Current vascular allograft procurement, cryopreservation and transplantation techniques in the Czech Republic

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Abstract

Background. Vascular allotransplantations are performed worldwide in selected patients suffering from vascular prosthesis infection or critical limb ischemia. Either fresh or cryopreserved vascular allograft may be used.

Objectives. In various points, we address several aspects (allograft procurement, cryopreservation and transplantation technique) of the program of vascular allotransplantations in the Czech Republic.

Materials and methods. Vascular grafts retrieval has been done within multiorgan harvests using no-touch technique. Very short time of cold ischemia is achieved due to close cooperation with Tissue Establishment where the following processing of cryopreservation is performed. Meeting all necessary quality criteria is a prerequisite for releasing grafts for clinical application. Standardized thawing protocol and surgical handling aims to minimize microfractures before implantation.

Results. Based on experimental and clinical work, the first validation of cryopreserved arterial and venous grafts for clinical use was performed between 2011 and 2013 in the Czech Republic. The development of storage of vascular tissue in banks was stimulated in 2000–2010 by the issue of EU directives and national harmonized norms, aimed at assurance of high quality and safety of cells and tissues used for transplantations in humans.

Conclusions. There are several crucial moments affecting final quality, including graft retrieval within a multiorgan harvest, short ischemic time, cryopreservation, and thawing technique used. The recommended surgical handling during implantation may also affect results and graft-related complications.

Key words: tissue banking, cryopreserved vascular allograft transplantation, operative procedures, graft procurement, cryopreservation
Introduction

Vascular allotransplantations are performed worldwide in selected patients suffering from vascular prosthesis infection or critical limb ischemia. Either fresh or cryopreserved vascular allograft may be used. In this paper, all the aspects and the up-to-date state of the transplantation program of cryopreserved vascular allografts in the Czech Republic introduced in 2011 are presented.

In this country, there is a network of licensed surgical facilities performing vascular graft retrieval as a part of multiorgan recovery. These participating centers – Transplant Centers or Vascular Surgery Centers – were licensed for this activity by the State Institute for Drug Control (SUKL) as Procurement Establishments (PE) tightly connected by agreements with the licensed Tissue Establishment (TE) – Tissue Bank of the University Hospital Hradec Králové, Czech Republic. All the licenses were granted after providing proof of full compliance of practice in these facilities with the strict safety and quality requirements established by the UE Directives 2004/EC, 17/2006/EC and 2006/86/EC, and the national harmonized legal norms: Act No. 296/2008 Coll. (Human Cell and Tissue Act) and Decree of the Ministry of Health No. 422/2008 Coll.1,2 Cryopreservation of collected grafts, subsequent storage at liquid nitrogen temperatures and quality control until the release of grafts for clinical application are the main duties of the tissue bank. This service is, however, accessible only for transplant centers involved in the program. Distribution of grafts is performed by a licensed company able to perform emergency and rapid transport of cryopreserved grafts in a vapor phase of liquid nitrogen to any destination in the Czech Republic.

Material and methods

Vascular graft procurement techniques

To meet the requirements of the SOP (standard operating procedure) provided by the PE with the aim to achieve the quality and safety of cryopreserved grafts, it is necessary to retrieve the blood vessels within multiorgan harvests.3 The responsible person at each PE, an experienced vascular surgeon, guarantees that all surgical procedures are performed according to accepted SOP and all required documentation is maintained at the PE and/or sent to the TE as well. They are also responsible for the education and training of all surgeons included in the list of persons competent to perform graft recovery, and for reporting on any incidents of SAR (serious adverse reaction) and SAE (serious adverse event) that may occur in connection with the graft retrieval. If possible, it is necessary to perform perfusion through the internal iliac artery. Ideally, the artery is cross-clamped distally from the point of the harvested vessels. During a multiorgan harvest, a no-touch technique is routinely used when operating on the arterial system – aortic bifurcation ranging from renal arteries to superficial femoral arteries (10 cm) with side branches at least 1 cm long. For the collection of 1-sided arteries, harvest is started from the external iliac artery to the popliteal artery. The saphenous vein is collected in total length (Fig. 1). The spectrum of blood groups is harvested with a preference for type 0. The tissue bank also keeps unusual grafts in limited quantities: carotid bifurcation, the aortic arch with head arteries, inferior vena cava, and iliac vein bifurcation, preferentially of blood type 0. The grafts are replenished as needed. A very short time of cold ischemia (hours) is achieved due to close cooperation with the TE, where grafts are also processed during nights and weekends.

