal) contamination, medical reasons and anatomical reasons. It

would be important to try to establish whether the medical and anatomical reasons leading to HV discard are being applied uniformly by all TEs. It would also be important to investigate further the microbiological contamination discards to establish whether the HV banking process can be amended to reduce the rate of such contamination. Harmonisation of the procurement and dissection techniques used, as well as the training of personnel, together with regular audit of the testing laboratories that TEs work with would help to decrease the discard rate of donated HVs by ensuring good uniform practice in all aspects of HV banking. This is subject to a separate study.

It is of note that only 7 of the 19 TEs reported HV discards secondary to BBV positivity in 2015 (data not shown). This may reflect either a higher prevalence of BBV positivity in the general donor population or a less detailed pre-donation donor assessment by these TEs. The latter certainly seems to be the case for at least one TE where 46% of all discards were due to BBV positivity – when contacted, TE D indicated that only a brief donor history was taken prior to tissue retrieval, relying instead on BBV testing and post-mortem results to assess suitability of the donated HVs. Further information, in particular the presence or absence of serious adverse events and/or reactions in recipients, is required to assess whether such practice impacts on donation safety or not; the risk of accepting "higher-risk" HV donors by TEs carrying out a less detailed donor assessment needs to be considered in the setting of the possibility of false negative results when relying mainly on BBV testing results [2-4].

All TEs carried out BBV testing although different TEs used different assays. NAT (nucleic acid test) testing for HIV, Hepatitis B and Hepatitis C was carried out by most but not all TEs, and all except one non-European TE tested for anti-HBc antibodies. Lack of information in this data-gathering exercise about the potential rate of serious adverse events/reaction (particularly as regards transplant transmitted infection rate) from the different TEs means that we cannot comment on the suitability or otherwise of the different testing regimes, although the risk of a transplant transmitted infection should be reduced by NAT testing as the window period is significantly reduced [5].

The staff responsible for carrying out heart retrieval for HV donation varied. Unsurprisingly, heart retrieval from MODs always included medical staff, however when the heart was being retrieved from a TOD, then the staff carrying out the retrieval included a varying combination of doctors, nurses and/or laboratory staff with one TE successfully carrying out retrieval using trained laboratory staff under medical supervision. There did not appear to be any correlation between the rate of HV discards and the type of retrieval team employed.

The order of heart retrieval varied between the different types of donors, with the heart often being the last organ to be retrieved from MODs, but the first or one of the first from TODs. It was of note that while most TEs had a higher microbiological contamination rate of HVs from TODs, this was not true in all cases (Figure 2). Further information is required to be able to reach definite conclusions about this as there were

too many variables: venue of retrieval; the length of time after circulatory arrest that tissue retrieval took place and the order of retrieval are all likely to have impacted the HV contamination rate. This level of detail was not part of this survey.

It is well documented that after cardiac arrest organs and tissue may be contaminated by endogenous organisms due to the transmigration of organisms from within the body [6], although this has been refuted by more recent literature [7]. The length of time between circulatory arrest and tissue retrieval of HV was not an issue for MODs as heart retrieval took place soon after cross-clamp or circulatory arrest as part of the organ retrieval process. However when HVs were being retrieved from a TOD, then retrieval took place at varying time points after the time of cardiac arrest. There was significant variation in the acceptable time limits that different TEs applied. There were too many variables that might have influenced the microbiological contamination rate of HVs to be able to draw definite conclusions from this data-gathering exercise, although there was a trend that the TE with the longest warm ischaemic time (12 hours after cardiac arrest) had one of the highest microbiological contamination rates of HVs, suggesting that cooling the donor body as soon as possible is important [8, 9].

Several of the TEs did not rinse the heart at the time of retrieval. The numbers in this data-gathering exercise are too small to reach definite conclusions, however there was a trend for a lower microbiological contamination rate of HVs in the TEs that did not rinse the heart at retrieval, something worth exploring further. Heart rinsing at the time of retrieval is carried out to remove any contaminants already present at the time of heart retrieval and also to remove any blood clots as the latter are thought to provide a good medium for bacterial growth[10]; however it is possible that the technique used for heart rinsing may have had an adverse impact on microbiological contamination.

Antibiotic decontamination protocols in use to decontaminate HVs varied significantly between the different TEs both in this data-gathering exercise and as previously reported by other authors [11-15]. The antibiotic cocktails used, the temperature at which decontamination was carried out and the lengths of time HVs were kept in antibiotics varied widely between different TEs. In the absence of further information as regards the presence or not of serious adverse events in recipients due to the presence of residual antibiotics it is impossible to comment whether the different protocols used were all equally effective in ensuring the microbiological safety of the HVs. This needs to be investigated further to try and establish best practice as regards HV decontamination.

The number and type of cultures taken and the culturing methods used also varied significantly between different TEs. It is highly likely that such different techniques would have led to different sensitivity in detecting contamination. Hence a low microbiological contamination rate quoted by some TEs could be due to a low sensitivity in identifying contamination rather than a genuinely low contamination rate [16]. Caution therefore needs to be exercised when interpreting the rate of microbiological contamination in isolation without attention to the numerous variables that may be influencing

that figure, including the potential of false negative culture results due to antibiotic treatment given to the donor in the hours prior to death and also the antibiotics used to decontaminate the HVs during processing [17, 18]. This requires further, more detailed work. It is important for individual TEs to be sure that the samples taken and the culture techniques used are sufficiently sensitive (using enriched culture media) to detect clinically significant contamination.

It is worth noting that only eight of the nineteen TEs cultured for Tuberculosis (TB). Further the length of culture for TB varied significantly between TEs. This is something that should be relatively straightforward to standardise. Testing HV donors for the presence of undiagnosed TB is important [19]. Culturing for TB for 40 hours or even 2 weeks is considered insufficient. Longer culture duration is required using tissue samples in specific culture media that has been shown to be suitable for the identification of the presence of TB. Expert microbiological opinion should be sought to ensure that TB cultures are done using appropriate culture media that is incubated for a sufficient length of time.

Further only just over half of the TEs carried out environmental monitoring during HV processing. The TEs that carried out environmental monitoring indicated that the environmental monitoring results were taken into account when deciding whether a particular HV would be released for transplant or not. Without carrying out environmental monitoring during HV processing it is impossible to assess whether the GMP (good manufacturing practice) environment has been maintained as required during the processing, leading to concerns re product safety [16].

CONCLUSIONS

This data-gathering exercise has highlighted that HV retrieval and processing are very variable with significant differences in processes between different TEs. There is a significant discard rate of HVs and many aspects of HV banking can and should be standardised through further collaborative work between TEs.

One weakness of this data-gathering exercise is the lack of information on the serious adverse events and/or reaction rates in recipients from the different TEs. Tissue vigilance should be encouraged by all TEs and clinicians to enhance the quality of the information available.

There are many aspects of HV banking that can be investigated further with the aim of validating and standardising practice. The main findings of this data-gathering exercise highlighted the significant variation in practice throughout the HV banking process - from the selection of the donor right through to storing the final product. All these factors need to be evaluated in a systematic manner to establish best practice. Following on from this initial exercise we also looked in greater detail at the microbiological testing

and decontamination protocols used by different TEs during HV banking. As a result we organised a HV Quality Round in 2017 as a first step in establishing an External Quality Assessment Scheme for HV banking, focusing in particular on the microbiological aspects of HV banking. The results of this Quality Round will be discussed in a separate publication.

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Barcelona Tissue Establishment
Birmingham Heart Valve Bank
Bloemfontein Homograft Bank, South Africa
Cardiovascular Tissue Establishment of Milan
Croatian Tissue and Cell Bank
Etablissement Française du Sang, Banque de Tissus et Cellules, Bordeaux
Etablissement Française du Sang, Banque de Tissus et Cellules, Lyon
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NHS Blood and Transplant, Tissue and Eye Services, England
Oxford Heart Valve Bank
Scottish National Blood Transfusion Service, Tissue, Cells and Advanced Therapeutics
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Tissue Establishment Lund, Sweden
Transplant Tissue Centre, Singapore

We can confirm that this work has been carried out in compliance with Ethical Standards.

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Informed consent is not relevant for this work.

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Figures

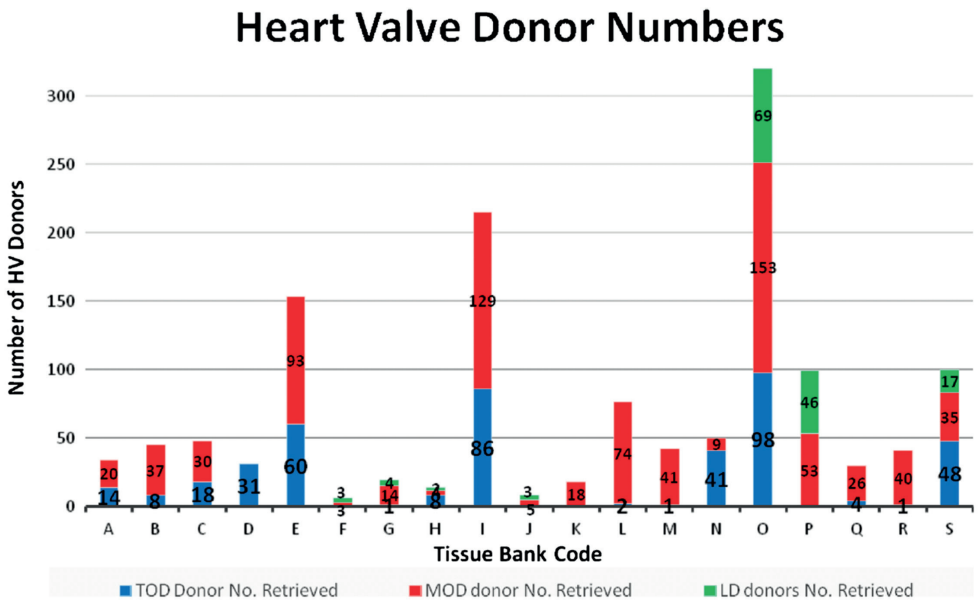

Figure 1. Donor numbers in the different TEs in 2015.

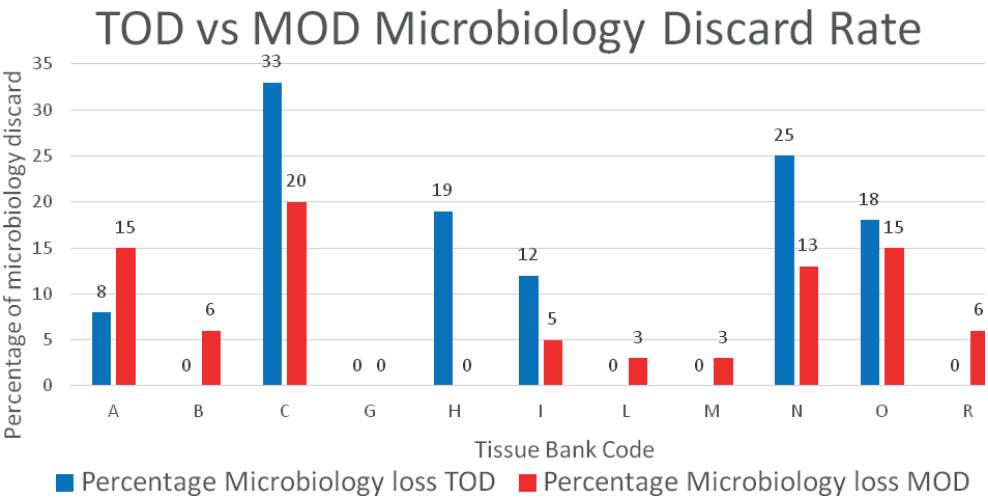

Figure 2. Comparing the rate of microbiological contamination of HVs from MOD donors and TOD in individual TEs that bank HVs from both types of donors.

Tables and Captions

Table 1. Details of blood borne virus testing carried out.

Testing		Number of TEs
Hepatitis B	HBsAg	19/19
	anti-HBc	18/19*
	anti-HBs	6/19
	anti-HBe/HBeAg	1/19
	NAT	16/19
Hepatitis C	anti-HCV	19/19
	NAT	16/19
HIV	Ag/Ab test	16/19
	anti-HIV	3/19
	NAT	16/19
Syphilis	Antibody assay	18/19
HTLV	Anti-HTLV I/II	13/19

*non-European TB

Table 2. Retrieval timings for HV donation from tissue-only donors.

TE Code	Retrieval Timings		TOD Microbiology Discard rate: (Total No. retrieved) % discarded
	Donor refrigerated	Donor not-refrigerated	
B	< 6hrs	< 6hrs	(7) 0%
G	< 4hrs - up to 24hrs	n/a	(1) 0%
C	< 6hrs - up to 24hrs	12hrs	(36) 33%
R	< 6hrs – up to 24hrs	15hrs	(2) 0%
O	< 6hrs – up to 24hrs	n/a	(196) 18%
A, I	< 6hrs - up to 48hrs	12hrs	(25) 8%, (132) 12%
Q	< 6hrs – up to 48hrs	24hrs	(1) 0%
D,M	< 6hrs – up to 48hrs	n/a	(53) 0%, (2) 0%
L	< 10hrs - up to 24hrs	13hrs	(4) 0%
N	< 12hrs - up to 24hrs	15hrs	(73) 25%
H	up to 24hrs	15hrs	(16) 19%

Table 3. Temperature of antibiotic decontamination.

TE Code	Temperature of Decontamination	Percentage HV Microbiology loss TOD+MOD+LD
B, C, D, F, H, I, J, K, L, O, P, S	4°C	5, 25, 0, 0, 11, 8, 20, 11, 3, 13, 5, 2%
E, G, M, Q, R	22°C	3, 0, 3, 6, 1%
A, N	37°C	13%, 23%

Table 4. Microbiological samples taken during HV processing.

TE Code	Tissue Sample	Rinse Fluid	Transport Fluid	Swabs	Cryoprotectant	Antibiotic Soln.	Percentage HV Microbiology loss TOD+MOD+LD
A	Yes	Yes	Yes	No	Yes	Yes	13%
B	Yes	Yes	Yes	No	Yes	No	5%
C	Yes	Yes	Yes	No	No	No	25%
D	Yes	No	No	No	No	No	0
E	Yes	No	No	No	No	No	3%
F	Yes	No	No	No	Yes	No	0
G	Yes	No	No	No	Yes	No	0
H	Yes	Yes	Yes	No	Yes	No	11%
I	Yes	No	No	Yes	No	No	8%
J	Yes	No	Yes	Yes	Yes	Yes	20%
K	Yes	Yes	Yes	Yes	No	No	11%
L	Yes	No	Yes	No	No	Yes	3%
M	Yes	Yes	No	No	No	No	3%
N	Yes	Yes	Yes	No	Yes	No	23%
O	Yes	No	Yes	No	No	No	13%
P	Yes	No	Yes	No	No	No	5%
R	Yes	Yes	Yes	No	No	No	1%
S	Yes	No	No	No	No	No	2%

CHAPTER 14

Validation of Microbiological Testing in Cardiovascular Tissue Establishments; Results of a Second International Quality-Round Trial

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ABSTRACT

Purpose: The performance of many laboratories can be evaluated by participation in external quality assessment (EQA) schemes. EQA allows for comparison of a laboratory's performance with a source outside the laboratory - either a peer group of laboratories or a reference laboratory.

Such EQA schemes do not exist in tissue banking despite the fact that Tissue Establishments (TE) perform very complex procedures. This paper describes the first ever EQA scheme in the field specifically assessing microbiological aspects in heart valve (HV) banking.

