Trasplante de Aloinjertos Valvulares y Vasculares

Implante, seguimiento y resultados a largo plazo de trasplantes de tejido valvular y vascular homólogo en una Comunidad Autónoma

Transplant of Vascular and Valvular Allografts

Implanting, follow-up and long-term results of vascular and valvular allografts transplants in an Autonomous Community.

Kerbi Alejandro Guevara Noriega.

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Kerbi Alejandro Guevara Noriega. M.Sc. M.D. FEBVS - NIE: Y0560881V

Parc Tauli University Hospital – Colaboración con: Organización Catalana de Trasplantes.

Tutor/Director: Prof. José Luis Pomar. Facultad de Medicina. Universidad de Barcelona. Hospital Clínic i Provincial Barcelona. Instituto Clínico del Tórax.

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This doctoral dissertation presented in Barcelona, Catalonia, is not more than one of the steps for which my medical career has passed. A career that I inescapably owe to the "Excellent University of Carabobo" and to Spain, the country that opened its doors to me at the right time. Without my passage through this wonderful country, much of what has been behind, including this dissertation, would not have been possible.

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SUMMARY.

Background

The transplantation of valvular and vascular allografts has evolved over the time. Despite being into the therapeutic options of two surgical specialties in our community, there is not an official registry for administrative and clinical control. The aim is to design the Registry of vascular and valvular allograft transplantation in Catalonia, Spain (ReVAC), as well as, to expose basic data about clinical outcomes. Special emphasis has been done in revascularization with arterial allografts of patients with Chronic Limb-threating Ischemia (CLI).

Materials and Methods

Three consecutives focal groups were made. Focal groups let to establish the administrative, technical and clinic necessities for the ReVAC. Basic data regards clinical outcomes is presented. Survival, primary patency, limb salvage and Major Adverse Limb Event-free (MALE-Free) rates are analyzed for arterial allografts. Survival rates and hemodynamic changes are analyzed for cardiac valves. Kaplan Meier curves are exposed. A specific subgroup is analyzed: Basic data related to clinical outcomes and predictive risk factors have been analyzed for patients with CLI and surgery with allografts. Statistical analysis was performed using SPSS Ver. 20 for Mac (Chicago, USA).

Results

A start-up online-access platform was created. ReVAC was divided into 3 levels; patient-related data, surgery performed and transplanted tissue. Online access is available through the website of applications of the “Generalitat de Catalunya”. 5-year primary outcomes are reported.

Revascularization to a distal target vessel was an independent positive predictive risk factor for a lower limb salvage rate and lower primary patency rate. Dyslipemia was related to a lower limb salvage rate and represents a risk factor involved in major adverse limb events.

Conclusion

ReVAC have been a useful tool for administrative and clinical control of allografts in Catalonia. Currently, basic data related to outcomes of arterial allografts are available reinforcing the guideline-supported indications but also opening a window for further analysis in other ones. For indications out of guidelines, arterial allografts seem to represent a suboptimal alternative although some selected patients could beneficiate of them. More studies are required to know the influence of immunological and clinical factors and for performing a better selection of patients.

RESUMEN.

Introducción.

El trasplante de aloinjertos valvulares y vasculares ha evolucionado a lo largo del tiempo. A pesar de estar en las opciones terapéuticas de dos especialidades quirúrgicas en nuestra comunidad, no hay un registro oficial para el control administrativo y clínico. El objetivo es diseñar el Registro de trasplante de aloinjerto vascular y valvular en Cataluña, España (ReVAC), así como exponer datos básicos sobre los resultados clínicos. Se ha hecho especial hincapié en la revascularización con aloinjertos arteriales en pacientes con isquemia critica de extremidades (CLI).

Materiales y métodos

Se realizaron tres grupos focales consecutivos. Los grupos focales permitieron establecer las necesidades administrativas, técnicas y clínicas para el ReVAC. Se exponen los primeros resultados clínicos de los aloinjertos vasculares y valvulares. Se analizan: la supervivencia, permeabilidad primaria, tasa libre de evento mayor adverso en la extremidad, y tasa de salvamento de extremidad en pacientes tratados con aloinjertos arteriales. Se analizan las tasas de supervivencia y los cambios hemodinámicos de las válvulas cardíacas trasplantadas. Los cálculos de tasas se realizan con Kaplan meier y las curvas son expuestas en el manuscrito. Se analiza un subgrupo específico: Datos de evolución clínica y factores predictivos de riesgo fueron calculados en pacientes con CLI que fueron tratados con aloinjertos. El análisis estadístico se realizó utilizando SPSS Ver. 20 para Mac (Chicago, USA).

Resultados

Se creó una nueva plataforma de acceso en línea. ReVAC se dividió en 3 niveles; datos relacionados con el paciente, cirugía realizada y tejido trasplantado. El acceso en línea está disponible a través del sitio web de aplicaciones de la *"Generalitat de Catalunya"*. Se informan resultados a 5 años. En el análisis de factores de riesgo se detectó que la revascularización a un vaso distal fue un factor de riesgo predictivo positivo independiente condicionando un tasa de salvamento de extremidad y para una tasa de recuperación de la extremidad inferior y una tasa de permeabilidad primaria más baja. La dislipemia se relacionó con una tasa de salvamento de la extremidad mas baja y también representa un factor de riesgo involucrado en una mayor tasa de eventos mayores relacionados a la extremidad revascularizada.

Conclusión

ReVAC ha sido útil para el control administrativo y clínico de los aloinjertos en Cataluña. Actualmente, se dispone de datos básicos relacionados con los resultados de los aloinjertos arteriales que refuerzan las indicaciones respaldadas por las guías clínicas, pero también abren una ventana para un análisis adicional de otras indicaciones. Para indicaciones fuera de las guías clínicas, los aloinjertos arteriales parecen representar una alternativa subóptima, aunque algunos pacientes seleccionados podrían beneficiarse de ellos. Se requieren más estudios para conocer la influencia de los factores inmunológicos y clínicos así como, para realizar una mejor selección de pacientes.

PREFACE

A PhD thesis about *arterial allografts* means a big challenge, especially in an area where the allografts still have the stigmas of their second stage. Catalonia is a privileged area to receive medical training and I had the chance of being trained in one of the most important hospital of this area. In fact, Parc de Salut Mar Barcelona, the place where my vascular surgery training was performed, is the largest consortium according the number of hospitalization beds but also is a place with a lot of skepticism about arterial allografts. In contrast, Hospital Clinic and Provincial Barcelona, the heart of academic medicine in the city, have performed surgeries with allografts and it has been known as the hospital of reference for donation and transplantation. Of course, this includes the donation and transplantation of tissues and, undoubtedly, the transplantation of vascular tissue.

Nevertheless, this PhD thesis goes beyond these hospitals. Ten centers were involved under the coordination of the Catalan Organization of Transplants. The main landmark was always to maintain the maximum possible objectivity and avoid that the project was affected by the subjectivities about allografts.

This PhD thesis has been developed and organized in order with the policy approved by the Doctoral Committee of the *Universitat de Barcelona.* Each step was taken according to ethical standards, respect for patients and their confidentiality and with the north of figuring out what extent the allografts are useful and at what point beliefs about their malfunction are true. All the published and no-published data have been obtained in accordance with the ethical guidelines of

the Declaration of Helsinki and local legislations, with particular emphasis on regulations regarding data privacy.

We recognize the allografts do not represent the ideal graft in vascular surgery and in our study would throw data against this type of tissue. However, we believe that they are grafts that need to be more studied and known. The creation of a registry for concentration of information and the encourage of starting a prospective analysis leads without doubt to have that better knowledge, as well as, to pose new questions and possible answers.

In the design process of a PhD thesis with such an ambitious aim, we needed to conform a multidisciplinary team under the coordination of the Catalan Organization of Transplants. This was a challenge mainly because it needs time and every task was done *ad honorem*, but also allowed us to have solid foundations to conduct the next steps of the study.

Preliminary results were more encouraging than those that finally were. However, at the end of the project there was a global idea of graft behavior and what are the next steps to follow to answer the questions that will arise along the way.

Undoubtedly, this project let me to improve my skills in research, statistics, and my knowledge in vascular grafts. Despite the obstructions put by some, the forces added in favor of the project allowed it to come to an end. New tools emerged with this project as well as, a lot of ideas that are now available to anyone who wants to continue with this line of research. Always for a better understanding that allows us to give the best to our patients.

PREFACIO

Una tesis doctoral sobre aloinjertos arteriales significa un gran desafío en un área donde los aloinjertos aún tienen los estigmas de su segunda etapa. Cataluña es un área privilegiada para recibir capacitación y entrenamiento médico y tuve la oportunidad de formarme en uno de los hospitales más importantes de esta área. De hecho, Parc de Salut Mar Barcelona, el lugar donde se realizó mi entrenamiento de Cirugía Vascular, es el consorcio más grande según el número de camas de hospitalización, pero también es un lugar con mucho escepticismo sobre los aloinjertos arteriales. En contraste, en el Hospital Clínico y Provincial de Barcelona, el corazón de la medicina académica en la ciudad, se han realizado cirugías con aloinjertos y ha sido conocido como el hospital de referencia para la donación y el trasplante. Por supuesto, esto incluye la donación de tejidos y, sin duda, el trasplante de tejido vascular.

Sin embargo, esta tesis doctoral va más allá de estos hospitales. Diez centros participaron bajo la coordinación de la Organización Catalana de Trasplantes. El reto principal fue siempre mantener la máxima objetividad posible y evitar que el proyecto se viera afectado por las subjetividades sobre los aloinjertos.

Esta tesis doctoral ha sido desarrollada y organizada de acuerdo con la política aprobada por el Comité de Doctorado de la Universitat de Barcelona. Cada paso se hizo de acuerdo con los estándares éticos, el respeto por los pacientes y su confidencialidad, siempre con el fin de determinar en qué medida son útiles los aloinjertos y en qué punto son ciertas las creencias sobre su mal funcionamiento. Todos los datos publicados y los no publicados se han obtenido de acuerdo

con las directrices éticas de la Declaración de Helsinki y las legislaciones locales, con especial énfasis en las regulaciones sobre privacidad de datos.

Reconocemos que los aloinjertos no representan el injerto ideal en la cirugía vascular y nuestro estudio arrojaría datos en contra de este tipo de tejido. Sin embargo, creemos que son injertos que necesitan ser más estudiados y conocidos. La creación de un registro para la concentración de información y el estímulo para comenzar un análisis prospectivo lleva sin duda a tener un mejor conocimiento, así como a plantear nuevas preguntas y posibles respuestas.

En el proceso de diseño de una tesis doctoral con un objetivo tan ambicioso, tuvimos que conformar un equipo multidisciplinario bajo la coordinación de la Organización Catalana de Trasplantes. Esto significó un gran desafío principalmente porque se requiere de mucho tiempo y cada tarea se realizó *ad honorem*, pero esta forma de trabajo nos permitió tener bases sólidas para llevar a cabo los siguientes pasos del estudio.

Los resultados preliminares fueron más alentadores que los que finalmente obtuvimos. Sin embargo, al final del proyecto se logró tener una idea global del comportamiento de este tipo de injertos y cuáles son los próximos pasos a seguir para responder a las preguntas que surgirán en el camino.

Sin lugar a dudas, este proyecto me permitió mejorar mis habilidades en investigación, estadísticas y mi conocimiento en injertos vasculares. A pesar de los obstáculos puestos por algunos, las fuerzas a favor del proyecto permitieron que llegara a su fin. Con este proyecto surgieron nuevas herramientas y muchas ideas que ahora están disponibles para cualquiera que quiera continuar con esta línea de investigación. Siempre para una mejor comprensión que nos permita dar lo mejor a nuestros pacientes.

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1. INTRODUCTION

Donation and transplantation of organs, tissues and cells is now an attractive and widely proven therapeutic option which involves practices that are accepted as rutinary such as kidney and liver donation; but it also involves less striking practices such as tissue transplantation. The latter can be considered vital for the survival of some patients[1].

Cryopreserved vascular and valvular tissue transplantation is one of the many therapeutic resources that each specialty, either vascular or cardiac surgery, has for its treatments.

As the vascular allografts concerns, the Nobel Prize in Medicine, Alexis Carrel, developed first projects in this field. Since then, a long way has been run and different stages can be highlighted[2,3]. Three stages have been described according the use and acceptance of theses grafts[1]. Nowadays, The Society for Vascular Surgery practice guidelines on the care of patients with an abdominal aortic aneurysm has recommended arterial allografts as an option of grafts for specific indications. European guidelines are more unspecific only clarifying that in-situ aortic reconstructions with vascular allografts have a lower rate of adverse events that extra-anatomical bypass[4,5]

In cardiac surgery, thanks to the introduction of the extracorporeal circulation in Minneapolis and Mayo Clinic by Gibbon, a new work field was created for the valvular graft use. A Spanish surgeon, Carlos Duran (LANCET 1962) in precarious facilities of the Radcliffe Infirmary of the University of Oxford, described in 1962 the sub-coronary transplant technique known as “orthotopic position technique” through a fresh bovine aortic valve transplantation in another living animal with good results. In the same year, in the antipodes, Donald Ross (LANCET 1962), and Sir Brian Barrat Boyes, two years

later (THORAX 1964) in London and Australia respectively, reported their first outcomes of the Duran technique in human beings. Nowadays, these types of transplants have been used uninterrupted especially in the pediatric population[6,7].

However, the emerging techniques in Vascular Surgery and Cardiac, as well as, the availability of new grafts and devices have limited the use of allografts.

In vascular surgery, endovascular techniques remain one of the most important choices when planning revascularization. Furthermore, we must also remember that autologous tissue in optimal conditions have been and will be over other types of graft because of its demonstrated superiority in surgical techniques[1].

Moreover, prosthetics grafts still are the second line resource in revascularization surgical procedures as Twine and McLain described it in a Cochrane review in 2010. Having this data in mind, arterial allografting is limited to specific cases mainly because they are hard to obtain, to store and without benefits for the industry[8].

In Cardiac Surgery, allografts have been more widely used and accepted. In fact, the most common valvular allograft indications are the endocarditis, the so-called Ross intervention, and the treatment of congenital cardiac pathologies in which these procedures could be vital.

A topic to be highlighted is that allografts go hand in hand with cryopreservation and processing techniques. It must be known that developments in dissection-explant, sterilization, conservation and processing of this type of graft (better known as cardiovascular allograft or homograft), and to the progress in logistics in coordination to organs and tissue transplantation have been notorious. Moreover, in the current age of the allografts, the use of either vascular or valvular allografts have change thanks to new trends in cryobiology and immunobiology, and the exact determination of

histocompatibility matching and safer immunosuppressive therapies has been reconsidered[9].

Nowadays, the problem lays in the lack of evidence about the correct behavior of allografts that, by being biologic tissue, are expected to act as a living structure with theoretical potential in the cardio-circulatory system.

Additionally, as a vascular allografts concern, despite current indications and acceptance vascular surgeons cannot forget the classical complications known during the second stage of its use, where there were cases with degenerative changes that resulted in aneurysmatic dilations, probably related to the receptor immunologic response. Back then, these mechanisms were intuited to be also related to poor conservatory and preparatory techniques of the biologic material[9,10].

In contrast, valvular allograft continues to show more advantages in hemodynamics and thromboembolic risk in comparison with the mechanical prosthetics in young active patients. Ross's technique has proved to be superior to the aortic allograft replacement due to the slower homologous allograft degeneration and its use is individualized for the patient demands.

Also, a problem is the failure to identify the immune response related to the vascular or valvular allograft, as well as, complications in a determined timeline and the durability of the transplant. Furthermore, there is a lack of scientific evidence in regarding the long-term outcomes. Currently, it is known the differences of emerging objective performance goals between allografts, autologous tissue or prosthetic materials, either Dacron or Polytetrafluorethylene (PTFE) that is the most common material used today[11,12].

2. HISTORICAL OVERVIEW OF VASCULAR ALLOGRAFTS

With more and more indications, the transplantation of vascular tissue is an emergent practice, which began in the early 20th century and has gone through several stages. Since the publication of preliminary experiences, its use was abandoned due to possible long-term complications and it has been taken up at the end of the 20th century for special indications and has been subject to in-depth analysis[2,3].

2.1 Stage 1

Although Hopfner described the possibility of performing the technique in 1903, the history of vascular tissue transplantation began in the hands of Alexis Carrel. This French biologist, physician and researcher is considered as a pioneer in vascular surgery. Carrel described surgical techniques used on animals by 1905. These techniques involved transplant of venous segments in arterial territories in mice and he described the process of arterialisation and exposition of the conservation of blood vessels for transplant with the intention of avoiding the need to find possible donors[3,13-15].

In 1906, there was an important publication describing the first two successful human cases of bypass using autonomous vessels. The first described a femoro-popliteal shunt with a femoral vein and the second was a resection of a popliteal syphilitic aneurysm and its subsequent replacement by a popliteal vein[11].

A year later, Lexer described the transplantation of an 8 cm vein segment after resection of a left subclavian artery aneurysm caused by dislocation of the humerus. These initial steps saw the beginnings of vascular tissue transplantation with autologous grafts[16].

Other isolated publications included the case described by Pirovano in 1910 of the first transplant of vascular allografts, which was not successful, and a case series reported by Moure in 1914, who described 17 transplants of venous allografts with good results[3,11].

In 1908, Carrel made a significant breakthrough by creating the first experimental blood vessel bank, leading to the Nobel Prize for Medicine in 1912 in recognition of his work on vasculature and the transplantation of blood vessels and organs. In the same year, he also published an article in which he demonstrated that an artery portion can be preserved and kept 'alive' in a chamber for several days or even weeks before transplantation. He found that blood vessels from dogs kept in a cold room can be transplanted successfully into cats and concluded that these methods could be applied to humans and there should be no delay to exploring this, but it was not until 1951 that Fontaine and Leriche founded the first bank of blood vessels for clinical use[3,11].

2.2 Stage 2

The second stage of vascular tissue transplantation is characterized by a significant decline in its use due to unsatisfactory long-term results. The poor results were possibly related to deficient preparation and preservation techniques. In this stage, there were cases of degenerative alterations with subsequent aneurysmal dilatation related to immunological responses. This led to an exploration of the use of synthetic prostheses[11].

In 1952, Voorhees et al. performed animal studies that demonstrated the patency of synthetic derivatives in vascular territory and translated its application into clinical practice. This technique had a rapid evolution. Among the materials used were Ivalon,

Orlon, nylon, Teflon and Dacron. In 1969, prostheses that are still in use today were developed by Gore, which developed the Teflon graft[11,16,17].

Prostheses were developed with the ideal aim being described in 1953 as: "biologically inert, with stable physicochemical properties, guaranteed sterilization, easy handling, non-carcinogenic and non-thrombogenic surface". These criteria were later modified by Moneta and Porter in 1995 when they described the ideal prosthesis as "strong, inexpensive and possible to be used throughout the patient's life, with easy and permanent insertion, biocompatible with the host, resistant to infection, with appropriate gauges, that remains permeable due to the visco-elastic properties resembling a natural artery without allowing blood or serum to escape, that does not degenerate or elicit an abnormal proliferative response of the vessel or tissue, non-thrombogenic or emboligenic, the one that does not occlude by flexing and does not damage the components of the blood". These criteria revealed large defects in the vascular allografts; however, no synthetic arterial substitute has ever been able to comply with all these conditions[18].

There were important achievements in this second stage, such as that of Gross in 1948, who replaced a segment of homologous aorta in a case of coarctation, establishing the technique as the first choice until the development of the prostheses. Similarly, Oudot in 1950 was the first to replace the aortic bifurcation and Dubost in 1951 the first to use a homograft after resection of an abdominal aneurysm[11,17].

During this stage, some groups, including the one led by DeBakey, published series of cases that have not been possible to match[11,17].

At present, thanks to advances in techniques of collection, processing and storage of the grafts, now called vascular allografts, and the progress in organ donation

and transplantation, cryobiology, immunobiology, histocompatibility determinations and immunosuppressive therapy, these grafts are being reconsidered for use in a greater number of indications, thus starting the third stage of vascular tissue transplantation. All this is in the context of the development of commercial prostheses as the first indication in almost all cases of vascular segment replacement[11].

2.3 Stage 3

Vascular transplantation began being used again with prudence and with limited indications. Among these indications was the replacement of complex arterial segments, complex vascular lesions, replacement of infected arterial prostheses and, although it is little studied, the use in vascular access for patients on hemodialysis. Mestres summarized the use of these grafts in three indications and confirmed long-term results. He described some benefits regarding the reduction of hospital mortality, a high patency rate and a minimal rate of reinfection and rupture up to 10 years' post-intervention[3,9,11,19].

In this third stage, immunology was the objective of research. In 1989, Koene described the role of adaptation in the acceptance of allografts. He concluded that the long-term survival of the allograft depends on these immune responses[20].

Previously Prendergast et al. observed immunological sensitivity and concluded that allografts generated immunological responses. More recently, the role that innate immunity and antibodies play in the rejection of grafts has been explored. So-called 'allograft vasculopathy' has been linked to a chronic inflammatory response mediated by natural killer cells, but carried out directly by donor-specific antibodies. Donor antibodies ultimately induce intracellular cascades that facilitate recruitment of

monocytes and neutrophils, damaging the transplanted tissue. This area is still being researched[21,22].

This gave rise to the experimental application of tissue transplantation and to the development of animal models. In 1983, Chow et al. performed the replacement of femoral arteries with cryopreserved tissues and compared the results with the replacement of autologous tissues, concluding that both techniques were similar[23].

In 1997, Neves et al. published their findings on mechanisms of degeneration of cryopreserved grafts, analysing them in sheep. They concluded that there is partial loss of the endothelium and lymphocytic invasion in the entire graft, despite which the grafts maintain their integrity and cellular viability after transplantation. They showed evidence of re-epithelialization of the graft and after a short period of neural degeneration, a reinnervation occurred. No statistically significant differences were found between transplants of fresh vascular tissue versus those that were cryopreserved[24].

Among the alternative studies in the translational field is the model on venous grafts treated with glutaraldehyde performed by Moura in 2009. He performed experiments in rabbits whose grafts were assessed macroscopically and microscopically at 24 hours, 14 and 28 days. In his conclusions, he suggested the need to expand studies in the field of autologous tissue transplantation, since he showed that there were no clear differences in the technique he proposed. He also explained that this technique could provide an important tool for human use[25].

Perhaps the three most recent translational works are those published by Sun et al. in 2010, Hwang et al. in 2011 and Olmos-Zúñiga et al. in 2016[26-28].