The vessels collected are immediately placed into a pre-cooled Celsior preservation solution (Genzyme, Cambridge, USA) supplemented with gentamicin and stored in sterile certified plastic jars (Medfor 250 mL; Medfor, Farnborough, UK). The jars are transported to the TE at the temperature of melting ice within 12 h after the harvest together with the documentation of the harvest and samples of the donor’s blood to perform serology tests in the licensed diagnostic laboratories (Department of Clinical Microbiology and Department of Clinical Immunology of the University Hospital Hradec Králové – UHHK).

Cryopreservation

Vascular graft cryopreservation is performed using the SOP required by the TE (Tissue Bank of the University Hospital Hradec Králové) fully licensed by a national competent authority.3,4 In the procurement and processing of vessels, only high-quality materials and drugs with approval for human use and meeting the requirements of the Directive of the European Parliament and Council No. 23/2004/EC are used.

After input control in the TE, the grafts are processed in a grade A clean room (according to the EU GMP classification) with a grade B background (Fig. 2 A,B) After decontamination using a modified van Katz5 method, the vessels are put into double sterile disposable plastic bags (Eva Bags; Maco Biotech, Eckbolsheim, France) containing 50 mL of a pre-cooled 6% solution of hydroxyethyl starch with molecular weight of 130,000 Da (Voluven 6%; Fresenius Kabi, Bad Homburg vor der Höhe, Germany) and mixed with an equal volume of the pre-cooled cryoprotective solution (20% dimethyl sulphoxide; WAK ChemieMedical GmbH, Steinbach, Germany) (Fig. 2C). The samples of the solution for bacteriological and mycological tests are taken from the collection solution and from the final package. The plastic bags are closed using heat sealing. The bags closed into outer metal cassettes are put into the freezing chamber of the programmable freezer and frozen at a rate of 1 K/min to −90°C.
(5 K/min to −150°C follows) (Fig. 2D). The grafts are stored until clinical use in the vapor phase of liquid nitrogen in the biological container equipped with an automatic filling system and continuous temperature monitoring (Fig. 2E).

**Quality criteria for release of grafts for clinical application**

The grafts can be released for clinical use by the responsible person from the TE only. The criteria for release are listed below:

- absence of contraindication for harvest in the clinical and anatomical diagnoses and patient’s medical history;
- good quality of the harvested tissue reported by the responsible person of the PE;
- absence of laboratory signs of infection as determined by the serology tests of the donor;
- absence of contamination of recovered grafts by pathogenic bacteria, molds or fungi;
- proof of sterility at the output control;
- absence of serious deviations from the SOP during retrieval, transportation, processing, and storage of grafts.

Reporting of SAR and SAE is connected with procurement, distribution and transplantation of grafts and is another important feature of the quality assurance system. A register for recording all clinical results achieved in all centers using cryopreserved grafts was established
M. Špaček, et al. Vascular allotransplantations in the Institute for Clinical and Experimental Medicine in Prague, Czech Republic, as a tool for evaluation of long-term results of vascular transplantation in the Czech Republic.

**Thawing**

After removal from the storage container, the cassettes with bags are transported to the operating room in the vapor phase of liquid nitrogen in a special Dewar vessel-dry shipper (Fig. 2F). In the operating room, the cassettes are removed from the shipper and placed into a refrigerator with temperature rising from +2°C to +8°C within 2 h. If some ice is still present after removal from the refrigerator, thawing can be completed at room temperature. Immediately after the ice melts, the vessels are aseptically removed from the bags and stored in the pre-cooled preservation solution (Celsior; Genzyme) until implantation.

**Surgical handling**

The surgical technique of vascular transplantation requires that side branches of the grafts be treated with Prolene sutures (Ethicon, Somerville, USA), avoiding any ligation or clipping. Under no circumstances should the allografts be cross-clamped – only the native vascular system of the patient can be cross-clamped during the operation. Proximal anastomosis is performed first. Later, under arterial pressure, the correction of sutured side branches is performed if needed. Afterwards, the graft is passed through the prepared tunnel, avoiding any rotation. Distal anastomosis of the bypass is performed. The surgical wounds are extensively drained.

**Results**

**Clinical application**

The first transplantation of a cryopreserved arterial allograft was performed in 2011. Five years of follow-up were uneventful (Fig. 3). A total of 87 cryopreserved vascular allografts were delivered for clinical application between 2011 and 2016 in the Czech Republic. A total of 59 saphenous vein grafts, 12 aortic bifurcations and 16 iliaco-femoro-popliteal (unilateral) grafts were used for bypass grafting. Our aim is to have all anatomical types of grafts in all blood groups (Table 1,2). A preference for grafts retrieved from donors with blood type 0 is evident, as an advantage in the case of a lack of anatomical types in TB stock.