Methods: Twenty-two TEs participated. Three HV tissue samples were sent to each participating TE - two contaminated with non-pathogenic micro-organisms and a third negative control. The aims were to isolate and identify the micro-organisms present and then to successfully decontaminate the HV tissue using the routine standard operating procedures of the TE.

Results: Eight of the TEs were able to isolate and identify all contaminating micro-organisms present and of these, five also successfully decontaminated the tissue; thirteen TEs failed to establish the identity of one or more of the contaminants; five TEs appear to have introduced contamination during the processing or testing of the tissue; and eight failed to successfully decontaminate the HV tissue.

Conclusion: This initiative provides TEs with an international benchmark for tissue product microbiology testing. It has identified significant variation in practice and in the ability of different TEs to identify the presence of contamination. There is now work ongoing with the aim of setting up a regular EQA scheme for HV banking.

Key Words: heart valves, banking, transplantation, microbiology, contamination

INTRODUCTION

For the cardio-thoracic surgical community, organ and tissue donation and transplantation are an important aspect of medical care. Donated tissues are regularly transplanted each year, providing life-saving (e.g. HVs, arterial grafts) and life-enhancing (e.g. veins, pericardium) treatment for thousands of patients [1].

Previous work by this group in 2016 identified significant differences in the methods used by TEs around the world in all aspects of HV banking, from donor selection and testing, tissue processing, and HV handling before implantation. It is unclear whether such variations in methodology are materially important to the safety and clinical effectiveness of the HVs.

It was therefore decided to investigate in more detail microbiological aspects of HV banking, including the microbiology testing and decontamination protocols used.

The method used to send out HV tissue purposefully contaminated with micro-organisms to different TEs around the world for them to isolate and identify the contaminating micro-organisms is based on the previously validated and published work of two of the authors (2,3).

METHODS

Thirty TEs from around the world were contacted, of which 25 (21 European, 4 Non-European) participated. HV tissue for this project was sourced from the European Homograft Bank (EHB) in Brussels. All donations were anonymised and had the necessary consent. All HVs used were confirmed to be sterile on microbiological culture. The HVs were cut into strips to ensure sufficient HV tissue for all participating TEs, and split up into three separate groups, sample A, sample B and sample C (see later).

Sample A was contaminated with *Sphingomonas paucimobilis* (Gram negative rod); sample B was the sterile negative control; and sample C was contaminated with *Roseomonas mucosa* (Gram negative coccobacilli) and *Staphylococcus hominis* (Gram positive cocci).

Three separate HV tissue samples were prepared for each TE, one each from sample A, B and C. The preparation of the samples was done at the EHB, using a clean room facility. The samples were packed in compliance with the European Directives for transportation of biomaterials (94/55EC). Two lots of samples were concurrently also sent to reference microbiology laboratories (one in Edinburgh and one in Rotterdam) as independent confirmation of the isolation and identification of the contaminating micro-organisms. The technique used was based on a previously published method that had been validated for different levels of contamination (high, medium and low), the transport medium

used, temperature of storage (room temperature) and transport duration (1 to 14 days) [2, 3].

The samples were sent out by registered delivery using a courier company that guaranteed delivery within 24 hours for European destinations and within 72 hours for non-European destinations. All participating TEs were asked to contribute a nominal charge to help cover the transport costs. The time of the organisers and equipment was provided free of charge.

Results were assessed against 4 microbiological criteria relevant in tissue banking: sampling methods, correct isolation and identification of micro-organisms, culture methods and decontamination protocols. Any deviations from these principles will be discussed.

RESULTS

Receipt of samples

Delivery of most samples was delayed, with only 2 samples being delivered within 24 hours within Europe, and a longer delay for the non-European samples, the maximum delay being 6 days. Four of the TEs also reported some damage and leakage of one or more of the sample tubes delivered. Of the 26 samples sent out, 24 were safely delivered to 23 TEs (one TE requested 2 sets of samples), whilst two samples were never received (held up at customs).

Most TEs managed to process the samples as soon as they were received. However four TEs delayed processing the samples by 3 days, and three delayed processing by five, ten and twenty-three days delay respectively for operational reasons. Twenty-two TEs completed the exercise and returned their results.

Microbiology reference laboratory results and interpretation

Sample A

The reference laboratory in Rotterdam received the tissue samples within 24-36 hours and processed them immediately. Using Bactec and then subculture onto plate culture media they were able to successfully isolate and identify the *Sphingomonas paucimobilis*, however on repeat sampling after 72 hours and again after 2 weeks, there was no growth of the micro-organism. The micro-organisms were still identifiable by Gram stain. This laboratory also tested the sample for the presence of antibiotics and found it to be positive.

The reference laboratory in Edinburgh received the tissue samples after 48 hours. Using chocolate and blood agar culture techniques they were not able to culture, isolate and identify the *Sphingomonas paucimobilis*. On reviewing these results it was concluded that the presence of antibiotics in sample A was interfering with the growth of the

Sphingomonas paucimobilis so that an acceptable result for Sample A would be either "no growth" or identification of the *Sphingomonas paucimobilis*.

Sample B
Both microbiology reference laboratories confirmed that this was a sterile sample. This sample was confirmed as a suitable negative control for this project.

Sample C
Both microbiology reference laboratories confirmed the presence of both *Roseomonas mucosa* and *Staphylococcus hominis*.

The Rotterdam microbiology laboratory again checked for the presence of antibiotics, and while there were antibiotics present in this sample they were not interfering with the growth of either of the two contaminating micro-organisms. This sample was therefore suitable for this project.

Results of participating TEs
The number and type of culture samples taken to look for potential contamination pre- and post-decontamination varies between the different TEs.

A summary of the pre-decontamination samples taken for culture by the 22 different TEs is shown in Table 1a. At the pre-decontamination stage most (n=19) TEs take tissue biopsies for culture; often (n=12) combined with a transport-fluid sample or other sample (swab/antibiotic cocktail (n=2)). Six TEs take only tissue biopsies and 1 TE samples only transport fluid. Two TEs do not routinely take any pre-decontamination samples.

Table 1b summarises the culture samples taken post-decontamination by the different TEs. Such samples serve as a final check for the potential presence of contamination. Twenty TEs sample the cryoprotectant in which the HVs are preserved, and 15 combine that with tissue biopsies; one TE takes a tissue biopsy and sample of the antibiotic cocktail and one TE only samples the antibiotic cocktail.

Pre-decontamination culture results
A summary of the pre-decontamination culture results from the individual TEs is presented anonymously in Table 2 - each TE is represented by a number assigned randomly. Table 2 provides details where the result returned by the TE is not the expected result i.e. TEs that appear to have contaminated their samples and TEs that did not manage to isolate and identify the micro-organisms present.

Decontamination protocols used
The antibiotic cocktails used and the temperature and duration of decontamination varies significantly between different TEs, with no two TEs using the same protocol. A

summary is provided in Table 3. It is worth noting that all TEs use antibiotic cocktails consisting of at least 3 different antibiotics from different antibiotic classes.

Post-decontamination microbiology culture results of participating TEs

Having decontaminated the HVs using their standard protocol, the TEs were asked to repeat microbiology culture of the HVs to check whether the HVs had been successfully decontaminated.

All TEs reported "no growth" post-decontamination of the HVs in sample A and sample B. Only twelve TEs reported "no growth" post-decontamination of the HVs in sample C. One TE (4) did not carry out post-decontamination testing of sample C due to the level of bioburden detected on the pre-decontamination cultures for this sample, which led to automatic discard of this HV. Eight TEs reported positive cultures post-decontamination i.e. failed to successfully decontaminate the HV tissue – please refer to Table 4.

Culture media used by participating TEs

The participating TEs were asked to provide details of the culture media used to isolate any contamination that may be present. There is significant variation in the type of media used and also in the protocols used when culturing tissue samples by the different TEs. Table 5 provides a summary of the ability of the different culture media used by the different TEs in isolating the different types of micro-organisms that may be present. All TEs use culture techniques that are able to isolate aerobic and anaerobic bacteria present, although 12 TEs use culture techniques that would not support the growth of fastidious or nutritionally-deficient micro-organisms. Nine TEs do not use culture techniques that are able to identify the presence of fungi.

DISCUSSION

Both the data-gathering exercise in 2016 carried out by the same group on HV banking, and this EQA in 2017, have been met with enthusiasm by TEs around the world with the number wishing to participate and eventually participating exceeding our expectations.

The courier company chosen to deliver the samples to the TEs unfortunately failed to live up to their guarantees of delivery of samples. However, from the results reported, the delay in sample delivery does not appear to have adversely affected the ability of TEs to correctly isolate and identify the micro-organisms present even when the sample was not processed until day 23 (TE 5) after preparation. The TEs reporting damage to the sample tubes did not have an issue with contamination – in fact none of the TEs who appear to have contaminated their samples reported any damage to their sample tubes.

Sampling error (i.e. failure to identify contamination that is present) in tissue banking is high (approximately 50%) if testing for the presence of contamination is done through

swabs and tissue biopsies only [4-6]. The sensitivity for isolating a contamination that is present can be increased to >90% if samples of the filtered transport fluid are also tested as well as a tissue biopsy [7, 8]. Most TEs in fact do sample both tissue biopsies and transport fluid. However this is not true of all TEs. In this EQA 6 TEs only tested tissue biposies, while one TE does not routinely take any pre-decontamination samples for culture. When deciding whether a particular HV is safe for clinical use or not it is important to be aware of whether there was any evidence of contamination on the tissue prior to attempting decontamination and the ID of such contaminating micro-organisms. HVs that have evidence of significant degrees of contamination particularly if contamination is with highly virulent micro-organisms that may be difficult to eradicate with antibiotics should be discarded irrespective of the culture results in the post-decontamination stage. There are a number of publications which underline the risk of false negative culture results (i.e. HV still contaminated with undetected micro-organisms) post-decontamination of tissue products when the pre-decontamination culture results are not taken into account [9-11].

In the post-decontamination stage, as a final sterility test of the HV tissue, most TEs take a sample of the cryoprotectant (DMSO) to check for the potential presence of contamination. DMSO has a toxic effect on micro-organisms and may therefore interfere with the successful isolation of contamination present leading to false negative culture results similar to the [12] effect of antibiotics, unless cultures are prolonged (2 weeks) and in validated antibiotic-neutralizing media. In order to minimise the risk of such false negative culture results in the post-decontamination stage it is important to include other samples besides the cryoprotectant when checking the sterility of the product, including tissue biopsies and rinsing fluid.

In this EQA potential contamination of the HV tissue from the donor or during the retrieval process are not relevant as the HV tissue used was known to be sterile and was "processed" in a controlled clean room environment before being artificially contaminated. In view of this, if a TE has reported the presence of micro-organisms other than the ones used to purposefully contaminate the HV tissue as part of this EQA, then these micro-organisms must have been introduced either during the HV processing by the individual TE or during sample culturing by the microbiological laboratory. From information provided in the previous data-gathering exercise that was carried out in 2016, TEs indicated that they process HVs in a grade A environment with either a grade B or grade C background (i.e. in controlled environments with laminar flow; refer to Eudralex Volume 4, Annex 1) [13]. This means that the risk of contamination during HV processing by the TE is low and usually by skin-flora from the operators, although cross contamination remains possible. On the other hand, contamination of the culture samples in the microbiology laboratory, either from the personnel or from the environment, is more likely. Microbiology laboratories may use open environments and non-sterile benches; the technicians processing the samples work in uncontrolled environments (e.g. near water taps), with no laminar flow or air-particle monitoring [14-17].

Sample A was contaminated with *Sphingomonas paucimobilis*. However it was retrospectively identified that in sample A there were residues of antibiotics that were interfering with the growth of the micro-organisms and even potentially killing the *Sphingomonas*. In view of this, this micro-organism could only be isolated if sample culture was carried out within 24 hours of the tissue samples being prepared, although the Gram negative rods could be identified microscopically for several days later and while these Gram negative rods weren't formally identified it is felt likely that they were the same micro-organism rather than a new contaminant. This means that cultures from sample A taken more than 24 hours after the samples were prepared would likely result in a negative culture result, as demonstrated and confirmed by the two microbiology reference laboratories. Any bacteria other than *Sphingomonas* isolated by the TEs at more than 24 hours from sample preparation must be a contaminant introduced by the TE or the microbiology testing laboratory.

Of the three TEs reporting a positive culture result for sample A, none of them reported damage to the sample tubes during transport. One of these 3 TEs reported the presence of a Gram negative rod (likely the *Sphingomonas*) but did not proceed to identify it. This was a correct result (cultured at <24 hours), and not a contaminant as the TE thought, and therefore considered a correct result for the purposes of this EQA, although ideally the micro-organism should have been fully identified. One TE reported the presence of a *Bacillus* species; the source of this contamination is likely to be *Bacillus* spores found in the environmental air; this type of contamination is most likely to have taken place in the microbiology laboratory. The third TE reported the presence of *Staphylococcus cohnii*, a skin commensal mostly found on the lower limbs, that is likely to have originated from the personnel handling the specimens, with the microbiology laboratory being the most likely source.

None of the TEs checked for the presence of antibiotics, which raises concern about the possibility of false negative culture results. Many donors will have received antibiotics before death which can lead to false negative culture results, unless the samples are cultured within a few hours in a blood culture medium that contains toxin and antibiotic neutralising resins. However most TEs rinse their tissue several times to remove any antibiotics that may be present, although most TEs have not confirmed or validated the effectivness of removing or neutralizing the antibiotics using this method.

Sample B was a negative control sample, also confirmed by both reference microbiology laboratories. Therefore any bacteria isolated in this sample would be a contaminant introduced either by the TE or the microbiology laboratory. Twenty of the 22 TEs reported "no growth" for this sample, while the remaining two TEs reported positive culture results: one TE (11) identified the presence of *Staphylococcus hominis*. This same TE reported "no growth" for sample C (which contained *Staphylococcus hominis*) leading to the possibility that this TE may have swapped their culture samples. The second TE reported the presence of *Propionibacterium acnes*, a skin commensal which may

have been introduced as a contaminant either by the TE or the medical microbiology laboratory.

Sample C was contaminated with *Roseomonas mucosa* and *Staphylococcus hominis*, also confirmed by both reference microbiology laboratories. Eight TEs isolated and correctly identified both contaminating micro-organisms (although one TE did not fully identify the *Staphylococcus hominis*, reporting the presence of coagulase negative *Staphylococci* (CNS) instead), these samples being processed between 48 and 552 hours after the samples were initially prepared.

A further seven TEs identified only the *Roseomonas* spp in their culture; these samples were processed between 48 and 192 hours after they were initially prepared (16 48 hours; 20 & 21 72 hours; 1, 12 & 18 144 hours; 15 192 hours). A possible reason for these TEs not identifying the *Staphylococcus hominis* could be heavy *Roseomonas* growth (reddish colonies) on the culture plates, which would make recognition of the *Staphylococci hominis* (small yellow-orange colonies) difficult.

One TE correctly identified the presence of the *Roseomonas* spp in sample C but also reported the presence of *Bacillus pumilis;* the only likely source of this contaminat are spores from the environmental air, most likely from the diagnostic microbiology laboratory. The presence of the *Bacillus pumilis* and *Roseomonas* are likely to have made identifying the *Staphylococcus hominis* also present difficult.

One TE reported the presence of *Staphylococcus schleiferi* for sample C; this is either a wrong identification or a contaminant from the microbiology laboratory given it is not a common CNS.