Sun et al. explained an improved technique for performing aortic transplants in a murine model and demonstrates pathognomonic changes of chronic rejection but with longer-term tissue survival rather than usual techniques[26].

Hwang et al. designed an experimental platform for the development of biocompatible microvasculature in rats and he showed that its model is potentially translatable and effective for future tissue engineering studies of small vessels[27].

Olmos-Zuñiga et al. published the hemodynamics, gasometrical and imaging results, as well as macroscopic and microscopic findings of the reconstruction of pulmonary arteries of dogs with lyophilized grafts (those not treated with glutaraldehyde) and cryopreserved arterial grafts. They suggested that the lyophilization techniques may play in favour of less antigenicity, as well as preventing thrombosis and calcification of the grafts. Finally, they concluded that the lyophilization without treatment with glutaraldehyde represents a feasible alternative with promising clinical results[28].

Among the described clinical cases is the correction of coarctation of the thoraco-abdominal aorta with autologous cryopreserved arterial graft performed in a 7-year-old boy with a correct postoperative ultrasound, doppler ultrasonography with no significant changes with respect to a healthy subject, correct clinical values and good quality of life after the procedure[29].

In 1998, an extensive study was published by Chiesa et al. that assessed differences between cryopreserved and fresh vascular tissues and concluded that there were no statistically significant differences between them. They described a 12-month tissue survival of 73% and reported the possibility of assessing ABO blood group compatibility among donors. This possible incompatibility has been dismissed in 2015

by Della Schiava et al., who considered that the immunological response may be related mainly to incompatibility of the major histocompatibility system. In this way, the clinical variables associated with the donor and recipient allografts become important[30,31].

The use classically associated with this type of graft is the replacement of infected prosthetic segments. This was described in 2004, where the replacement of infected grafts in the infrarenal aorta by vascular allografts was presented in a series conducted over 14 years[32].

Kieffer et al. concluded that vascular allografts, in the short, as well as, long term are at least similar in behavior to other replacement techniques in terms of the management of infra-renal prosthesis infections. They also found that most of the complications associated with this type of grafts are avoidable with an adequate cryopreservation process. Previously in 2001, Leseche et al. had commented on the usefulness of the use of vascular allografts in prosthetic infections, and in 1996, Koskas et al. documented 6 years of experience replacing infected prostheses from 83 cases with several postoperative complications, but with a limb survival rate of 100%[33,34].

In 2009, Brown et al. published their mid-term results for arterial reconstruction with cryopreserved vascular tissue in cases of prosthesis infections. They presented a series of 52 patients followed up over 10 years that showed that the replacement of infected vascular prostheses by vascular allografts was a viable alternative. They stated that with adequate cryopreservation, allografts are resistant to reinfection, thrombosis and aneurysmal dilatation and recommended a long-term study to evaluate whether this technique is the most successful, effective and safe[35].

More recently, in Greece, Locati et al. published a short series of 18 patients where 25 infected prostheses were replaced in different areas such as femoro-popliteal, aorto-iliac, and subclavian, concluding that these techniques are very useful in this indication since these grafts seem to have a greater resistance to infection[36].

In 2010, a German team published an 8-year follow-up of patients treated with cryopreserved arterial homografts using exposure to C-reactive protein and leukocytes as analytical parameters to monitor during the immediate postoperative period[37].

They also proposed that platelets and body temperature were important clinical parameters in the postoperative period. The team reported an 81% survival of transplanted tissue and free of re-interventions at 3 years. In the remaining 19% of patients, there were occlusions, stenosis, aneurysmal degenerations and graft-duodenal fistulas. It was concluded that the vascular allografts were a useful alternative[37].

A more unusual use as pulmonary artery augmentation in a lung transplant has been described by Pablo Rueda et al. in 2005. They performed enlargement of the pulmonary artery in a case of inadequate organ extraction using an aortic artery allograft and concluded that the technique was useful to avoid the loss of the organ[38].

The use of a graft in vascular access construction for hemodialysis was described in 2016 by Ha et al. as an alternative for immediate dialysis and with a survival rate comparable with other types of grafts[39].

However, not all published results are so positive. In Italy in 2011, Ravenni et al. reported a case of total calcification of a homologous vascular graft used in the replacement of the aortic root in a 66-year-old man[40].

Similarly, Minga Lowampa et al. published a series of 103 patients with replacement of prostheses infected by allografts whose short-term results were unfavorable with a high rate (29%) of postoperative complications, such as graft thrombosis, anastomotic pseudoaneurysm, aneurysmal degeneration and graft rupture. However, the authors comment on methods that could improve these results[41].

There are limited data on the long-term evolution of vascular allografts. The research continues with a focus on advances in cryopreservation, immunology and alternative tissue preparation[1].

3. ALLOGRAFTS IN GUIDELINES

Regards vascular allografts, differences between arterial and venous allografts must be stablished. Venous allografts have not any guideline-supported indication currently. Their acceptance is low and few publications have arisen in the last decade.

Arterial allografts continue being investigated and several researches have been published recently. At present, only one indication of vascular allografts has been established in clinical guidelines. The European Society of Vascular Surgery and the American Society of Vascular Surgery have considered allografts as a suitable alternative for replacement of infected grafts in aortic position[4,5].

Other out-of-guidelines indications have been published recently. Arterial allografts have been used as resource technique in vascular access for hemodialysis. In CLI, allografts have been described as suitable conduit with similar outcomes to prosthetic grafts. Despite the wide use around the world, all of these uses are out of guidelines. Each case, indication, patient and territory should be assessed individually, with more data and studies needed to clarify the most appropriate indications.

On the other hand, Cardiac valves have been constantly used since 1960s until today. Cardiac valve has a wide acceptance and several recent publications have been published. However, no clear indication has been stablished in the guidelines.

4.- VASCULAR ALLOGRAFTS FOR CHRONIC LOWER LIMB-THREATING ISCHEMIA.

Revascularization of a lower limb with critical ischemia is the only alternative that has been shown to reduce the symptoms, either rest pain, non-healing ulcers or established necrosis. Revascularization surgery has also demonstrated to be the best option for improving limb salvage rate. In vascular surgery, currently, the indication of revascularization should be assessed for open surgery or endovascular procedures.

Endovascular techniques have gained wide acceptance as primary and secondary treatment of critical ischemia of the lower extremities. Despite a large number of established indications and many other emerging indications, many patients require open surgery using lower extremity bypass (LEB) to try to ensure therapeutic success.[41,42]

The autologous saphenous vein has been widely accepted as the preferred graft for revascularization of the lower limbs. In patients requiring a lower extremity bypass, it is estimated that 20 to 45% do not have a single segment of autologous saphenous vein available for the bypass. Previous coronary or lower limb revascularization, varicose veins of the lower limbs, previous surgery of varicose veins and/or inadequate quality of the native vessel due to diameter or length are the main reasons why patients could confront this scenario. [43,44,45]

In this scenario, the vascular surgeon must consider the use of alternative grafts, the main options being autologous veins from less usual location, synthetic prostheses and vascular allografts. However, for aorto-iliac location, there are several alternative grafts, most of them with excellent results. In this location, advantages of allografts over other grafts are limited to a lower re-infection rate. For this reason, allografts are

included into international guidelines as a suitable option for aorto-iliac infected graft replacement.

For femoral territory, we could consider that allografts are resistant to infection more than other grafts. However, no clear indication has been stablished and other grafts are widely used rather allografts. Endovascular revascularization has demonstrated to play a important role for femoral superficial artery revascularization. No indications of allografts have been stablished for superficial femoral artery revascularization and most of the available grafts have better outcomes.

Several groups have considered that PTFE grafts for distal vessel target revascularization is incorrect and few groups have used allografts for it. However, there is not enough data available to support this indication.

5.- THE ROSS PROCEDURE AND THE CURRENT CARDIAC VALVE REPLACEMENT SURGERY.

5.1 Ross Procedure and Valvular Allografts.

Ross procedure is a surgical technique where the aortic valve is replaced with the autologous pulmonary valve and this latter is replaced in turn by an allograft. Beginning of this technique date back to 1967 when Donald Ross performed first time this surgery. However, initially, ross technique did not gain widespread popularity until a modification to the technique was introduced in late 1980s. At that moment, Ross procedure experienced a revival when the knowledge of surgeons applying coronary buttons for aortic root operations promoted pulmonary autograft replacement using the full-root technique. This was further facilitated by the publication of excellent midterm results in the 1990s. In the modality of the Ross procedure described here, the complete pulmonary root is implanted into the aortic position after the aortic root is removed, including the re-implantation of the coronary arteries buttons into the neoaortic root. Today, most surgeons perform the root replacement technique, whereas the use of the initial sub-coronary implantation technique is rarely applied[46].

Nowadays, the aortic valve prosthesis is well developed with many brands and options available. The surgical technique has been constantly improved. However, patients with mechanical prostheses are exposed to permanent anticoagulation, restricted hemodynamics and a higher risk for thromboembolism and endocarditis[46].

In this scenario, the aortic valve replacement with patient's own pulmonary valve play an important role. Through this technique, the patient is not exposed to permanent anticoagulation and good long-term functional results have been observed,

even in combined procedures and complex clinical presentations, which encourage continual use[46].

Against the aortic valve replacement with autograft (pulmonary valve), most surgeons have no intention of taking additional morbi-mortality throughout the learning curve and causing pulmonary valve disease. Moreover, concerns were expressed whether the pulmonary valve withstands the hemodynamic stress of the aortic position[46,47].

Currently, experts recommend the Ross technique because the pulmonary autograft demonstrates low rates of degeneration, endocarditis and thromboembolism for a period >20 years compared with current biological or mechanical replacement valves. The Ross procedure was associated with better aortic valve hemodynamics. The technique represents a good option that can be performed safely in pediatric patients with aortic valve disease, even in a small-volume center. Moreover, Ross Technique is considered a durable solution for multilevel left ventricular outflow tract obstruction in a highly complex patient population with high incidence of previous procedures[46,48,49].

5.2 State-of-art in valve replacement.

Currently, there has been a significant decrease in industrialized countries thanks to better management of rheumatic fever. However, developing countries still have a substantial number of cases due to this reason[50].

Nevertheless, industrialized countries have increased their life expectancy, and this increasing elderly population leads to an increased rate of degenerative valve disease (DVD). Even though the VHD or DVD are not considered as a public health problem, a significant number of patients require an answer to their problem in an ever-changing field of the cardiac surgery[51].

It is essential to highlight that left-sided valves still represent the main indication of surgery for valve replacement being the aortic valve the most common followed by the mitral valve. Currently, at the moment of offering a solution to the patient, either, open or percutaneous procedures are available, and specific indication has been established for each one[52].

In this field, cardiologists and surgeons had joined forces to write the Valvular Heart Disease Guidelines for the first time in 2012 when the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS) published an unavoidable document to read when we are talking about VHD in Europe[51].

In 2017, ESC/EACTS updated the guidelines due to new evidence on percutaneous interventional techniques and risk stratification concerning the timing of intervention in VHD. Simultaneously, the American College of Cardiology/American Heart Association (ACC/AHA) also published guidelines for VHD. Many differences among two major society guidelines have been described and, even, a small number of the guideline recommendations seem contradictory. The author of these reviews about these differences considers more randomized trials are required to clarify recommendations[53,54].

From our perspective, guidelines and their recommendations in any specialty is a just a guide to facilitate decision making of health professionals in their daily practice but every indication, clinical decision and options offered to the patients must be individualized and taken by caregiver and physician according to the specific case.

Particularly in Valve heart disease management, the medical option is still an indication in specific cases, percutaneous procedures have dramatically increased in

number and indications, and the surgical approach of repair versus replace is dependent upon a variety of factors, including surgical experience, valve morphology, surgical risk, mechanism of the affectation, anticoagulation planning, and patient age.

5.3 Summarizing surgical indications for valve replacement.

Aortic stenosis (AS) is the most common valvular disorder in Europe, and the 2017 ESC/EACTS guidelines consider balloon aortic valvotomy, transcatheter aortic valve implantation (TAVI), or surgical aortic valve replacement (SAVR) as suitable options in patients requiring intervention[51,55].

For establishing indications, symptomatic versus asymptomatic patients must be differentiated. Indications could be summarized as follow:

- Intervention is indicated in symptomatic patients with severe, high-gradient aortic stenosis (mean gradient >_40 mmHg or peak velocity >_4.0 m/s). It is also indicated if severe low-flow, low-gradient (<40 mmHg) aortic stenosis with reduced ejection fraction and evidence of flow (contractile) reserve excluding pseudo-severe aortic stenosis

- Patients with low-flow, low-gradient (<40 mmHg) aortic stenosis have an indication of surgery if they have normal ejection fraction after careful confirmation or if they have a reduced ejection fraction without flow (contractile) reserve, particularly when CT calcium scoring confirms severe aortic stenosis.

- Symptomatic severe AS demands intervention in nearly all clinical circumstances. However, quality of living, benefits for the patient, comorbidities or surgical risk must be analyzed before indicating the surgery.

- Asymptomatic patients have indications only if severe aortic stenosis and systolic LV dysfunction (LVEF) not due to another cause, an abnormal exercise test showing symptoms on exercise related to aortic stenosis or an abnormal exercise test showing a decrease in blood pressure below baseline

- In Asymptomatic patients should indicated a surgery if they have low surgical risk and very severe aortic stenosis defined by a Vmax >5.5 m/s, or severe valve calcification and a rate of Vmax progression >_0.3 m/s/year or markedly elevated BNP levels confirmed by repeated measurements without other explanations or in cases of severe pulmonary hypertension without other explanation[51].

Specific details about open surgery or percutaneous, or concomitant aortic valve surgery at the time of other cardiac/ascending aorta surgery procedure are beyond this review, and we strongly recommend to review the 2017 ESC/EACTS guidelines[51].

Regarding Aortic Regurgitation, every symptomatic patient or anyone with acute Aortic Regurgitation requires surgical intervention, and the latter often demands emergent surgical intervention. Asymptomatic patients have an indication of surgery if resting Left Ventricular Ejection Fraction (LVEF) <50%. Also, should be considered the surgery for asymptomatic patients with resting ejection fraction >50% with severe LV dilatation: LVEDD >70 mm or LVESD >50 mm (or LVESD >25 mm/m2 BSA in patients with small body size). Surgery is indicated in patients undergoing CABG or surgery of the ascending aorta or another valve[51,55].

Mitral regurgitation (MR) remains the second most common indication for valve surgery in Europe. Patients with acute severe mitral regurgitation must be provided for urgent surgical intervention according to the 2017 ECS/EACTS guidelines. In contrast,

for deciding about surgery in chronic MR, symptomatic and asymptomatic patients must be differentiated[51,55].

In symptomatic patients with MR the surgery if indicated if LVEF >30%. For Asymptomatic patients, surgery is indicated in asymptomatic patients with LV dysfunction (LVESD >_45 mm and/or LVEF 60%) and atrial fibrillation secondary to mitral regurgitation, as well as, patients with pulmonary hypertension. Indication of Surgery should be considered in asymptomatic patients with preserved LVEF (>60%) and LVESD 40–44 mm when a durable repair is likely, surgical risk is low, the repair is performed in a heart valve center and presence of flail leaflet or significant Left Atrium (LA) dilatation in sinus rhythm[51,55].

The guidelines exposed recommendation IIa and IIb for specific techniques, either, valve repair, valve replacement or percutaneous procedures. However, these details go beyond our scope, and we strongly recommend to review the ECS/EACTS guidelines for this information.

For patients with Mitral stenosis, the scenario is different because surgery has been displaced by Percutaneous mitral commissurotomy (PMC). Currently, only symptomatic patients who are not suitable for PMC or asymptomatic patients with unfavorable anatomic and clinical characteristics undergo surgery. For all the other patients with moderate or severe mitral stenosis requiring intervention, PMC is the mainstay[51,55].

Regarding, left-sided valves, the 2017 ES/EACTS guidelines recommend surgery for symptomatic patients with severe tricuspid stenosis and in patients with severe tricuspid stenosis who are undergoing left-sided surgery. In cases of tricuspid regurgitation (TR), indications of surgery have been established for symptomatic

patients but also should be considered in asymptomatic patients when progressive Right Ventricle (RV) dilatation or decline of RV function is observed as well as, patients with severe primary tricuspid regurgitation undergoing left-sided valve surgery. Mildly symptomatic (or asymptomatic) patients with progressive RV dysfunction and severe primary tricuspid regurgitation[51,55].

5.4 Grafts for valve replacement.

Once the indication of valve replacement has been established according to the guidelines or because individualization of the case lead to think this is the best option, surgeons will have a wide range of options to choose. However, this selection seems to be related to geographical areas, personal experiences, research conducted in specific locations, regional/national guidelines and/or an attempt to adjust the grafts to the patient.

Guidelines limit the choice between a mechanical and a biological valve and this selection is made on the basis on the risk of anticoagulation-related bleeding and thromboembolism with a mechanical valve versus the risk of structural valve deterioration with a bioprosthesis. Although the life expectancy and lifestyle of the patient are considered for this selection, the guideline does not go beyond to thought the emerging alternatives.

Allografts are one of these alternatives to grafts. At the moment, cryopreserved human heart valve allografts still represent almost perfect substitutes for heart valves. In fact, valve allografts have wide acceptance, and they have been in several indications. However, the most common procedure where the valve allografts are used is the Ross Procedure. This technique dates back to 1967 when Donald Ross transferred the patient's pulmonary valve into the aortic root. An allograft replaces the pulmonary

valve. Since the 60s, excellent long-term results and the possibility of combination with other technique have encouraged to continue this technique[56].

However, the Ross procedure is a complex operation; careful patient selection and experienced surgeons are mandatory requirements to achieve satisfactory results[56].

In Europe, several groups have worked with allografts, and new variants have been proposed with the aims of creating a graft with improved durability compared to routinely used valve substitutes. The most significant and more exciting proposal involving allografts and tissue-engineered aortic valve (TEV) came in 2013 from the Department of Cardiothoracic, Transplant and Vascular Surgery at the Hannover Medical School. This project has evolved and currently is called ARISE. Now, it is a European Commission funded project, led by the Hannover Medical School, nine hospitals, six tissue banks and an innovative biotechnology company providing the decellularization service came together for the world-wide first prospective study on cell-free allografts for aortic valve replacement[57].

After extensive preclinical work, and their first publication in 2013 performed in sheep, the group leader, Haverich et al. have used decellularized allogenic heart valve matrices for aortic valve replacements (AVR) based on compassionate use in 34 patients with tentative assessment showing favorable initial clinical results. However, transferring this regenerative approach to routine clinical application necessitates controlled prospective clinical trials which are lacking to date[57].

Simultaneously, Tissue-engineering Heart Valve (TEHV) has emerged as an interesting alternative to find a solution for the increasing demand of cardiac valves. TEHV have centered on its research lines in creating an ideal scaffold to be seeded by cells which are expected to proliferate to resemble a natural human heart valve.

Scaffolds materials can be classified into natural and synthetic. Natural scaffolds are decellularized xenografts purified from animal valves and the synthetics as they are called come mainly from polycarbonate urethane and polyether urethane. To avoid an immune reaction, cells to seed in the scaffold must come from autologous source[58].

Combination of the natural and synthetic scaffold has also been developed. Decellularized bovine pericardium extracellular matrix modified with synthetic polymers by coating the structure with a layer of Polycaprolactone-Chitosan (PCL-CH) nanofibers have been previously described as an attractive hybrid scaffold with superior mechanical properties and promising results[59].

Tissue-engineered heart valves is an increasing alternative with the hope of eventually to develop an ideal and clinically suitable cardiac valve replacement to cover the growing demand. Currently, TEHV is considered the only technology is working on the potential creation of tissues analogous to a native human heart valve, with longer sustainability and fewer side effects[58].

Other exciting alternatives to valve replacement are the xenografts. In this regard, an interesting valve highlight. The Melody transcatheter Valve (Medtronic, Minneapolis, MN, USA) is a heart valve from a cow's vein that has been attached to a wireframe which has demonstrated to be safe and effective in pediatric patients with excellent short- and mid-term follow-up hemodynamic results. However, their approved indications are limited to treat bioprosthetic valves dysfunction, mainly in pulmonary position[60].

The Melody transcatheter Valve (Medtronic, Minneapolis, MN) has emerged in the era of percutaneous cardiac valves. Other percutaneous alternative is Sapiens3 from

Edward Lifesciences (Edwards, Irvine, CA, USA). However, Transcatheter Valve replacement is not the scope of this review, and we will not go deep in this alternative.

Among the xenografts, also highlight The Edwards Inspiris Resilia® valve (Edwards Lifesciences, Irvine, CA, USA) which is bovine pericardial tissue transformed by a preservation technique which was primarily designed as the aortic valve, but because of availability of small size have been tested in children. Currently, The Edwards Inspiris Resilia® valve (Edwards Lifesciences, Irvine, CA, USA) have been accepted to be potentially used in mitral position in children but there are not robust clinical trials, and more studies are required[61].

Regarding synthetic valve replacement, the classical concept is that by implanting a mechanical valve the patient will require permanent anticoagulation. However, preclinical assessment of a trileaflet mechanical valve has been performed by Lapeyre et al. They have tried to develop a mechanical valve which will not require permanent anticoagulation[62,63].

Finally, it is essential to highlight that the decellularization technique and valves fully built with polyurethane are being developed. However, until having enough trials and studies, these options are also too young to extract conclusions.

6.- HYPOTHESIS.

Synthetic prostheses represent, for various reasons, the graft of first choice in orthotopic and extra-anatomical revascularization of patients without suitable autologous grafts. Our study raises the hypothesis that vascular and valvular allografts represent a useful and sometimes unique alternative for the treatment of cardiovascular pathologies. Allografts, as tissue of biological origin, have special and unbeatable properties in cases of infection, especially in case of prosthetic infection, as well as, congenital valvular and vascular anomalies in which the size and necessity of minimal adaptation to growth make them indispensable, in arterial leads where, due to their caliber and possibly their limited distal bed, synthetic grafts have been demonstrated to have an excessive rate of occlusion.

The careful monitoring of transplanted cardiovascular tissues is essential to know the evolution in the medium and long term of this therapy. Therefore, the Catalan Transplant Organization (OCATT) of the Autonomous Community of Catalonia, Spain, through the Vascular Transplant Commission, promoted the creation of an exhaustive Official Registry with data about autologous cardiovascular tissue transplants performed in that community and in this way, advance in the knowledge of the long-term evolution of these cardiovascular substitutes. The creation of this database, as well as, the collection and detailed analysis of the data obtained constitute the central core of the information required to carry out this doctoral thesis.