Although the first experimental transplantations of vascular allografts or xenografts were performed more than 100 years ago and the mechanisms of freezing damage and cryoprotection have been known since the middle of the last century, some theoretical and clinical aspects of this surgical procedure still remain unsolved.
Some authors describe good results achieved using transplantation of fresh arterial grafts with immunosuppression.\textsuperscript{8,10} Some research groups\textsuperscript{11} point out the advantages of transplantation of cryopreserved grafts, such as the low probability of infection transmission and low immunogenicity leading to limited cellular and humoral rejection – in contrast to the acute rejection of fresh grafts which leads to progressive degeneration of elastic fibers and connective tissue and aneurysm formation if immunosuppression is not used. Other authors\textsuperscript{12,13} are convinced that certain cryopreservation protocols are responsible for early ruptures of grafts that may occur even intraoperatively and are always associated with life-threatening complications. It seems, however, that such serious adverse events are less likely to occur if cryopreservation protocols based on equilibrium and slow freezing are used. Such procedures are followed by, e.g., the European Homograft Bank in Brussels (EHB)\textsuperscript{14,15} and other centers, including our TE.\textsuperscript{16,17}

It must not be forgotten, however, that not only the freezing protocol itself but also the pre-freezing history of the graft may be responsible for such events. Our previous study in dogs\textsuperscript{18} showed that hypothermic storage of vessels in physiological saline for several days lead to considerable edema of the vessel wall. This finding shows the enhanced probability of vessel wall injury caused by crystal formation during freezing. For this reason, we consider it very important to use exclusively organ preservation solutions for intermediate hypothermic storage immediately after vascular graft harvest and to strictly control the timespan between the graft harvest in the PE and start of the cryopreservation procedure in the TE.\textsuperscript{17}

Achieving relatively stable ice structures by slow freezing does not, however, guarantee complete avoidance of the devitrification phenomena during thawing, as demonstrated by Pegg et al. in experiments in rabbits.\textsuperscript{19} They proved that the formation of microfractures in arteries caused by devitrification during fast thawing was responsible for graft rupture. This finding led us to the implementation of a slow-thawing protocol. In addition, injury caused by recrystallization is prevented in our practice by strict use of a cold chain based on the use of liquid nitrogen temperatures for storage and transport of grafts till thawing before use in the operating room. This is in contrast to the practice of some TEs,\textsuperscript{14} which use temperatures of –80°C for transport and even allow intermediate storage of grafts at these temperatures directly in cooperating surgical departments if the graft is not used immediately. In our practice, the graft is always sent back to the TE in the transport cryocontainer if the surgical intervention in the patient must be postponed for unexpected reasons.

Long-term storage of cardiovascular grafts in cryobanks was introduced before 2000.\textsuperscript{14,15,20} Development of the storage of vascular tissue in these banks was stimulated in 2000–2010 by the issue of EU Directives and national harmonized norms such as Act No. 296/2008 Coll in the Czech Republic\textsuperscript{1,4} or the Tissue Act in Germany,\textsuperscript{2} aimed at the assurance of high quality and safety of cells and tissues used for transplantation in humans. This law caused radical changes in the standard procedures used in the tissue banks, including the recovery and processing of vascular tissue.\textsuperscript{12,4,13} In contrast to Germany, where the use of fresh grafts was practically stopped, Czech law allows the use of fresh grafts in the regimen of organ transplantation regulated by Act No. 285/2002 Coll. within 48 h after harvest.

There is also a difference between the required purity of the environment in the graft processing areas. While Czech law permits the use of grade A environments (according to the EU GMP classification) with a grade C

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Table 1. Distribution of blood groups in venous cryopreserved grafts

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<th>Blood group</th>
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<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>Total</th>
<th>Rate [%]</th>
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<tr>
<td>Total</td>
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<td>14</td>
<td>3</td>
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Table 2. Distribution of blood groups in arterial cryopreserved grafts

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<td>9</td>
<td>14</td>
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<td>28</td>
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background, German law requires the same environmental conditions as in manufacturing of sterile medicinal products, i.e., a grade A processing area with a grade B background. As the TE of the UHHK has been retrofitted in compliance with the standards of the International Society for Pharmaceutical Engineering, it is able to assure this high level of quality of the environment that is regarded as standard in other Western European countries. National law may also set some restrictions of the use of grafts. While in some countries, the free sale of grafts to surgical departments is possible, in Germany the use of cryopreserved vascular grafts is strictly limited to clinical trials only. The situation in the Czech Republic is somewhere between these 2 extremes; the use of both fresh and cryopreserved grafts is limited to accredited transplantation or vascular transplantation centers.

References