One TE reported the presence of *Staphylococcus hominis* together with *Stenotrophomonas maltophilia* for sample C. The latter could either be a wrong identification or possibly a contaminant, most likely introduced by the medical microbiology laboratory, as this micro-organism is normally found in water systems and the microbiology laboratory is the only site during HV processing where there would be direct contact with a water system.

Four TEs reported "no growth" for sample C – one of these four does not routinely carry out pre-decontamination tissue cultures (although did culture the samples sent for the purposes of this EQA) and two of the TEs probably use media that are insufficient to identify all potential contaminants. The fourth TE may has swapped sample C with sample B, explaining their return of "no growth" for this sample (see earlier).

When considering the decontamination protocols used, the different TEs used very variable protocols with variable antibiotic cocktails at variable temperatures for variable lengths of time. The important question is whether the antibiotic cocktail used is likely

to be effective against the skin, mucosal and environmental contaminants that are likely to be present on HVs.

In order to have effective antibiotic decontamination of HVs, the antibiotic cocktail used needs to have broad effectiveness against aerobic and anaerobic bacterial and fungal micro-organisms.

The concentrations of antibiotics used by the different TEs varies significantly. When compared to the doses normally used clinically to treat patients, all TEs are using very high concentrations of antibiotics. Only three TEs use antibiotic concentrations that do not exceed the dose used in clinical patients (i.e. the therapeutic dose) by three-fold. The smallest antibiotic doses used by five of the TEs is around 10-times a therapeutic dose and the remaining 17 TEs use antibiotic doses that are more than 20-times higher than therapeutic doses, in some cases up to more than 50-times higher. Such doses are very difficult to rinse or neutralise leading to concerns about potential false negative culture results after antibiotic decontamination and may have toxic effects on the tissue, something that is a concern for all antibiotics, but is a particular concern when using Aminoglycosides and Amphotericin [18] at high doses.

The temperature at which decontamination is carried out also varies between different TEs (4°C, room temperature or 37°C). Bacteria that grow on humans, both commensal and pathogenic micro-organisms, are "mesophilic" i.e. they grow best at a temperature of 20 to 40°C. Below 20°C such micro-organisms grow much slower, their enzymes cannot mediate chemical reactions, so that eventually the viscosity of the cell-interior brings all activity to a halt. All human bacteria (and human cells) slow down and eventually die at low temperatures. Most bacteria do not grow at all, or minimally so, at 4°C which means that betalactam antibiotics (all penicillins and cephalosporines) cannot be effective at low decontamination temperatures [19]. Even protein-attacking antibiotics are only effective against growing bacteria when protein synthesis is taking place and not at low temperatures. Any static/cidal effect on the micro-organisms at low temperatures therefore is not being caused by the antibiotics but by the temperature/medium and other factors in the liquid that the HVs are in.

The length of time the HVs are left in the antibiotic cocktail for decontamination also varies between the different TEs. Killing of bacteria is mostly done within the first hour of contact with antibiotics at 35°C with no additional benefit beyond 6 hours at this temperature [20]. This temperature has been shown to lead to a 100% antibiotic cidal effect for several antibiotic cocktails; this is not the case for decontamination carried out at 4°C (10)

Post-decontamination, 13 TEs reported negative cultures, while 8 TEs reported persistently positive cultures with the same micro-organisms that had been present in the pre-decontamination cultures. The most likely cause for persistent growth after

antibiotic decontamination is the lack of antibiotic activity at the low temperatures used during decontamination by seven of the TEs.

A further identified reason for failure of decontamination is that *Roseomonas* is resistant to Cephalosporins, Piperacillin/Tazobactam and Polymyxin/Colistin. Five of the 8 TEs that failed to successfully decontaminate the HVs use only Polymyxin or Polymyxin in combination with a cephalosporin. Further one TE left the HVs in antibiotics for only 5 hours (at 37°C); this is possibly too short a period to be fully effective against slow growing and slimy bacteria in tissue.

Finally consideration needs to be given to the potential of residual antibiotics leading to false negative post-decontamination culture results. Hence the importance of proper rinsing or neutralisation [21] before carrying out repeat tissue culture.

When selecting the media used to culture tissue samples TEs need to ensure that the media will be able to isolate all potentially contaminating micro-organisms, including aerobic, anaerobic and also fungal contaminants. Fastidious bacteria are common on skin, mucosa and in the environment: for example *Corynebacteria* and *Propionibacterium* from the skin, *Stenotrophomonas* from the environment and *Neisseria* and *Streptococci* from the mucosa amongst others. In order to isolate such potential contaminants it is necessary to use enriched media (such as blood culture media). Enriched media are also required in order to be able to successfully isolate many streptococci and other mucosal inhabitants as well as non-fermenting Gram negative bacteria from the environment (Pseudomonas-like). A different medium needs to be added to allow growth of anaerobes (an oxygen free medium with extra iron). Blood culture-media is also able to neutralise toxins and antibiotics that may be present [22, 23] so that growth of micro-organisms may be possible even when antibiotics are present in the tissue (although this still needs to be validated).

Contamination from the air, especially by spores, is very common. Since many such spores are fungal, culture at a lower (20°C) temperature is required to isolate such micro-organisms. When looking for potential contamination of HVs it is therefore important to use media that supports fungal growth to ensure no contamination of the tissue occurs during processing.

Table 5 provides a summary of the suitability or otherwise of the culture media used by the TEs in isolating the different types of potentially contaminating micro-organisms. As can be seen from this table more than 50% of the TEs do not use culture media that would allow the isolation of fastidious bacteria or fungi. This would particularly be a concern if the antibiotic cocktail used for decontamination did not provide sufficient broad coverage that is effective against all types of micro-organisms, including against potential fungal contaminants. On the other hand there are reports of positive bacterial results post-implantation without resultant clinical infection [24, 25], suggesting that low numbers of commensals (but not environmental micro-organisms and spores) that

may be present on HVs are not necessarily detrimental to the recipient, although it is impossible to confirm this in a research trial, since avoidance of infection transmission remains the aim.

An important question to consider is whether culturing at temperatures other then 30-37°C is necessary or not. Only fungi and bacteria that survive at human body temperature (30-35°C) can be pathogenic. At temperatures <30°C some micro-organisms die straight away. However there remains a concern that toxins and spores from such micro-organisms can cause problems in the recipient, leading to the conclusion that TEs should aim to identify any potential contaminants that may be present to be able to minimise the risk to recipients as much as possible.

One weakness of this work is that hardly any TE carry out recipient follow-up. It is important to establish whether the reported differences in the decontamination procedures used are leading to clinical problems or not.

CONCLUSIONS

This was an excellent collaborative initiative following on from our previous data-gathering exercise on HV banking. There is evidence of significant differences in practice between different TEs. While a number of TEs were able to successfully isolate and identify all contaminating micro-organisms present and then successfully decontaminate the HV tissue, a fair number of TEs failed to isolate and identify one or more of the contaminants due to the use of culture media that do not support all types of potential contaminants that may be present or invalid identification of the micro-organisms. Further some TEs, or their microbiology laboratories, appear to have introduced contamination during their processing or testing of their samples. Also, a number of TEs were unable to fully decontaminate the HV tissue, likely due to insufficient antibiotic cover and/or the incubation temperature at which decontamination was carried out; and none of the TEs checked for the residual presence of antibiotics.

Following on from this initiative we now aim to work towards establishing a regular External Quality Assessment scheme for HV banking, and potentially other tissue products, with the aim of standardising tissue banking and improving the clinical safety of tissue products.

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Corlife, OHG, Hannover Germany
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Ethical approval was not required.

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Tables and Captions

Table 1a. Summary of the pre-decontamination samples taken by TEs for culture

Number of TEs	Tissue biopsy	Transport fluid	Antibiotic cocktail	Swabs	No samples taken
11	+	+			
1	+	+	+		
1	+			+	
6	+				
1		+			
2					+
Total : 22	19	13	1	1	2

Table 1b. Summary of the post-decontamination samples taken by TEs for culture (final check for potential of contamination)

Number of TEs	Tissue biopsy	Cryoprotectant	Antibiotic cocktail	Rinsing fluid
12	+	+		
2	+	+	+	
1	+	+		+
1	+		+	
2		+		
2		+		+
1		+	+	
1			+	
Total: 22	16	20	5	3

Table 2. Summary of culture results submitted by the participating TEs.

	TE code	Wrong/ insufficient results	No. TEs with correct results	No pre-decontamination sampling	Insufficient Media	Other potential cause
Sample A			20*			
Contamination	2	*Bacillus spp*				Possible air contamination ML**
	10	*Staph.cohnii*				TE or ML** contamination
Sample B			20***			
Contamination	11	*Staph.hominis*				Cross contamination or sample B and C switched
	10	*Propionibact.acne*				TE or ML** contamination
Sample C			8****			
No growth	2	No growth		+		
	11	No growth			+	
	13	No growth			+	
	28	No growth				unknown
Only one micro-organism identified	14	*Roseomonas* spp				Possible ML** error
	16	*Roseomonas* spp				Possible ML** error
	1	*Roseomonas* spp				Possible ML** error
	21	*Roseomonas muc*				Possible ML** error
	20	*Roseomonas muc*				Possible ML** error
	12	*Roseomonas gilardi*				Possible ML** error
	23	*Roseomonas* spp				Possible ML** error
One micro-organism identified + contamination	3	*Roseomonas* spp+ *Bacillus pumilis*				Possible air contamination ML**
	4	*Staph.hominis* + *Stenotrophomonas*				Possible environmental contamination ML**
Contamination	10	*Staph.schleiferi*				Possible false identity ML**

TE - Tissue Establishment; No.- Number; ML - Microbiology laboratory

*1 TE correctly identified the presence of non-fermemtative Gram-negative rods in sample A but did not fully identify this micro-organism, the other 19 TEs reported "no growth" for sample A; 1 TE (13), concerned that they had been unable to identify any micro-organisms in the samples, also carried out a Gram stain of the samples (Gram stain not being part of their normal protocol) – the Gram stain identified the presence of Gram negative bacilli in the sample. TE 13 reported that the samples had been received after six days and the tube of sample A was leaking.

**ML - probable error (e.g. not recognised *Staph. hominis* amongst the *Roseomonas*) or contamination of the culture samples occurred in the diagnostic medical microbiology laboratory

***20 TBs reported "no growth" including TE 13 which again carried out a Gram stain of the samples (Gram stain not being part of their normal protocol), the Gram stain being negative on this sample i.e. no micro-organisms present.

****8 TEs identified both the *Roseomonas* and the *Staph hominis*; TE 13 again carried out a Gram stain on the sample (Gram stain not being part of their normal protocol) – the Gram stain confirmed the presence of micro-organisms.

Table 3. Summary of the decontamination protocols used by the 22 TEs including the following information: number of TEs using a particular class of antibiotics, the number of TEs carrying out decontamination at a particular temperature and the duration in antibiotics during decontamination and how many TEs rinse off the antibiotics or not.

	Micro-organism cover of antibiotic cocktail				Temperature of decontamination			Duration in antibiotics			Rinsing of antibiotics	
	Gram +ve	Gram -ve	Anaerobic	Fungi	4˚C	22˚C	37˚C	12 hr	24 hr	48 hr	Yes	No
No.* TEs** with sufficient cover	19	14	9	13	15	3	4	4	14	4	15	7
No.* TEs** with no cover	3 insufficient	8 insufficient	13	9								

Gram +ve - organisms that stain positively (blue) in a Gram stain (i.e. all staphylococci and streptococci)
Gram -ve – organisms that stain negatively (red) in a Gram stain (i.e. all aerobic rods such as E.coli, Klebsiella spp, Pseudomonas spp a.o.)
*No. - number **TE - Tissue Establishment

Table 4. Culture results post-antibiotic decontamination for sample C for TEs that failed to fully decontaminate the HVs

TE Code	Culture results	Temperature of decontamination	*Staph. hominis* Ab. coverage	*Roseomonas* Ab. coverage	Ab. Incubation Time (hours)	Possible reasons for positive cultures post-decontamination
1	*Roseomonas*	22˚C	sufficient	sufficient	24	Low temperature
2	*Staph. hominis*	4˚C	sufficient	dubious	12	Low temperature
6	*Roseomonas*	4˚C	sufficient	dubious	24	Low temperature
8	*Roseomonas*	37˚C	sufficient	sufficient	5	Too short for slow slimy growing bacteria
11	*Rhodococcus equi* + *Staph. hominis**	4˚C	sufficient	dubious	40	Low temperature +wrong identification
16	*Roseomonas*	4˚C	sufficient	dubious	24	Low temperature
19	*Roseomonas*	22˚C	sufficient	sufficient	18	Low temperature
21	*Roseomonas*	4˚C	sufficient	sufficient	24	Low temperature

Ab. - antibiotic
**Roseomonas* is a gram negative coccobacillus that is resistant to Cephalosporins and Piperacillin/Tazobactam and also resistant to Polymyxin. It can be difficult to differentiate *Roseomonas* from *Staph. hominis*.

Table 5. Summary of ability of culture media used to identify different types of micro-organisms

TE* code	Gram +ve bacteria		Gram –ve bacteria		Anaerobes	Fungi
	Robust‡	Fastidious‡	Robust‡	Fastidious‡		
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3	+	-	+	-	+	+
4	+	-	+	-	+	+
5	+	+	+	+	+	-
6	+	-	+	-	+	+
7	+	-	+	-	+	+
8	+	+	+	+	+	-
9	+	-	+	-	+	+
10	+	-	+	-	+	-
11	-	-	+	-	+	-
12	+	-	+	-	+	+
13	+	-	+	-	+	+
14	+	+	+	+	+	-
15	+	-	+	-	+	+
16	+	-	+	-	+	-
17	+	+	+	+	+	+
18	+	+	+	+	+	+
19	+	+	+	+	+	-
20	+	-	+	-	+	-
21	+	+	+	+	+	-
22	+	+	+	+	+	+
Total	21+/1-	10+/12-	22+	10+/12-	22+	13+/9-

*TE – Tissue Establishment

‡Robust vs Fastidious micro-organisms: robust micro-organisms are ones that are able to grown on general media without needing the additon of special nutritional supplements or conditions, as different to fastidious micro-organisms.

CHAPTER 15

Discussion and Conclusion

Differences in applied methods and their outcomes can be determined in different areas of cardiac surgery. By comparing the identified differences, cardiac surgery centres and cardiovascular tissue establishments can gain insight into the quality of their activities and clinical outcomes. To this end, various registries and databases in which data from many international centres are collected and shared, are used. When the comparison provides a negative result, for example showing a higher morbidity, more frequent infectious infections or indicates shortages of cardiovascular allografts, there will be room for improvement. By periodic benchmarking of their outcomes and analysing the results, the individual centers can concentrate on the elimination of identified areas for improvement. As a result, the outcomes improved and the differences with other organisations are minimised or eliminated. In this way they arrive at the best possible 'best practices'.

Benchmarking of outcomes in cardio-thoracic surgery at European level

In **Chapter 2** we presented a survey of literature concerning the influence of registry data on the outcomes of heart surgery. The hypothesis was that there are hundreds of studies that claim that the registries investigated contribute to quality improvement, but that the causal relationship is often not demonstrated.