HIPÓTESIS.

Las prótesis sintéticas representan, por diversas razones, el injerto de primera elección en la revascularización ortotópica y extra anatómica en pacientes sin tejido autólogo disponible. Nuestro estudio plantea la hipótesis de que los aloinjertos vasculares y valvulares representan una alternativa útil y en ocasiones única para el tratamiento de patologías cardiovasculares seleccionadas al tratarse de tejido de origen biológico, lo que le confiere propiedades especiales e inmejorables en casos de infección, especialmente protésica, anomalías congénitas valvulares y vasculares en las que el tamaño y necesidad de adaptación mínima al crecimiento las hacen indispensables y en derivaciones arteriales donde, por su calibre y posiblemente su limitado lecho distal, injertos sintéticos se han demostrado con excesiva tasa de oclusión.

El seguimiento minucioso de los tejidos cardiovasculares trasplantados es esencial para conocer la evolución a medio y largo plazo de esta terapéutica. Por tanto, la Organización Catalana de Trasplantes (OCATT) de la comunidad autónoma de Cataluña, España, mediante la Comisión de Trasplante Vascular, impulsó la creación de un Registro Oficial exhaustivo de datos sobre los trasplantes de tejido cardiovascular autólogo realizados en dicha comunidad y de esta manera avanzar en el conocimiento de la evolución a largo plazo de estos sustitutos cardiovasculares. La creación de dicha base, así como la obtención y el análisis pormenorizado de los datos obtenidos constituyen el núcleo central de la información requerida para llevar adelante esta tesis doctoral.

7. OBJECTIVES

The objective of this research is: To know the long-term evolution of the series of patients transplanted with autologous vascular tissues carried out in the autonomous community of Catalonia, Spain. For this, it is necessary to design a regional registry to collect information on patients transplanted with autologous vascular tissue.

To describe the long-term evolution of patients transplanted with homologous vascular allografts.

To characterize the population transplanted with vascular allografts according to:

Indication for transplant.

Vascular segment used for transplantation.

To know the vascular complications associated with vascular allograft transplantation.

- Primary patency rate.
- Secondary patency rate.
- Graft occlusion rate.
- Graft re-infection rate.
- Graft rupture rate.
- To know outcomes of valvular allografts

- To know specific types of malfunctioning of valvular allografts.

To know the medical history in patients transplanted with vascular allografts.

- Associated comorbidity prior to transplantation.

- New pathologies specific related to the transplant.

To know the survival rate in patients transplanted with vascular allografts.

- Global survival rate

- Transplant-related survival rate.

To compare results with the known alternatives at the moment.

- To compare permeability rate with synthetic prostheses.

- To compare rate of reinfection with synthetic prostheses.

- To compare rate of thrombosis with synthetic prostheses

- To describe alternatives to synthetic prostheses and homologous grafts

Literature review of techniques of extraction, processing, storage and conservation of vascular tissues.

- To review of conventional preservation techniques

- To review the processing and storage of vascular tissue samples.

- To review of cryopreservation techniques

- Description of the cryopreservation technique used.

- To discriminate complications according to the time lapse of the cryopreserved tissue.

Design a regional network for collecting information about patients transplanted with autologous vascular tissue.

Design a regional network for registration and monitorization of vascular transplants.

- Creation of a database/registry.

- Start operating the *"Registry of vascular and valvular allografts transplant in the autonomous community of Catalonia, Spain (ReVAC)"*.

- Retrospective data collection of arterial allografts transplanted in the autonomous community.

- Design of a proposal for prospective monitoring of new implants of homologous vascular tissue in the autonomous community.

OBJETIVOS

El objetivo de la tesis es, en consecuencia, "conocer la evolución a largo plazo de los pacientes trasplantados con tejido cardiovascular autólogo en la Comunidad Autónoma de Cataluña mediante la creación de un registro retrospectivo de carácter obligatorio (e intencionadamente prospectivo cara al futuro)* y analizar los resultados con el fin de verificar la bondad de la hipótesis acerca de la utilidad de dicho tejido en situaciones especiales".

* No existe evidencia alguna de que exista en la actualidad ninguna base de datos ni registro similar que permita llevar a cabo un estudio como el propuesto.

Describir la evolución a largo plazo de los pacientes trasplantados con tejido cardiovascular homologo.

Caracterizar a la población de trasplantados con aloinjertos cardiovasculares vasculares según:

Indicación del trasplante

Segmento vascular o posición valvular trasplantados

Conocer las complicaciones cardiovasculares asociadas al trasplante de aloinjertos cardiovasculares

- Conocer la tasa de permeabilidad primaria.

- Conocer la tasa de permeabilidad secundaria.

- Conocer la tasa de trombosis

- Conocer la tasa de reinfección

- Conocer la tasa de ruptura

- Conocer la durabilidad de los injertos valvulares

- Definir las causas de disfunción de los mismos.

Conocer la morbilidad asociada y generada

- Comorbilidad asociada previa al trasplante.

- Nuevas patologías originadas después de trasplante

Conocer la Mortalidad asociada

- Tasa de mortalidad relacionada al trasplante

- Tasa de mortalidad no relacionada al trasplante.

Comparar resultados con los antecedentes conocidos al momento.

- Comparar tasa de permeabilidad con prótesis sintéticas.

- Comparar tasa de reinfección con prótesis sintéticas.

- Comparar tasa de trombosis con prótesis sintéticas

- Describir alternativas a las prótesis sintéticas e injertos homólogos

- Definir las eventuales ventajas de los aloinjertos valvulares frente a xenoinjertos o a las prótesis artificiales

Revisión Bibliográfica de técnicas de extracción, procesamiento, almacenamiento y conservación de tejidos vasculares.

- Revisión de las técnicas de preservación iniciales y las convencionales actuales

- Revisión del procesamiento y almacenamiento de muestras de tejido cardiovascular. Bancos de Tejidos en Cataluña y su referencia a otros en el España, en Europa o en los EEUU.

- Revisión sobre técnicas de Criopreservación modernas

- Descripción de la técnica de Criopreservación utilizada en los injertos trasplantados en nuestra Comunidad Autónoma.

- Discriminar complicaciones según lapso de tiempo del tejido criopreservado.

Diseño de una red de alcance medio para recogida de información de pacientes trasplantados con tejido cardiovascular autólogo en Cataluña.

- Estructurar la base de datos.

- Recogida de datos retrospectiva de los injertos valvulares y segmentos arteriales trasplantados en la Comunidad Autónoma.

- Activar un nuevo Registro de Trasplante de Tejido Cardiovascular en Cataluña

- Diseño de Protocolo para seguimiento prospectivo de nuevos implantes de tejido cardiovascular homólogo en la comunidad autónoma.

8. METHODOLOGY

8.1 Creation of "Registry of vascular and valvular allografts transplant in the autonomous community of Catalonia, Spain (ReVAC)".

In June 2013, the Catalan organization of transplant supported the creation and design of the Registry of vascular and valvular allografts transplant in the autonomous community of Catalonia, Spain (ReVAC). Administrative and legal authorization were obtained during the first trimester of 2014.

A total of 10 medical centers were involved in the study: Centro Médico Teknon, Hospital Universitario Vall d´Hebron, Hospital Universitario Germans Trias I Pujol, Fundación Puigvert, Hospital de la Santa Creu i Sant Pau, Hospital Universitario de Bellvitge, Hospital Universitario Sant Joan de Deu, Hospital materno-infantil Vall d´Hebron, Hospital Clínic i Provincial de Barcelona, Hospital Juan XXIII Tarragona.

The phase 1 corresponds to the design of the Registry of vascular and valvular allografts transplant in the autonomous community of Catalonia, Spain (ReVAC)". A second phase for analysis of the collected data was performed.

Phase 1: Registry design. For its conception, the "Work focal groups technique" was performed. 3 sub-groups were organized according to clinical, administrative and technical requirements. In this phase, all of the different hospitals were involved.

Focal group 1: Relevant variables

Performed under the direction of the Catalan Transplant Organization (OCATT) and integrated by 14 persons involving faculty doctors and health system directors. The members were; Director of the OCATT, coordinator of tissue and cells donation in the OCATT, medical director of bank tissue in the Catalan community, administrative

coordinator in the OCATT, the president of the advisory committee for vascular tissue procurement and transplant, 2 vascular surgeons, 2 cardiac surgeons from Catalan hospitals and 1 additional cardiovascular surgeon from the national tissue transplant coordination, an informatic engineer, the director of an allograft tissue bank, and the 2 persons directly charged of the ReVAC creation(JLP: Deputy director of Cardiovascular surgery Department and KAGN: Vascular Surgeon, PhD candidate, MSc in Donation and transplantation of organs, tissues and cells, and specialized in translational research).

The focus and scope of this focal group were:

1. Presentation of each member in the research team
2. Demonstration of the project to be developed by the research team
3. Open forum for ideas and criteria for variable selection
4. Registry scope and delimitation.
5. Voting and establishing of the registry characteristics and its variables

Focal group 2: Informatics design of the registry

Once the variables were established, the second focal group was created with the intention of deciding the technical aspects and data resources of the registry. Guided by an informatics engineer from the OCATT who was a participant in the focal group 1. The other members of the group were the 2 persons directly charged of the ReVAC creation (JLP and KAGN). 2 additional members were selected from the Focal Group 1

The focus and scope of this focal group were:

1. Technical feasibility of the ReVAC creation and selection of informatic characteristics.

2. Technical aspects and resources required for its operation.

Focal group 3: Data collection plan and design

The focus and scope of this focal group were:

1. To design the data gathering strategy and the format for preliminary results exposition.
2. To create a strategy for contacting patients to reduce data/information lost.

The third focal group involved the same persons of the second one. This focal group emphasized in the planning for obtaining the information from medical histories/reports. Patient history data privacy was assessed and ethical statements were reviewed.

8.2 Data collection and introduction to the "Registry of vascular and valvular allografts transplant in the autonomous community of Catalonia, Spain (ReVAC)".

The entire data collection was supported by The Catalan Organization of Transplants (OCAT: Organización Catalana de Trasplantes). Before visiting each hospital, the authorities of the Tissue Transplant Department contacted to the Hospitals and Chief of departments involved. An in-hand copy of this communication was kept for avoiding delays.

For data collection, The Catalan Organization of Transplants (OCAT: Organización Catalana de Trasplantes) gave fully support and two member of the tissue transplant department work together the 2 persons directly charged of the ReVAC creation (JLP and KAGN) for visiting each hospital. KAGN coordinated the whole data collection.

There was a standardized form for data collection. Only a test with in-hand or digital report were considered as valid. KAGN and any of the other member of data collection team gathered information from the medical reports. After that, the information was compared. In case of any doubt, the medical report was assessed for a third person for solving the doubts. If the doubt persisted, an interview was appointed for the patient and the information was asked the patient in the interview.

Due to the retrospective nature of this study, the follow-up was based on the examinations and visits recorded to the patient in his reference center. It was considered valid to any visit, ultrasound test or hospital admission. The follow-up was considered as ended at the date of the last visit to the vascular surgery department in the reference center, except for survival analysis that was used the last visit registered in any department or hospital in the autonomous community of Catalonia. If in any case the last contact of the patient was beyond to 1 month, the patient was contacted by phone or an appointment was scheduled for the patient. After 1 month without information, the patient was considered as lost.

An additional review of the processing and cryopreservation technique was performed to know the specific protocol related to the analyzed allografts. Also, a systematic review of Ziegler et al. was used for comparison with other grafts.

8.3 Data gathering from the "Registry of vascular and valvular allografts transplant in the autonomous community of Catalonia, Spain (ReVAC)".

After the ReVAC creation, all the available data about vascular and valvular transplant of the Catalan community was available for analysis. ReVAC allowed knowing the arterial segments and cardiac valves transplanted in Catalonia and patient follow-ups.

An initial agreement let to analyze the information related to this doctoral dissertation. Because the registry also allows scientific productions through data gathering, the Catalan Organization of Transplants have the option of asking for permission to analyze additional information. Data of patients with a transplanted cryopreserved vascular segment or cardiac valves that were distributed by Catalan tissue banks were analyzed between January 1995 and December 2015. Data was collected between January and December 2015. The last record was introduced in December 2015. Later, due to administrative and budget reasons, no new cases have been introduced to the ReVAC.

8.4 Definitions

For arterial allografts and lower limb revascularization, infra-inguinal was considered to any bypass with proximal anastomosis originating below the inguinal ligament. Therefore, a supra-inguinal bypass was considered to any bypass with proximal anastomosis originating above the inguinal ligament.

Chronic Limb-threating Ischemia (CLI) was defined as ischemic rest pain, nonhealing ulceration, gangrene or necrosis requiring an imminent major amputation

Primary patency was defined as the interval from the time of surgery (bypass placement) to any intervention designed to maintain or re-establish patency or to the thrombosis of the bypass.

Limb salvage rate was defined as the interval from the time of surgery to any ipsilateral major amputation.

According with the objective performance goals for lower extremity revascularization of the Society for Vascular surgery, we defined the major adverse limb event (MALE) as any major amputation, major reintervention, including thrombectomy or bypass revision. Major adverse event related to the patient was considered any major ipsilateral amputation, ipsilateral limb reoperation o revascularization, cardiovascular event requiring hospitalization, or death.

For infection, only was cases with a positive culture of the graft available in the medical reports were considered as valid. Any other suspected case without confirmation was not considered valid.

8.5 Ethical Statement.

Authors state that no human or animal experiments have been performed for this research, no personal patient data is exposed, and there is no conflict of interests.

This project has been submitted to ethical evaluation which was carried out by the ethics committee of the "Hospital Clinic i Provincial de Barcelona". No funding sources.

Data introduced in the ReVAC is confidential. The registry is available through the website of the Catalan Government (*Generalitat de Catalunya)*. A specific authorization from the health department of *"Generalitat de Catalunya"* is required for gathering information from the registry. Personal and digital data in Spain is protected according to the Organic Law for protection of personal data and guarantee of digital rights (BOE-A-2018-16673). The review was conducted according to in-effect regulations and being respectful of the confidentiality of the patient information.

8.6 Statistical Analysis.

Statistical analysis was performed using SPSS Ver. 20 for Mac (Chicago USA). The descriptive data is presented in relative and/or absolute frequencies.

According the objectives, the whole cohort of patients transplanted with vascular allografts was analyzed. Information about indication and vascular segment transplanted are showed in absolute and relative frequencies.

Demographical and clinical variables such age, sex and comorbidity (arterial hypertension, diabetes mellitus and dyslipidemia) were recorded.

For vascular allografts, cumulative patient survival, limb salvage rate, and graft patency rates were assessed by the Kaplan-Meier method at 30 days, and annually until 5 years. Additional analysis of overall occlusion and infection rate was performed.

Considering the clinical relevance, a specific analysis for infra-inguinal revascularization was included and patency rate, limb salvage rate and Major Adverse Limb Event-free (MALE-free) rate related to the patient was also assessed. A second analysis was performed in this group, which included a Cox regression model to determine risk factors for survival, primary patency rate, limb salvage rate and MALE-free rate at 5 years.

The $P<0.05$ level was used to determine statistical significance. 95% confidence intervals were selected for hazard ratios.

8.7 Literature review about tissue cryopreservation and conservation.

A PubMed Medline search was conducted through December 2015 to identify all reviews, guidelines, trials and meta-analysis related to tissue cryopreservation and conservation techniques. For inclusion, the publication had to be written in English, or Spanish. The MeSH term “blood vessels” and “cryopreservation” (and various synonyms) were used. Reference lists of the papers initially detected were manually searched to identify additional relevant reports.

For analysis, studies had to contain sufficient and clear information about the process of conservation and cryopreservation. One investigator (KAGN) selected the articles and analyze them. Methodological quality of the selected papers was assessed. Finally, the most common ideas about cryopreservation and conservation were extracted and exposed in the results. Processing and cryopreservation techniques used for Catalan tissue banks were also reviewed and exposed in the results.

9. RESULTS

Focal group 1 results: Design and basic aspects of the "Registry of Vascular and valvular allografts in an autonomous community of Spain, Catalonia (ReVAC)".

The overall product was the conception of an online access platform for related vascular segments and cardiac valves transplant data in the "Autonomous Community of Catalonia" divided into 3 levels (figure 1).

1. Patient data: Invariable in-time patient data and follow up status:
2. Surgery data per se: Information about the transplant surgery: In case of reoperation, every surgery was recorded as a new episode.
3. Transplanted tissue data per se: Information regarding tissue characteristics and evolution/outcomes

9.1 Registry of Vascular and Valvular Transplant in Catalonia.

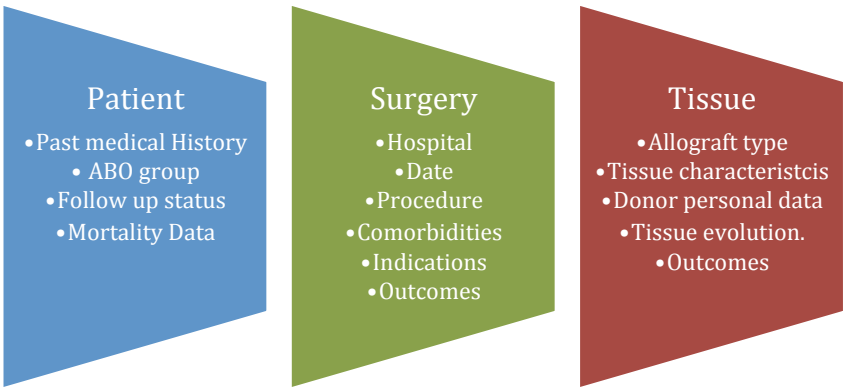

Figure 1. Structural design of the ReVAC

Focal group 2 results: Online-access platform for the registration of data about arterial segments and cardiac valves transplantation in the "Comunitat Autonoma de Catalunya". ReVAC Platform was divided into 3 levels: patient, surgery, and tissue. (figure 1). The username and password access were assigned and controlled by the

department of health of the “Generalitat de Catalunya”. Personal and medical history was protected according to local and national law, as well as, according to the ethical standards of the “Organización Catalana de Trasplantes” (OCATT). The access to the platform is available from any computer with access to the Internet through salut.gencat.cat.

Focal group 3 results: A schedule for collecting data was designed. Data collection started in January 2015 and finished in December of 2015. After this, the ReVAC online platform started working and the preliminary data was processed. Only information about adults was analyzed.

Finally, a regional network for registration and monitorization of vascular allografts transplants was created. The final product is the ReVAC, a database acting as autonomic registry for Catalonia. The ReVaC started working in December 2015 and currently is available for data gathering. However, due to administrative reasons, no new cases have been introduced after December 2015. All the data related to vascular allografts and cardiac valves distributed by Catalan tissues banks before 2015 is available for analysis in the ReVAC.

After the preliminary assessment of results, a follow-up protocol was designed:

A.- Pre-Transplant tests: Must be added blood group, Rh Test, Reactive Protein C (RPC) and Globular Sedimentation Rate (GSR).

B.- Doppler Ultrasound: 1month, 6 months, 12 months and then annually.

C.- Clinical Assessment: Pre-discharge, 1 month, 3months, 6 months, 12 month and then annually.

D.- AngioTC for failing graft: Suspicion from doppler ultrasound.

E.- Post-Transplan Test: Reactive Protein C and Globular Sedimentation Rate daily until day 5 and then weekly until week 5.

9.2 Allografts Transplantation.

A total of 1084 records are available in the ReVAC. However, only 397 registries have valid information for analysis.

From the 397 patients were transplanted: 229 (57,7%) with arterial segments and 168 (42.3%) cardiac valves. From the 229 arterial segment patients, 82.1% (188) were adults and 17.9% (41) were pediatric patients. The 47.62% (80) of cardiac valve patients were adults and 52.38% (88) were pediatric patients (Table 1). Distribution according to gender is presented in table 2 (Table 2)

Type of transplant		**Patients**	**%**
Vascular (arterial segments)	Adults	171	82.10
	Pediatrics	41	17.90
Valvular (cardiac valves)	Adults	80	47.62
	Pediatrics	85	52.38

Table 1. Type of transplant and age distribution.

	Pediatric Valves	**Pediatric Segments**	**Adult Valves**	**Adult Valves**
Male.	45 (52.9%)	23 (43.9%)	55 (68.8%)	136 (72.3%)
Female.	40 (47.1%)	18 (56.1%)	45 (31.2%)	52 (27.7%)
Total	85	41	80	188

Table 2. Gender distribution of patients transplanted with allografts.

9.2.1 Arterial segments

171 adults were transplanted with a mean age of 69.1 +/- 11.08 years whose 72.3% (125) were males. The surgery indication was chronic ischemia grade III and IV of inferior extremities without autologous great saphenous veins available as conduit for the surgery in 79.2% (149) of the cases, infected prosthesis replacement or infected revascularization was the indication in 9.9% (17), previous allograft replacement represented 5.3% (9) and complex vascular lesion correction 3.5% (6). Other indications under surgeon criteria were performed in 4.1% of the patients.

Indication	Absolute Frequency	Relative Frequency (%)
Chronic Limb-threating Ischemia	149	79.2
Replacement Infected Grafts	17	9.0
Vascular Allograft Replacement	9	4.8
Complex Vascular Lesion	6	3.2
Vascular Access for Hemodialysis	3	1.6
Other Indications.	4	2.2

Table 3. Indication of transplantation of vascular allografts.

The arterial segment transplanted was aorto-iliac in 15 cases (8.8%), long segment involving femoral and popliteal artery without distal vessels in 131 cases (76.6%), 17 (9.9%) additional cases involved femoral and popliteal artery with distal vessels associated. In the other cases, the specific segment used for transplantation was not identified. In the pediatric cohort, femoral artery represents 28 cases (68.3%). 13 cases (31.7%) have not information about the segment transplanted.

9.2.2 Cardiac Valves

80 adult patients were transplanted with a mean age of 46,9 +/- 11,51 years, 68,8% (55) men. Surgical indication was aortic stenosis 36,3% (29), aortic insufficiency 22,5% (18), double aortic lesion 12,5% (10), endocarditis 10% (8), aortic valve aneurysm dilation 3,8% (3), Fallot tetralogy reintervention 3,8% (3) and prosthetic infections 2,5% (2). Other specific indications were performed in 6 patients

9.3 Outcomes and Complications associated with allografts transplantation.

9.3.1 Vascular allografts.

Outcomes and complications of vascular allografts are exposed by locations. Infra-inguinal revascularization was performed in 149 (87.13%) patients and supra-inguinal in 15 (8.77%). Other cases (7:4.1%) correspond to surgeries not involving the lower extremities.