Numerous publications conclude that a reduction in morbidity and mortality in the clinical units of the participants is demonstrated. However, studies demonstrating the direct causal link between the use of cardio-thoracic surgery-oriented registers and improvement of clinical hospital outcomes are extremely scarce. In this study, we wanted to analyse the causal relationship between the use of cardio-thoracic surgery-oriented registers and improvement of clinical outcomes. In a systematic literature review, publications were selected that demonstrate the use of registry data to obtain consolidated quality improvements. After analysing 2990 scientific publications, 6 studies filled the inclusion criteria. The selected studies acknowledged that benchmarking of data with registries was used for targeted and methodologically organised improvement in cardio-thoracic departments. Combined with the impact of the applied methods on healthcare, their results show quantifiable improved local results over time. An eye-catching observation is that the United States has a big lead over Europe. This leads to the conclusion that Europe has some catching up to do.

Benchmarking in the field of therapy through support hearts.

Chapters 3 and 4 show the state of affairs with regard to the use of "Mechanical Circulatory Support" or MCS in centres participating in the EUROMACS Registry. The implanted devices provide mechanical support for the heart, and, as a result the blood circulation, in the long term. MCS is often used for patients who are at risk of dying on the waiting list for heart transplantation ("bridge to transplant") or, as an alternative,

for patients with end-stage heart failure who are not eligible for a heart transplant. The second EUROMACS report, presented in Chapter 4, shows that most devices are used to support the function of the left ventricle of the heart: 2646 implants. In this situation they are referred to as "Left Ventricular Assist Devices, LVADs". In addition to this, 114 Right Ventricular Assist Devices (RVADs) were implanted to support the right ventricle; 81 to support both ventricles (BiVAD); and 31 as "total artificial heart" (TAH) implants. In 36% of the patients an MCS device was implanted to assess whether the patient would be eligible for a heart transplant ("bridge-to-candidacy"). This was immediately apparent in 28% of patients with heart failure ("bridge-to-transplant"). There are two forms of pump technology that are used in support hearts: pumps that pulsate and pumps that rotate continuously. In this study, the ratio was 68 (3%) and 2214 (97%), respectively. Furthermore, it was found that the total population of patients consisted of 18% women and 82% men. This is important for further research as shown in **chapter 6**.

For those who do not qualify for heart transplantation, generally or due to age and/or additional illnesses, an artificial heart is permanently implanted as a last resort ("destination therapy"): 16%. In this category, most patients were older than 65 years.

The development of the mechanical support therapy for circulation is relatively new. Although the devices have undergone technical improvements in recent years, the therapy frequently results in serious complications: in the first 3 months after implantation, 647 serious complications were reported and 1404 after more than 3 months from implantation. Infections (41.2%) are the most common serious complications of therapy, followed by bleeding (21%) and malfunction of the supporting heart, often due to thrombosis (22%) and neurological incidents (15%).

In the second EUROMACS report we also looked at the long-term results of MCS and the long-term clinical results. We only observed the results of "mainstream" devices with continuously rotating pumps. The survival of 2268 adult patients (older than 17 years) with an average follow-up time of 379 days was 86% after 30 days, 66% after 1 year, 53% after 2 years; and 42% after 3 years.

The report provides a breakdown of those survival rates by age, stage of heart failure (INTERMACS level) and therapy strategy (bridge to transplant, destination therapy etc.).

It was of great importance to investigate the chance of competing outcomes. In the case of supporting heart therapy this means that the frequency of heart transplantation influences survival with an MCS device. At the end of the first 6-month period, 5.4% of the observed population (n = 2044) were transplanted and 27.4% had died. In 65.2% of patients, the device still functioned after 6 months, while the heart of 1.5% of this group recovered so that the assist device could be explanted. **Chapter 7** focuses on the latter phenomenon.

The importance of these analyses is that heart failure programs participating in the EUROMACS registry, as well as programmes that do not participate, can compare their outcomes with accumulated European results. With this second report we have created a benchmark for therapy with MCS in Europe. Furthermore, we benchmarked the results with those of the American registry, INTERMACS. This showed that considerable differences in results could be observed. The 3-year survival of patients with an LVAD and of patients with a BiVAD was 44% and 21% respectively in the EUROMACS database and 58% and 40% in that of INTERMACS. If the European outcomes are real and not biased by the different types of patients, non-compliance of the provided data, and/or differences in definitions between Europe and the United States, then the causes of the poorer outcomes in Europe should be further investigated. Possibly, the differences in patient categories, the extent of illnesses and additional disorders, in addition to specific selection criteria and other therapeutic applications, contributed to the observed differences.

These differences are also observed in **Chapter 4** in which data regarding MCS therapy in children are analysed. The use of MCS in children differs from adults in that 36% of the implanted devices are pulsating. In children younger than 10 years the number of implanted pulsating devices is 84%. This is due to the limited range of suitable devices for small children; there is only one supplier, other than the mainstream manufacturers who only make rotating pumps, which also produce small sizes.

One of the most striking differences with the situation on the American continent (in this case the United States and Canada) is the waiting time for children with heart failure on a donor heart for transplantation. The North American data, collected in the Pedimacs Report, where almost 50% of the children could undergo a transplant within 6 months, was only 33% in Europe. The competitive outcomes, however, show a more favourable picture for European patients with regard to death: 15% after 1 year and 17% after 2 years. These percentages are relatively low and give good prospects for assistive heart therapy in Europe. In both the Pedimacs and in the EUROMACS report, neurological complications were the main cause of death 30% versus 24%.

With this study we have been able to create a benchmark for child heart specialists. This report provides a first insight into the results of supporting heart therapy in children in Europe. As with the adults, the Benchmark with data from the Pedimacs report raises possible differences with regard to treatment methods. These questions, like those asked in the report on the results of adults with supporting hearts, should be the subject of further studies.

In **Chapter 5** we added the data of the EUROMACS registry to those from the International Society for Heart and Lung Transplantation Mechanical Circulatory Support (IMACS) registry database. In the report, data from 14,062 patients from different parts of the world are accumulated and analysed. The 1- and 2-year survival of all patients was 80% and 70% respectively. The 3-year survival of patients with an LVAD

equipped with a continuously rotating pump was 61%, which is better than the results reported in the second EUROMACS report, but also better than those in the seventh INTERMACS report. For the first time, a comparison was also made between MCS with pulsating and MCS with continuously rotating devices. Although the pulsating devices represent only a relatively small amount of the total number of implants (3%), the survival compared to continuously rotating devices over the 3-year term was better: 79.6% versus 62.4%. An important finding in the analysis we did for the IMACS report is that kidney function was measured 2 days before implantation of an MCS device. Patients undergoing dialysis had higher preoperative urea and creatinine levels and higher premature death. As in the EUROMACS cohort, bleeding and infection were the most important major complications of therapy.

With regard to the IMACS registry, the conclusion is justified that the data, partly due to its quantity, is an important benchmark for analyses of outcomes of MCS therapy on an international level.

In **Chapter 6** we present a study of the differences in results of LVAD devices in men and women, using data from the EUROMACS database. 151 (15.6%) of the patients studied were female and 815 (84.4%) male. Moreover, although heart failure is more frequent in women than in men, the likelihood of being treated with an assist device is lower. This study attempts to identify the factors that influence the survival of women and men. In doing so, we looked at factors that play a preoperative role and at the other hand at what influences outcomes peri- and post-operatively. It is also striking that in the selection of patients who are eligible for an LVAD, women are later in time eligible for implantation as compared to men. This is despite the fact that in the final selection, heart failure is more advanced in women than in men.

During the peri-operative period, it appeared that women, after implantation of an LVAD, required more frequent support of the right ventricle. It is possible that the size of the assist device and the type play a role here. At the time of the study, there was only one manufacturer offering a smaller LVAD in terms of size. However, this type is associated with thrombosis formation. Postoperatively, women also stayed in intensive care for longer than men, while the ventilation period was longer too. An analysis of peri-operative and long-term outcomes has shown that the number of serious complications such as bleeding, arrhythmia and right heart failure was greater in numbers than in men. Also, the mortality rate for women undergoing LVAD implantation is 7.7% higher after 1 year than in men (24.5% and 16.8% respectively) and 7.3% higher after 2 years (32.1% and 26.8%).

The number of publications regarding differences in selection and treatment by gender when applying MCS therapy is still very limited. This study has provided a benchmark that contributes to the insight that it is necessary to further investigate the causes of the observed differences. Moreover, the clear timing and practical consequences for the clinical application of MCS therapy in women must be included.

In **Chapter 7** we investigate patients whose MCS device has been explanted after adequate recovery of the heart muscle and, if so, what the long-term outcomes are. Data from the EUROMACS registry were also used for this study. 49 patients were identified in the registry with the LVAD successfully removed. As explantation, in addition to heart transplantation and death, is an end point in EUROMACS, the follow-up data after explantation are unfortunately not in EUROMACS. That is why the practitioners of the patients concerned were approached with a questionnaire. This questionnaire had a response rate of 57% (28 patients, 17.9% female) and provided information on primary outcomes as well as relevant clinical data after explantation. The long-term outcomes of the investigated patient group were excellent with 92% 4-year survival with no major complications and or little need for renewed LVAD implantation or heart transplantation. This study is one of the first studies to show results at a European level based on data from multiple centers, for patients of whom the LVAD has been removed. As such, the data is a reference for necessary future studies. It is recommended that in new follow-up studies, the clinical data should continue to be recorded even after the MCS device has been explanted and, moreover, to add biological and pathological data to the database.

In **Chapter 8** we present a comparison between patients who have undergone surgery on the tricuspid valve during the implantation of an LVAD. In patients with heart failure, in which the left ventricle should be supported with an assist device, leakage of the tricuspid valve is more frequent, current guidelines recommend that surgery be performed on the tricuspid valve when an LVAD is implanted. As there is hardly any good clinical literature on this, we have performed an extensive analysis using data from the EUROMACS database and show that such an intervention does not necessarily lead to significantly better results in the short or longer term. A good selection of patients with leakage of the tricuspid valve may possibly give better results. Prospective, randomized studies are required for this.

Benchmarking in the field of tissue transplants, organizational forms, costs and quality

Chapters 9 and 10 form part of a wide-ranging study for the European Commission into applications for the clinical use of human tissues in the European Union. The selected chapters relate to economic and organisational aspects of processes that aim to acquire, process, preserve and distribute human tissues for transplantation.

The studies that are presented give an overview of tissues that are used in various specialties as a replacement for a diseased tissue in the patient with the aim of taking over the original function of the diseased tissue.

In order to be able to make a non-donated post-mortem human tissue available to a surgeon for implantation in a patient, investments in people and resources must be

made. Chapter 10 explains which aspects play a role in setting up a process aimed at making human tissues available for transplantation. Since the European Directives in the field of quality and safety of human tissues came into force, investments in clean rooms have become necessary. The conditions in those clean rooms are comparable to similar units in pharmaceutical production companies. In addition to the costs of the cleanrooms, there are costs that are accumulated in the process from donation to transplantation. Chapter 10 shows that process. Ultimately, the total of investment and processing costs is spread over the tissues to be distributed. The different ways of passing on costs, if not from a public or private organisational setting, leads to the obstacles identified in Chapter 11 in achieving a balance between supply and demand.

For use in cardiac surgery we have focused on human heart valves, also known as homografts, and on other tissues that are transplanted in cardiac surgery to replace the recipient's own tissue such as arteries, veins and pericardium. Important findings with regard to factors that obstruct a sufficient supply of donor tissue for use in cardiac surgery in a number of European countries are presented in **Chapter 10**. In those countries where explicit permission (opting-in) for donation is required, the additional effort that is required to obtain permission from relatives of the donor is a further obstacle in obtaining sufficient heart valve and other tissues. A better exchange could take place at European level so that the supply of human implants is better balanced with the demand from the clinics. The obstacles identified are summarised as follows: giving priority to national patient recipients over those from other European countries; differences in donor selection criteria; deviating virological and microbiological test regimes; divergent legislation; and costs of the process included in the price of the tissue be passed on. When determining the costs and charging them to the receiving hospital, the question is whether the tissue establishment that receives, processes and distributes the tissue is a public or private organisation. It can also play a role in this if the tissue establishment is part of another (public law) hospital, an independent foundation or an organisation financed with shareholder capital.

The report from the European Commission takes stock of the figures from 2012 and 2013, showing no increasing trend in availability or in the number of implantations of cardiovascular tissues. One of the causes is the aforementioned obstructing factors for obtaining suitable grafts. On the other hand, more industrially produced valves and bio-prostheses are produced, using bovine or porcine tissues. By innovating the production methods, those bio-prostheses are getting better and better.

Our conclusion is that there is a decreasing trend in the use of cardiovascular donor tissues, but that there will always be a - albeit lower - demand. For the long term, we expect better long-term results due to the application of new techniques such as decellulating heart valves before implantation.

Benchmarking methods and outcomes of cardiovascular tissue establishments

In **Chapter 11** we present detailed findings from a study of 18 different cardiovascular tissue institutions. These institutions received an average of 1672 donor hearts per year (range 1640-1700). On average, 709 usable heart valves were preserved from those donor hearts. The number of distributed valves differs greatly per tissue establishment: from a minimum of 4 to a maximum of 79 aortic valves and from 17 to 243 for pulmonary valves per year. The reasons for these differences are largely due to divergent selection criteria in these institutions. The application of those criteria leads to much higher rejection rates than we believe is necessary. Very large methodological differences in decontamination of heart valves raise the question of the substantiation of those methods and demonstrate the need for standardisation of these methods.

Chapter 12 is the first step towards the need to validate decontamination methods, as found in Chapter 11. The starting point for this quality round, in which microbiological methods and their results are examined in tissue establishments, is that cardiac surgeons must be able to trust that the methods used are effective and that tissue grafts are sterile. The participating institutions, 19 of which are situated in the European Union, and one in Singapore, each received 2 pieces of tissue that we had contaminated with our known micro-organisms. The task of the participants was to identify and decontaminate those micro-organisms with the locally used method. Then it had to be tested whether the tissues were free of micro-organisms and to indicate whether the institution in question would release the tissue, if it were to be a heart valve, for implantation.

This study shows in detail the large differences between both the nature and concentration of antibiotics used, as well as a wide spread in the temperature used (20° C to 30° C) and whether or not antibiotics were washed out after the decontamination procedure.

Ultimately, the decontamination method in 13 tissue establishments was not effective enough to perform adequate decontamination. Moreover, it could be established that many unnecessary process steps were carried out. 17 tissue establishments could save costs by applying a more logical design of their processes.

The exercise by means of a quality round in this study is in fact a benchmarking tool that makes best practices visible. This study also demonstrates once again that harmonisation of methods is necessary to prevent the loss of scarce tissue. If a stage can be reached in which tissue establishments can rely on each other's methods, exchange at European level can be optimised.

The research presented in **Chapter 13** is in line with that of Chapter 12. Where Chapter 12 relates to the outcomes of microbiological research and decontamination methods of micro-organisms, Chapter 13 focuses on mapping factors that lead to the rejection of donated hearts or tissues of hearts. The research among 15 European and 4 non-

European organisations showed that all 19 cardiovascular tissue establishments accept multi-organ donors. 4 limit themselves to these and do not accept other donors. Most other (15) also accept donor hearts from deceased people who are only tissue donors. So-called domino hearts, which are hearts of patients undergoing a heart transplant, and whose sick heart sometimes still has one or more good heart valves, were accepted by 8 institutions. Another factor that influences the quantity of donated tissues is the upper age limit set on donors. This varies from 40 years to 70 years. We also saw great variation in the anamnestic study, in which 4 respondents indicated that the medical history is never examined, 7 did not contact the GP more closely and 1 establishment does not use pathology data.