From those with infra-inguinal revascularization, 108 (72.5%) had a distal vessel anastomosis. Distal target vessel was the anterior tibial artery in 42 (38.8%), peroneal artery 34 (31,5%), posterior tibial artery 23 (21,3%), tibial-peroneal trunk 6 (5,6%), and dorsalis pedis artery 3 (2.8%) cases. A femoro-popliteal bypass was performed in the remaining 41 (27.5%) cases of infra-inguinal revascularization.

From those with supra-inguinal revascularization, in 9 (60%) cases an aorto-femoral revascularization was performed. 2 (13.3%) iliac territory, 1 (6,7%) aortoplasty

9.3.1.1 Results for supra-inguinal revascularization

The 1-year and 5-year MALE-Free rate rate for patients transplanted with arterial allografts in the supra-inguinal territory was 40% and 13.3% respectively. Primary patency rates of the supra-inguinal allograft were 86.7% at 12 months and 72.2% at 60 months. Secondary patency rates were 79.2% at 12 months and 67.1% at 60 months. Limb salvage rate was 93.3% and 62.2% at 12 and 60 months. (Table 4)

Supra-inguinal	**12 months**	**24 months**	**36 months**	**48 months**	**60 months**
Survival	60%	60%	52.5%	45%	37.5%
Primary Patency	86.7%	72.2%	72.2%	72.2%	72.2%
Limb Salvage	93.3%	93.3%	93.3%	93.3%	62.2%
MALE-Free	40%	33.3%	26.7%	20%	13.3%

Table 4. Results of supra-inguinal revascularization with arterial allografts.

9.3.1.2 Results for infra-inguinal revascularization

After 36-month follow-up, more than 20% of patients transplanted with arterial allografts for femoropopliteal revascularization got lost. Only 1-year and 3-year rates are exposed. The 1-year and 3-year MALE-Free rate for patients transplanted with arterial allografts in femoropopliteal territory was 60.4% and 50.1% respectively. Primary patency rates of the femoropopliteal revascularization were 78.8% at 12 months and 64.8% at 36 months. Limb salvage rate was 83.4% at 12 and 36 months.

Femoropopliteal	12 months	24 months	36 months
Survival	87.5%	78.8%	75.1%
Primary Patency	78.8%	64.8%	64.8%
Limb Salvage	83.4%	83.4%	83.4%
MALE-Free	60.4%	54.2%	50.1%

Table 5. Results of femoropopliteal revascularization with arterial allografts

When a distal target vessel revascularization was performed, 1-year and 5-year MALE-Free rate was 52.5% and 18.2% respectively. Primary patency rates for distal target vessel revascularization with arterial allografts was 53.5% at 12 months and 34.1% at 60 months. Limb salvage rate was 59.5% and 43.4% at 12 and 60 months.

Distal Target V.	12 months	24 months	36 months	48 months	60 months
Survival	92.4%	85.1%	71.9%	66.1%	55.6%
Primary Patency	53.5%	42.5%	39.2%	36.9%	34.1%
Limb Salvage	59.5%	49.3%	46.1%	46.1%	43.4%
MALE-Free	52.5%	39%	28.5%	25%	18.2%

Table 6. Results of distal vessel target revascularization with arterial allografts.

9.4 Infrainguinal revascularization in patients with chronic limb-threating ischemia.

A total of 149 patients with Chronic Limb-threatening Ischemia (CLI) who underwent infra-inguinal surgical reconstruction with cryopreserved arterial allografts. The population was composed of 108 men (72.5%), with a mean age of 70.1 +/- 10.7 years old at the time of the surgery. Clinical characteristics of this group are exposed in

Table 7. One hundred two patients (68,5%) had a grade IV ischemia according to the Fontaine classification (ulcer, necrosis or tissue loss), 1 vascular segment was used in 83 patients (55.7%), 2 segments in 54 patients (36.2%) and only 12 patients (8.1%) required 3 or more segments.

	Patients	%
Age >65 years old	108	72.5
Age >75 years old	43	28.9
Diabetes Mellitus	72	48.3
Arterial Hypertension	100	67.1
Hyperlipidemia	62	41.6
Smokers	95	63.8
Renal Failure	13	8.7

Table 7. Clinical characteristics of patients with CLI and transplanted with vascular allografts.

There was not a specific protocol about antiplatelet or anticoagulation medication and it was determined by surgeon discretion and/or according to the comorbidities of the patient.

In the overall follow-up, 37 patients (24.8%) required a reoperation, 78 patients (52.3%) present an occlusion of at least 1 arterial segment, 9 patients (6%) had a graft infection and 8 patients (5.4%) had a graft dilation, and only 1 spontaneous rupture was observed.

9.4.1 30-days Results.

Overall 30-days mortality was 0.7%. Primary patency rate, limb salvage rate and MALE-free rate were 89.3%, 89.9%, and 87.9% respectively. A total of 4 patients (2.7%) required a reoperation in the ipsilateral extremity. Two patients (1.34%) had a coronary event, 9 patients (6.04%) had a non-treated occlusion of at least one transplanted arterial segment and in 1 (0.7%) case a spontaneous rupture of the graft occurred.

9.4.2 5-year results.

The cumulative 1-year, 3-year and 5 year survival rates were 91%, 72.3% and 54.2% respectively. The primary patency had a decline from 54% at 1 year to 46.1% at 3 years. 5-year primary patency rate was 38.6%. High percentage of lost patients were observed at 5 years for this end-point (21.48%) (Figure 2, Figure 3, Table 8).

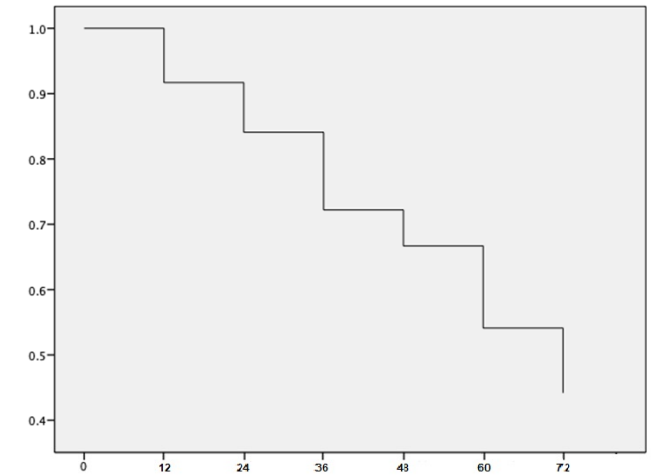

Figure 2. Survival Rate in patients with CLI revascularized with arterial allografts.

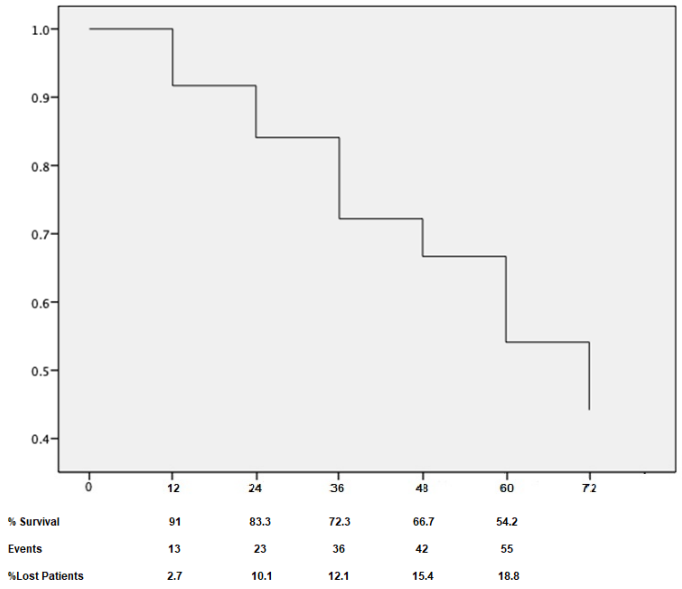

Figure 3. Primary patency rate in patients with CLI revascularized with arterial allografts.

	PP	LSR	SR	MALE-free
30 days	89.3	89.9	99.3	87.9
1-year	60.4	65.7	91	54.7
2-year	48.8	57.6	83.3	43
3-year	46.1	55	72.3	33.6
4-year	43.8	55	66.7	27.7
5-year	38.6**	50.2**	54.2	21.5

Table 8. Outcomes of patients with CLI and LEB with Cryopreserved Allografts.

*PP: Primary Patency rate, LSR: Limb Salvage Rate. SV: Survival Rate, MALE: Major adverse limb event rate. **High percentage of lost patients.

Regard the limb salvage rate, evolved from a 65.7% at 1 year to a 3-year limb salvage rate of 55%. 4-year limb salvage rate was 55%. 5-year limb salvage rate was 50.2%, however, 20.13% of patients were lost of follow-up for this end-point at 5 years (Figure 4, Table 8).

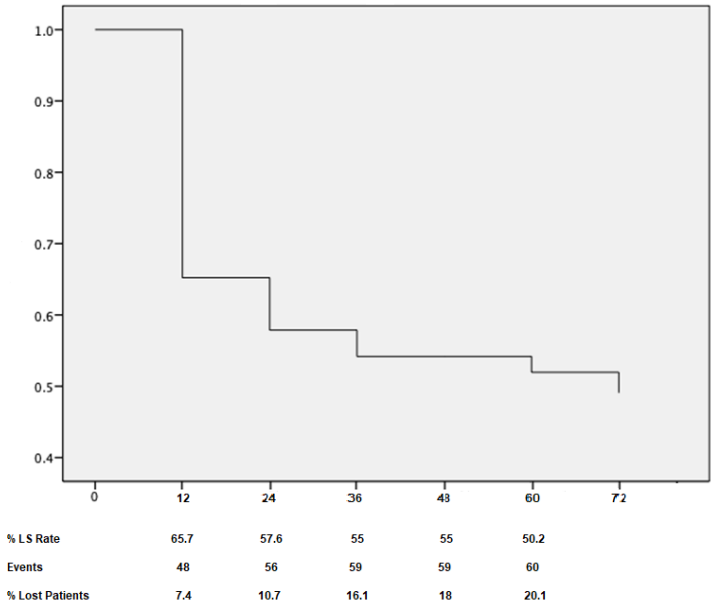

Figure 4. Limb Salvage Rate in patients with CLI revascularized with arterial allografts.

MALE-free rate declined from 54.7% at 1 year to 33.6% at 3 years. 5-year MALE free rate was 21.5% (Figure 5, Table 8).

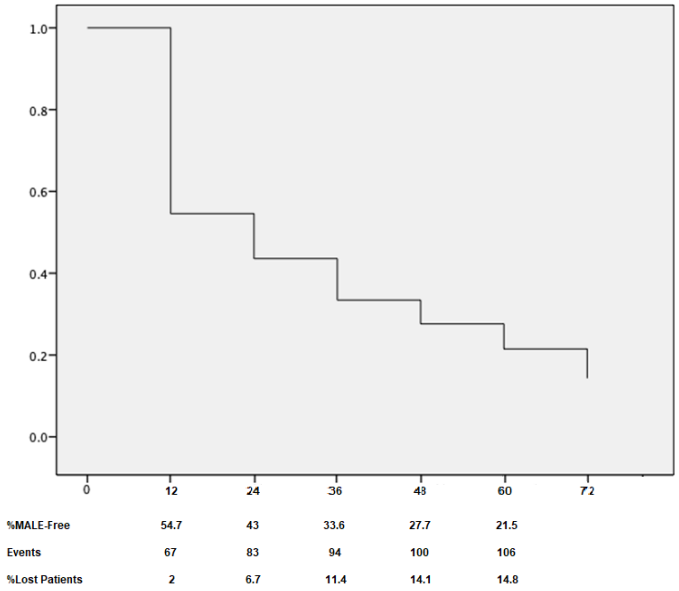

Figure 5. Major Adverse Limb Events-Free Rate in patients with CLI revascularized with arterial allografts.

At 3-year cut-point, two additional aneurysmal dilations and five grafts infection occurred in this period. Also, 2 additional stenoses in at least 1 transplanted arterial segment were demonstrated.

After multivariable Cox proportional hazards, regression analysis including all the clinical and demographic characteristics, the revascularization with a distal target vessel was an independent positive predictive risk factor for a lower limb salvage rate (HR: 3.241, 95% Confidence Interval (CI): 1.677-7.115, p=0.003) and lower primary patency rate (HR: 2.242, 95% CI: 1.206 – 4.169, p=0.011). Dyslipemia was an independent positive predictive risk factor involved in major adverse limb events (HR: 1.480, 95% CI: 1.014 – 2.160, p=0.042) and for a lower limb salvage rate (HR: 1.968, 95% CI: 1.141 – 3.396, p=0.015).

Arterial hypertension was a protective factor for MALE (HR: 0.608, 95% CI: 0.415 - 0.885, p=0.008) and involved in a better primary patency rate (HR: 0.498, 95% CI: 0.312 - 0.794, p=0.004)

Smoking habit (HR: 1.661, 95% CI: 1.278 - 2.558, p=0.021) and the age of the patient at time of the surgery (HR: 1.302, 95% CI: 1.090 - 1.570, p=0.007) were associated as risk factor for mortality.

9.5 Medical history of patients treated with allografts

9.5.1 Vascular Allografts

Table 9 shows a summary of main comorbidities in patients transplanted of vascular allografts

	Patients	**%**
Hypertension	114	66.7
Diabetes Mellitus	78	45.6
Dyslipidemia	71	41.5
Tobacco use	112	65.5
Renal Disease	17	9.9

Table 9. Medical history of patients transplanted of Vascular Allografts

9.5.2 Cardiac Valves:

Table 10 shows a summary of main comorbidities in patients transplanted of valvular allografts

	Patients	%
Hypertension	31	38.8
Diabetes Mellitus	10	12.5
Dyslipidemia	23	28.8
Smoking	31	38.8

Table 10. Medical history of patients transplanted of Vascular Allografts

Owing to the retrospective nature of this study, it was impossible to stablish causality factors to assure that vascular allografts transplantation is related to new pathologies except for hemodynamic changes of transplanted valves. Hemodynamic changes in transplanted allograft valves are showed in table 11

Cardiac Valves.	12 months	24 months	36 months	48 months	60 months
Survival	98.6%	98.6%	98.6%	98.6%	97.1%
Stenosis-Free	98.6%	98.6%	95.5%	94%	84%
Insufficiency-Free	98.6%	98.6%	97.1%	94%	81.1%

Table 11. Results of survival and hemodynamic changes in patients transplanted with valvular allografts.

9.6 Survival rate in patients transplanted with allografts.

9.6.1 Vascular Allografts

The survival rate for patients transplanted with arterial allografts in the supra-inguinal territory was 60% at 12 months and 37.5% at 60 months. The survival rate for

patients transplanted with arterial allografts in femoropopliteal territory was 87.5%% at 12 months and 75.1% at 36 months. Survival rates for distal target revascularization was 92.4% at 12 months and 55.6% at 60 months. Only 3 death can be directly related to the transplant. (Table 4, Table 5, Table 6 and Table 8)

9.6.2 Cardiac Valves

Survival rate of patients transplanted with valvular allograft was 98.6% and 97.1% at 12 and 60 months respectively (Table 11). Two intraoperative death and 2 additional cases could be attributed to the transplant.

9.7 Cryopreservation technique – Literature review.

Figure 6 shows the process of selection of articles for this systematic review. The search strategy yielded 216 before applying filters. Abstracts of these 216 articles were reviewed looking for relevant publications. From these, 15 articles were included for analysis. After filters application a total of 3 additional articles were found.

No randomized controlled trial has been performed; therefore, comparison between cryopreservation techniques are not available in the literature. No meta-analysis of cryopreservation techniques of vascular allografts has been reported. No guidelines about protocols of cryopreservation of vascular allografts are available in the literature.

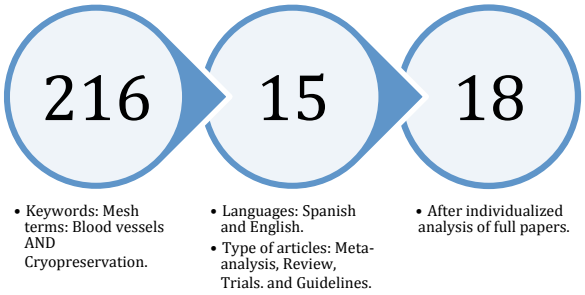

Figure 6. Literature review about cryopreservation

9.7.1 Conservation and Cryopreservation technique of Vascular Allografts

Three vascular allograft preservation methods were used in the selected studies: cryopreservation, cold storage, and glutaraldehyde preservation.

Regards Cryopreservation, initial technique was described by Müller-Schweinitzer E, in 1988: The best recovery after thawing of frozen stored canine veins was obtained on tissues which had been frozen slowly to -70 degrees C and stored in liquid nitrogen while being immersed in Fetal Calf Serum (FCS) containing 1.8 mol/l dimethyl sulfoxide (DMSO). The same method of cryopreservation was applied to store samples of human veins. Comparison of the pharmacological tests yielded an excellent correlation between the parameters determined on frozen/thawed and unfrozen human veins. Müller-Schweinitzer concluded that freezing isolated blood vessels may be considered an effective means of preserving and storing vascular tissues for pharmacological investigations.

In 1997, Mesa et al. described a technique including the initial processing in which the graft was taken from the femoral artery and washed in physiologic solution at

4°C. The vessel was placed in a 10 percent solution v/v of fetal calf serum (FCS) in RPMI 1640 at a final concentration of 10 percent v/v of DMSO in 1.5 ml tubes at 4°C.

In contrast to Müller-Schweinitzer E., The graft was placed in a freezer at -20°C for 2 hours, then transferred to a freezer at -80°C. At the moment of implantation, the graft was then placed in a solution based in RPMI 1640, FCS at a concentration of 10 percent v/v and a temperature of 4°C for slow warming.

In 2001, At the Italian Homograft Bank at *Centro Cardiologico*, an additional variation of the technique was described. They ratified the technique for initial manipulation by keeping the vascular homograft at +4 degrees C for 96 hours on average with antibiotics. However, after this phase the tissue is frozen according to a homogeneous and controlled thermic decrease and stored at -150 degrees C/-180 degrees C in fumes of liquid nitrogen till the moment of their employment allowing a long-term conservation.

The newest cryopreservation technique has been described from Cryolife (Cryolife, Inc Kennesaw, Ga), a commercial brand. The main difference is about the temperature. Cryopreservation is performed in a dimethyl sulfoxide– containing solution by using a programmable control-rate freezer. Storage temperatures ranged from -120°C to -196°C.

In 2000, the concept of vitreous cryopreservation was introduced. After in-vivo studies of vitrified vessel segments in an autologous transplant model, this technique showed no adverse effects of vitreous cryopreservation compared with fresh tissue grafts. However, this technique has not been total acceptance because in 2006 new results suggest that vitrification maintains both elastic and viscous components of the mechanical properties of vascular grafts, which is positively correlated with their

functional patency. In contrast, damage caused during cryopreservation significantly affects the overall tensile strength and elasticity of the vessel, the dynamic properties, and appears to significantly affect the viscous component of the vessel wall, which is likely reduce the patency of the graft for transplantation purposes.

More recently, the introduction of cryoprotectants and scalable nanowarming technology for 1- to 80-ml samples using radiofrequency-excited mesoporous silica-coated iron oxide nanoparticles have been introduced. Only experimental studies are available and there is not evidence of clinical application until yet.

Cold preservation has been dismissed after introduction of cryopreservation techniques. Glutaraldehyde preservation has been used mainly for venous allografts and Human Umbilical Veins (HUV).

About the consequences of cryopreservation to the vascular tissues, it is important to highlight that freezing and thawing of blood vessels can cause substantial injury to cells and tissues. Cryopreservation of vascular tissues leads to certain loss of both smooth muscle contractility and endothelial function which is a key regulator of vascular homeostasis. Endothelial cells interact continuously with blood components and with the structure of the vessel itself, maintaining both vascular tone and antithrombotic properties. The rates of cooling to and rewarming from the storage temperature, the selection of the vehicle solution and cryoprotectant agents, the manner of cryoprotectants addition to and removal from the tissue, all these steps may influence the degree of injury sustained by the tissue during cryopreservation.

Freezing tissue without cryoprotective additives generally induces severe cell damage and only few if any cells survive. Additionally, during cooling to subzero temperatures water tends to flow out of the cell to freeze externally and the cell shrinks

during this process. If cooled too fast, cells are injured by the formation of intracellular ice crystals; if cooled too slowly water will freeze externally, and the cells will be injured by the "solution-effect,". As a consequence of changes in both temperature and concentration of extracellular salts, cell membranes will be injured.

Consequences to the vascular tissue are not directly related to the time of cryopreservation but to the application of a correct technique for cryopreservation.

9.7.2 Processing, Conservation and Cryopreservation technique of Vascular Allografts used by Catalan Tissue Banks.

The vascular allografts distributed by the tissue banks of Catalonia (Spain) are extracted in cases of multi-organic donation by centers authorized by the Catalan Organization of Transplantation, Spain (in Catalan: Organització Catalana de Transplantaments: OCATT). Only arterial allografts were included for analysis.

After their collection, allografts were transferred to the tissue bank under sterile conditions and immersed in a preservation solution.

Preservation Solution: Allografts must be totally submerged in the preservation solution: Saline 0.9%, Wisconsin and nutrient solution RPMI 1640, TC199. Collins solution or ringer lactate alone must be avoided.

In no case, the ischemia time was greater than 24 hours (if the body has been refrigerated within the first 6 hours since asystole) or greater than 12 hours if the body has not been refrigerated.

Dissection and initial tissue evaluation were performed in laminar flow chambers under sterile techniques. In all the cases, a thorough inspection of the anatomical characteristics is performed and all the grafts were immersed in a solution of cellular nutrient medium with antibiotics at the lowest effective concentration. They were kept under refrigeration for 12 to 30 hours. A sample of vascular tissue is taken for pre-antibiotic microbiological control.

For cryopreservation, 10 ml of DMSO and 10% human albumin solution were used. The cryopreservation program used reduces the temperature of the tissues from 1ºC/min to a temperature of -60ºC and then from 3-4ºC/min to -100ºC. The maximum programmed temperature deviations accepted was 10ºC.