Striking differences were noted in other important quality-determining processes, such as donor cooling, accepted post-mortem time and virological tests. Again, the decontamination methods and the temperatures used in them were found to be different and correlation with rejection rates was observed.

The conclusions of the research presented in Chapter 13 into the factors that play a role in rejecting hearts and tissues encouraged us to organise a quality round again.

In **Chapter 14** we report the findings of the second quality round in which the microbiological methods used in cardiovascular tissue settings are examined in more detail than before. 25 organisations were involved in the survey (21 of them from Europe). 3 contaminated tissue samples were sent to each participating institution. Two of the samples (sample A and sample C) were infected with micro-organisms known to us. The third sample (sample B) was sterile and intended as a control for negative testing. 20 of the 22 institutions tested sample A correctly and only 8 tested sample C correctly. In the sterile sample B, 2 institutions still found micro-organisms; an evaluation showed that the cause is probably the result of cross-contamination and/or swapping of samples. Once again it was suspected that low decontamination temperatures are often ineffective, an analysis of the results of the institutions that proved unable to decontaminate sample C was sufficient. The low temperature (4° C to 22° C) was the cause of the failure.

The large variation found in donor selection criteria, working methods, decontamination methods and assessment criteria led us to initiate a recurring external objective quality assessment. Its purpose must lead to standardisation and improvement of the clinical quality of tissues for implantation.

Future perspective

In many countries, national databases with data and outcomes of cardio-thoracic procedures are used to accumulate and compare data. This process is still ongoing in some countries. It is expected that in Europe the development of an instrument of benchmarking for hospitals and surgeons providing cardio-thoracic care will be realised in the coming years and play an important role in demonstrating quality. In addition, they must demonstrate that they are able to meet the justified expectation that they, as healthcare providers, can actually deliver, according to the definition: "quality of care is the degree to which health services for individuals and populations increase the likelihood of desired health outcomes and are consistent with current professional knowledge". This is not exclusively limited to benchmarking based on numbers. Tools such as standardised feedback reports and collaborative groups in which differences in methods and results are frequently analysed and discussed at European level have proven to add to the improvement of outcomes. For the success of registries and the resulting improvement initiatives, the objectivity and good governance of the responsible organisation are of great importance. Additionally, adequate structural financing of costs must guarantee long-term maintenance of the registry.

In view of the growing population of heart failure patients worldwide, and taking into account that further innovations are being implemented, it is expected that the implantation of MCS devices will increase in the long term. In this thesis it is already suggested that further improvement of the results can be expected through early selection of patients who are eligible for an assist device.

Further developments and innovations in the field of support MCS devices are to be expected. The devices will become smaller, which is beneficial for children, young adults and women. Complications due to advancing technological innovation, such as minimal invasive or percutaneous implantations, and/or from full support to partial support will decrease unwanted complications. In addition to this, much can be gained by investigating which factors can improve the long-term outcomes for women. The largest number of complications occurs as a result of infections. Their occurrence is largely caused by the porte d'entrée that is formed by the external power supply of the support hearts. The first results of a fully implantable artificial heart were recently presented. The external power supply took place by means of induction. However, many problems still have to be solved before this technique can be used as standard. In particular, issues such as heat development affecting the skin and other organs of the patient are a point of attention.

Cardiac surgeons must be able to trust that human allogeneic tissue is available for transplantation and that it is also safe for the patient. As there is progressive insight within the European Union about the different methods used in the member states, the international allocation of cardiovascular grafts can be improved. The tissue establishments that collaborate in the international benchmarking project are looking

to eliminate factors that influence quality by setting up an affiliation with NEQAS (National External Quality Assessment Service) an independent, now international, consortium that focuses on improving test results in laboratories. Eventually the observed differences in outcomes will disappear and the certainty that the quality of the tests is reliable will increase.

The use of human heart valve tissue will generally decrease, for specific indications decellulated tissues will be preferred, although due to the high processing costs, no total substitution of traditionally processed heart valves can be expected.

Conclusion

This dissertation contributes to the knowledge of bridging negative differences in outcomes through analysis and providing methods for improvement. Benchmarking methods providing more detailed insight where there is specific room for improvement are presented. Literature research combined with analyses from registries and quality rounds shows how we can contribute to the knowledge about improving clinical outcomes and preventing complications. Reports and studies using the data from the EUROMACS database reflect the European state of affairs regarding the results of implantation of Mechanical Circulatory Support devices in adults and children. Moreover, these results can be compared with outcomes on a global level and it can be determined that adult mortality is higher. North American data on the course of supporting heart therapy in children show that although the chance of a heart transplant in Europe is smaller, the results in Europe show a longer survival on assist devices. Additionally, it was found that a comparison between the results of MCS therapy is worse in women compared to that of men. This observation does not only occur with MCS therapy and therefore deserves priority in further research. The long-term outcomes of patients whose own heart muscle could function independently after explantation of an assist device show that the results in those patients are very good. To better understand the factors that play a role, further research is also indicated for this group of patients. With the study of the effect of surgical correction of the tricuspid valve during the implantation of an LVAD, it has been shown that there is no significant improvement in results.

For the first time, Europe-wide insight has been provided into the landscape of human tissues for transplantation. Our contributions to the European Commission's report show where there are bottlenecks in the quantity and quality of the processes that lead to the adequate availability of grafts at international level. This is an impetus for the European Commission to develop further policy.

Four studies that are in line with each other show which methods are used by tissue establishments in acquiring and processing cardiovascular tissues for transplantation. Areas for improvement were identified through consensus meetings and benchmarking.

Given the high participation rate of the organisations, it can be expected that best practices can be realised in the long term and with the help of external standards.

Finally, it is hoped that this thesis contributes to the work of cardiac surgeons and cardiologists who are responsible for the clinical care of patients.

CHAPTER 16

Summary/Samenvatting
Dankwoord/Acknowledgements
PhD Portfolio
List of publications
About the Author

Summary/Samenvatting

SUMMARY

Increasingly, health care providers must demonstrate that they are able to meet the justified expectation that they can actually deliver results of care according to the definition: "quality of care is the degree to which health services for individuals and populations increase the likelihood of desired health outcomes and are consistent with current professional knowledge".

Measuring the outcomes of health services as such doesn't provide adequate information about the quality of services of an individual provider. It's when we compare the outcomes of that provider with those of other providers, offering similar services, that the results can be analysed in perspective. In this way best practises can be defined and the relative outcomes of an individual provider of (health) services can be determined. This process is called benchmarking. (Chapter 1)

In this thesis different benchmarking studies in the field of cardio-thoracic surgery are presented.

The instruments and platforms, used in these studies, in order to objectively assess outcomes are databases and registries. In addition professional societies and peer-organisations play a key role in organising focus-meetings and workshops indicating the possible causes, and directions to solve the negative differences.

A systematic literature review (Chapter 2) of benchmarking studies showed that the causal relationship between the use of registries and increased quality could be proven. Benchmarking provided tangible evidence of measurable qualitative improvement of outcomes. All studies provided additional instruments to come to best practises.

Heart failure is an important cause of morbidity and mortality. Ultimately, heart transplantation was -and perhaps is- the therapy of choice for heart failure patients. Since more than 2 decades, a technological innovation in the form of Mechanical Circulatory Support (MCS) in the form of blood-pumps has made it possible to support the heart, and therewith the blood circulation of these patients. Several studies about the use of MCS are presented in this thesis (Chapters 3-8). Since the common denominator of this thesis is the benchmarking of methods and outcomes, these studies provide benchmarks based on registry data, specifically of the European EUROMACS registry.

Two studies (Chapters 3 and 4) provide reports on the application of MCS respectively in adult and in paediatric patients. The reports show the applied practises in the field of MCS by the accumulation of base-line data. Follow-up analyses analyse the factors contributing to short- and long-term outcomes.

Such data have been made available on a global level too. One chapter (Chapter 5) of this thesis is a study of the International Society for Heart and Lung Transplantation

Registry for Mechanical Circulatory Support (IMACS Report), in which, besides data from EUROMACS, contributions of similar data from other parts of the world are brought together. The source of these data is mostly from the United States, while data from countries such as Canada, Japan, Singapore and Australia are integrated as well. The IMACS report offers multiple benchmarks with respect to device strategies, risk-factors, adverse events and predictors for mortality.

A difference between female and male patients in terms of selection for as well as outcomes of MCS therapy was demonstrated as part of this thesis (Chapter 6). While heart failure is more frequent in women, only 15.6% of the patients in the EUROMACS database was female, and short- as well as long-term mortality appeared to be higher. One of the causes may be the limited availability of smaller devices (only 1 manufacturer offered smaller devices at the time of the study). The results of the study have provided a benchmark for future analysis of the differences in outcomes in female patients.

Another benchmark has been set in the paper (Chapter 7) about patients whose MCS device has been explanted after adequate recovery of the heart muscle, showing that weaning from an MCS device shows excellent results, and that only a minority of the patients suffer from a relapse or significant heart failure.

The EUROMACS registry enabled us to compare different strategies when it comes to patients with leakage of the tricuspid valve. While some studies underlined the necessity to repair the tricuspid valve during valve implantation. The study in this thesis (Chapter 8) demonstrates that such an intervention doesn't necessarily lead to better results, and that an improved patient selection may possibly give better results.

The transplantation of human tissues (allografts), focused on cardio-vascular allografts, comprises several chapters of this thesis (Chapters 9 and 10). First, a wide-range study, carried out for the European Commission, looks into the clinical use. Qualified as "replacement tissues" cardiovascular allografts are implanted in those patients with illnesses or malfunction of the heart-valves or arteries. The causes can be congenital or acquired, such as by infections or sepsis.

To make replacement tissues available for transplantation they must be donated by a deceased donor. Contrary to organ donation, in which the short time between donation and transplantation is a qualitative factor, tissues can be stored for a longer period of time. This enables tissue establishments to evaluate the quality of the tissue in different ways.

The presented studies give an insight in the different methods that are applied. Rates of donations and transplantations are quantified for countries in the EU, while cross-border activities have been chartered. A breakdown of factors influencing the availability of sufficient grafts learns that in countries with an opting-in system, the additional

efforts to obtain permission for donation is threshold to overcome to get sufficient grafts to cover the clinical need.

These studies also provide an economic evaluation into the costs, generated because of the necessary quality and safety requirements. Although donated for altruistic reasons, the processing and investment costs require compensation of the hospital, or the patient's insurance once the graft is transplanted.

Our conclusion is that there is a decreasing trend in the use of cardiovascular donor tissues, but that there will always be a - albeit lower - demand. For the long term, we expect better long-term results due to the application of new techniques such as decellularized heart valves before implantation.

Four chapters (Chapters 11-14) of this thesis consist of studies focusing on different methods used by tissue establishments and their outcomes.

Contamination of the implanted tissue graft can lead to severe complications in the vulnerable recipient. In order to determine that no micro-organisms are transferred to the recipient, tests are carried out. In a first study (Chapter 12) we observed great differences in methods and results of the different tissue establishments in Europe and in third countries. And concluded that there was a need for an international standard.

A second study (Chapter 12) made a quantitative inventory of numbers of donations and transplantations as well of methods used in the processes from donation, excision, decontamination and storage. The observed differences triggered us to execute a "quality round" in which the tissue establishments were asked to determine which micro-organisms could be found in samples that were purposely contaminated. From the 20 participating establishments, 13 appeared to use methods that were insufficiently effective to perform adequate decontamination. Moreover, some methods could save a substantial level of costs if they would re-design their processes in a more logical way.

The conclusion was that, in order to come to best practises, harmonisation of methods was, and is an necessity to enable exchange of grafts on a European level. Moreover, surgeons -in the interest of their patients- must be able to rely on the methods used.

In an additional study (Chapter 13) we focused on mapping the factors that lead to the rejection donation of heart or tissues of hearts from donors. This time, great differences were found in e.g. donor management, age limits and the acceptance of non-organ donors, thus limiting of the availability of tissues for transplant. The decontamination methods and the temperatures were again found to be different and correlation with rejection rates of potential donors was observed.

The last study (Chapter 14) reports the results of a 2nd quality round in tissue establishment in which from 21 European and 4 third countries participated. The instruction was

to identify micro-organisms in each of 3 samples. Repeatedly, some establishments provided incorrect results. What became clear though is that low temperatures of 4° C to 22 C° are inadequate to decontaminate, thus setting a standard and a benchmark for applied methodologies in cardiovascular tissue establishment.

In a future perspective (Chapter 16) we stated that the necessity to come to national and international European registries that provide adequate data for benchmarking is increasing. In order to demonstrate that health care providers can deliver the health outcomes that are desired by society, they must prove, by benchmarking and transparent publication of their outcomes, that their results and outcomes are consistent with current professional knowledge. As for patients who need MCS several factors will determine future results. One is the early selection of eligible patients, and another is that devices will become smaller, which will benefit women and children. A third is the development of peripherals such as apps that enable monitoring patients at a distance, on-line, that enable clinicians to detect adverse events in an early stage.

The tissue establishments that collaborate in the international benchmarking project are looking to eliminate factors that negatively influence quality by setting up an affiliation with NEQAS (National External Quality Assessment Service) an independent, now international, consortium that focuses on improving test results in laboratories. Eventually the observed differences in outcomes will disappear and the certainty that the quality of the tests is reliable will increase.

The use of human heart valve tissue will generally decrease, for specific indications decellularized tissues will be preferred, although due to the high processing costs, no total substitution of traditionally processed heart valves can be expected.

SAMENVATTING

In toenemende mate moeten zorgverleners aantonen dat zij in staat zijn te voldoen aan de gerechtvaardigde verwachting dat zij daadwerkelijk resultaten van zorg kunnen leveren volgens de definitie: “kwaliteit van zorg is de mate waarin gezondheidsdiensten de kans op resultaten in overeenstemming met de huidige professionele kennis de gewenste gezondheid voor individuen en bevolkingsgroepen vergroten”.

Het meten van de resultaten van gezondheidsdiensten als zodanig biedt geen adequate informatie over de kwaliteit van de diensten van een individuele aanbieder. Het is wanneer we de resultaten van die aanbieder vergelijken met die van andere aanbieders die vergelijkbare diensten leveren, dat de resultaten in perspectief kunnen worden geanalyseerd. Op deze manier kunnen best practices worden gedefinieerd en kunnen de relatieve resultaten van een individuele aanbieder van (gezondheids-) diensten worden bepaald. Dit proces wordt benchmarking genoemd. (Hoofdstuk 1)

In dit proefschrift worden verschillende benchmarkingstudies op het gebied van cardio-thoracale chirurgie gepresenteerd.

De instrumenten en platforms die in deze studies worden gebruikt om de resultaten objectief te beoordelen, zijn databases en registries. Daarnaast spelen professionele verenigingen en peer-organisaties een sleutelrol bij het organiseren van focusbijeenkomsten en workshops waarin de mogelijke oorzaken en oplossingsrichtingen voor de negatieve verschillen.

Een systematisch literatuuronderzoek (Hoofdstuk 2) van benchmarkingstudies toonde aan dat het causale verband tussen het gebruik van registries enerzijds en anderzijds een verhoogde kwaliteit kon worden aangetoond. Benchmarking leverde tastbaar bewijs op van meetbare kwalitatieve verbetering van de resultaten. Alle studies hebben aanvullende instrumenten opgeleverd om tot best practices te komen.

Hartfalen is een belangrijke oorzaak van morbiditeit en mortaliteit. Uiteindelijk was harttransplantatie de therapie bij uitstek voor patiënten met hartfalen. Sinds meer dan twee decennia heeft een technologische innovatie in de vorm van Mechanische Circulatory Support (MCS), in de vorm van steunharten, het mogelijk gemaakt om het hart en daarmee de bloedcirculatie van deze patiënten te ondersteunen. Verschillende studies over het gebruik van steunharten worden gepresenteerd in dit proefschrift (Hoofdstukken 3-8). Aangezien de gemeenschappelijke noemer van dit proefschrift de benchmarking van methoden en resultaten is, bieden deze studies benchmarks op basis van registry-gegevens, met name van het Europese EUROMACS-register.

Twee studies (hoofdstukken 3 en 4) geven rapporten over de toepassing van steunharten bij respectievelijk volwassen patiënten en kinderen. De rapporten tonen de toegepaste werkwijzen op het gebied van MCS door de accumulatie van gegevens m.b.t.

voorgeschiedenis en peri-operatieve gegevens. Door middel van follow-up analyses worden de factoren die bijdragen aan de resultaten op korte en lange termijn manifest.

Dergelijke gegevens zijn ook op mondiaal niveau beschikbaar gesteld. Een hoofdstuk (Hoofdstuk 5) van dit proefschrift is een studie van de International Society for Heart and Lung Transplantation Registry for Mechanical Circulatory Support (IMACS Report), waarin, naast gegevens van EUROMACS, bijdragen van vergelijkbare gegevens uit andere delen van de wereld worden samengebracht. De bron van deze gegevens is meestal afkomstig uit de Verenigde Staten, terwijl gegevens uit landen zoals Canada, Japan, Singapore en Australië ook zijn geïntegreerd. Het IMACS-rapport biedt meerdere benchmarks met betrekking tot implantaat strategieën, risicofactoren, ongewenste voorvallen en voorspellers voor mortaliteit.

Een verschil tussen vrouwelijke en mannelijke patiënten in termen van selectie voor, en uitkomsten van, steunhart-therapie werd aangetoond als onderdeel van dit proefschrift (Hoofdstuk 6). Hoewel hartfalen vaker voorkomt bij vrouwen, was slechts 15,6% van de patiënten in de EUROMACS-database vrouwelijk en bleek zowel de korte- als de lange termijn sterfte hoger te zijn. Een van de oorzaken kan de beperkte beschikbaarheid van kleinere apparaten zijn (slechts 1 fabrikant bood op het moment van het onderzoek kleinere apparaten aan). De resultaten van de studie hebben een benchmark opgeleverd voor toekomstige analyse van de verschillen in uitkomsten bij vrouwelijke patiënten.

In dit proefschrift (hoofdstuk 7) is een benchmark gezet voor patiënten bij wie het steunhart is geëxplanteerd na voldoende herstel van de hartspier, waaruit blijkt dat in dergelijke gevallen het verwijderen van een steunhart uitstekende resultaten oplevert en dat slechts een minderheid van de patiënten een terugval heeft of aanzienlijk hartfalen ontwikkelt.

Het EUROMACS-register stelde ons in staat om verschillende strategieën te vergelijken als het gaat om patiënten met lekkage van de tricuspidalisklep. Sommige studies onderstreepten de noodzaak om de tricuspidalisklep te repareren tijdens implantatie van een steunhart. De studie in dit proefschrift (hoofdstuk 8) laat zien dat een dergelijke interventie niet noodzakelijkerwijs tot betere resultaten leidt en dat een verbeterde selectie van patiënten mogelijk eerder betere resultaten oplevert.

De transplantatie van menselijke weefsels (allografts), gericht op cardiovasculaire allo-transplantaten, omvat verschillende hoofdstukken van dit proefschrift (hoofdstukken 9 en 10). Ten eerste wordt in een breed onderzoek, uitgevoerd voor de Europese Commissie, gekeken naar het klinische gebruik. Gekwalificeerd als “vervangingsweefsel” worden cardiovasculaire allografts geïmplanteerd bij patiënten met ziekten of storingen van de hartkleppen of slagaders. De oorzaken kunnen aangeboren of verworven zijn, zoals door infecties of sepsis.

Om vervangende weefsels beschikbaar te maken voor transplantatie moeten ze worden gedoneerd door een overleden donor. In tegenstelling tot orgaandonatie, waarbij de korte tijd tussen donatie en transplantatie een kwalitatieve factor is, kunnen weefsels voor een langere periode worden bewaard. Hierdoor kunnen weefselinstellingen de kwaliteit van het weefsel op verschillende manieren evalueren.

De gepresenteerde studies geven inzicht in de verschillende methoden die worden toegepast. Tarieven van donaties en transplantaties worden gekwantificeerd voor landen in de EU, terwijl grensoverschrijdende activiteiten in kaart zijn gebracht. Een uitsplitsing van factoren die de beschikbaarheid van voldoende transplantaten beïnvloeden, leert dat in landen met een opting-in-systeem de extra inspanningen om toestemming voor donatie te krijgen een drempel zijn om te om voldoende transplantaten te krijgen teneinde de klinische behoefte te dekken.

Deze studies bieden ook een economische evaluatie van de kosten, gegenereerd vanwege de noodzakelijke kwaliteits- en veiligheidseisen. Hoewel gedoneerd om altruïstische redenen, vereisen de verwerkings- en investeringskosten een vergoeding van het ziekenhuis of de verzekering van de patiënt zodra het transplantaat is getransplanteerd.

Onze conclusie is dat er een dalende trend is in het gebruik van cardiovasculaire donorweefsels, maar dat er altijd een - zij het minder - vraag zal zijn. Voor de lange termijn verwachten we betere resultaten als gevolg van de toepassing van nieuwe technieken zoals het decelluleren van hartkleppen vóór implantatie.

Vier hoofdstukken (hoofdstuk 11-14) van dit proefschrift bestaan uit studies die zich richten op verschillende methoden die worden gebruikt door weefselinstellingen en de resultaten daarvan.

Besmetting van het geïmplanteerde weefseltransplantaat kan leiden tot ernstige complicaties bij de kwetsbare ontvanger. Om te bepalen dat er geen micro-organismen op de ontvanger worden overgedragen, worden tests uitgevoerd. In een eerste studie (hoofdstuk 11) hebben we grote verschillen waargenomen in methoden en resultaten van de verschillende weefselinstellingen in Europa en in derde landen, en concludeerden dat er behoefte was aan een internationale standaard.

Een tweede studie (hoofdstuk 12) maakte een kwantitatieve inventaris van het aantal donaties en transplantaties en van methoden die worden gebruikt in de processen van donatie, excisie, decontaminatie en opslag. De waargenomen verschillen hebben ons ertoe aangezet een ‘kwaliteitsronde’ uit te voeren waarin de weefselinstellingen werd gevraagd om te bepalen welke micro-organismen konden worden gevonden in monsters die opzettelijk waren besmet. Van de 20 deelnemende instellingen bleken er 13 methoden te gebruiken die onvoldoende effectief waren om adequate decontaminatie uit te voeren. Bovendien zouden sommige weefselinstellingen een aanzienlijke hoeveelheid kosten

kunnen besparen als ze hun processen op een logischer manier zouden herontwerpen en inrichten.

De conclusie was dat, om tot best practises te komen, harmonisatie van methoden een noodzaak was en is om uitwisseling van allografts op Europees niveau mogelijk te maken. Bovendien moeten chirurgen - in het belang van hun patiënten - kunnen vertrouwen op de gebruikte methoden.

In een aanvullend onderzoek (hoofdstuk 13) hebben we ons gericht op het in kaart brengen van de factoren die leiden tot het niet accepteren van hart of hartweefsels van donoren. Deze keer werden grote verschillen gevonden in b.v. donormanagement, leeftijdsgrenzen en de acceptatie van niet-orgaandonoren, waardoor de beschikbaarheid van weefsels voor transplantatie wordt beperkt. De ontsmettingsmethoden en de temperaturen bleken opnieuw verschillend te zijn en correlatie met de reden waarom potentiële donoren werden afgewezen werd waargenomen.

De laatste studie (hoofdstuk 14) rapporteert de resultaten van een 2e kwaliteitsronde in weefselinstellingen waaraan 21 Europese en 4 derde landen hebben deelgenomen. De instructie was om micro-organismen in elk van de 3 monsters te identificeren. Herhaaldelijk leverden sommige vestigingen onjuiste resultaten op. Wat echter duidelijk werd, is dat lage temperaturen van 4° C- 22° C onvoldoende zijn om te ontsmetten, waardoor een norm en een benchmark wordt vastgesteld voor toegepaste methodologieën voor instellingen die cardiovasculair weefsel bewerken.

In een toekomstperspectief (hoofdstuk 15) hebben we gesteld dat de noodzaak om naar nationale en internationale Europese registries te komen die voldoende gegevens voor benchmarking verstrekken, toeneemt. Om aan te tonen dat zorgaanbieders de door de maatschappij gewenste gezondheidsresultaten kunnen leveren, moeten zij door benchmarking en transparante publicatie van hun resultaten aantonen dat die resultaten consistent zijn met de huidige professionele kennis. Wat betreft patiënten die een steunhart nodig hebben, zullen verschillende factoren de toekomstige resultaten bepalen. Een daarvan is de vroege selectie van in aanmerking komende patiënten, en een andere is dat apparaten kleiner zullen worden, wat vrouwen en kinderen ten goede zal komen. Een derde is de ontwikkeling van randapparatuur, zoals apps waarmee patiënten op afstand online kunnen worden gevolgd, waardoor artsen in een vroeg stadium bijwerkingen kunnen detecteren.

De weefselinstellingen die samenwerken in het internationale benchmarkproject proberen factoren te elimineren die de kwaliteit negatief beïnvloeden door een samenwerkingsverband op te zetten met NEQAS (National External Quality Assessment Service), een onafhankelijk, nu internationaal consortium dat zich richt op het verbeteren van testresultaten in laboratoria. Uiteindelijk zullen de waargenomen verschillen in uitkomsten verdwijnen en zal de zekerheid dat de kwaliteit van de testen betrouwbaar is toenemen.

Het gebruik van menselijk hartklepweefsel zal in het algemeen afnemen, voor specifieke indicaties zullen gedecelluleerde weefsels de voorkeur hebben, hoewel vanwege de hoge verwerkingskosten geen totale vervanging van traditioneel bewerkte hartkleppen kan worden verwacht.

Dankwoord/Acknowledgements

Dit proefschrift, en in het bijzonder de studieresultaten die in de verschillende hoofdstukken zijn opgenomen, is het resultaat van samenwerking met veel mensen in de medische wereld en daarbuiten. Ik wil graag iedereen bedanken die met mij de inspanningen hebben geleverd om de resultaten van ons werk te kunnen publiceren. Zonder hen zou dit proefschrift niet to stand zijn gekomen. Ik hoop dat ze tevreden zijn met het resultaat. Een aantal mensen wil ik in het bijzonder bedanken.

In de eerste plaats mijn promotor, **Prof. dr. Ad Bogers**. Beste Ad, onze samenwerking gaat decennialang terug. Een van mijn vroegste herinneringen is de overdracht van de verantwoordelijkheden voor de toenmalige Rotterdamse hartkleppenbank van je voorganger aan jou. Het gebeurde in een etablissement aan de Zweth, hetgeen, naar jouw verbazing, destijds de gebruikelijk locatie was voor besprekingen over de samenwerking met betrekking tot het preserveren van hartkleppen. Na jouw aantreden werden de bijeenkomsten zakelijker en effectiever. Ik zal jouw diplomatieke optreden rond de grote financiële verschillen tussen het Erasmus MC en Stichting BIS niet licht vergeten. In die periode werd de Rotterdamse hartkleppenbank de grootste van Europa en de in Rotterdam gehanteerde methodes werden overgenomen door Europese collega's. Je steunde toen mijn initiatief om de internationaal waargenomen verschillen in preservatietechnieken bespreekbaar te maken in zgn. consensus meetings. De presentatie en onderbouwing van de verschillende werkwijzen was het begin van benchmarking dat nu het onderwerp is van dit proefschrift.

Na mijn vertrek bij Stichting BIS zette ik dit werk voort en met jouw steun kon ik resultaten gaan publiceren. Ik ben je dankbaar dat je mij de mogelijkheid hebt geboden om mijn publicaties in dit proefschrift te presenteren.

Mijn copromotor **dr. Kadir Caliskan**. Beste Kadir, sinds onze eerste ontmoeting in Praag, in 2012, hebben wij samen gestalte kunnen geven aan de verder opbouw van de EUROMACS database. Daaruit zijn mooie studies voortgekomen en ik verwacht dat er nog zeker een aantal zal volgen. Dankzij jou heeft de reikwijdte van het onderzoek met onze data internationaal ingang gevonden onder cardiologen op het gebied van hartfalen. Recent hebben we weer initiatieven ontwikkeld die uiteindelijk zullen bijdragen aan inzichten die leiden tot verbetering van de klinische resultaten in de cardiologie en in de hartchirurgie. Ik zie uit naar onze verder samenwerking. Jij bent degene geweest die mij heeft gestimuleerd om te werken aan "een kroon op mijn werk" zoals je het noemde. Die kroon is gemaakt en ligt hier nu ingekaft voor de lezer om te aanschouwen. Ik ben je veel dank verschuldigd.

Prof. dr. Mark Hazekamp. Beste Mark, mijn (her)aantreden in Leiden, eind jaren 80, viel ongeveer samen met jouw begin als thoraxchirurg. Het gebruik van homografts, in het bijzonder in kinderen met aangeboren hart(klep)-afwijkingen bracht ons op elkaars pad. We werkten ook samen om te proberen de continuïteit van de congenital database te waarborgen. Hopelijk zal dat uiteindelijk een positief resultaat hebben. Dank voor de samenwerking en voor je bereidheid om plaats te nemen in de kleine commissie.

Prof. dr. Bart Meyns, beste Bart, vanuit je positie als lid van de EACTS EUROMACS Committee, en sinds 2017 als voorzitter, heb je mij gesteund bij het tot stand brengen van onderzoek en samenwerking tussen de verschillende Europese ziekenhuizen die steunharten implanteren. Je nuchtere aanpak heeft ervoor gezorgd dat de registry groeit en, ook dat ook degenen die aanvankelijk kritisch over EUROMACS waren, zich hebben aangesloten. Hartelijk dank voor dit alles en in het bijzonder ook voor je bereidheid om plaats te nemen in de kleine commissie.

Prof. dr. Hanneke Takkenberg, beste Hanneke, De Rotterdamse Hartkleppenbank opende op 24 augustus 1988 en niet lang daarna leerde ik je kennen als onderzoekster in het vakgebied van de homografts. Jouw analyses werden hoog gewaardeerd en jouw aanwezigheid en presentaties tijdens de consensus meetings hebben mij gesteund in mijn pogingen d.m.v. benchmarking de verschillende werkwijzen en de resultaten daarvan kritisch te vergelijken. Ik waardeer je huidige werkzaamheden bijzonder en ben vereerd dat je in de kleine commissie hebt willen plaatsnemen.

Prof. dr. Örjan Friberg, dear Örjan, our joint efforts to develop the EACTS Adult Cardiac Database into a benchmarking tool that is accepted all over Europe as an instrument to improve outcomes seem to have some success. Since many can learn from the achievements from you and your colleagues from Swedeheart, I hope to continue our cooperation even after you step down as chair of the ACD task force. It is an honor for me that you're a member of my PhD committee.