After reaching this temperature, the graft was extracted from the chamber and introduced into the vats of liquid nitrogen. Tissues not used for clinical applications were used as control for mechanisms that verify that the established parameters have been properly followed. Storage is carried out in large capacity liquid nitrogen tanks monitored to maintain the proper level of liquid nitrogen. All tissues are validated 1 month after storage to be included in the bank's availability lists and distributed to hospitals upon request.

When an implantation center needs an allograft, the tissue or tissues that best fit the request received are selected and the information is prepared so that the implantation center can decide its acceptance. If the graft is accepted, the delivery is organized so that the tissue is in the implantation center on the date planned for the intervention. The tissue is transferred in boxes of porexpan of 30x30 cm, which is filled with half of carbonic snow and placed, on the cryopreserved graft, followed by an additional layer of dry snow.

9.7.3 Considerations for implantation

Prior to use, the grafts were thawed slowly in a sterile solution at 20 ° C and microbiological samples are taken prior to implantation.

Senior vascular surgeons performed the surgery. In no case was immunosuppression or anticoagulation intentionally used after implantation. No distal vein patches were used in any case.

9.8 Vascular Allografts versus other grafts.

A direct comparison between grafts is considered incorrect because of several factors affecting the outcomes. Anticoagulation, antiaggregating therapy, medical history of patients, groups selected for analysis, exclusion de specific group of patients and etcetera could affect the final outcomes of any cohort. However, a simple comparison of ratios could be exposed but for a deeper analysis, each publication must be reviewed.

In 2011, Ziegler et al. published a compilation of reported data about primary outcomes of several types of grafts. In this review were excluded data focused on re-operative interventions. From these data, and the publications analyzed during the writing of this dissertation, we have built a table to compare allografts versus other vascular grafts.

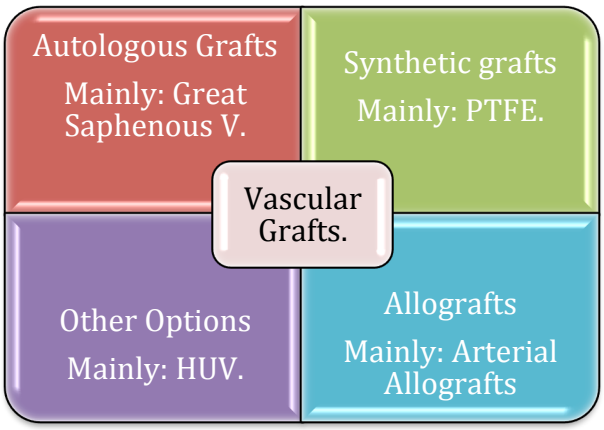

Figure 7. Vascular allografts versus other grafts.

Primary Patency								
Author	**Year**	**Graft**	**Territory**	**1y**	**2y**	**3y**	**4y**	**5y**
Guevara-Noriega	***2019***	***Allografts***	***Suprainguinal***	***87***	***72***	***72***	***72***	***72***
Jackson	2000	Autologous Vein	Femoro-Popliteal	78	71	58	58	-
Gwan-Chul	2012	Dacron	Suprainguinal	-	-	-	-	86
Prager	2003	Dacron	Suprainguinal	-	-	-	-	89
Jonhson	1999	Dacron	Extra-Anatomical	79	-	63	-	50
Foster	1986	Dacron	Aorto-femoral	-	-	48	-	32
Cintora	1980	Dacron	Suprainguinal	-	-	-	90	-
Prager	2003	PTFE	Suprainguinal	-	-	-	-	88
Jonhson	1999	PTFE	Extra-Anatomical	77	-	62	-	47
Cintora	1980	PTFE	Suprainguinal	-	-	-	97	-
Guevara-Noriega	***2019***	***Allografts***	***Femoro-Popliteal***	***79***	***65***	***65***	***-***	***-***
Suckow	2013	Autologous Vein	Femoro-Popliteal	70	-	-	-	-
Kram	1991	Autologous Vein	Femoro-Popliteal	-	-	-	-	74
Jensen	2007	Dacron	Femoro-Popliteal	-	70	-	-	-
Devine	2004	Dacron	Femoro-Popliteal	71	-	54	-	46
Robinson	2003	Dacron	Femoro-Popliteal	36	36	-	-	-
Post	2001	Dacron	Femoro-Popliteal	-	-	64	-	-
Green	2000	Dacron	Femoro-Popliteal	78	-	65	-	45
Robinson	1999	Dacron	Femoro-Popliteal	70	-	56	-	47
Daenens	2009	PTFE-Heparine	Femoro-Popliteal	92	83	-	-	-
Jensen	2007	PTFE	Femoro-Popliteal		57	-	-	-
Devine	2004	PTFE	Femoro-Popliteal	62	-	44	-	35
Robinson	2003	PTFE	Femoro-Popliteal	56	47	-	-	-
Post	2001	PTFE	Femoro-Popliteal	-	-	61	-	-
Jackson	2000	PTFE	Femoro-Popliteal	58	47	36	32	
Green	2000	PTFE	Femoro-Popliteal	80	-	63	-	43
Robinson	1999	PTFE	Femoro-Popliteal	72	-	52	-	52
Kram	1991	PTFE	Femoro-Popliteal	-	-	-	-	55
Guevara-Noriega	***2019***	***Allografts***	***Distal Vessel Target***	***54***	***43***	***39***	***37***	***34***
Avgerinos	2015	Autologous Vein	Distal Vessel Target		47			32
Suckow	2013	Autologous Vein	Distal Vessel Target	72	-	-	-	-
Avgerinos	2015	Alternative Vein	Distal Vessel Target		24			23
Furuyama	2018	PTFE	Distal Vessel Target	75	65	60	-	-
Avgerinos	2015	PTFE	Distal Vessel Target		43			38

Table 12. Allografts versus other grafts: Primary Patency.

Major Adverse Limb Events-Free Rate								
Author	**Year**	**Graft**	**Territory**	**1y**	**2y**	**3y**	**4y**	**5y**
Guevara-Noriega	***2019***	***Allografts***	***Suprainguinal***	***40***	***33***	***26***	***20***	***13***
Guevara-Noriega	***2019***	***Allografts***	***Femoro-Popliteal***	***60***	***54***	***50***	***-***	***-***
Tsujimura	2019	Endovascular:Innova	Femoro-Popliteal	82	-	-	-	-
Uhl	2017	Autologous Vein	Femoro-Popliteal	76	-	72	-	69
Uhl	2017	Heparine PTFE	Femoro-Popliteal	74	-	64	-	55
Guevara-Noriega	***2019***	***Allografts***	***Distal Vessel Target***	***53***	***39***	***29***	***25***	***18***
Ziza	2015	Venous Allografts	Distal Vessel Target	65	-	43	-	28

Table 13. Allografts versus other grafts: Major Adverse Limb Event-Free Rate.

Limb Salvage Rate								
Author	**Year**	**Graft**	**Territory**	**1y**	**2y**	**3y**	**4y**	**5y**
Guevara-Noriega	***2019***	***Allografts***	***Suprainguinal***	***93***	***93***	***93***	***93***	***62***
Guevara-Noriega	***2019***	***Allografts***	***Femoro-Popliteal***	***83***	***83***	***83***	**-**	**-**
Uhl	2017	Autologous Vein	Femoro-Popliteal	78	-	57	-	48
Curi	2002	Autologous Vein	Femoro-popliteal		81			
Jackson	2000	Autologous Vein	Femoro-popliteal	-	-	-	81	-
Lawson	1999	Autologous Vein	Femoro-popliteal	-	92	-	-	-
Kram	1991	Autologous Vein	Femoro-popliteal					78
Uhl	2017	Heparine PTFE	Femoro-Popliteal	74	-	51	-	28
Curi	2002	PTFE	Femoro-popliteal		79			
Jackson	2000	PTFE	Femoro-popliteal	-	-	-	56	-
Kram	1991	PTFE	Femoro-popliteal					78
Guevara-Noriega	***2019***	***Allografts***	***Distal Vessel Target***	***60***	***49***	***46***	***46***	***43***
Suckow	2013	Autologous Vein	Distal Vessel Target	86	-	-	-	-
Lawson	1999	Autologous Vein	Distal Vessel Target	**-**	85	**-**	**-**	**-**
Ziza	2015	Venous Allografts	Distal Vessel Target	83	-	70	-	53
Harris	2001	Allografts	Distal Vessel Target	66	-	62	-	-
Stonebridge	2000	PTFE	Distal Vessel Target	53	44	-	-	-

Table 14. Allografts versus other grafts: Limb Salvage Rate

Survival Rate								
Author	**Year**	**Graft**	**Territory**	**1y**	**2y**	**3y**	**4y**	**5y**
Guevara-Noriega	***2019***	***Allografts***	***Suprainguinal***	***60***	***60***	***53***	***45***	***38***
Guevara-Noriega	***2019***	***Allografts***	***Femoro-Popliteal***	***88***	***79***	***75***	**-**	**-**
Tsujimura	2019	Endovascular:Innova	Femoro-Popliteal	82	-	-	-	-
Uhl	2017	Autologous Vein	Femoro-Popliteal	81	-	60	-	47
Uhl	2017	Heparine PTFE	Femoro-Popliteal	80	-	61	-	43
Curi	2002	Autologous Vein	Femoro-popliteal	-	94	-	-	-
Curi	2002	PTFE	Femoro-popliteal	-	78	-	-	-
Guevara-Noriega	***2019***	***Allografts***	***Distal Vessel Target***	92	85	72	66	56
Ziza	2015	Venous Allografts	Distal Vessel Target	85	-	80	-	75
Harris	2001	Allografts	Distal Vessel Target	91	-	63	-	-
Stonebridge	2000	PTFE	Distal Vessel Target	66	61	-	-	-

Table 15. Allografts versus other grafts: Survival rate.

10. DISCUSSION

10.1 REVAC Design and Creation.

We have proposed to create a cryopreserved arterial segment and cardiac valve transplant autonomous registry based on clinical information. Any official, multicenter and digitalized data related to vascular allografts or cardiac valves has not been reported to date yet. Clinical information aroused from this registry will be useful to guide future research lines, as well as, its design by itself could help to other governmental entities to create other registries.

Catalonia is the second-ranked autonomous community in Spain. In our country, few centers perform surgeries with allografts because this kind of tissue requires amazing coordination for extraction, huge infrastructures for having a wide available stock and tremendous operative capacity to allocate them. To encourage a constantly growing of this structure, or reduce to a more pragmatic level, specific information must be available. The design and creation of a registry is a step forward to know outcomes, to precise the cases where they result useful, and eventually, cover the specific demand of these tissues by adjusting the structure to the actual demand.

According to the retrospective nature of this registry, some limitations were evident when analyzing outcomes. Different follow-up schemes, lack of information in the medical reports, the presence of two convergent languages in the autonomous community, the absence of funding for the project and the involvement of 10 centers were some of the main challenges to be dealing with. Establishing of minimum standards to analyze every case, telephonic interviews with patients, appointments for ultrasound follow-up, an international team working ad honorem, health authorities

involvement and a doctoral dissertation behind this project were the tools to go ahead with it.

All these problems, lead to the necessity to strengthen the registry data with prospective cases and quality standards must be established by the governmental entity, in this case, the Catalan Transplant Organization. Thanks to the ReVAC, authorities will have the change of reach it, however, a budget issue is still on the table.

Registries created from retrospective data have limitation at the moment of to analyze data. Incomplete medical reports and lack of information are the greatest problems. ReVAC and its online platform will let the prospective introduction of data, and therefore, to avoid data missing of new cases. However, this task requires budget and coordination that are the barriers to overcome in the future to improve the quality of data available in the registry. Authorities and physicians must work together to success in this task.

In 1989, the European Homograft Bank (EHB) was built and 6 countries participated in the project. Spain was not one of them. Several difficulties were detected at the beginning. However, after 20 years of experience, collaboration between the EHB and the Transplant Coordination is satisfactory. Donor selection criteria are discussed with the transplant coordinator; whereas, implantation indication, with the implanting surgeon[73].

We hope that after a short period of time, the Catalan Organization of Transplants be able of reach this level of coordination between centers and other health authorities. ReVAC would let to have enough clinical and administrative information to take better decisions and stablish new protocols.

One of the current problems of EHB is that not always is able to meet demands for the cryopreserved valves and arterial segments. Nowadays, in Catalonia, this is not a problem. However, in case of a better coordination, and better knowledge of outcomes by physicians, the demand could increase. For coordinating new protocols with the intention of increase number of procurements and to stablish a better distribution network, ReVAC will be a useful tool, and the improving of the data quality in the registry will let to take better decisions. Cardiovascular and Vascular surgeons need to play more active roles towards facilitating this end[73].

10.2 Arterial segments

Vascular allografts carry with a stigma from the bad results observed in their first steps. Consequently, only a clear indication is covered by guidelines, the replacement of infected grafts in aortic position because after several series and publications, they have shown a lower reinfection rate and to be a reliable alternative. However, the first aspect to consider is that most of the bad results of allografts were observed in other territories and indications.

Recent publications have shown the role of the Natural Killer cells in the allograft rejection. The recently called "Allograft vasculopathy" has been linked to chronic inflammatory response mediated by Natural Killers. Also, specific antibodies against the donor induce intracellular mechanisms that recruit monocytes and leukocytes injuring the transplanted tissue producing graft degeneration. The clinical expression of this mechanism could be occlusion, aneurysmal degeneration, and rupture[74].

That is why despite this project involve cases out of the guideline-supported indication, and therefore, the information provided must be carefully analyzed. In a new era where immunosuppressive therapy, ABO interaction, and antibodies mechanism of action can be analyzed and controlled, the clinical behavior showed previously by allografts in the past could be different in the current scenario.

The data showed in this research correspond to transplant of arterial allograft without specific immunosuppression or control of these factors. Even so, the rates, which can be interpreted in any way, suggest that in some cases the arterial allografts made the work. These factors conditioning the outcomes must be known and controlled for future analysis.

Moreover, if we consider the hypotheses in which it is assumed that vascular allograft represents a viable alternative is because is a biologic tissue that behaves like the normal autologous tissue and, additionally, we control that mentioned factors, arterial allografts could represent a reliable alternative. However, to our knowledge, there are not publications involving arterial allografts and immunological factors control. Thus, the first step is to show results in large series like this and to incorporate the new performance gold standard in vascular surgery, as major adverse limb event rate (MALE).

It is important to highlight that survival rates, as well as, patency, limb salvage, and MALE-free rates are linked to multiple factors regardless the grafts selected for revascularization, especially in the “vascular patient”, and therefore, some comparison between grafts must be done.

We exposed results of arterial allograft in supra-inguinal territory where few publications are published, none of them with MALE-free rate as an endpoint. For

femoropopliteal and distal target vessel revascularization, the known primary patency rate of PTFE, the most commonly used graft, is around 51% and 25% respectively. If we compare this result with our 43% and 34%, at least more wide studies are justified[75].

From a different point of view, regarding to the supra-inguinal territory, where the primary indication is the infected prosthesis replacement, primary patency at 5 years in our cohort (72,2%) resembles the one observed in the superficial femoral vein reconstruction also called Neoaortoiliiac System (NAIS) in which the primary patency at 5 years is close to 75%, making allograft a viable alternative. However, as it was previously exposed, this already is an indication supported by the European Society of Vascular Surgery Guideline[4,76].

Moreover, the axilo-femoral bypass has a 5-year patency rate of 55% approximately for the bifemoral technique and 14% for the uni-femoral technique according to the New England Cardiovascular Surgery Society. The summary is that extra-anatomic surgery is not better than arterial allograft in this territory. Nevertheless, extra-anatomic still have some indications because is a less invasive technique useful in very complex and comorbid patients[77,78].

However, not only primary patency matter but also survival and infection rate must be considered in infected aortic graft replacement. Kieffer et al. showed that allografts have a considerable annual mortality rate reduction compared to another type of grafts and its use is limited due to the availability of tissue. Arterial allograft has been shown that has the lowest reinfection rates only being overcome by NAIS[79].

Regarding the Limb Salvage rate, Allograft and NAIS represented the best alternative with lower amputation rate and mortality according to Smeds et al when compared different therapeutic alternatives in infected prostheses. Other publications

have reinforced use of allografts or NAIS in front of extra-anatomical revascularizations due to a better limb salvage rate[78,80,81].

In the infra-inguinal revascularization, it is known that while more distal the anastomosis, less primary patency. Therefore, it is necessary to differentiate allograft used in the femoral-popliteal with the ones with distal vessel anastomosis.

In our cohort, the primary indication is the chronic ischemia grade III and IV without great saphenous vein available in either of lower extremities. Despite, autologous graft has shown superior results in this territory, when lack of autologous veins, other grafts must be considered. PTFE and Dacron have shown to be acceptable alternatives[82].

In patients with critical limb ischemia and distal vessel target revascularization, our results demonstrated 5-year primary patency similar to PTFE (34% allografts versus 25% PTFE). We can consider that factors affecting result could determine outcomes regardless of the graft used. However, more studies controlling immunological factors are justified. For limb salvage and MALE-free rate comparison, there are not comparable cohorts to extract valid conclusions[83,84].

Major adverse limb event is a current objective performance goal. In our cohort, it is important to highlight that after 5 years between 81,2% and 86,7% (according to revascularized territory) of our patients presented a MALE. At first sight, this could be discouraging. However, due to the complexity of the so-called “vascular patient” and their multiple systemic comorbidities, these results are according to the expected evolution. Dead cause and future analysis should be researched in future publications[85].

10.2.1 Arterial Segment for Treatment of Chronic Limb-Threating Ischemia.

The transplantation of arterial allografts has been exposed in the recent literature as an attractive alternative in patients with chronic limb-threating ischemia and no suitable good-quality autologous saphenous vein. However, considering that allografts have gone through several stages, it is mandatory to be cautious in this regard[1].

An historical overview is required to figure out the current role of arterial allografts. In the mid-19th century, due to cases of graft calcification, disruption or occlusion, the arterial allografts were abandoned. However, changes in cryopreservation techniques and immunobiology lead to new studies and, therefore, the allografts were reconsidered as a therapeutic option[1].

Currently, it is widely commented by vascular surgeons that allografts have significant complications. In our series, classical complications as rupture or dilation were observed in an impressive lower rate than expected. This could be explained by an improvement in cryopreservation techniques, but more studies should be conducted, controlling specific variables related to the cryopreservation technique[86].

Chronic Limb-threatening Ischemia (CLI), the most advanced expression of atherosclerotic pathology in the lower limbs has been associated with complications that go beyond the major adverse limb events affecting the revascularized extremity. CLI requires the planning of surgery in order of improve the symptoms and the limb salvage rate. After this surgery, it is widely known that an alarming high rate of cardiovascular events and major adverse limb events, including reinterventions, could be needed[87,88].

Our large retrospective review found large differences in the outcome according to the end-point being analyzed. All this information is relevant when the physician

exposes the therapeutic options to the patient who must understand the process by which he will go through in the years following the surgery for resolution of the CLI

That is why our group has analyzed the rate of major adverse limb events in the patient as an indicative measure of the route that the patient will go through in the next years after revascularization[87,88].

Nevertheless, it is important to highlight 2 important points, the first one is that there are no previous studies comparing MALE-free rate in patients with CLI surgery with allografts, which does not allow us to compare with other therapeutic options or even with revascularization using another type of grafts. The second one is that the high 5-year rate of 78.5% of major adverse limb events is a clear indicator of an appalling evolution of the patients.

Although there is not available data in the literature to compare MALE rates with other types of revascularization or graft, several factors are involved in these results. Our study reinforces the role of dyslipemia as a risk factor but also it is important to consider the nature of the atherosclerotic pathology as a systemic disease.

The association of these three factors (Chronic Limb-threatening Ischemia, the absence of autologous vein and surgery) may lead us to assume that these patients have a complex management and comorbidity including atherosclerotic disease in other territories.

Although patient complexity could be an explanation for this this appalling evolution, another factors, such as the type of graft chosen for revascularization, or the selection of an open or endovascular technique could also be important. In the absence of similar publications, we cannot make comparisons and new studies are needed to clarify this influence.

Regarding limb salvage rate, which is one of the most studied end-points in cases of lower limb revascularization and more information is available, Ziegler et al. published a pool data from papers in English from 1990 to 2009 (excluding those series related to reintervention) and he exposed long-term limb salvage rates ranging from 57%-70% for PTFE and 89% in revascularizations with autologous saphenous vein. When comparing to our results where the 5-year limb salvage rate is 50.2%, it could represent an overall inferiority of allografts in front to the PTFE for this outcome[75].

When looking for the source of the information for the paper of Ziegler et al. we find the publication of Berlakovich et al. This publication excludes from the analysis all patients with technical error, which was considered as any case with occlusion in the first 72 hours. Also, these patients were treated with anticoagulation or antiaggregation systematically in all the cases. Some other sources of this publication only include femoro-popliteal bypass[75,89].

Our cohort consisted of patients revascularized to distal territory in 74.2% of the cases, we included any occlusion or event even happening in the first 24 hours and only 79% of our patients were taking antiaggregation or anticoagulation drugs. Although this could explain our lower limb salvage rate, the difference between cohorts makes them not comparable. Also, it is important to consider that our data at 5 years for this end-point is weak due to high percentage of lost patients, and therefore, more studies are required[89].

To our knowledge, there is not any publication before exposing a 5-year limb salvage rate in patients with CLI revascularized with arterial allografts. There are no series exposing results to 5 years in those patients requiring lower limb revascularization with no autologous saphenous vein available.

About the primary patency, our cohort showed a 5-year rate of 38.6%, clearly inferior to the known rates for autologous saphenous vein and PTFE prosthesis, which are round about 72% and 44%, respectively. The nature of the allografts and the so-called "vasculopathy of allografts" could be a conditioning factor for a greater rate of occlusion in the short and long term. Unfortunately, the immunosuppressive therapy has no effect on the permeability of cryopreserved allografts, as demonstrated by Carpenter et al.[90].

Seon-Hee Heo et al. published results of a 10-year cohort comparing autologous vs. PTFE and showed a primary patency rate near 42% without statistically significant differences (P = 0.330). Almost all the patients in the Seon-Hee Heo group who were treated with PTFE were systematically anticoagulated and the revascularization was landed in the popliteal artery. Therefore, controlling factors related to coagulation or aggregation could be a strategy for revascularizations with allografts that could traduce in better primary patency. Nevertheless, is important to consider the comorbidity of the "vascular patients" and the consequences of this strategy[91].

Arm vein autograft has also been studied in revascularisation surgery. These conduits have demonstrated primary patency of 42% at 3 years. When comparing with our 43.8%, no clear indications of arm vein over allograft can be stablished. As neither of these grafts are used very commonly, it would be difficult to perform a study to properly compare them[75,92].

In term of survival rate, our results are consistent with the average evolution of patients with critical limb ischemia. Our data demonstrated that smokers and older patients could have a worse evolution. A wide sample for risk stratification by age could answer which is the cut-point to consider as a risk factor. Also, smoking cessation must

be recommended to patients revascularized with allografts. Although this is an acceptable recommendation to improve the health of any patient, especially "vascular patients".

Finally, our analysis of predictor factors correlates with the known literature reporting that the more distal the revascularization the less success rate. According to our results, distal revascularization has a lower primary patency and limb salvage rate. This could be because the patient who requires distal revascularization with an allograft due to the absence of native vein is precisely the one with aggressive pathology and/or who has exhausted other options that have a better outcome.

10.3 Cardiac Valves

Since the firsts publications of cryopreserved antibiotic incubated autologous cardiac valves until today, cryopreserved valves have been considered perfect substitutes due to its optimal hemodynamic properties, infection resistance and long-term outcomes which are widely known. Nowadays, newest publications are oriented to described better preservation techniques.

Our study shows consistent data about short and mid-term survival. The 5-year survival rate of 97% match with those results of Waszyrowski et al. (1997) and discretely better to Ganguly et al (2004), Vuran et al. (2012) and Rouchi et al (2016:). These differences could be determined by surgical techniques, cohort characteristics, and cryopreservation techniques. At a valvular level, it is still unknown if this immunologic response against the graft requires long-term use of immunosuppression[93-97].

Biologic plausibility indicates that affected tissue loses its hemodynamic characteristics and trends to stenosis or insufficiency. However, our data confirm and reinforce previous publications about long-term hemodynamic changes.

Since the Ross technique is an accepted technique around the world, our research can add few data to the previously known information about valvular allograft.

Our cohort presented 5-year stenosis and insufficiency rates of 84% and 81.1% respectively. Despite the rates, this does not mean mortality, and therefore more studies about the evolution of the hemodynamic changes are required.

Cardiovascular tissue banks that were our reference previously worked in a multicenter study published by De by et al in 2012 in which stable activity of the graft was studied for 4 years. An increasing tissue demand and publications were observed. ReVAC is a necessary tool for the design of new clinical and administrative strategies in case of that increasing demand reach to our area[98].

11. CONCLUSION

ReVAC has permitted, for the first time, to the Catalan community in Spain to collect and analyze the outcomes of cryopreserved arterial segments and cardiac valves. This pioneering project has left a great tool available which would allow monitorization and quality assessment of cryopreserved tissue in this community, and most important, there is available data of 5 years of transplant surgeries in Catalonia.

A three-level registry was designed and created. Its scheme could be reproducible for the creation of similar databases and registries. ReVAC is available for the authorized staff through the website for applications of the "Generalitat de Catalunya".

Survival, primary patency, limb salvage and MALE-free rates have been analyzed for arterial segments in Supra-inguinal and Infra-inguinal revascularization. Results are wide and susceptible to analysis.

Only one guideline-supported indication is currently done for allografts, the replacement of infected aortic grafts. However, information about lower extremities revascularization is useful to address future project, which is required in order to reconsider new or specific indications.

Cryopreserved and surgical techniques are determinants in the global survival rate in valvular allograft transplants. Hemodynamic changes are expected complications of a valvular allograft. However, these changes do not traduce in mortality and the overall survival rate is good.

Lack of availability, costs and surgeon preferences could be a determinant factor in the limitation of this practice. Even though, favorable results indicate that valvular

allograft transplant is a good alternative. Our group considers that its use should be done according to each patient needs.

Several recent publications have exposed cryopreserved arterial allografts as an attractive alternative in patients with critical ischemia requiring revascularization in the absence of autologous saphenous vein. However, our results do not demonstrate superiority of allografts over other grafts in term of limb salvage. Survival rate in patients revascularized with allografts is consistent with the expected for patients with CLI. Primary patency of allografts for infra-inguinal revascularization seem to be lower than autologous vein and PTFE but similar to arm veins. In the absence of a comparable cohort, it is not possible to compare the rate of major adverse events in the patient or the MALE rate of these patients in respect to another type of graft. Classical complications of vascular allografts seem to be related to older cryopreservation techniques and they are that frequent as expected. More studies are needed to clarify outcomes of arterial allografts.

11.1 Contrast of hypothesis.

Synthetic protheses are second line for cardiac and vascular surgeons. However, in vascular surgery, allografts are the first option for replacement of infected grafts in aortic position, and an option considered for revascularization of patients with chronic limb-threating ischemia.

Nevertheless, a deeper analysis of predictive factors must be performed in order of better choosing the patients to be revascularized with allografts.

Allografts have a lower infection rate when comparing with other grafts. It was demonstrated in our results but simultaneously several publications also concluded the

same. In the period while our study was performed, a guideline-supported indication arose due to this characteristic of allografts.

Cardiac valves have demonstrated an excellent hemodynamics results and its use are wide including the Ross procedure, as well as, selected cases of congenital heart defects.

Regarding ReVAC, the registry let to have the first autonomic database with information about allografts performance. Data extracted from the ReVAC let us to clarify some aspect about the outcomes of theses grafts, and, in the future, additional conclusion could be extracted.

12. PUBLICATIONS

1.- Historical overview of vascular allografts transplantation.

Published in Vascular and Endovascular Reviews: 2019

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2.- Cryopreserved Allografts for treatment of Chronic Limb-threatening Ischemia in patients without Autologous Saphenous Veins.

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Cryopreserved Allografts for treatment of Chronic Limb-threatening Ischemia in patients without Autologous Saphenous Veins.

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Guevara-Noriega KA, [1,2], Lucar-Lopez Gustavo Adolfo [3], Pomar Jose Luis [4]

[1] Research and Clinical Staff at Vascular Surgery Department. Centro Médico Teknon. Barcelona, Spain.

[2] Vascular Surgery Staff. Consorci Sanitari Parc Tauli, Sabadell, Spain.

[3] Orthopedic Surgery. Hospital of Mataro, Maresme, Spain

[4] Cardiovascular Surgery Department. Hospital Clinic i Provincial, Barcelona, Spain.

Correspondence:

Kerbi Alejandro Guevara-Noriega MD, MSc.

Institution: Clínica Teknon Barcelona, Barcelona, Spain

Calle Bilbao 110, Piso 8 Puerta 6 Barcelona, España, 08018.

Tel.: +34 628-888-896

Correo electrónico: kerbiguevara@hotmail.com

ABSTRACT

BACKGROUND

Revascularization is the best alternative to reduce symptoms and to improve the limb salvage rate in patients with Chronic Limb-threatening Ischemia (CLI). Alternative grafts as synthetic prostheses and allografts must be considered for patients without a suitable autologous graft (SAG). Our aim is to evaluate outcomes of cryopreserved allografts used as a vascular conduit for bypass surgery in the infra-inguinal territory.

METHODS.

Retrospective analysis (January 1995 to January 2014) of the Registry of vascular and valvular allografts transplant in the autonomous community of Catalonia, Spain (ReVAC) was performed for identifying patients with CLI who required infra-inguinal bypass with cryopreserved arterial allografts. Statistical analysis was performed using SPSS Ver. 20 for Mac (Chicago, USA).

RESULTS.

A total of 149 patients with CLI (mean age of 70.1 years) were analyzed. 102 patients (68.5%) had a grade IV lesion (Fontaine classification). In the overall follow-up, 24.8% of patients required a reintervention. Overall graft occlusion, infection and dilation rate were 52.3%, 6% and 5.4% respectively. Overall 30-days mortality was 0.7%. 5-year primary patency rate and limb salvage rate were 38.6% and 50.2% respectively. Survival rate at 5 years was 54.2%. Major Adverse Limb Event (MALE)-free rate was 21.5% at 5 years. Revascularization to a distal target vessel was an independent positive predictive risk factor for a lower limb salvage rate and lower

primary patency rate. Dyslipemia was related to a lower limb salvage rate and represents a risk factor involved in major adverse limb events.

CONCLUSION

Arterial allografts seem to represent a suboptimal alternative although some selected patients could beneficiate of them. 5-year results are disappointing and more studies are required to know other predictor factors for a better selection of patients.

KEYWORDS: Cryopreserved Allografts, MALE, Chronic Limb-threatening Ischemia, Vascular Transplant, Vascular Tissue.

BACKGROUND

Revascularization of a lower limb with critical ischemia is the only alternative that has been shown to reduce the symptoms, either rest pain, non-healing ulcers or established necrosis. Revascularization surgery has also demonstrated to be the best option for improving limb salvage rate. In vascular surgery, currently, the indication of revascularization should be assessed for open surgery or endovascular procedures. Endovascular techniques have gained wide acceptance as primary and secondary treatment of critical ischemia of the lower extremities. Despite a large number of established indications and many other emerging indications, many patients require open surgery using lower extremity bypass (LEB) to try to ensure therapeutic success.

The autologous saphenous vein has been widely accepted as the preferred graft for revascularization of the lower limbs. In patients requiring a lower extremity bypass, it is estimated that 20 to 45% do not have a single segment of autologous saphenous vein available for the bypass. Previous coronary or lower limb revascularization, varicose veins of the lower limbs, previous surgery of varicose veins and/or inadequate quality of the native vessel due to diameter or length are the main reasons why patients could confront this scenario.

In this scenario, the vascular surgeon must consider the use of alternative grafts, the main options being autologous veins from less usual location, synthetic prostheses and vascular allografts.

Prostheses, which are an important resource, date back to 1952 when Voorhees et al. performed animal studies that demonstrated the patency of synthetic derivatives in vascular territory. However, since 1969 to our days, the most clinically relevant graft

have been the so-called Expanded Polytetrafluoroethylene (PFTE) graft (Gore Medical, Flagstaff, Arizona, US), developed by Bill and Vieve Gore.

Currently, PTFE is the first-choice prosthesis among most vascular surgeons and its results have been validated in multiple studies, placing PTFE as a useful alternative with acceptable permeability in the below-the-knee territory. Arterial allografts, although with few studies, have proven to be a valuable alternative to synthetic prostheses in cases where the autologous saphenous vein is not available.

It is assumed that arterial allografts represent a viable alternative because it is a biological tissue whose behavior must be like the autologous tissue. However, long-term results in terms of permeability and major adverse limb event rate in the patient are linked to many factors, being determinant the nature of the graft and the revascularized territory.

Our intention is to evaluate the results of vascular tissue transplantation in the infra-inguinal territory by selecting cases from the "Registry of vascular and valvular allografts transplant in the autonomous community of Catalonia, Spain (ReVAC)". Long-term results will be expressed in terms of permeability, limb salvage rate, survival rate, rate of major adverse limb events, and major events in the patient. There are no previous studies evaluating the latter two end-points in patients treated with cryopreserved arterial segments.

METHODS

We performed a retrospective analysis of the "Registry of vascular and valvular allografts transplant in the autonomous community of Catalonia, Spain (ReVAC)"

which includes cases from 14 hospitals in the region of Catalonia, Spain. This registry includes cases from January 1995 to January 2014. Data introduced in the ReVAC is confidential. The registry is available through the website of the Catalonian Government (*Generalitat de Catalunya)*. A specific authorization from the health department of *"Generalitat de Catalunya"* is required for gathering information from the registry. Personal and digital data in Spain is protected according to the Organic Law for protection of personal data and guarantee of digital rights (BOE-A-2018-16673). The review was conducted according to in-effect regulations and being respectful of the confidentiality of the patient information.

This registry was queried to identify all patients who had undergone infra-inguinal bypass and Chronic Limb-threatening Ischemia (CLI) grade III or IV.

Demographical and clinical variables such age, sex and comorbidity (arterial hypertension, diabetes mellitus and dyslipidemia) were recorded.

Due to the retrospective nature of this study, the follow-up was based on the examinations and visits recorded by the patient in his reference center. The follow-up was considered as ended at the date of the last visit to the vascular surgery department in the reference center, except for survival analysis that was used the last visit registered in any department or hospital in the autonomous community of Catalonia.

Vascular Allografts

The vascular allografts distributed by the tissue banks of Catalonia (Spain) are extracted in cases of multi-organic donation by centers authorized by the Catalan

Organization of Transplantation, Spain (in Catalan: Organització Catalana de Transplantaments: OCATT). Only arterial allografts were analyzed.

After their collection, allografts were transferred to the tissue bank under sterile conditions and immersed in a preservation solution. In no case, the ischemia time was greater than 24 hours (if the body has been refrigerated within the first 6 hours since asystole) or greater than 12 hours if the body has not been refrigerated.

Dissection and initial tissue evaluation were performed in laminar flow chambers under sterile techniques. In all the cases, a thorough inspection of the anatomical characteristics is performed and all the grafts were immersed in a solution of cellular nutrient medium with antibiotics at the lowest effective concentration. They were kept under refrigeration for 12 to 30 hours. A sample of vascular tissue is taken for pre-antibiotic microbiological control.

For cryopreservation, 10 ml of DMSO and 10% human albumin solution were used. The cryopreservation program used reduces the temperature of the tissues from 1ºC/min to a temperature of -60ºC and then from 3-4ºC/min to -100ºC. The maximum programmed temperature deviations accepted was 10ºC.

After reaching this temperature, the graft was extracted from the chamber and introduced into the vats of liquid nitrogen. Tissues not used for clinical applications were used as control for mechanisms that verify that the established parameters have been properly followed. Storage is carried out in large capacity liquid nitrogen tanks monitored to maintain the proper level of liquid nitrogen. All tissues are validated 1 month after storage to be included in the bank's availability lists and distributed to hospitals upon request.

When an implantation center needs an allograft, the tissue or tissues that best fit the request received are selected and the information is prepared so that the implantation center can decide its acceptance. If the graft is accepted, the delivery is organized so that the tissue is in the implantation center on the date planned for the intervention. The tissue is transferred in boxes of porexpan of 30x30 cm, which is filled with half of carbonic snow and placed, on the cryopreserved graft, followed by an additional layer of dry snow.

Prior to use, the grafts were thawed slowly in a sterile solution at 20 ° C and microbiological samples are taken prior to implantation.

Senior vascular surgeons performed the surgery. In no case was immunosuppression or anticoagulation intentionally used after implantation. No distal vein patches were used in any case.

End-points

Our end-points were primary patency, survival rate, limb salvage, major limb adverse event (MALE) and major event related to the patient. 5-year rates are exposed. Overall graft occlusion, infection, rupture and dilation rates were considered as clinically relevant, and therefore, rates for these outcomes are exposed.

Maximum acceptable rate of patients lost was stablished at 20% (in higher rates, we consider that data is not extrapolable).

Definitions

We considered infra-inguinal to any bypass with proximal anastomosis originating below the inguinal ligament. CLI was defined as ischemic rest pain, nonhealing ulceration, gangrene or necrosis requiring an imminent major amputation

Primary patency was defined as the interval from the time of surgery (bypass placement) to any intervention designed to maintain or re-establish patency or to the thrombosis of the bypass.

Limb salvage rate was defined as the interval from the time of surgery to any ipsilateral major amputation.

According with the objective performance goals for lower extremity revascularization of the Society for Vascular surgery, we defined the major adverse limb event (MALE) as any major amputation, major reintervention, including thrombectomy or bypass revision. Major adverse event related to the patient was considered any major ipsilateral amputation, ipsilateral limb reoperation o revascularization, cardiovascular event requiring hospitalization, or death.

For infection, only was cases with a positive culture of the graft available in the medical reports were considered as valid. Any other suspected case without confirmation was not considered valid.

Ethical Statement

The authors state that no human or animal experiments have been performed for this research, no patient data appears in this article, and there are no conflict of interests.

This project has been submitted to ethical evaluation which was carried out by the ethics committee of the “Hospital Clinic i Provincial de Barcelona”. No funding sources.

Statistical analysis

Statistical analysis was performed using SPSS Ver. 20 for Mac (Chicago USA). The descriptive data is presented in relative and/or absolute frequencies. Cumulative patient survival, limb salvage rate, graft patency rate, MALE-free rate related to the patient were assessed by the Kaplan-Meier method at 30 days, and annually until 5 years. A second analysis was performed which included a Cox regression model to determine risk factors for survival, primary patency rate, limb salvage rate and MALE-free rate at 5 years.

The $P<0.05$ level was used to determine statistical significance. 95% confidence intervals were selected for hazard ratios.

RESULTS:

In the “Registry of vascular and valvular allografts transplant in the autonomous community of Catalonia, Spain (ReVAC)” there were a total of 149 patients with Chronic Limb-threatening Ischemia (CLI) who underwent infra-inguinal surgical reconstruction with cryopreserved arterial allografts. The population was composed of 108 men (72.5%), with a mean age of 70.1 +/- 10.7 years old at the time of the surgery.

Clinical characteristics are exposed in Table 1. One hundred two patients (68,5%) had a grade IV ischemia according to the Fontaine classification (ulcer, necrosis or tissue loss), 1 vascular segment was used in 83 patients (55.7%), 2 segments in 54 patients (36.2%) and only 12 patients (8.1%) required 3 or more segments.

There was not a specific protocol about antiplatelet or anticoagulation medication and it was determined by surgeon discretion and/or according to the comorbidities of the patient. Distal target vessel is exposed in Table II.

In the overall follow-up, 37 patients (24.8%) required a reoperation, 78 patients (52.3%) present an occlusion of at least 1 arterial segment, 9 patients (6%) had a graft infection and 8 patients (5.4%) had a graft dilation, and only 1 spontaneous rupture was observed.

30-days Results.

Overall 30-days mortality was 0.7%. Primary patency rate, limb salvage rate and MALE-free rate were 89.3%, 89.9%, and 87.9% respectively. A total of 4 patients (2.7%) required a reoperation in the ipsilateral extremity. Two patients (1.34%) had a coronary event, 9 patients (6.04%) had a non-treated occlusion of at least one transplanted arterial segment and in 1 (0.7%) case a spontaneous rupture of the graft occurred.

5-year results.

The cumulative 1-year, 3-year and 5 year survival rates were 91%, 72.3% and 54.2% respectively. The primary patency had a decline from 54% at 1 year to 46.1% at 3 years. 5-year primary patency rate was 38.6%. High percentage of lost patients were observed at 5 years for this end-point (21.48%) (Figure 1, Figure 2, Table III).

Regard the limb salvage rate, evolved from a 65.7% at 1 year to a 3-year limb salvage rate of 55%. 4-year limb salvage rate was 55%. 5-year limb salvage rate was 50.2%, however, 20.13% of patients were lost of follow-up for this end-point at 5 years (Figure 3, Table III).

MALE-free rate declined from 54.7% at 1 year to 33.6% at 3 years. 5-year MALE free rate was 21.5% (Figure 4, Table III).

At 3-year cut-point, two additional aneurysmal dilations and five grafts infection occurred in this period. Also, 2 additional stenoses in at least 1 transplanted arterial segment were demonstrated.

After multivariable Cox proportional hazards, regression analysis including all the clinical and demographic characteristics, the revascularization with a distal target vessel was an independent positive predictive risk factor for a lower limb salvage rate (HR: 3.241, 95% Confidence Interval (CI): 1.677-7.115, p=0.003) and lower primary patency rate (HR: 2.242, 95% CI: 1.206 – 4.169, p=0.011). Dyslipemia was an independent positive predictive risk factor involved in major adverse limb events (HR: 1.480, 95% CI: 1.014 – 2.160, p=0.042) and for a lower limb salvage rate (HR: 1.968, 95% CI: 1.141 – 3.396, p=0.015).

Arterial hypertension was a protective factor for MALE (HR: 0.608, 95% CI: 0.415 - 0.885, p=0.008) and involved in a better primary patency rate (HR: 0.498, 95% CI: 0.312 - 0.794, p=0.004)

Smoking habit (HR: 1.661, 95% CI: 1.278 - 2.558, p=0.021) and the age of the patient at time of the surgery (HR: 1.302, 95% CI: 1.090 - 1.570, p=0.007) were associated as risk factor for mortality.

DISCUSSION

The transplantation of arterial allografts has been exposed in the recent literature as an attractive alternative in patients with no suitable good-quality autologous saphenous vein. However, considering that allografts have gone through several stages, it is mandatory to be cautious in this regard.

An historical overview is required to figure out the current role of arterial allografts. In the mid-19th century, due to cases of graft calcification, disruption or occlusion, the arterial allografts were abandoned. However, changes in cryopreservation techniques and immunobiology lead to new studies and, therefore, the allografts were reconsidered as a therapeutic option.

Currently, it is widely commented by vascular surgeons that allografts have significant complications. In our series, classical complications as rupture or dilation were observed in an impressive lower rate than expected. This could be explained by an improvement in cryopreservation techniques, but more studies should be conducted, controlling specific variables related to the cryopreservation technique.

On the other side, a low infection rate has been considered as one of the strong point of allografts. For that reason, European society of vascular surgery and the American society of vascular surgery accepted allografts as a suitable alternative for

replacement of infected grafts in aortic position. Although our study does not include cases of aortic surgery, our results show as well a low infection rate.

To sum up, the so-called classical complications seem to show that beliefs about allografts are not totally right. However, survival rate, primary patency rate, limb salvage rate and MALE-free rates must be analyzed to have a better understanding of the behavior of arterial allografts.

Conte et al. consider the MALE rate as clinically relevant outcome, and they suggest that the introduction of this measure could be a key strategy to assess the evolution of revascularization surgeries in patients with CLI. To our knowledge, our cohort is pioneer in using this end-point in patients with CLI revascularized with arterial allografts.

Chronic Limb-threatening Ischemia (CLI), the most advanced expression of atherosclerotic pathology in the lower limbs has been associated with complications that go beyond the major adverse limb events affecting the revascularized extremity. CLI requires the planning of surgery in order of improve the symptoms and the limb salvage rate. After this surgery, it is widely known that an alarming high rate of cardiovascular events and major adverse limb events, including reinterventions, could be needed.

Our large retrospective study found large differences in the outcome according to the end-point being analyzed. All this information is relevant when the physician exposes the therapeutic options to the patient who must understand the process by which he will go through in the years following the surgery for resolution of the CLI

That is why our group has analyzed the rate of major adverse limb events in the patient as an indicative measure of the route that the patient will go through in the next years after revascularization.

Nevertheless, it is important to highlight 2 important points, the first one is that there are no previous studies comparing MALE-free rate in patients with CLI surgery with allografts, which does not allow us to compare with other therapeutic options or even with revascularization using another type of grafts. The second one is that the high 5-year rate of 78.5% of major adverse limb events is a clear indicator of an appalling evolution of the patients.