Prof. dr. Kitty Jager, ongeveer 25 jaar geleden leerden we elkaar kennen toen we met gezamenlijk werkten aan de verbetering van de orgaan- en weefseldonatie procedures in Nederland. Nadat je BIS verliet ben je onderzoek gaan doen op het gebied van de renale informatica en renale epidemiologie. Daarbij maak je gebruik van verschillende Europese registers die inzicht geven in incidentie en behandeling van eindstadia van nierziekten bij kinderen en adolescenten. In het gebruik van registries zie ik een overeenkomst met een groot aantal studies in dit proefschrift, en bij het schrijven van hoofdstuk 2 kon ik gebruik maken van de bevinding van een onderzoek van een van jouw promovenda. Ik was vereerd en trots dat ik bij je inaugurele rede aanwezig mocht zijn, en ben je dankbaar dat je nu in mijn promotiecommissie hebt willen plaatsnemen.

Prof. dr. Domenico Pagano, dear Domenico, thank you for your willingness to be part of my PhD committee. I'm honored to have the visionary and initiator of the EACTS Quality Improvement Project (QUIP) as a member of my PhD committee. In one of your publications you quoted "If you want to go fast, go alone. If you want to go far, go together". Bringing this in practice, you have created a great ACD team that is able to realise the EACTS's quality improvement goals. I'm happy to be part of it.

Prof. dr. Osama Soliman. Beste Osama, de risk score die jij ontwikkelde op basis van EUROMACS data heeft de trend gezet naar verder onderzoek in de samenhang tussen LVAD implantatie en rechter-hart falen. In de nabije toekomst kunnen we hopelijk

de EuroEchoVAD studie op de rit zetten om de invloed van de LVAD op de rechterhart functie te visualiseren en inzichten te verkrijgen die uiteindelijk de patiënten met hartfalen ten goede komen. Hartelijk dank dat je in mijn grote commissie hebt willen plaatsnemen.

Egbert Ottevanger. Beste Eg, eigenlijk ben je al 40 jaar mijn paranymf, vanaf de tijd dat we bij Eurotransplant werkten en waar we naast ons werk menige goodwill reis hebben ondernomen. Daarna, terwijl jij wetenschappelijk onderzoek deed en ik bij de Nationale Ziekenhuisraad werkte, produceerden we jaarverslagen met Appleworks op de Apple IIc, waarbij jij de genius was en ik de productie medewerker. Ik heb toen veel van je geleerd. Door de tijd heen zijn we dicht bij elkaar gebleven en het doet me genoegen dat we ook in 2020 nog steeds samen leuke (startups) en aanverwante projecten doen. Ik heb bewondering voor je doorzettingsvermogen in slechte tijden en ik ben blij dat je nog steeds mijn paranymf en vriend bent en hopelijk nog lang blijft.

Arlinke Bokhorst. Beste Arlinke, de psycholoog die rond jouw indiensttreding, destijds bij BIS, de compatibiliteit van onze karakters onderzocht waarschuwde ons dat ons wederzijdse ondernemerszin en enthousiasme om de organisatie verder uit te bouwen ons té positief zou kunnen beïnvloeden. Dat is ook regelmatig gebeurd, en we hebben ons inderdaad vaak moeten beheersen om niet teveel tegelijk te willen doen. Samen hebben we véél initiatieven ontplooit en heel veel projecten tot een goed einde gebracht. Ook de interne organisatie stond door onze inspanningen op hoog niveau. Jij en je medische staf zorgden ervoor dat onze reputatie internationaal een voorbeeld vormden voor vele collega-organisaties. Ook als je ziek was stond werkte je, alsof er niets aan de hand was, door ter wille van de doelen die we hadden gesteld, chapeau! Ik ben je heel veel dank verschuldigd voor alles wat je deed om onze samenwerking en onze organisatie te doen welslagen. Ik ben er trots op dat je mijn paranymf wil zijn.

Pieter Petit. Beste Pieter, dankzij de goede samenwerking met jou heb ik de draad m.b.t. de consensus meetings en het benchmarken van decontaminatie methoden en technieken weer op kunnen pakken. Zo zijn we beiden aan onze vierde (of vijfde?) carrière begonnen. Ik had je in het verleden aan het werk gezien tijdens de onderzoeken die je deed met de artsen bij BIS. Dat resulteerde steevast in een proefschrift en promotie van die mannen waarin het detecteren van micro-organismen en het onschadelijk maken daarvan voor de recipiënten van transplantaten het centrale onderwerp was. En uiteindelijk heb je zelf ook een promotiefeest gegeven. Nu heb ik zelf mogen ervaren hoe systematisch je te werk gaat bij de opzet van zo'n onderzoek, en ik dank het aan jou dat ik de studies naar methoden in cardiovasculaire weefselbanken die zijn opgenomen in dit proefschrift heb kunnen publiceren.

Prof. dr. Roland Hetzer in the 1990s we were able to develop a homograft laboratory to cover the needs of the Deutsches Herzzentrum Berlin and, in a European setting, to address the demand for safe grafts from yourself and from your international colleagues. Your initiative to found the European Foundation of Tissue Banks, and giving me

the opportunity to become director, enabled me to further develop the scheme of benchmarking methodologies in cardiovascular tissue banking. Additionally, this led to your invitation to me to become director of EUROMACS as well. Again, this appointment enabled me to make another important step in my career. Both organisations appeared to be an excellent basis for the analysis of data and for the publication of the results. I regret that we disagreed about the best move for EUROMACS, nevertheless I wish to express my gratitude to you. I have great respect for your lifetime achievements.

Here, I also wish to thank my late friend, **Norbert Franz**. Norbert and I enjoyed a true European friendship that started in the 1970s when Norbert was working at Station 37B at the Medizinische Hochschule Hannover. Later, in the interest of the DHZB and BIS Foundation we cooperated to influence the decision making of the German legislators with respect to organ donor and tissue legislation. The connection with Prof. Hetzer was established thanks to Norbert, and I owe it to Norbert that a good relation with the DHZB and BIS Foundation flourished during a great many years. We dearly miss you Norbert.

I am grateful to the chairmen and the committee members of EUROMACS who give great support and stimulate the work I do for the EACTS MCS registry: **Prof. Jan Gummert, Prof. Bart Meyns,** now member of the doctoral committee, as is **dr. Kadir Caliskan**, **Prof. Paul Mohacsi, Prof. Steven Tsui, dr. Felix Schönrath, Prof. Daniel Zimpfer, dr. Michel Morshuis, Prof. Ivan Netuka, Prof. Pascal Leprince, Priv. Doz. Evgenij Potapov, Prof. André Vincentelli, Prof. Finn Gustafsson, Prof. Gregorio Rábago,** and **Prof. Francesco Musumesci.**

Thomas Höhn and **Mehran Moazami Goudarzi**, it was a pleasure to work with you as co-directors of the Foundation of European Tissue Banks. Your matter-of-fact approach of the activities and position of the foundation has been of great help to me when striving to materialise the goals of the foundation. Several of the tissue-banking studies and publications have been made possible thanks to the fact that you created the right circumstances. Finally, we were able to terminate the foundation's activities in an orderly manner. **Mehran**, I'm happy that our friendship survived the foundation.

Special thanks to **Prof. dr. Rainer Sundmacher** for the establishing our fruitful relationship which resulted in the cornea transplantation of many of your patients. In times of trouble you provided tremendous help when the Netherlands had an organisational problem. In your position of Director of the Augenklinik der Heinrich Heine Universität Düsseldorf you decided that the Lions Hornhautbank Nordrhein Westfalen would process the donated corneas from the Netherlands. Today, when one looks at the website of the Hornhautbank the enormity of that work is still visible in the statistics. Our good relation and the mutual trust also lead to the successful investment in research. We jointly worked to influence the Bundestag to draft legislation that guaranteed a good position for cornea transplantation activities. In the Ausschuss of the Bundesärztekammer we achieved to establish new guidelines, serious business, but we

also had great fun from time to time. Equally in the board of BIS Foundation in which you functioned a great many years. Thank you for your lasting friendship.

Prof. dr. Thomas Reinhard, the studies that you did during your time at the HHU taught me that scientific sub-structuring of our work is of utmost importance. It was a pleasure to see subsequent publications coming from your pen. In Freiburg you continued to do so, moreover you've shaped the Klinik für Augenheilkunde to a leading clinic and foremost research unit in ophthalmology. With great interest I read your annual reports and I have great admiration for what you have achieved. Thank you for all you have done in the years we cooperated.

Natalie Delesalle, Merci beaucoup de m'avoir inspiré. Votre exemple m'a incité à réaliser des études collaboratives nationales et européennes sur les contrôles microbiologiques des tissus et sur les contrôles qualité.

Patrick McBrayer has been instrumental to, and assisted me setting up, the allograft donation and transplantation programme under the auspices of BIS Foundation. Besides a successful working relationship, we became close friends, and we're like brothers to one another. You honored me by being my best man. For more than 30 years we exchange almost daily literary preferences, film recommendations and great recipes. And…we continue to working together; the recipe to reach old age? Thank you Pat.

Martí Manyalic: from the first moment we met there was an understanding between us about the fact that we could join forces to create an international co-operation to jointly allocate tissue allografts to those clinicians who needed them for their patients. And we built up a network for that purpose. Also, we became brothers, had social get-togethers while at the same time we elaborated plans to improve the quality of our work. We organised successful multi-national projects, symposia and publications, the results of which were clear steps in the improvement processes of tissue establishments in Europe and in third countries. Moltes gràcies germà.

Boy van de Wiel, docent en vriend, je was en bent voor mij de guru van het inrichten van organisaties rondom processen. Jarenlang bracht ik de methoden die je doceerde aan Business School Nederland in praktijk. De gevolgen waren: Goede focus op de behoeften van de klanten, een lerende organisatie, goed gedefinieerde ruimte voor innovatie (20%) en goed georganiseerde processen om te komen tot strategie- en beleid, om maar een paar verworvenheden te noemen. Ik mis ons gezamenlijke golfwedstrijden en gezelligheid, hopelijk heb ik daar meer tijd voor na de Hora Est.

Prof. dr. José Luis Pomar, our mutual friend Martí introduced us some 30 years ago and we managed to extend the activities of the Criobarna cardio-vascular tissue bank while always maintaining -thanks to you and your co-workers- a high level of quality. In 2013, in your capacity of president of the EACTS, an additional dimension was added to our co-operation, when you and Pieter Kappetein decided to continue the EUROMACS

registry and my position as director under the auspices of the EACTS. Now that you're chairman of the of the International Cooperation Committee of EACTS we have good reasons, not that we need any, to continue our friendship.

Prof. dr. Pieter Kappetein, heel veel dank voor het vertrouwen dat je in me hebt gesteld door de overname van EUROMACS waaraan ik hiervoor refereerde. Door de vrijheid die je mij gunde om de registry verder te ontwikkelen zijn we nu in staat om veel wetenschappelijke output te genereren. Daarenboven heb je het als secretary general van de EACTS mogelijk gemaakt om een nieuw platform te creëren waarin jouw vakgenoten, zowel degenen die implantaties van steunharten bij volwassenen, alsook die bij kinderen, implanteren, kunnen samenkomen om op basis van data best practises na te streven.

Mijn collega-promovendi **dr. Rahat Muslem** en **Stan Antonides,** ik dank voor jullie inbreng in de verschillende publicaties over respectievelijk benchmarking en van analyses met EUROMACS data. Ook ben ik **Kevin Veen en Yunus Yalcin** erkentelijk voor de goede samenwerking. Mijn dank betreft natuurlijk ook in het bijzonder **dr. Sakir Akin** die altijd enthousiast doorpakt en die zijn mateloze energie verdeelt tussen zijn gezin, de cardiologie en steeds weer nieuw onderzoek. Onze reis naar de Turkse VAD centra heeft onze vriendschap voor altijd beklonken. Dank je wel voor de opdracht in jouw proefschrift "Theo, you are the next". Die opdracht is hiermee vervuld.

Mijn grote dank gaat vooral ook uit naar degenen die mij gedurende mijn carrière hebben gestimuleerd en gesteund. In de eerste plaats wil ik wijlen **Henk Schippers** en **dr. Guido Persijn** bedanken voor hun nooit aflatende steun en hun onvergetelijke bijdrage aan mijn carrière. **Henk Schippers** nam mij in 1974 bij Eurotransplant als werkstudent in dienst om -naar zijn voorbeeld- al tijdens de studie iets nuttigs te doen en geld te verdienen om te voorzien in mijn levensonderhoud. Later, toen Henk voorzitter werd van Stichting BIS was hij altijd, ook als het soms wat minder goed ging, positief stimulerend. Zijn raad "het bestuur zorgt voor jou, zorg jij goed voor je mensen" heb ik getracht in praktijk te brengen. Het is aan anderen te oordelen of dat ook is gelukt. Van **Guido Persijn** heb ik de grondbeginselen van het wetenschappelijk veldwerk geleerd. Voor zijn promotie onderzoek reden we stad en land af en haalden we, soms onaangekondigd (het was nog in het tijdperk van voor de AVG-wet), informatie uit statussen en archieven van bloedbanken voor de bewijsvoering dat 3 transfusies konden bijdragen aan een langere overleving van getransplanteerde nieren in de ontvangers. In zijn functie als bestuurder van Stichting BIS heb ik van Guido zeer gewaardeerde steun en advies mogen krijgen.

Arnoud Slooff. Een betere adjunct en tegelijkertijd manager innovatie heb ik me niet kunnen denken. Je nam destijds een behoorlijk stuk werk van mijn schouders, je steunde me door dik en dun, maar was ook kritisch als dat nodig was. Ik ben je daar zeer erkentelijk voor.

Mijn collega's en teamgenoten bij Stichting BIS (helaas kan ik me niet alle namen herinneren waarvoor mijn verontschuldigingen):

Alimieke Bol, Remmelt Veen, Nicole van Nierop (de beste secretaresse ooit)**, Ruud Deijkers, Eveline Abbekerk, Audrey Laven, Herman Kaptijn, Stephan Vehmeijer, Huibert Tjabbes, Heiman Wertheim, Hajo Bruining, Fieke Wijffels, Manon Hanekamp, Saskia Tuinstra, Gijs Patijn, Josien Wolterbeek, Carmen van der Pol, Arlette de Voogd, Igor van den Brand, en dan toch ook Jurriaan van den Brand, Crispijn van den Brand, Charlotte van Koesveld, Tim Bredehorn, Paul van der Eerden, Jan Willem Mazel, Pol Huysmans, Arno Starrenburg, Els Erich, Joris van der Putten, Charlotte Robertson, Annette Barkhof, Martijne van 't Riet, Marit Maatman, Thirza Horn, Prof. Heiman Wertheim, Corné Otto, Hélène van Paridon, Jenneke Litjens, Ben Schmidt, Marnix de Roos, Els Janssens, Noor Holsboer, Stan Antoine Bel, Caroline Dorrepaal, Rijk Spijkerboer, Stan Vos, Leonard Corion, en Lieke Welling,** ik dank jullie allemaal voor de goede samenwerking van destijds. Jullie hebben allemaal bijgedragen aan de kwaliteit van werken en aan de successen die we samen konden boeken.

Lieve kinderen, **Esther, Felix en Frédérique**, ik ben heel erg trots op wat jullie doen en wat jullie tot nu toe hebben bereikt en ik weet zeker dat jullie nog veel tot stand gaan brengen. Ik hou ontzettend veel van jullie. Het spijt me dat ik er niet altijd voor jullie was vanwege het werk dat ik deed.