Although there is not available data in the literature to compare MALE rates with other types of revascularization or graft, several factors are involved in these results. Our study reinforces the role of dyslipemia as a risk factor but also it is important to consider the nature of the atherosclerotic pathology as a systemic disease.

The association of these three factors (Chronic Limb-threatening Ischemia, the absence of autologous vein and surgery) may lead us to assume that these patients have a complex management and comorbidity including atherosclerotic disease in other territories.

Although patient complexity could be an explanation for this this appalling evolution, another factors, such as the type of graft chosen for revascularization, or the selection of an open or endovascular technique could also be important. In the absence of similar publications, we cannot make comparisons and new studies are needed to clarify this influence.

Regarding limb salvage rate, which is one of the most studied end-points in cases of lower limb revascularization and more information is available, Ziegler et al. published a pool data from papers in English from 1990 to 2009 (excluding those series related to reintervention) and he exposed long-term limb salvage rates ranging from 57%-70% for PTFE and 89% in revascularizations with autologous saphenous vein.

When comparing to our results where the 5-year limb salvage rate is 50.2%, it could represent an overall inferiority of allografts in front to the PTFE for this outcome.

When looking for the source of the information for the paper of Ziegler et al. we find the publication of Berlakovich et al. This publication excludes from the analysis all patients with technical error, which was considered as any case with occlusion in the first 72 hours. Also, these patients were treated with anticoagulation or antiaggregation systematically in all the cases. Some other sources of this publication only include femoro-popliteal bypass.

Our cohort consisted of patients revascularized to distal territory in 74.2% of the cases, we included any occlusion or event even happening in the first 24 hours and only 79% of our patients were were taking antiaggregation or anticoagulation drugs. Although this could explain our lower limb salvage rate, the difference between cohorts makes them not comparable. Also, it is important to consider that our data at 5 years for this end-point is weak due to high percentage of lost patients, and therefore, more studies are required.

To our knowledge, our publication is the first exposing a 5-year limb salvage rate in patients with CLI revascularized with arterial allografts. There are no series exposing results to 5 years in those patients requiring lower limb revascularization with no autologous saphenous vein available.

About the primary patency, our cohort showed a 5-year rate of 38.6%, clearly inferior to the known rates for autologous saphenous vein and PTFE prosthesis, which are round about 72% and 44%, respectively. The nature of the allografts and the so-called "vasculopathy of allografts" could be a conditioning factor for a greater rate of occlusion in the short and long term. Unfortunately, the immunosuppressive therapy has

no effect on the permeability of cryopreserved allografts, as demonstrated by Carpenter et al.

Seon-Hee Heo et al. published results of a 10-year cohort comparing autologous vs. PTFE and showed a primary patency rate near 42% without statistically significant differences (P = 0.330). Almost all the patients in the Seon-Hee Heo group who were treated with PTFE were systematically anticoagulated and the revascularization was landed in the popliteal artery. Therefore, controlling factors related to coagulation or aggregation could be a strategy for revascularizations with allografts that could traduce in better primary patency. Nevertheless, is important to consider the comorbidity of the "vascular patients" and the consequences of this strategy.

Arm vein autograft has also been studied in revascularisation surgery. These conduits have demonstrated primary patency of 42% at 3 years. When comparing with our 43.8%, no clear indications of arm vein over allograft can be stablished. As neither of these grafts are used very commonly, it would be difficult to perform a study to properly compare them.

In term of survival rate, our results are consistent with the average evolution of patients with critical limb ischemia. Our data demonstrated that smokers and older patients could have a worse evolution. A wide sample for risk stratification by age could answer which is the cut-point to consider as a risk factor. Also, smoking cessation must be recommended to patients revascularized with allografts. Although this is an acceptable recommendation to improve the health of any patient, especially "vascular patients".

Finally, our analysis of predictor factors correlates with the known literature reporting that the more distal the revascularization the less success rate. According to

our results, distal revascularization has a lower primary patency and limb salvage rate. This could be because the patient who requires distal revascularization with an allograft due to the absence of native vein is precisely the one with aggressive pathology and/or who has exhausted other options that have a better outcome.

CONCLUSION

Several recent publications have exposed cryopreserved arterial allografts as an attractive alternative in patients with critical ischemia requiring revascularization in the absence of autologous saphenous vein. However, our results do not demonstrate superiority of allografts over other grafts in term of limb salvage. Survival rate in patients revascularized with allografts is consistent with the expected for patients with CLI. Primary patency of allografts for infra-inguinal revascularization seem to be lower than autologous vein and PTFE but similar to arm veins. In the absence of a comparable cohort, it is not possible to compare the rate of major adverse events in the patient or the MALE rate of these patients in respect to another type of graft. Classical complications of vascular allografts seem to be related to older cryopreservation techniques and they are that frequent as expected. More studies are needed to clarify outcomes of arterial allografts.

13. DISCLOSURES

None.

14.- SPANISH SUMMARY

Visión histórica de los aloinjertos

Primera Etapa.

La donación y el trasplante de órganos, tejidos y células presenta en la actualidad un gran auge que involucra prácticas terapéuticas aceptadas como comunes y de primera indicación como la donación riñón e hígado; pero también involucra prácticas un poco menos llamativas como el trasplante de tejidos.

El trasplante de tejido vascular cuenta cada vez con más indicaciones. Se inició a principios del siglo XX y ha pasado por varias etapas; desde la publicación de experiencias preliminares, posteriormente se abandonó su uso al describirse complicaciones a largo plazo para retomarse de nuevo a finales de siglo en indicaciones especiales y sujetas a un profundo análisis.

A pesar que en 1903, Hopfner había descrito la posibilidad de realizar experimentalmente la técnica, la historia del trasplante de tejido vascular nace con Alexis Carrel. Biólogo, médico e investigador de origen francés, considerado actualmente como un pionero en la cirugía vascular. En 1905 describe técnicas quirúrgicas en animales desde trasplantar segmentos venosos en territorios arteriales y describir el llamado proceso de arterialización hasta exponer sus logros en la conservación de vasos sanguíneos para trasplantar y evitar la espera de un posible donante.

En 1906, aparece publicado en *Siglo Medico* la descripción de 2 casos de derivación con utilización de vasos autólogos. Goyanes realizó una derivación Femoro-poplíteo con vena femoral y en el segundo se describe la resección de un aneurisma

sifilítico poplíteo y posterior reemplazo por vena poplítea, resultando ser los dos primeros casos con éxito en el ser humano.

Un año después, Erich Lexer, Profesor de Cirugía en Königsberg, describe el trasplante de un segmento de 8 centímetros de vena safena autóloga después de la resección de un aneurisma de arteria subclavia izquierda causado por la luxación del húmero. De esta forma quedan descritos los primeros pasos en el trasplante de tejido vascular autólogo y se inicia la primera etapa del trasplante de tejido vascular.

Otros casos aislados de publicación son el descrito por Pirovano en 1910 (Primer trasplante de aloinjertos vasculares) aunque sin éxito y Moure en 1914, quien registró 17 trasplante de injertos venosos con buenos resultados.

En 1908, Alexis Carrel realiza un avance importante al crear el primer banco de vasos sanguíneos de uso experimental, que condujo sus investigaciones a ser galardonadas en 1912 con el premio Nobel de Medicina en reconocimiento a su trabajo acerca de sutura vascular y trasplante de vasos sanguíneos y de órganos.

Este mismo año, también publica un artículo donde demuestra que una porción de arteria puede conservarse y mantenerse “viva” en una cámara fría durante varios días o incluso semanas antes del trasplante. Comenta que vasos sanguíneos de perros conservadas en una cámara frigorífica pueden ser trasplantadas con éxito en gatos y concluye afirmando que la aplicación estos métodos son trasladables a humanos y en todo caso no debe tardar. Pero no es hasta 1951 cuando Fontaine y Leriche fundaron el primer banco de vasos sanguíneos para uso clínico.

Segunda Etapa.

La segunda etapa de los trasplantes de tejido vascular se caracteriza por un declive importante en su uso, por lo poco satisfactoria que resultó a largo plazo su utilización, posiblemente debido a las técnicas deficitarias de preparación y conservación. Se describieron casos de alteraciones degenerativas con posterior dilatación aneurismática, relacionadas a respuestas inmunológicas. Todo ello sumado al nacimiento de los estudios con prótesis sintéticas.

En relación a las prótesis, el primer acontecimiento data de 1952 cuando Voorhees, Jaretzky y Blakemore realizan estudios en animales y demuestran la permeabilidad de derivados sintéticos en territorio vascular, sin tardar en trasladar su aplicación a la práctica clínica. Esta técnica presenta una rápida evolución. Entre los materiales que se utilizaron figura el Ivalón, Orlón, Nylon, Teflón y Dacron. En 1969 surge la prótesis de mayor trascendencia y uso clínico actualmente en manos de Gore, quien a partir del teflón obtiene la llamada membrana de Politetrafluoretileno Expandido ó PFTE.

Desde su nacimiento las prótesis intentaron ser elaboradas bajo condiciones ideales que en 1953 se describieron como: “biológicamente inerte, de propiedades físico-químicas estables, de esterilización garantizada, fácil manejo, no cancerígena y con superficie no trombógena”; criterios que posteriormente fueron modificados por Moneta y Porter en 1997 cuando describen la prótesis ideal como aquella “que sea fuerte, no costosa y susceptible de utilizarse durante toda la vida del enfermo, de inserción fácil y permanente, biocompatible con el huésped, resistente a la infección, con calibres apropiados, que permanezca permeable con propiedades visco elásticas semejantes a una arteria natural sin dejar escapar sangre o suero, que no degenere, sin

suscitar una respuesta proliferativa anormal del vaso o tejido circundante, no trombogénica ni embolígena, que no se ocluya al flexionarse ni dañe los componentes de la sangre".

Estos criterios dejaban en evidencia grandes defectos de los aloinjertos vasculares; sin embargo, ningún sustituto arterial sintético lograba, ni logra en la actualidad, cumplir con todas estas condiciones.

No obstante, hay logros importantes en esta segunda etapa como el de Gross en 1948, quien reemplaza por primera vez un segmento de aorta homóloga en un caso de coartación, estableciendo la técnica como la primera elección hasta la aparición de las prótesis. Así, Oudot en 1950 fue el primero en reemplazar la bifurcación aórtica y Dubost en 1951 el pionero en emplear un homoinjerto tras la resección de un aneurisma abdominal.

Durante esta etapa, algunos grupos como el liderado por DeBakey publicaron series de tan grande tamaño que incluso en la actualidad no se han logrado igualar.

En la actualidad, gracias a los avances en técnicas de recolección, procesamiento, conservación y almacenamiento de injertos (ahora denominados aloinjertos vasculares) y al progreso en la donación y trasplante de órganos, criobiología, inmunobiología, determinaciones de histocompatibilidad y terapéutica inmunosupresora, están siendo reconsiderados para su uso cada vez en mayor número de indicaciones, inaugurando así la tercera etapa del trasplante de tejidos vasculares. Todo ello en el marco de la existencia de prótesis comerciales que son actualmente la primera indicación en casi la totalidad de casos de sustitución de segmentos vasculares.

Tercera Etapa.

Se inicia nuevamente su uso con cautela y con indicaciones limitadas. Dentro de estas indicaciones está el reemplazo de segmentos arteriales complejos, lesiones vasculares complejas, sustitución de prótesis arteriales infectadas y aunque, poco estudiada, la utilización en accesos vasculares para paciente en hemodiálisis, entre otros usos particulares descritos. Carlos Mestres ha resumido el uso de los mismos en 3 indicaciones que resulta interesante estudiar a largo plazo y confirmar sus resultados con respecto a la reducción de la mortalidad hospitalaria, alta tasa de permeabilidad y mínima tasa de reinfección y rotura a 10 años.

En esta tercera etapa, la inmunología es objetivo de investigaciones, por ejemplo Koene RA en 1989 describió el rol de la adaptación en la aceptación de aloinjertos. Concluyó que la supervivencia a largo plazo del aloinjerto depende de estas respuestas inmunológicas. Previamente Prendergast FJ et al. en el trabajo titulado *"Vein to Artery Allografs"* exponían la experiencia observada en sensibilidad inmunológica y concluyeron que los aloinjertos generaban respuestas inmunológicas.

Mucho más reciente ha sido la descripción de esta respuesta inmunológica. Y exposición del papel que juegan las células de la inmunidad innata y anticuerpos en el rechazo de estos injertos. La ahora llamada "vasculopatía de los aloinjertos" se ha vinculado a la respuesta inflamatoria crónica mediada por Linfocitos Natural Killers, pero llevada a cabo directamente por anticuerpos específicos contra el donante que finalmente inducen cascadas intracelulares facilitando reclutamiento de monocitos y neutrófilos y dañando el tejido trasplantado. Nuevas líneas de investigación se mantienen abiertos en esta dirección.

Estos trabajos dieron origen a la aplicación experimental de trasplante de tejido y al desarrollo de modelos animales y plataformas de investigación. En 1983, Chow SP et al. realizó el reemplazo de arterias femorales por tejidos criopreservados y comparó con reemplazo de tejidos autólogos, concluyendo que ambas técnicas tienen un comportamiento similar.

En Portugal 1997, Neves et al. publicaron sus avances acerca de mecanismos de degeneración de los injertos criopreservados, analizándolos en un modelo experimental de ovejas. Concluyeron que existe pérdida parcial del endotelio e invasión linfocítica de todo el injerto, a pesar de lo cual los injertos mantienen su integridad y viabilidad celular después del trasplante. Evidenciaron la re-epitelización del injerto y tras un corto periodo de degeneración neural acontece uno de reinervación. Es de resaltar que no se encontraron diferencias estadísticamente significativas entre los trasplantes de tejido vascular fresco con respecto a aquellos que fueron criopreservados.

Dentro de los estudios alternativos en el campo traslacional está el modelo sobre injertos venosos tratados con glutaraldehído que realizó Moura en 2009 en conejos cuyo injertos se valoraron macroscópica y microscópicamente a las 24 horas, 14 y 28 días. Sugiere en sus conclusiones, la necesidad de ampliar estudios en materia de trasplante de tejidos autólogos ya que evidencia que no hay diferencias claras en la técnica propuesta por él. Igualmente expuso que el tema podía proveer una importante herramienta para el uso en humano.

Tres de los trabajos de índole traslacional más recientemente publicados son los de Hong Sun en 2010, Seong-Jun Hwang en 2011 y Olmos-Zúñiga en 2016. El primero de ellos explica una técnica mejorada para realizar trasplantes aórticos en modelo murino y demuestra cambios patognomónicos de rechazo crónico, pero con

supervivencia del tejido a más largo plazo que las técnicas habituales. Por otro lado, Hwang diseñó una plataforma experimental para el desarrollo de microvasculatura biocompatible en ratas y demostró que su modelo resulta potencialmente trasladable y efectivo para futuras investigaciones en ingeniería de tejidos de vasos de pequeño tamaño.

Olmos-Zuñiga et al. publica los resultados hemodinámicos, gasométricos, imagenológicos y hallazgos macroscópicos y microscópicos de la reconstrucción de arterias pulmonares de perros con injertos liofilizados (no tratados con glutaraldehído) e injertos arteriales criopreservados. Sugiere que las técnicas de liofilización pueden jugar a favor de una menor antigenicidad, así como prevenir trombosis y calcificación de los injertos. Finalmente concluyen que la liofilización sin tratamiento con glutaraldehído representa una alternativa factible con resultados clínicos prometedores.

En práctica clínica, se han publicado casos aislados o serie de casos. Entre las aplicaciones que se describen se encuentra la corrección de coartación de aorta toraco-abdominal con injerto autólogo arterial criopreservado, realizado en un niño de 7 años, con postoperatorio correcto, ecografía doppler control a corto plazo sin cambios significativos con respecto a un sujeto sano y con valores clínicos y correcta calidad de vida posterior al procedimiento.

Así mismo, en 1998 se publicó en Annals of Vascular Surgery una seria amplia que valoraba diferencias entre tejidos vasculares criopreservados y frescos donde se concluyó que no había diferencias estadísticamente significativas entre ellos. Describen una supervivencia del tejido de 73% a los 12 meses e informan de la posibilidad de valorar compatibilidad ABO entre los donantes. Esta posible incompatibilidad ha sido desestimada en 2015 por Della Schiava et al, quienes consideran que la respuesta

inmunológica puede estar vinculada principalmente a incompatibilidad del sistema mayor de histocompatibilidad. De esta manera empiezan a cobrar importancia las variables clínicas asociadas al paciente donante y receptor de aloinjertos vasculares.

Clásicamente, este tipo de injertos se han utilizado en la sustitución de segmentos protésicos infectados. Así se puede evidenciar en el Journal of Vascular Surgery de Mayo 2004, donde se presenta el reemplazo de injertos infectados en la aorta infrarrenal por aloinjertos vasculares. Con un periodo de estudio de 14 años, presentan lo que puede ser la serie más amplia y de más largo seguimiento publicada en Europa.

Durante este periodo Kieffer E. et al. en Paris concluyen que los aloinjertos vasculares son, a corto y largo plazo, al menos similares en comportamiento con respecto al uso de otras técnicas de reemplazo en cuanto al manejo de infecciones de prótesis infrarrenal, así como que la mayoría de las complicaciones asociadas a este tipo de injertos son evitables con un adecuado proceso de criopreservación. Previamente en 2001, Leseche G. et. al habían comentado la utilidad del uso de aloinjertos vasculares en infecciones protésicas y anteriormente en 1996, Koskas et al. exponían 6 años de experiencia sustituyéndose prótesis infectadas de 83 casos con varias complicaciones postoperatorias pero con una tasa de supervivencia de extremidades de 100%.

En Chicago 2009, Katherine Brown et al. publicaron sus resultados a mediano plazo de la reconstrucción arterial con tejido vascular criopreservado en casos de infecciones de prótesis. Presentaron una serie de 52 pacientes en un plazo de 10 años que coloca a la sustitución de prótesis infectadas por aloinjertos vasculares como una alternativa viable y describe que, con una adecuada criopreservación, los aloinjertos son resistentes a reinfección, trombosis y dilatación aneurismática; sin embargo,

recomiendan un estudio a largo plazo que valore si resulta esta técnica como la más exitosa, eficaz y segura.

Más recientemente, en Grecia, Locati et al. publicaron una serie corta de 18 pacientes donde se sustituyeron 25 prótesis infectadas en distintos territorios (femoro-poplíteo, aorto-ilíaco, y subclavio) concluyendo que son técnicas de gran utilidad en esta indicación, ya que estos injertos parecen tener mayor resistencia a la infección.

En 2010, un equipo alemán liderado por Bisdas T, publicó un seguimiento a 8 años de pacientes tratados con homoinjertos arteriales criopreservados y expone la proteína C reactiva y los leucocitos como parámetros analíticos para monitorizar el postoperatorio inmediato de estos injertos, y propuso las plaquetas y temperatura corporal como parámetros clínicos de importancia en el postoperatorio. Reporta un 81% de sobrevida del tejido trasplantado a los 3 años y libre de re-intervenciones. En los restantes, describe oclusiones, estenosis, degeneraciones aneurismáticas y fístulas injerto-duodenales. Concluye que es una alternativa útil.

Aplicaciones más inusuales como el aumento de la arteria pulmonar en un trasplante pulmonar han sido descritas por Pablo Rueda en 2005. Utilizando tejido aórtico realizó la ampliación de la arteria pulmonar en un caso de inadecuada extracción del órgano y concluyó que la técnica resultaba útil para evitar la pérdida del órgano.

El uso como injerto en la construcción de acceso vascular para hemodiálisis fue descrito en 2016 por Tae-Yong Ha et al. como una alternativa para diálisis inmediata y con una supervivencia de la técnica comparable con otro tipo de injertos.

No obstante, no todos los resultados publicados son tan positivos. En Italia 2011, Ravenni G et al. reportaron un caso de total calcificación de un injerto vascular homólogo utilizado en el reemplazo de la raíz aórtica en un hombre de 66 años.

En esta misma línea, Minga Lowampa E et al. publican una serie de 103 pacientes con sustitución de prótesis infectadas por aloinjertos cuyos resultados a corto plazo son desfavorables con una alta tasa (29%) de complicaciones postoperatorias (trombosis del injertos, pseudoaneurisma anastomótico, degeneración aneurismática y ruptura del injerto entre otras). Sin embargo, los autores apuntan maniobras que podrían mejorar estos resultados.

Aloinjertos en las guías de práctica clínica.

En lo que respecta a los aloinjertos vasculares se deben establecer diferencias entre los aloinjertos venosos y arteriales. Los aloinjertos venosos no tienen actualmente ninguna indicación apoyada por guías de práctica clínica. Su aceptación es baja y pocas publicaciones han surgido en la última década.

Los aloinjertos arteriales continúan siendo investigados y varias publicaciones han surgido recientemente. En la actualidad, solo se ha establecido una indicación de aloinjertos vasculares en las guías clínicas. La Sociedad Europea de Cirugía Vascular y la Sociedad Americana de Cirugía Vascular han considerado a los aloinjertos como una alternativa adecuada para la sustitución de injertos infectados en posición aórtica.

Otras indicaciones fuera de guías clínicas han sido publicadas recientemente. Los aloinjertos arteriales se han utilizado como técnica de recurso en el acceso vascular para hemodiálisis. En pacientes con isquemia critica, los aloinjertos se han descrito como injertos adecuados y con resultados similares a las prótesis. A pesar del uso generalizado en todo el mundo, todos estos usos están fuera de guías de práctica clínica. Cada caso, indicación, paciente y territorio debe evaluarse individualmente, y más datos y estudios son necesarios para aclarar las indicaciones correctas.

Por otro lado, las válvulas cardíacas se han utilizado constantemente desde los años 60 hasta hoy. La válvula cardíaca (aloinjerto) tiene una amplia aceptación y se han publicado varias investigaciones recientes. Sin embargo, no se ha establecido una indicación clara en guías de práctica clínica.

Aloinjertos en la isquemia critica de extremidades inferiores.

La revascularización de una extremidad inferior con isquemia crítica es la única alternativa que ha demostrado reducir los síntomas, ya sea dolor en reposo, úlceras que no cicatrizan o necrosis establecida. La cirugía de revascularización también ha demostrado ser la mejor opción para obtener una más alta tasa de salavamento de la extremidad. En cirugía vascular, actualmente, ante un procedimiento de revascularización se deben evaluar la alternativa endovascular y cirugía abierta.

Las técnicas endovasculares han ganado una amplia aceptación como tratamiento primario y secundario de la isquemia crítica de las extremidades inferiores. A pesar de un gran número de indicaciones establecidas y muchas otras indicaciones emergentes, muchos pacientes requieren cirugía abierta con bypass de extremidades inferiores para tratar de garantizar el éxito terapéutico.

La vena safena autóloga está ampliamente aceptada como el injerto preferido para la revascularización de las extremidades inferiores. En pacientes que requieren un bypass de la extremidad inferior, se estima que del 20 al 45% no tiene un solo segmento de vena safena autóloga disponible para ello. La revascularización previa de coronarias, de extremidades inferiores, varices de extremidades inferiores, la cirugía previa de várices y/o la calidad inadecuada del vaso nativo debido al calibre o la longitud

necesaria son las principales razones por las que los pacientes podrían enfrentar este escenario.

En este escenario, el cirujano vascular debe considerar el uso de injertos alternativos, ya que las principales opciones son venas autólogas de lugares menos usuales, prótesis sintéticas y aloinjertos vasculares. Sin embargo, para la revascularización aorto-ilíaca, existen varios tipos de injertos y técnicas alternativas, la mayoría de ellos con excelentes resultados. En esta ubicación, las ventajas de los aloinjertos sobre otros injertos se limitan a una tasa de reinfección más baja. Por este motivo, los aloinjertos se incluyen en las directrices internacionales como una opción adecuada para el reemplazo de injertos infectados aorto-ilíacos.

Para el territorio femoral, podríamos considerar que los aloinjertos son resistentes a la infección más que otros injertos. Sin embargo, no se ha establecido una indicación clara y otros injertos se utilizan ampliamente en lugar de aloinjertos. La revascularización endovascular ha demostrado jugar un papel importante en la revascularización de la arteria superficial femoral. No se han establecido indicaciones de aloinjertos para la revascularización de la arteria femoral superficial y la mayoría de los injertos disponibles tienen mejores resultados.

Varios grupos han considerado que la prótesis de PTFE para la revascularización de troncos distales es una técnica incorrecta. Pocos grupos han usado aloinjertos para ello. Sin embargo, no hay suficientes datos disponibles para apoyar esta indicación

Válvulas cardiacas criopreservadas y el procedimiento de Ross.

El procedimiento de Ross es una técnica quirúrgica en la cual la válvula aórtica se reemplaza con la válvula pulmonar autóloga y esta última se reemplaza a su vez por un aloinjerto. El inicio de esta técnica se remonta a 1967 cuando Donald Ross realizó por primera vez esta cirugía. Sin embargo, inicialmente, la técnica de Ross no ganó una gran popularidad hasta que se introdujo una modificación a la técnica a fines de los años ochenta. En ese momento, el procedimiento de Ross experimentó un resurgimiento cuando el conocimiento de los cirujanos que aplicaban "*botones coronarios*" para las operaciones de la raíz aórtica decidieron realizar un reemplazo de autoinjerto pulmonar utilizando la técnica de "*raíz completa*".

Hoy en día, la mayoría de los cirujanos realizan la técnica de reemplazo de raíz, mientras que el uso de la técnica de implantación sub-coronaria inicial rara vez se aplica.

La prótesis de válvulas aórticas está bien desarrollada con muchas marcas y opciones disponibles. La técnica quirúrgica ha sido constantemente mejorada. Sin embargo, los pacientes con prótesis mecánicas están expuestos a anticoagulación permanente, hemodinámica restringida y un mayor riesgo de tromboembolismo y endocarditis.

En este escenario, el reemplazo de la válvula aórtica con la válvula pulmonar propia del paciente juega un papel importante. A través de esta técnica, el paciente no está expuesto a la anticoagulación permanente y se han observado buenos resultados funcionales a largo plazo, incluso en procedimientos combinados y presentaciones clínicas complejas, todo ello fomenta el uso continuo.

En contra la sustitución de la válvula aórtica con autoinjerto (válvula pulmonar), la mayoría de los cirujanos no tienen intención de tomar morbi-mortalidad adicional

debida a la curva de aprendizaje o por deterioro de la válvula pulmonar. Además, se expresaron preocupaciones sobre si la válvula pulmonar resiste el estrés hemodinámico de la posición aórtica.

Actualmente, los expertos recomiendan la técnica de Ross porque el autoinjerto pulmonar muestra tasas bajas de degeneración, endocarditis y tromboembolismo durante un período> 20 años en comparación con las válvulas de reemplazo biológicas o mecánicas actuales. El procedimiento de Ross se asoció con una mejor hemodinámica de la válvula aórtica. La técnica representa una buena opción que se puede realizar de manera segura en pacientes pediátricos con enfermedad de la válvula aórtica, incluso en un centro de pequeño volumen de casos. Además, la técnica Ross se considera una solución duradera para la obstrucción multinivel del tracto de salida del ventrículo izquierdo en pacientes muy complejos quienes tienes cirugías previas.

Registro de Trasplante Cardiovascular de Cataluña.

Para la creación del "Registro de trasplantes de aloinjertos vasculares y valvulares en la comunidad autónoma de Cataluña, España (ReVAC)" se utilizó la ténica de grupos focales. La idea surgió en Junio de 2013 bajo la coordinación de la Organización Catalana de Trasplantes y los Drs. Jose Luis Pomar Moya-Prats y Kerbi Alejandro Guevara Noriega, en el marco de la realización de esta tesis doctoral. Las autorizaciones administrativas y legales se obtuvieron durante el primer trimestre de 2014.

Un total de 10 centros médicos participaron en el estudio: Centro Médico Teknon, Hospital Universitario Vall d'Hebron, Hospital Universitario Germans Trias i Pujol, Fundación Puigvert, Hospital de la Santa Creu i Sant Pau, Hospital Universitario de Bellvitge, Hospital

Universitario Sant Joan de Déu, Hospital materno-infantil Vall d´Hebron, Hospital Clínic i Provincial de Barcelona, Hospital Juan XXIII Tarragona.

Se ejecutaron 2 fases: La fase 1 corresponde al diseño del ReVAC y en la segunda fase se realizó el análisis de los datos recogidos.

Fase 1: Diseño de registro. Para su concepción, se realizó la "técnica de grupos focales de trabajo". Se organizaron 3 subgrupos según los requisitos clínicos, administrativos y técnicos. En esta fase, todos los diferentes hospitales estuvieron involucrados.

Grupo focal 1: Variables relevantes

Grupo focal 2: Diseño informático del registro.

Grupo focal 3: Plan de recolección de datos y diseño.

La recopilación de datos fue apoyada por la Organización Catalana de Trasplantes. Hubo un formulario estandarizado para la recolección de datos. Solo se consideró válida una prueba con informe en mano o digital. KAGN y otros 2 miembros del equipo de recopilación de datos recopilaron información de los informes médicos.

Debido a la naturaleza retrospectiva de este estudio, el seguimiento se basó en los exámenes y visitas registrados al paciente en su centro de referencia. Se consideró válido para cualquier visita, prueba de ultrasonido o ingreso hospitalario.

Se realizó una revisión de la técnica de procesamiento y criopreservación realizada por los bancos de tejidos de Cataluña para conocer el protocolo específico relacionado a los aloinjertos analizados. Además, una revisión sistemática de Ziegler et al. se utilizó para la comparación con otros injertos y se expusieron datos comparativos en tablas.

Los autores afirman que no se han realizado experimentos con seres humanos o animales para esta investigación, no se exponen datos personales de pacientes y no hay conflicto de intereses.

Este proyecto ha sido sometido a una evaluación ética realizada por el comité de ética del "Hospital Clinic i Provincial de Barcelona". No hay fuentes de financiación.

Los datos introducidos en el ReVAC son confidenciales. El registro está disponible a través de la página web de la Generalitat de Catalunya. Se requiere una autorización específica del departamento de salud de "Generalitat de Catalunya" para recopilar información del registro. Los datos personales y digitales en España están protegidos de acuerdo con la Ley Orgánica para la protección de datos personales y la garantía de derechos digitales (BOE-A-2018-16673). La revisión se llevó a cabo de acuerdo con las regulaciones vigentes y respetando la confidencialidad de la información del paciente.

El análisis estadístico se realizó utilizando SPSS Ver. 20 para Mac (Chicago USA). Los datos descriptivos se presentan en frecuencias relativas y / o absolutas. De acuerdo con los objetivos, se analizó toda la cohorte de pacientes trasplantados con aloinjertos vasculares. La información sobre la indicación y el segmento vascular trasplantado se muestran en frecuencias absolutas y relativas.

Se registraron variables demográficas y clínicas como edad, sexo y comorbilidad (hipertensión arterial, diabetes mellitus y dislipidemia). Para los aloinjertos vasculares, la supervivencia acumulada del paciente, la tasa de recuperación de la extremidad y las tasas de permeabilidad del injerto se evaluaron mediante el método de Kaplan-Meier a los 30 días y anualmente hasta los 5 años. Se realizó un análisis adicional de la oclusión global y la tasa de infección.

Teniendo en cuenta la relevancia clínica, se incluyó un análisis específico para la revascularización infrainguinal y también se evaluó la tasa de permeabilidad, la tasa de recuperación de la extremidad y la tasa libre de eventos mayores en la extremidad. Se realizó un segundo análisis en este grupo, que incluyó un modelo de regresión de Cox para determinar los factores de riesgo para la supervivencia, la tasa de permeabilidad primaria, la tasa de recuperación de la extremidad y la tasa de eventos mayores en la extremidad.

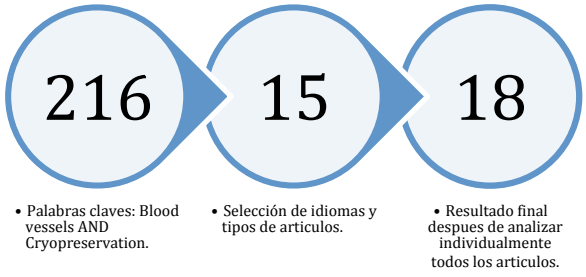
Se usó el nivel de $P < 0.05$ para determinar la significación estadística. Se seleccionaron intervalos de confianza del 95% para los índices de riesgo.

Revisión bibliográfica sobre criopreservación y conservación de tejidos.

Se realizó una búsqueda en PubMed Medline sobre publicaciones hasta diciembre de 2015 con la intención de identificar todas las revisiones, guías de práctica clínica, ensayos y metanálisis relacionados con la criopreservación de tejidos y las técnicas de conservación. Para su inclusión, la publicación tenía que estar escrita en inglés o español. Se utilizaron el término MeSH "blood vessels" y "cryopreservation" (y sinónimos). Las listas de referencias de los artículos detectados inicialmente se

analizaron manualmente para identificar publicaciones adicionales que resultaran relevantes.

Para su análisis, los estudios debían contener información suficiente y clara sobre el proceso de conservación y criopreservación. Un investigador (KAGN) seleccionó los artículos y los analizó. Se evaluó la calidad metodológica de los trabajos seleccionados. Finalmente, las ideas más comunes sobre criopreservación y conservación se extrajeron y expusieron en los resultados.



Trasplantes realizados

Un total de 1084 registros están disponibles en el ReVAC. Sin embargo, solo 397 registros tienen información válida para el análisis. De los 397 pacientes trasplantados: 229 (57,7%) fueron segmentos arteriales y 168 (42,3%) válvulas cardíacas. De los 229 pacientes trasplantados con segmentos arteriales, el 82,1% (188) eran adultos y el 17,9% (41) eran pacientes pediátricos. El 47,62% (80) de los pacientes con válvula cardíaca eran adultos y el 52,38% (88) eran pacientes pediátricos

Un total de 171 adultos fueron trasplantados con segmentos arteriales con una edad promedio de 69.1 +/- 11.08 años, el 72.3% (125) eran hombres. La indicación quirúrgica fue isquemia crónica grado III y IV de extremidades inferiores sin venas safenas autólogas disponibles como conducto para la cirugía en el 79,2% (149) de los

casos, el reemplazo de prótesis infectada o la revascularización infectada fue la indicación en el 9,9% (17). el reemplazo previo de aloinjerto representó el 5.3% (9) y la corrección de la lesión vascular compleja 3.5% (6). Otras indicaciones bajo criterios del cirujano se realizaron en el 4,1% de los pacientes.

El segmento arterial trasplantado fue aorto-ilíaco en 15 casos (8,8%), segmento largo que involucra arteria femoral y poplítea sin vasos distales en 131 casos (76,6%), 17 (9,9%) casos adicionales con arteria femoral y poplítea con vasos distales asociados. En los otros casos, no se identificó el segmento específico utilizado para el trasplante. En la cohorte pediátrica, la arteria femoral representa 28 casos (68,3%) y 13 casos (31.7%) no tienen información sobre el segmento trasplantado.

Se trasplantaron 80 pacientes adultos de válvulas cardiacas con una edad media de 46,9 +/- 11,51 años, 68,8% (55) hombres. La indicación quirúrgica fue estenosis aórtica 36,3% (29), insuficiencia aórtica 22,5% (18), lesión aórtica doble 12,5% (10), endocarditis 10% (8), dilatación del aneurisma de la válvula aórtica 3,8% (3), reintervención de la tetralogía de Fallot 3,8% (3) e infecciones protésicas 2,5% (2). Otras indicaciones específicas se realizaron en 6 pacientes.

Los resultados y las complicaciones de los aloinjertos vasculares están expuestos por nivel anatómico revascularizado. La revascularización infrainguinal se realizó en 149 pacientes (87.13%) y suprainguinal en 15 (8.77%). Otros casos (7: 4,1%) corresponden a cirugías que no involucran las extremidades inferiores. Tablas y curvas de Kaplan meier son expuestos en la sección de resultados. Los resultados hemodinámicos son de las válvulas cardiacas trasplantadas son expuestos en tablas en la sección de resultados.

La tasa de supervivencia de los pacientes trasplantados con aloinjertos arteriales en el territorio suprainguinal fue del 60% a los 12 meses y del 37,5% a los 60 meses. La tasa de supervivencia de los pacientes trasplantados con aloinjertos arteriales en el territorio femoropoplíteo fue del 87,5% a los 12 meses y del 75,1% a los 36 meses. Las tasas de supervivencia para la revascularización distal fueron del 92,4% a los 12 meses y del 55,6% a los 60 meses. Sólo 3 muertes pueden estar directamente relacionadas con el trasplante

La tasa de supervivencia de los pacientes trasplantados con aloinjerto valvular fue del 98,6% y del 97,1% a los 12 y 60 meses, respectivamente. Dos muertes intraoperatorias y 2 casos adicionales podrían atribuirse al trasplante.

Protocolo de seguimiento de pacientes trasplantados con aloinjertos arteriales.

A.- Pruebas previas al trasplante: deben agregarse el grupo sanguíneo, grupo Rh, proteína C reactiva (PCR) y velocidad de sedimentación globular (VSG).

B.- Ultrasonido Doppler: 1 mes, 6 meses, 12 meses y luego anualmente.

C.- Evaluación clínica: Pre-alta, 1 mes, 3 meses, 6 meses, 12 meses y luego anualmente.

D.- AngioTC para injerto con alteraciones hemodinámicas detectadas con ultrasonido doppler.

E.- Prueba postrasplante: PCR y VSG diariamente hasta el día 5 y luego semanalmente hasta la semana 5.

Datos importantes a considerar.

Existen datos limitados de la evolución a largo plazo de este tipo de injertos. En este marco, las investigaciones prosiguen y grupos de investigación intentan ponerse a la vanguardia del tema, así como también se han centrado en lograr importantes avances en criopreservación, inmunología y alternativa de preparación del tejido.

Solo el reemplazo de prótesis aortica infectada ha sido establecida como indicación en guías de práctica clínica. Por tanto, se debe valorar individualmente cada caso, indicación, paciente y territorio siendo necesario más datos y estudios para esclarecer las indicaciones más adecuadas.

Las publicaciones recientes han mostrado el papel de las células *Natural Killers* en el rechazo de aloinjerto. La recientemente llamada "vasculopatía de aloinjerto" se ha relacionado con la respuesta inflamatoria crónica mediada por *Natural Killers*. Además, los anticuerpos específicos contra el donante inducen mecanismos intracelulares que reclutan monocitos y leucocitos que lesionan el tejido trasplantado y producen degeneración del injerto. La expresión clínica de este mecanismo podría ser la oclusión, la degeneración aneurismática y la ruptura.

Estas respuestas inmunológicas podrían condicionar la evolución de los aloinjertos trasplantados y mas estudios con control de estos factores es necesario para esclarecer este punto en particular.

A nivel clínico, expusimos resultados del trasplante de aloinjertos arteriales en territorio suprainguinal donde se conocen pocas publicaciones, ninguna de ellas con “tasa de eventos mayores de la extremidad” como endpoint. Para la revascularización del territorio femoropoplíteo y distal, la tasa de permeabilidad primaria conocida de PTFE, el injerto más comúnmente utilizado, es de alrededor del 51% y el 25%,

respectivamente. Si comparamos este resultado con nuestro 43% y 34%, se justifican al menos más estudios amplios

Sin embargo, no solo es importante la permeabilidad primaria, sino también la supervivencia y la tasa de infección en el reemplazo de injerto aórtico infectado. Kieffer et al. mostró que los aloinjertos tienen una considerable reducción de la tasa de mortalidad anual en comparación con otro tipo de injertos y su uso está limitado debido a la disponibilidad de tejido. Se ha demostrado que el aloinjerto arterial tiene las tasas de reinfección más bajas solo superadas por la técnica NAIS, ello ha conllevado a su aceptación como injerto de remplazo en infecciones protésicas en territorio aórtico.

La tasa de evento mayor de la extremidad intervenida es un nuevo endpoint sugerido por Michael Conte. En nuestra cohorte, es importante resaltar que después de 5 años entre 81,2% y 86,7% (según territorio revascularizado) presentó un evento mayor en la extremidad intervenida. A primera vista, esto podría ser desalentador. Sin embargo, debido a la complejidad del llamado "paciente vascular" y sus múltiples comorbilidades sistémicas, estos resultados están de acuerdo con la evolución esperada indistintamente del tejido/injerto utilizado para revascularizar. Sin embargo, un análisis más detallado está justificado y la causa de muerte es otro aspecto a estudiar con más detalle.

Conclusion

ReVAC ha permitido, por primera vez, que Cataluña, España recopile y analice los resultados de los segmentos arteriales y válvulas cardíacas criopreservados. Este proyecto pionero ha dejado una gran herramienta disponible que permitiría la monitorización y la evaluación de la calidad del tejido criopreservado en esta

comunidad, y lo más importante, hay datos disponibles de los resultados a 5 años de cirugías de trasplante en Cataluña.

Un registro de tres niveles fue diseñado y creado. Su esquema podría ser reproducible para la creación de bases de datos y registros similares. ReVAC está disponible para el personal autorizado a través del sitio web para las aplicaciones de la "Generalitat de Catalunya".

Se han analizado las tasas de supervivencia, permeabilidad primaria, recuperación de extremidades y eventos mayores en la extremidad intervenida en pacientes trasplantados con segmentos arteriales en varias localizaciones anatómicas. Considerando el uso amplio a nivel mundial, los resultados son variados y susceptibles de análisis.

En la actualidad, solo se realiza una indicación respaldada por guías de práctica clínica (europeas y Americanas) para los aloinjertos arteriales, el reemplazo de los injertos aórticos infectados. Sin embargo, la información sobre la revascularización de las extremidades inferiores es útil para abordar proyectos futuros, que se requieren para reconsiderar indicaciones nuevas o específicas.

Las técnicas de criopreservación y técnicas quirúrgicas son determinantes en la tasa de supervivencia global en los trasplantes de aloinjerto valvular. Los cambios hemodinámicos son complicaciones esperadas de un aloinjerto valvular. Sin embargo, estos cambios no se traducen en mortalidad y la tasa de supervivencia general es buena.

La falta de disponibilidad, los costos y las preferencias del cirujano podrían ser un factor determinante en la limitación de esta práctica. Aún con resultados favorables que indican que el trasplante valvular de aloinjerto es una buena alternativa, algunos

grupos prefieren otras técnicas para evitar exponerse a la curva de aprendizaje. Nuestro grupo considera que su uso debe hacerse de acuerdo a las necesidades de cada paciente.

Varias publicaciones recientes han expuesto aloinjertos arteriales criopreservados como una alternativa atractiva en pacientes con isquemia crítica que requieren revascularización en ausencia de vena safena autóloga. Sin embargo, nuestros resultados no demuestran la superioridad de los aloinjertos sobre otros injertos en términos de tasa de salvamento de la extremidad. La tasa de supervivencia en pacientes revascularizados con aloinjertos es consistente con la esperada para pacientes con CLI. La permeabilidad primaria de los aloinjertos para la revascularización infrainguinal parece ser inferior a la vena autóloga y al PTFE, pero similar a las venas del brazo. En ausencia de una cohorte comparable, no es posible comparar la tasa de eventos adversos mayores en la extremidad intervenida de estos pacientes. Las complicaciones clásicas de los aloinjertos vasculares parecen estar relacionadas con las técnicas más antiguas de criopreservación y no son tan frecuentes como se esperaba. Se necesitan más estudios para aclarar los resultados de los aloinjertos arteriales.

Contraste de hipótesis.

Las prótesis sintéticas son de segunda línea para los cirujanos cardíacos y vasculares. Sin embargo, en la cirugía vascular, los aloinjertos son la primera opción para el reemplazo de injertos infectados en posición aórtica, y una opción considerada para la revascularización de pacientes con isquemia crónica que amenaza la extremidad.

Sin embargo, se debe realizar un análisis más profundo de los factores predictivos para elegir mejor a los pacientes que deben revascularizarse con aloinjertos.

Los aloinjertos tienen una tasa de infección más baja cuando se comparan con otros injertos. Se demostró en nuestros resultados, pero simultáneamente varias publicaciones también concluyeron lo mismo. En el período en que se realizó nuestro estudio, surgió una indicación apoyada por una guía debido a esta característica de los aloinjertos.

Las válvulas cardíacas han demostrado excelentes resultados hemodinámicos y su uso es amplio, incluido el procedimiento de Ross y casos seleccionados de defectos cardíacos congénitos.

Con respecto a ReVAC, el registro permite tener la primera base de datos autónoma con información sobre el rendimiento de los aloinjertos. Los datos extraídos del ReVAC nos permiten aclarar algunos aspectos sobre los resultados de estos injertos y, en el futuro, se podría extraer una conclusión adicional.

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