Fijn dat jullie goede partners hebben in **Jelle, Claire en Frank**, en ik ben gelukkig vanwege het feit dat we allemaal samen met **Salomon** en **Jonathan** een grote familie vormen.

Lieve **Marie-Thérèse**, ik heb het met jou wel heel erg goed getroffen. Je bent mooi en je hebt goede smaak, we hebben veel overeenkomstige belangstellingssferen, genieten daar samen veelvuldig van, we lachen veel en kunnen ook van mening verschillen. "Het is stil waar het nooit waait" is een wijsheid die je van je ouders hebt meegekregen. "We zijn een héél goed setje" zeggen we vaak. Voor de kinderen zette je jezelf, en nog steeds, met grote betrokkenheid in. Ik ben trots op jou vanwege je professionele instelling in het werk dat je doet en natuurlijk op je initiatief waardoor destijds Interplast Holland gestalte kon krijgen, en waarin je nog steeds veel van je eigen tijd investeert. Terecht werd je daarvoor geridderd. Veel geduld bracht je op gedurende de tijd dat ik achter, nee vóór, het computerscherm doorbracht om aan dit proefschrift en andere publicaties te werken. We hebben tijdens onze lange wandeltochten véél plannen ontwikkeld en zijn bezig die te realiseren. Dank je voor je liefde, ik hou ook veel van jou, en héél veel dank voor je steun tijdens mijn werk en ook voor alles dat ons leven zo prettig maakt.

Phd Portfolio

PhD Portfolio

Name PhD student	Theo M.M.H.de By
Erasmus MC department:	Cardiothoracic Surgery
PhD period:	2010 – 2019
Title thesis:	International Benchmarking in Cardio-Thoracic Surgery. Mechanical Circulatory Support and Heart Valve Banking
Promotors:	A.J.J.C. Bogers
Co-promotor:	K. Caliskan

ACADEMIC EDUCATION

1975-1978	Bachelor of Science, Rijksuniversiteit Leiden, the Netherlands
1995-1997	Master of Business Administration, Business School Nederland, The Netherlands
1979	Hospital Sciences, Rijksuniversiteit Utrecht, the Netherlands

Oral presentations

Foundation of European Tissue Banks, Schiedam, the Netherlands	2010	1,2
Foundation of European Tissue Banks, Berlin, Germany	2011	1,2
6th World Congress on Tissue Banking, Barcelona, Spain	2011	1,2
The 1st Sino-German Summit Forum on Cardiac Surgery, Shanghai, China	2012	1,2
European Eye Bank Association, Freiburg Germany	2011	1,2
European Association of Tissue Banks, Hannover, Germany	2016	1,2
Portuguese and Brazilian Transplant Congress, Porto, Portugal	2016	1,2
European Association of Tissue Banks, Treviso, Italy	2017	1,2
European Association of Tissue Banks, Lund, Sweden	2014	1,2
European Association of Tissue Banks, Brussels, Belgium	2013	1,2
European Association of Tissue Banks, Barcelona, Spain	2012	1,2
International Society of Rotary Blood Pumps, Yokohama, Japan	2012	1,2
International Society of Rotary Blood Pumps, Split, Croatia	2015	1,2
European Society for Artificial Organs, Leuven, Belgium	2015	1,2
8e TRIP symposium Biovigilance, Utrecht, the Netherlands	2015	1,2
SFCTCV Annual Meeting, Nantes, France	2015	1,2
DGTHG, Freiburg, Germany	2014	1,2
DGTHG, Freiburg, Germany	2015	1,2
EUMS, Berlin, Germany	2018	1,2
ETS, Bern, Switzerland	2014	1,2
British Association of Tissue Banks, Oxford, England	2012	1,2
International Society of Heart and Lung Transplantation, San Diego, USA	2014	1,2
VIIIth Congress of the Czech Society for Cardiovascular Surgery, Brno, Czech Republic	2018	1,2

EACTS Annual Meeting, Vienna, Austria	2013	1,2
EACTS Annual Meeting, Milan, Italy	2014	1,2
EACTS Annual Meeting, Amsterdam, the Netherlands	2015	1,2
EACTS Annual Meeting, Barcelona, Spain	2016	1,2
EACTS Annual Meeting, Vienna, Austria	2017	1,2
EACTS Annual Meeting, Milan, Italy	2018	1,2
Société Française de Chirurgie Thoracique et Cardio-Vasculaire, Rennes, France	2019	1,2
International Society for Mechanical Circulatory Support, Bologna, Italy	2019	1,2
3rd EACTS Mechanical Circulatory Support Summit, Berlin, Germany	2018	1,2
4th EACTS Mechanical Circulatory Support Summit, Prague, Czech Republic	2019	1,2
Teaching		
MCS workshop, Berlin, Germany	2013	0,6
MCS workshop, Berlin, Germany	2014	0,6
MCS workshop, Berlin, Germany	2015	0,6
MCS workshop, Berlin, Germany	2016	0,6
MCS workshop, Berlin, Germany	2017	0,6
MCS workshop, Berlin, Germany	2018	0,6
MCS workshop, Berlin, Germany	2019	0,6
VAD Coordinators Training Course Berlin	2015	0,6
VAD Coordinators Training Course Berlin	2016	0,6
VAD Coordinators Training Course Berlin	2017	0,6
DTI Training Course, Venice, Italy	2011	0,6
DTI Training Course, Barcelona, Spain	2012	0,6
International conferences		
6th World Congress on Tissue Banking, Barcelona, Spain	2011	1,5
The 1st Sino-German Summit Forum on Cardiac Surgery, Shanghai, China	2012	1,5
European Association of Tissue Banks, Hannover, Germany	2016	1,5
8e TRIP symposium Biovigilance, Utrecht, the Netherlands	2015	1,5
European Association of Tissue Banks, Treviso, Italy	2017	1,5
European Association of Tissue Banks, Lund, Sweden	2014	1,5
European Association of Tissue Banks, Brussels, Belgium	2013	1,5
European Association of Tissue Banks, Barcelona, Spain	2012	1,5
British Association of Tissue Banks, Oxford, England	2012	1,5
European Training Symposium (ETS), Bern, Switzerland	2012	1,5
European Training Symposium (ETS) Bern, Switzerland	2014	1,5
DGTHG, Freiburg, Germany	2014	1,5
EUMS, Paris, France	2015	1,5

EUMS, Paris, France	2016	1,5
EUMS, Bad Oeynhausen, Germany	2017	1,5
EUMS, Berlin, Germany	2018	1,5
EUMS, Paris, France	2018	1,5
EACTS Annual Meeting, Barcelona, Spain	2012	1,5
VIIIth Congress of the Czech Society for Cardiovascular Surgery, Brno, Czech Republic	2018	1,5
EACTS Annual Meeting, Vienna, Austria	2013	1,5
EACTS Annual Meeting, Milan, Italy	2014	1,5
EACTS Annual Meeting, Amsterdam, the Netherlands	2015	1,5
EACTS Annual Meeting, Barcelona, Spain	2016	1,5
EACTS Annual Meeting, Vienna, Austria	2017	1,5
EACTS Annual Meeting, Milan, Italy	2018	1,5
EACTS Annual Meeting, Lisbon, Portugal	2019	1,5
International Society of Heart and Lung Transplantation, Prague, Czech Republic	2012	1,5
International Society of Heart and Lung Transplantation, Montreal, Canada	2013	1,5
International Society of Heart and Lung Transplantation, San Diego, USA	2014	1,5
International Society of Heart and Lung Transplantation, Nice, France	2015	1,5
International Society of Heart and Lung Transplantation, Washington D.C., USA	2016	1,5
International Society of Heart and Lung Transplantation, San Diego, USA	2017	1,5
International Society of Heart and Lung Transplantation, Nice, France	2018	1,5
International Society of Heart and Lung Transplantation, Orlando, USA	2019	1,5
Gordon Research Conference, Castelldefels, Spain	2019	1,5
International Society for Mechanical Circulatory Support, Bologna, Italy	2019	1,5
4th EACTS Mechanical Circulatory Support Summit, Prague, Czech Republic	2019	1,5
	Total	**102,3**

List of publications

1. **De By TMMH,** Antonides CFJ, Schweiger M, Sliwka , Davies B, Berger, Hübler M, Özbaran M, Maruszewski B, Napoleone CP, Zimpfer, Antretter H, Meyns B, Miera O. The European Registry for Patients with Mechanical Circulatory Support (EUROMACS): second EUROMACS Paediatric (Paedi-EUROMACS) Report; submitted.

2. Akin S, Soliman O, **de By TMMH**, Muslem R, Schönrath F, Meyns B. ICU mortality following LVAD implantation. Intensive Care Medicine; doi.org/10.1007/s00134-020-05939-1.

3. Antonides CFJ, Schoenrath F, **de By TMMH**, Muslem R, Veen K, Yalcin YC, Netuka I, Gummert J, Potapov E, Meyns B, Özbaran M, Schibilsky D, Caliskan K. Long-term outcome of patients after successful LVAD explant: A EUROMACS Study. ESC Heart Failure doi:10.1002/ehf2.12629.

4. Veen KM, Caliskan K, **de By TMMH**, Mokhles MM, Soliman OI, Mohacsi P, Schoenrath F, Gummert J, Paluszkiewiecz L, Netuka I, Loforte A, Pya Y, Takkenberg JJM, Bogers AJJC. Outcomes after tricuspid valve surgery concomitant with left ventricular assist device implantation in the EUROMACS registry: A propensity score matched analysis. Eur J Cardiothorac Surg 2019; doi:10.1093/ejcts/ezz208.

5. Vierecke J, Gahl B, **de By TMMH**, Antretter, Beyersdorf F, Caliskan K, Krachak V, Loforte A, Potapov E, Schoenrath F, Stockman B, Vanderheyden M, Meyns B, Gummert J, Mohacsi P. Results of Primary Biventricular Support: An Analysis of Data from the EUROMACS-Registry. Eur J Cardiothorac Surg 2019 2019 Dec 1;56(6):1037-1045.

6. Zahra S, Galea G, Jashari R, Petit P, **de By TMMH**. Significant variation in heart valve banking practice. Eur J of Clin Microbiol Infect Dis. 2019 Aug;38(8):1491-1498.

7. Zahra S, Galea G, Jashari R, Petit P, **de By TMMH**. Validation of microbiological testing in cardiovascular tissue establishments: results of a second international quality-round trial. Eur J Clin Microbiol Infect Dis. 2019 Aug;38(8):1481-1490.

8. Løgstrup BBL, Nemec P, Schönrath F; Gummert J, Pya Y, Potapov E, Netuka I, Ramjankhan F, Parner ET, **de By TMMH**, Eiskjær H. Heart failure etiology and risk of right heart failure in adult left ventricular assist device support: The European Registry for Patients with Mechanical Circulatory Support (EUROMACS). submitted.

9. Hannan M, Xie R, Cowger J, Schueler S, **de By TMMH**, Dipchand AI, Chu VH, Cantor RS, Koval CE, Krabatsch T, Hayward CS, Nakatani T, Kirklin JK. Epidemiology of infection in mechanical circulatory support: A global analysis from the ISHLT Mechanical Assisted Circulatory Support Registry. *J Heart Lung Transplant* 2019,38:364-373.

10. **De By TMMH**, Schweiger M, Waheed H, Berger, Hübler M, **Özbaran, Maruszewski BI, Napoleone CP**, Meyns B, Miera O. The European Registry for patients with mechanical circulatory support (EUROMACS): first EUROMACS PEDIATRIC (Pedi-EUROMACS) report. *Eur J Cardiothoracic Surg. 2018 Nov 1;54(5):800-808.*

11. **De By TMMH**, Mohacsi P, Gahl B, Zittermann A, Krabatsch T, Gustafsson F et al. The European Registry for Patients with Mechanical Circulatory Support (EUROMACS) of the European Association for Cardio-Thoracic Surgery (EACTS): second report. Eur J Cardiothorac Surg 2017; *Eur J Cardiothoracic Surg. 2018 Feb 1;53(2):309-316.*

12. Kirklin JK, Xie R, Cowger J, **de By TMMH**, Nakatani T, Schueler S, Taylor R, Lannon J, Mohacsi P, Gummert J, Goldstein D, Caliskan K, Hannan MM. Second Annual Report from the ISHLT Mechanically Assisted Circulatory Support (IMACS) Registry, j.healun.2018.01.1294.

13. Aslam S, Xie R, Cowger J, Kirklin J, Chu V, Schueler S, **de By TMMH**, Gould K, Morrissey O, Lund L, Martin S, Goldstein D, Hannan M. Bloodstream Infections in MCSD Recipients in the IMACS Registry: Epidemiology, Risk Factors, and Mortality. J.healun.201804.006.

14. **De By TMMH**, McDonald C, Süßner S, Davies J, Heng WL, Jashari R, Bogers A, Petit P. Validation of microbiological testing in cardiovascular tissue banks: results of a quality round trial. Eur J Cardiothorac Surg 2017; doi:10.1093/ejcts/ezx178.

15. Zuk K, Gahl B, Susac M, Vierecke J, **de By TMMM**, Stephan Windecker, Englberger L, Carrel T, Hetzer R, Gummert J, Mohacsi P. Midterm mechanical circulatory support: comparison of single-centre data with the EUROMACS registry. Eur J Cardiothorac Surg 2017;51:127–34.

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About the author

Theo de By was born on August 24th, 1951 in Eindhoven, the Netherlands. He accumulated several diplomas (Mulo-A, Mulo-B, HBS-A) and graduated from the Aloysius College at The Hague in 1971. While working at Eurotranplant International Foundation as well as at other employers, he studied western sociology from 1975 and obtained a bachelor degree in 1978, after which he followed the master program Sociology of Economics and Organisations at the Free University in Amsterdam. He finalised the Hospital Sciences Master program at the State University in Utrecht in 1980, which resulted in his first publication about the costs of kidney transplantation as compared to those of haemodialysis.

From 1995 to 1997 Theo studied at Business School Nederland in Heukelum, the Netherlands, and obtained the Master of Business Administration degree. His master thesis was dedicated to successfully influencing the German legislator with respect to the enlargement of possibilities for transplantation of human tissues.

From 1981 to 1988 he fulfilled different functions for the National Board of Hospitals, Utrecht, the Netherlands, where he was secretary general of the IZZ foundation for health insurance for health care workers, and co-responsible for the financing of the introduction for a new salary system in 750 institutions.

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From 1988 to 2010 he was managing director of Bio Implant Services Foundation in Leiden, the Netherlands.

From 1992 to 2005 he was member of the project management team of Beta Cell Transplant, Brussels, Belgium.

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From 2001-2010 he was vice-chairman of the supervisory board of 't Lange Land Ziekenhuis, a regional hospital in Zoetermeer, the Netherlands.

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Since 2012 Theo is director of the EUROMACS Registry, which was initially founded in Berlin and continued in 2014 under the auspices of the European Association for Cardio-Thoracic Surgery (EACTS), Windsor, United Kingdom. In 2015 he also became project manager of the EACTS Quality Improvement Programme with focus on the development of benchmarking using the European Adult Cardiac Database.
Theo is married to Marie-Thérèse de Bakker. He has 3 children: Esther-Viviënne, Felix and Frédérique, and 2 grandchildren: Salomon and Jonathan.

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IT'S A
HABIT